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Mechanisms of fast signaling in GABAergic interneurons

Peter Jonas[1]

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Parvalbumin-expressing basket cells (BCs) play a key role in the function of neuronal networks. They contribute to fast feedback and feedforward inhibition and also are involved in the generation of network oscillations in the gamma frequency range. BCs operate as fast signaling devices, showing a fast excitatory input, a fast-spiking action potential phenotype, and a fast inhibitory output on their target cells. We examined the mechanisms of the fast inhibitory output of BCs, using paired recordings between synaptically connected BCs and granule cells (GCs) in the dentate gyrus of acute hippocampal slices and organotypic slice cultures. We first identified the type of Ca^{2+} channel involved in transmitter release, using selective peptide blockers. Transmitter release at BC output synapses was exclusively dependent on P/Q-type Ca^{2+} channels. As these channels show fast gating, their selective involvement will contribute to fast signaling. We further attempted to identify the Ca^{2+} sensor of exocytosis, using mice in which synaptotagmin 1, the putative Ca^{2+} sensor at excitatory synapses, was genetically deleted. Whereas the phasic component of excitatory synaptic transmission at GC-BC synapses was abolished, inhibitory synaptic transmission at BC-GC synapses in organotypic slice culture was maintained. As the time course of transmitter release in knockout synapses was similar to that in wild-type synapses, these results suggest that the non-synaptotagmin 1 Ca^{2+} sensor may also contribute to rapid signaling. Finally, we probed the coupling distance between Ca^{2+} source and Ca^{2+} sensor, using Ca^{2+} chelators with different on rates. To deliver Ca^{2+} chelators at defined concentrations, pipette perfusion was combined with paired recording in acute slices. Transmitter release was sensitive to millimolar concentrations of the fast Ca^{2+} chelator BAPTA, but was almost insensitive to the slow Ca^{2+} chelator EGTA. Modeling the concentration dependence of the chelator effects revealed a coupling distance in the range of 10 – 20 nm.Furthermore, modeling showed that tight coupling promotes rapid signaling. As inhibitory synapses between interneurons are thought to be particularly important for the generation of gamma oscillations, we studied the properties of BC-BC synapses in paired recordings. We found that the kinetics of synaptic transmission at BC-BC synapses were even faster than that at BC-GC synapses. Computational analysis revealed that fast signaling at BC-BC synapses can promote the generation of gamma frequency oscillations in interneuron networks. These results provide an example how we are beginning to understand the relation between synaptic properties and complex functions of neuronal networks.

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Bartos M, Vida I, Jonas P (2007) Synaptic mechanisms of synchronized gamma oscillations in inhibitory interneuron networks. Nature Reviews Neuroscience 8:45-56.

Epilepsy and Its Models: Progress or Wrong Track?

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Epilepsy is, by itself, not a disease. Because epilepsy has many causes, it should be thought of as a chronic condition of the brain which results in the repeated occurrence of epileptic seizures. Since this is so, a huge variety of animal models exist. They range from acute causes such as applying a substance on the brain (i.e. penicillin) to initiating a status epilepticus resulting in an alteration of the hippocampus which is similar to the one found in humans suffering from mesial temporal lobe epilepsy. Thus, the latter is viewed as being a model of temporal lobe epilepsy and is therefore studied intensively. From human studies, it is clear that the major alteration of the hippocampus, however, takes place before seizures occur (initial "hit"). This epileptogenic development finally results in the epileptogenic hippocampus. Using knocked-out animals, it became clear that the temporary up-regulation of calcium channels might be the decisive reason for this epileptogenicity.

However, this finding does not solve the epilepsy problem. As previously mentioned, many disturbances of the central nervous system result in epilepsy and, in most of them, an epileptogenic period cannot be seen. However, this back and forth studying of animals and humans opens a unique possibility since the validity of animal models can be tested in correlation with the patient's disease, which is available as a specimen from epilepsy surgical procedures. From these data, it became obvious that one of the critical phenomena in pharmaco-resistant epilepsies might be the altered receptor function for the often used drug, Carbamazepine. The in-depth research in epilepsy has just recently opened; epileptogenicity, pharmaco-resistance and genetic alterations of ion channels and receptors are currently being closely examined. A clear-cut, single result cannot be expected because of the many reasons for an increased epileptogenicity of the brain. However, it should be kept in mind that the paroxysmal depolarization shift first described in 1964 as being the cellular basis of epileptic activity is still the final path where all epilepsy processes end. The inference of this is still a valuable aim for the improved treatment of epilepsy patients.

Electrical Microstimulation and fMRI

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Electrical stimulation (ES) of the brain has been performed for over 100 years, and although some might say it is a crude technique for understanding the detailed mechanisms underlying different neural computations, microstimulation has made significant contributions to our knowledge in both basic and clinical research. Recently there has been resurgence in its use in the context of electrotherapy and neural prostheses. For example, ES has made it possible to at least partially restore hearing to deaf patients by delivering pulses via implanted electrodes to different regions of the cochlea. Stimulation of the basal ganglia is remarkably effective in restoring motor function to Parkinson's patients, and microstimulation of the geniculostriate visual pathway is regarded by some as a very promising (future) method for making the blind see again.

Yet, the methodology still suffers from at least two fundamental problems; (a) we do not always know exactly what is being stimulated when we pass currents through the tissue; and (b) stimulation causes activation in a large number of areas even outside the stimulation site, making it difficult to isolate and evaluate the behavioral effects of the stimulated area itself. Microstimulation during fMRI (esfMRI) could provide a unique opportunity to visualize the networks underlying electrostimulation-induced behaviors, to map neuromodulatory systems, or to develop electrotherapy and neural prosthetic devices. Last but not least, esfMRI can offer important insights into the functional neurovascular coupling.

In my talk, I shall discuss findings from recent and on-going work on signal propagation during electrical stimulation. These findings not only offer some insights into functional neurovascular coupling and the interpretation of negative hemodynamic responses, but also reveal some interesting properties of the cortical microcircuits and the way they could propagate incoming population signals.

Cellular mechanisms underlying ß-amyloid generation and its implications for Alzheimers disease

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The human brain is essentially a network of billions of nerve cells that communicate through specialized connections called synapses. Changes in the strength of these synapses, termed synaptic plasticity, are essential for learning and memory. Synaptic dysfunction leads to neurodegeneration observed in age-related disorders such as Alzheimers disease (AD). The disease presents itself with two distinguishing features in terms of its pathogenesis, one, the formation of neurofibrillary tangles, and the other, amyloid plaques. These plaques contain the ß-amyloid peptide (Aß), which either in the plaque-associated form or in its soluble oligomeric form is thought to set in a cascade of events that eventually leads to neurodegeneration. The reduction of amyloid, even in small measures, has been shown to alleviate the disease, and is the basis for much of the research attempting to understand and cure the disease. The neurotoxic Aß peptide is derived from a large type I transmembrane protein, the amyloid precursor protein (APP). APP is cleaved sequentially by two enzymes termed ß- and γ-secretase leading to the formation of the Aß peptide. The key players in the processing of APP, i.e. ß-, γ-secretase and the substrate APP itself, are all membrane associated and hence are subjected to regulation by the lipid environment and membrane trafficking. Our work showed that these amyloidogenic cleavages occur in early endosomes followed by the routing of the cleaved product to late endosomes. Subsequently, Aß peptides can be released from the cells via the novel exosomal pathway. Exosomal proteins were found to accumulate in the plaques of AD patient brains suggesting a role in the pathogenesis of AD. Moreover, targeting a transient state analog, ß-secretase inhibitor to endosomes inhibited the secretase more efficiently than its soluble counterpart suggesting a novel therapeutic strategy.

MRI-based diagnosis of neurodegeneration using support vector machines

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Alzheimer's Disease (AD) is the commonest form of dementia. Definite diagnosis can only be made through neuropathological examination, usually after death. Conversely, potential treatments are likely to be most beneficial at the earliest stages of degeneration. Structural Magnetic Resonance Imaging has proven useful in diagnosing dementia but relies on the expertise of neuroradiologists which is often unavailable outside specialised centres.
In an international research project, we used support vector machines (SVM), a multivariate and supervised machine learning technique to aid clinical diagnosing. These algorithms combine information from several brain regions and learn to separate e.g. AD from healthy aging from a training dataset with known diagnosis.
There is an extensive amount of information in an MRI scan, most of which is unrelated to the disease process. The extraction of relevant information (feature extraction) is therefore an essential step for these methods to work successfully. Imaging segmentation into grey matter, white matter and cerebrospinal fluid was combined with spatial normalisation before entering data into linear SVM.
Patients suffering from AD were successfully separated from healthy aging and from another form of dementia in around 90% of times. Interestingly, SVM trained with data from one imaging centre performed accurate classification of data from another centre acquired using different imaging hardware. In a direct comparison with radiologists SVM were found to be at least as accurate as experienced neuroradiologists.
Results of these studies are encouraging and suggest an application of such methods to the clinical setting. Future studies should focus on less pre-selected data from patients seen in clinical practise. New methodological developments should facilitate the inclusion of additional clinical data (e.g. demographic information or basic neuropsychological testing). In addition, similar to diagnosing by radiologists, a level of diagnostic confidence should be assigned to the automatic classification.

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Semiconductor Chips for Neurophysiology

Peter Fromherz[1]

[1]Department of Membrane and Neurophysics, Max Planck Institute for Biochemistry, Martinsried-München

A dream in neurophysiology is a selective noninvasive stimulation and recording of myriads of individual nerve cells in an intact brain with a resolution of 10 micrometers and 0.1 milliseconds. At present, no method can be envisaged to implement that dream. The idea of our work is more restricted. We tried to develop an experimental technique that allows a supervision of thousands of neurons in dissociated cell culture and in cultured or acute brain slices with the goal to understand the dynamics of neuronal networks in those artificial situations. Considering the required resolution in space and time, we found it suggestive to take advantage of the highly developed technology of semiconductor chips.

In extensive studies, we elucidated the nature of signal transduction from the microelectronics of chips to the microionics of neurons and vice versa. On the biological side, we considered the levels of recombinant ion channels, of individual nerve cells from invertebrates and mammals as well as of brain tissue using retinae and cultured or acute slices. On the technological side, we studied individual microelectronic devices with capacitors and transistors of silicon chips for stimulation and recording, respectively, as well as arrays with a myriad of devices in highly integrated CMOS chips.

In all systems, the coupling of ionic and electronic currents is mediated by extracellular voltages without Faradaic current. Crucial for an efficient coupling is the geometry and electrical resistance of cell-chip and tissue-chip junctions that were investigated by optical techniques and by measurements of the intrinsic Johnson noise.

Whereas the interfacing of large neurons from leech and snail was rather straightforward, the interfacing of rat neurons was established only recently. The interfacing of networks with defined geometry – controlled by chemical, topographic or galvanotropic guidance – proved to be difficult. An interfacing of random networks on CMOS chips with a high density of capacitors and transistors appeared to be more promising to achieve two-way supervision of learning networks with neurons from snail and rat.

With respect to an interfacing of neuronal tissue, retinae with their planar structure are most closely related to cultured cells. Stimulation and recording were implemented for an epiretinal configuration of CMOS chips. Two-way interfacing was also achieved for cultured brain slices from rat hippocampus. Using CMOS chips, we recorded time-resolved maps of field potentials in the CA3 and CA1 region as well as of LTP and LTD. With acute slices, time-resolved maps of oscillations (epileptiform, theta) were recorded.

The well-understood biophysics of neuroelectronic interfacing is a basis for the development of neurosensoric and neuroprosthetic devices. The resolution of the method in space and time is unexcelled such that new questions may be addressed with respect to the spatiotemporal correlation in neuronal systems with planar structure.

Ref.: P. Fromherz, Joining Microelectronics and Microionics: Nerve Cells and Brain Tissue on Semiconductor Chips. *SolidState Electronics* 52 (2008) 1364.

The fly's self and its brain

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A growing arsenal of genetic tools has opened the doors to studying the brain in the living fly *Drosophila melanogaster*. What are we to find there beyond sensory integration and motor programming? Intuitively, we treat most animals as *subjects* granting them vision and memory, pain and sleep. Assume for a moment this intuition to be right, what, then, is it in the brain that makes the fly a subject? What is the nature of 'self-ness'? For an answer we have to look at properties such as 'autonomy', 'identity' and 'agency'. Even the simplest living creature is a highly integrated system, separated from its environment in many sophisticated ways. Most of the processes occurring in it do not transgress its boundaries. Secondly, its genetic endowment and its life history make it significantly unique, asking for preservation of its identity. Third, like most animals the fly is an agent, it generates motor activity. It therefore will encounter two kinds of sensory events, those due to this activity and those not. The lecture will describe how the fly's self shows up in stationary flight at the torque meter, and will give first examples of how this characteristic property of an animal is related to the neural circuitry and molecular properties of its brain.

Neuroscience of primate intellectual evolution

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Several recent studies report how laboratory-raised, nonhuman primates exposed to tool use can exhibit intelligent behaviors, such as imitation and reference vocal control, that are never seen in their wild counterparts. Tool-use training appears to forge a novel cortico–cortical connection that underlies this boost in capacity, which normally exists only as latent potential in lower primates. Although tool-use training is patently non-naturalistic, its marked effects on brain organization and behavior could shed light on the evolution of higher intelligence in humans.

We trained Japanese monkeys to use tools, an advanced cognitive function monkeys do not exhibit in the wild, and then examined their brains for signs of modification. Following tool-use training, we observed neurophysiological, molecular genetic and morphological changes within the monkey brain that enables to incorporate tools into their own body schema. Despite being 'artificially' induced, these novel behaviours and neural connectivity patterns reveal overlap with those of humans. Thus, they may provide us with a novel experimental platform for studying the mechanisms of human intelligence, for revealing the evolutionary path that created these mechanisms from the 'raw material' of the non-human primate brain, and for deepening our understanding of what cognitive abilities are and of those that are not uniquely human. On these bases, we propose a theory of '*intentional niche construction'* as an extension of natural selection in order to reveal the evolutionary mechanisms that forged the uniquely intelligent human brain, by which functions our cultures are formed.

As our ancestors' array of tools and tool-modified environment increased in size and complexity, selective pressure would have favoured individuals that were more adept at acquiring and mastering them. Although each step in this evolution might have been a simple association, even after just a few such steps, it may have produced something beyond a mere association. One such candidate could be the concept of the *'self*', which would have emerged through the self-objectification process to incorporate tools into their user's body schema. Thus, tool-induced environmental construction became 'intentional', which remarkably accelerated the speed of evolution. With the advent of '*intentional niche construction'*, the direction of evolution was no longer passively determined by the natural selection. Now the organisms themselves could decide how the environment should and could be remade. And, of course, each new iteration of the human-altered civilized environment influenced the development of (as well as the selection pressures on) the next generation's brains.

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T1-6A Evidences for glycinergic control of cortical migration
Denise Gabrielle Denter, Aurel Bogdan Sava, Nicolas Heck, Werner Kilb, Heiko J. Luhmann

T1-7A Scratch2 in neurogenesis of the mammalian brain
Vanessa Paul, Anastassia Stoykova

T1-8A Common Cadherin-Based Adhesive Cues Behind Neuroangiogenesis?
krishna K, Christoph Redies

T1-9A Nucleotides as potential cell cycle modulators of basal stem cells in the olfactory epithelium
Thomas Hassenklöver, Detlev Schild, Ivan Manzini

T1-10A A similar set of genes patterns the vertebrate neural plate and the insect head
Gregor Bucher, Nico Posnien

T1-11A Relation between granule cell dispersion, neurogenesis and the spread of epileptiform activity in the hippocampus
Ute Häussler, Lena Bielefeld, Martin C. Müller, Christian Garbers, Carola A. Haas

T1-12A Hypoxia Precedes Vascular Endothelial Growth Factor Up-regulation and Angiogenesis in a Mouse Model of Temporal Lobe Epilepsy

Carola A. Haas, Martin C. Müller, Susanne Huber , Matthias Osswald

T1-13A Sim1 is a novel regulator in the differentiation of a mouse serotonergic neuron subpopulation
Eleni Roussa, Nadja Osterberg, Michael Wiehle, Oliver Oehlke, Cheng Xu, Chen-Ming Fan, Kerstin Krieglstein

T1-14A Polysialic Acid in Dopaminergic System Development
Miriam Schiff, Claudia Grothe, Rita Gerardy-Schahn, Herbert Hildebrandt

T1-15A Sip1 controls progenitor fate and timing of production of cortical neurons and astrocytes
Anjana Nityanandam, Eve Seuntjens, Amaya Miquelajauregui, Sandra Goebbels, Klaus-Armin Nave, Danny Huylebroeck, Victor Tarabykin

T1-16A Characterization of primary neurospheres generated from mouse ventral rostral hindbrain.
Nadja Osterberg, Eleni Roussa

T1-17A Characterization of neurosphere-generating cells in the developing peripheral nervous system.
Ellen Binder, Hermann Rohrer

T1-18A Loss of p75 affects adult neurogenesis within the adult dentate gyrus of mice
Oliver von Bohlen und Halbach, Klaus Unsicker

T1-19A Functional Analysis of Neuroblastoma Phox2b Mutations in Immature Sympathetic Neurons
Tobias Reiff, Konstantina Tsarovina, Hermann Rohrer

T1-20A Analysis of neural stem cell identity in the larval brain of *Tribolium castaneum*
Nikolaus Bernhard Dieter Koniszewski, Hendrikje Hein, Gregor Bucher

T1-1B The bHLH transcription factor Hand2 is essential for the maintenance of noradrenergic properties in differentiated sympathetic neurons
Mirko Schmidt, Shengyin Lin, Manuela Pape, Uwe Ernsberger, Matthias Stanke, Kazuto Kobayashi, Marthe Howard, Hermann Rohrer

T1-2B Haloperidol and atypical neuroleptic drugs increase glutamate sensitivity in neurally differentiated NTera2 cells as revealed by calcium imaging
Bernhard Reuss, Liane Dahm, Fanny Klugmann, Angeles Gonzalez Algaba

T1-3B Reelin-induced phosphorylation of cofilin in neuronal processes is required for the directional migration of neurons
xuejun chai, Eckart Förster, Shanting Zhao , Hans Bock, Michael Frotscher

T1-4B Postnatal brain overgrowth in Bassoon-mutant mice involves increased hippocampal cell numbers and aberrant proliferation
Alexandra Heyden, Frank Angenstein, Bettina Kracht, Constanze Seidenbecher, Eckart Gundelfinger

T1-5B Phospho-cofilin, induced by Reelin signaling, is involved in the proper positioning of sympathetic preganglionic neurons in the spinal cord
Marie Therese Krueger, Shanting Zhao, Xuejun Chai, Hans H Bock, Michael Frotscher

T1-6B Defining in vitro conditions for enrichment of stem-like cell population in primary human brain tumor cultures

Ulf Dietrich Kahlert, Jaroslaw Maciaczyk, Guido Nikkhah

T1-7B Distinct effects of post-weaning environmental enrichment and adult wheel running on neurogenesis and synaptic turnover in the hippocampus, subiculum and entorhinal cortex of mice.
Andrea Schäfers, Keren Grafen, York Winter

T1-8B Development of the sensory innervation in the antenna of the grasshopper, Schistocerca gregaria
Tatjana Kleele, Zsofia Herbert, Bertram Niederleitner, George S. Boyan

T1-9B Integration of ES cell derived neurons into pre-existing neuronal circuits
Franziska Neuser, Martin Korte

T1-10B Neuropeptide and serotonin expression in the adult and developing central complex of the grasshopper, *Schistocerca gregaria*
Zsofia Herbert, Neval Kapan, Sandra Rauser, George Boyan

T1-11B Tamoxifen and Raloxifen change the amount and the subcellular localisation of the Gap Junction Connexin 43 in NTera-2/D1 Embryonal Carcinoma Cells
Liane Valerie Dahm, Fanny Klugmann, Bernhard Reuss

T1-12B Pax6 Promotes Neurogenesis in Human Neural Stem Cells
Therése Kallur, Ramiro Gisler, Olle Lindvall, Zaal Kokaia

T1-13B Betulinic acid changes the amount and subcellular localization of Cx43 in human NTera-2/D1 cells
Fanny Klugmann, Liane Dahm, Bernhard Reuss

T1-14B Dopaminergic differentiation of immortalized neural progenitors of the cell line CSM14.1 in vitro
Birthe-Christine Eckhoff, Stefan Jean-Pierre Haas, Grit Lessner, Oliver Schmitt, Andreas Wree

T1-15B Proliferative response of distinct precursor subpopulations and neurogenesis after cortical infarcts in the young and aged dentate gyrus.
Josephine Walter, Silke Keiner, Julia Oberland, Otto W. Witte, Christoph Redecker

T1-16B Self-renewal and differentiation in neural stem cell cultures are regulated by gp130-dependent signaling
Matthias Kirsch, Alexandra Skorupa, Hans-Dieter Hofmann

T1-17B Gene Regulation of Tenascin C and its Isoforms in the Developing Mouse Central Nervous System and Neural Stem Cells
Ursula Theocharidis , Alexander von Holst, Andreas Faissner

T1-18B Intracellular signalling and cellular remodelling induced by Epidermal Growth Factor in the adult neurogenic subventricular zone *in vivo*
Kristine Gampe, Monika Brill, Stefan Momma, Nanette Messemer, Magdalena Götz, Herbert Zimmermann

T1-19B Optical modulation of neuronal differentiation of mouse embryonic stem cells expressing Channelrhodopsin-2
Albrecht Stroh, Hsing-Chen Tsai, Li Ping Wang, Feng Zhang, Jenny Kressel, Alexander

Aravanis, Nandhini Santhanam, M. Bret Schneider, Arthur Konnerth, Karl Deisseroth

T1-1C Mapping of the embryonic isoform of the microtubule associated protein tau in the adult rat brain
Torsten Bullmann, Max Holzer, Wolfgang Härtig, Thomas Arendt

T1-2C Transforming Growth Factor ß Induces Neurogenesis through Activation of Nedd9
Nicole Buettner, Sandra Ahrens, Kerstin Krieglstein, Tanja Vogel

T1-3C S-phase marker - 5-bromo-2-deoxyuridine bioavailability after intraperitoneal injection.
Juraj Ševc

T1-4C Role of the *Tshz1* gene in olfactory bulb development
Elena Rocca, Carola Griffel, Carmen Birchmeier, Alistair Garratt

T1-5C Directed neural differentiation using protein nanoarrays on tissue-like soft substrates
Christian P. Gojak, Kerry L. Tucker, Joachim P. Spatz

T1-6C Effects of human amyloid precursor protein on hippocampal neurogenesis in transgenic mice housed in enriched environment.
Nicole Naumann, Uwe Ueberham, Thomas Arendt, Ulrich Gärtner

T1-7C Lipid phosphate phosphatases control cortical layering during embryonic development
Tanja Velmans, Arne Battefeld, Jan Baumgart, Nicolai E. Savaskan, David N. Brindley, Wouter H. Moolenaar, Robert Nitsch, Ulf Strauss, Anja U. Bräuer

T1-8C From the Matrix to the Nucleus – The Sam68 gene family in neural stem cells
Sören Moritz, Stefanie Lehmann, Andreas Faissner, Alexander von Holst

T1-9C Glial differentiation in the developing dentate gyrus of reeler mice
Bianka Brunne, Shanting Zhao, Joachim Herz, Michael Frotscher, Hans Bock

T1-10C A crucial role for primary cilia in cortical morphogenesis
Kerry Lee Tucker, Kerstin Hasenpusch-Theil, Humphrey Athelstan Roy Gardner, Igor Kitanovic, Vera C. Hirschfeld-Warneken, Christian P. Gojak, Karin Gorgas, C. Lulu Bradford, Joachim Spatz, Stefan Wölfl, Thomas Theil, Marc August Willaredt

T1-11C Expression pattern of the 473HD epitope on dividing cortical progenitors: a novel evidence for regulation of neuron generation during corticogenesis
Anika Weber, Swetlana Sirko, Andreas Faissner

T1-12C Histone deacetyltransferase-mediated control of forebrain neurogenesis: Analyzing the role of specific HDACs through RNA interference
Kathrin Weissmüller, Kerry Lee Tucker

T1-13C Functional analysis of Svet1 (Unc5h4) gene in the developing mouse neocortex
Elena Kvashnina, Vadim Beilinson, Victor Tarabykin

T1-14C shRNA-mediated knockdown of TNAP affects proliferation and differentiation in adult neural stem cell cultures
Matthias Ernst Ulrich Stanke, Boris Albuquerque, Christoph Leib, Mathias Ritter, Herbert Zimmermann

T1-15C Sonic hedgehog is a polarized signal for motor neuron regeneration in adult zebrafish.
Catherina G Becker, Michell M Reimer, Veronika Kuscha, Inga Sörensen, Thomas Becker

T1-16C Expression and functional relevance of RPTPß/ζ Isoforms in progenitors of the developing visual retina
Manuela Besser, Andreas Faissner

T1-17C Protein-Tyrosine Phosphatase MEG2 is Spatially and Temporally Regulated in the Developing Retina and is Expressed in the Retinal Stem Cell Niche
Jacqueline Reinhard, Andrea Horvat-Bröcker, Andreas Faissner

T1-18C After traumatic brain injury cells of the oligodendroglial lineage differentiate into protoplasmatic astrocytes
Anja Scheller, Johannes Hirrlinger, Frank Kirchhoff

T1-19C The identification and cell biological characterization of the neural stem/progenitor cells throughout hippocampus at early stages of embryonic development
Swetlana Sirko, Anika Weber, Andreas Faissner

Poster Topic

T2: Axon and Dendrite Development, Synaptogenesis

T2-1A Levels and regional expression patterns of major histocompatibility complex (MHC) class I genes in the brain of the common marmoset monkey (*Callithrix jacchus*)
Adema Ribic, Gabriele Flügge, Lutz Walter, Eberhard Fuchs

T2-2A Formation of GABAergic synapses occurs without the involvement of dendritic protrusions
Corette J Wierenga, Nadine Becker, Tobias Bonhoeffer

T2-3A The actin binding protein profilin1 is critical for mouse CNS development
Jan Kullmann, Sebastian Wiesner, Emerald Perlas, Reinhard Fässler, Eckhard Friauf, Walter Witke, Marco Rust

T2-4A Development of the nitric oxide/cyclic GMP signalling pathway in the nervous system of the locust embryo
Nicole Böger, René Eickhoff, Giorgio P. Martinelli, Gerd Bicker, Michael Stern

T2-5A Carbon monoxide organises NO-dependent neuronal chain migration.
Sabine Knipp, Gerd Bicker

T2-6A Pre-synaptic Vesicle Exocytosis in Human Model Neurons Generated by Spherical Aggregate Culture Method
Million Adane Tegenge, Gerd Bicker

T2-7A SynCAM1 Overexpression Increases the Number of Excitatory Synapses, *in vivo*
Alexander J. Krupp, Elissa M. Robbins, Thomas Biederer, Valentin Stein

T2-8A The role of SATB2 and CTIP2 in cortical connectivity and the elucidation of their downstream pathways
Paraskevi Sgourdou, Olga Britanova, Camino de Juan Romero , Manuela Schwark, Amanda Cheung , Zoltan Molnar , Victor Tarabykin

T2-9A Calneurons provide a Calcium-threshold for trans-Golgi network to plasma membrane trafficking
Marina Mikhaylova, Parameshwar Pasham, Thomas Munsch, Shashi Suman, Karl-Heinz Smalla, Eckart Gundelfinger , Yogendra Sharma, Michael Kreutz

T2-10A The histone deacetylase SIRT2 in axonal outgrowth and pathfinding
Kai Volker Harting, Ruwin Pandithage, Bernhard Lüscher, Bernd Knöll

T2-11A BDNF/ephrin-modulated neuronal motility relies on SRF-dependent gene expression
Christin Meier, Bernd Knöll

T2-12A Fast synaptic signalling as a guiding cue for migrating precursors of cerebellar cortical inhibitory interneurons?
Annika Kristina Wefers, Christian Haberlandt, Christian Steinhäuser, Karl Schilling, Ronald Jabs

T2-1B *Srf* mutant mice display Reeler-like phenotypes including hippocampal cell/fibre delamination and aberrant dendritic branching
Christine Carina Stritt, Bernd Knöll

T2-2B Analysis of neuronal membrane dynamics using farnesylated PA-GFP
Anne Gauthier, Roland Brandt

T2-3B Role of the microtubule associated tau protein in the shaping of neuronal dendrites
Corina Emilia Barbu, Torsten Bullmann, Jens Gerdelmann, Max Holzer, Thomas Arendt

T2-4B Survival Promoting Peptide/ Y-P30 enhances axon growth by activating Syndecan-3 signaling.
Suvarna Dash-Wagh, P. Landgraf, M R Kreutz, H C Pape, P. Wahle

T2-5B *In Vivo* Imaging of Spontaneous Activity Patterns in the Developing Mouse Visual Cortex
Friederike Siegel, Christian Lohmann

T2-6B Global deprivation of brain-derived neurotrophic factor in the CNS reveals area-specific requirement for dendritic growth
Marta Zagrebelsky, Anita Drezniak, Stefanie Rauskolb, Yves-Alain Barde, Martin Korte

T2-7B Specific role of ProfilinIIa as a mediator of structural plasticity in mature hippocampal neurons
Kristin Michaelsen, Kai Murck, Marta Zagrebelsky, Brigitte M. Jockusch, Martin Rothkegel, Martin Korte

T2-8B MOLECULAR AND CELLULAR CHARACTERIZATION OF METHYLMERCURY AND SELENIUM SYNAPTOTOXICITY IN THE DEVELOPING HIPPOCAMPUS OF RATS AND MICE
Johannes Vincenz Hradsky, Ute Kreher, Katharina Braun, Richard Nass

T2-9B The IRM proteins and their involvement in optic lobe development
Martin Helmstädter, Birgit Ahrens, Kokil Chaudhary, Karl-Friedrich Fischbach

T2-10B Counter-balancing of Eph forward and ephrin reverse signaling explains topographic guidance of retinal ganglion cell axons
Christoph Gebhardt, Martin Bastmeyer, Franco Weth

T2-11B Mapping the ‘synaptome’ of spontaneous synaptic calcium transients reveals distance dependent patterns of synaptic activation in dendrites of developing hippocampal neurons
Thomas Kleindienst, Claudia Roth-Alpermann, Tobias Bonhoeffer, Christian Lohmann

T2-12B Paternal care is essential for dendritic and synaptic development of pyramidal neurons in the somatosensory cortex
Josephine Pinkernelle, andreas Abraham, katja Seidel, carina Helmeke, katharina Braun

T2-1C Investigation of developing nerve fibres in mouse embryos by ultramicroscopy
Nina Jährling, Martin Körte, Klaus Becker, Edgar R. Kramer, Reto Weiler, Hans-Ulrich Dodt

T2-2C Neuroligin1 regulates presynaptic maturation
Nina Wittenmayer, Thomas Kremer, Frederique Varoqueaux, Nils Brose, Thomas Dresbach

T2-3C Nogo-A/RTN4-A regulates dendritic growth in developing primary hippocampal neurons.
Christine E Bandtlow, Sabrina Khantane, Gerald J. Obermair, Marta Zagrebelsky

T2-4C Calsyntenin-1 and APP specify distinct endosomal compartments in the axonal growth cone
Martin Steuble, Bertran Gerrits, José-Maria Mateos, Peter Streit, Peter Sonderegger

T2-5C The RNA-Binding Protein MARTA2 Regulates Dendritic Targeting of MAP2 mRNAs
Stefan Kindler, Krishna H. Zivraj, Monika Rehbein, Janin Schütt, Katrin Falley, Friedrich Buck, Michaela Schweizer, Dietmar Richter, Hans-Jürgen Kreienkamp

T2-6C Nitric Oxide Regulates Development of Zebrafish Motoneuron Axons
Sophie Ann Bradley, Jonathan Robert McDearmid

T2-7C Neuroligin 2 Drives Postsynaptic Differentiation at Inhibitory Contacts Through Gephyrin and Collybistin
Alexandros Poulopoulos, Gayane Aramuni, Guido Meyer, Ingo Paarmann, Nils Brose, Weiqi Zhang, Frédérique Varoqueaux

T2-8C 3D Quantification of Morphological Alterations of Coincidence Detector Neurons in the Medial Superior Olive of Gerbils During Late Postnatal Development
Philipp Lothar Rautenberg, Benedikt Grothe , Felix Felmy

T2-9C Neuronal M6-proteolipids are required for neurite extension
Patricia de Monasterio-Schrader, Ursula Fünfschilling, Agnieszka Zofia Burzynska, Leda Dimou, Matthias Klugmann, Klaus-Armin Nave, Hauke B. Werner

T2-10C Neuronal bHLH Transcription Factors NEX and NDRF are Essential Regulators of Cortical Axon Tract Formation
Ingo Bormuth, Tomoko Yonemasu, Maike Gummert, Sandra Goebbels, Victor Tarabykin , Klaus-Armin Nave, Markus H. Schwab

T2-11C Imaging the outgrowth of spinal nerves in Semaphorin3A mutant mouse embryos
Isabel Brachmann, Silke Herzer, Kerry Lee Tucker

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T3: Developmental Cell Death, Regeneration and Transplantation

T3-1A 6-hydroxydopamine lesions of nigrostriatal neurons in the mouse induces tyrosine hydroxylase expression in striatal neurons
Stefan Jean-Pierre Haas, Andreas Hilla, Dinah Reinhardt, Oliver Schmitt, Andreas Wree

T3-2A Control of neuronal apoptosis by electrical activity
Antje Golbs, Nicolas Heck, Jyh-Jang Sun, Heiko J. Luhmann

T3-3A ADAM17 overexpression induces angiogenesis by increasing the proliferation of pericytes during chicken brain microvessel development<
Juntang Lin, Cornelius Lemke, Jiankai Luo, Christoph Redies

T3-4A Differential expression of apoptosis inhibitor survivin in rat olfactory epithelium
Elke Weiler, Denis Sokolski

T3-5A Hippocampal Slices as Model for Evaluation of Neuronal Stem/Progenitor Cells to Identify Regenerative Therapies in Bacterial Meningitis
Sandra Hofer, Denis Grandgirard, Kevin Oberson, Stephen L. Leib

T3-1B The role of serum response factor in axonal regeneration
Sina Stern, Bernd Knöll

T3-2B A potent anti-NgR antibody with ligand-blocking properties does not robustly ameliorate functional deficits in two rat models for spinal cord injury
Mario Mezler, Bernhard K Mueller, Achim Moeller, Reinhold Mueller, Axel H Meyer, Martin Schmidt, Tariq Ghayur, Eve Barlow, Alfred Hahn, Jean-Chretien Norreel, Laszlo Szabo, Hans Schoemaker

T3-3B A novel synthetic silica hydrogel for culturing rat hippocampal neuronal cells in 3D
Angela Cheung, George Attard, William Gray, Philip Newland

T3-4B Ciliary neurotrophic factor stimulates axon outgrowth of mature retinal ganglion cells directly via the JAK/STAT3- and PI3K-signaling pathways and indirectly via MAPK/ERK-signaling pathway dependent glial activation
Adrienne Müller, Thomas G Hauk, Jieun Lee, Ralf Marienfeld, Dietmar Fischer

T3-1C BMP signaling induces transdifferentiation of the NR into RPE
Astrid Vogel-Höpker, Jörg Steinfeld, Paul Layer

T3-2C Suppression of fibrous scarring following spinal cord injury results in different axon growth behaviour of various spinal cord fiber tracts
Nora Schiwy, Nicole Brazda, Veronica Estrada, Hans Werner Müller

T3-3C Use of different gel matrices as implants after scar resection in chronic spinal cord injury to promote axonal regeneration
Veronica Estrada, Nicole Brazda, Nora Schiwy, Christine Schmitz, Hans Werner Müller

T3-4C Glucose-dependent insulinotropic polypeptide (GIP) and its receptor (GIPR): cellular localization, lesion-affected expression, and impaired regenerative axonal growth.
Frank Bosse, Bettina Alexandra Buhren, Marcia Gasis, Bernard Thorens, Hans Werner Müller

T3-5C THERAPEUTIC EVERY-OTHER-DAY FASTING IMPROVES RECOVERY FROM CERVICAL AND THORACIC SPINAL CORD INJURIES
Wolfram Tetzlaff, W. Plunet, F. Streijger, M. Jeong, C. Lam, J. Plemel, J. H. T. Lee, J. Liu

Poster Topic

T4: Neurotransmitters, Retrograde messengers and Cytokines

T4-1A Studies on a collembolan brain: Neuroanatomy and Immunocytochemistry
Martin Helmut Georg Kollmann, Wolf Huetteroth, Joachim Schachtner

T4-2A Does Nitric Oxide affect neuronal plasma membranes and morphological differentiation?
Sven Hippe, Christian Grote-Westrick, Rolf Heumann

T4-3A Neuronal integration of neurotransmitter inputs in *Drosophila* Kenyon cells
Davide Raccuglia, Uli Müller

T4-4A Taurine specifically activates GABAergic networks in the developing cerebral cortex
Aurel Bodgan Sava, Heiko J. Luhmann, Werner Kilb

T4-5A Action of NOS inhibitors and an NO scavenger on the expression of aggression in the cricket *Gryllus bimaculatus* (De Geer)
Almuth Maas, Klaus Schildberger, Paul A. Stevenson

T4-6A SIFamide in the brain of the honeybee
Sabine Kreissl, Jens Bierfeld, C. Giovanni Galizia

T4-1B Modulation of a locust flight steering muscle by octopamine and tyramine
Bettina Stocker, Heike Wolfenberg, Hans-Joachim Pflüger

T4-2B New, cryptic peptide derived from the rat neuropeptide FF precursor
Piotr Suder, Jolanta Helena Kotlinska, Jerzy Silberring

T4-3B How does Tan maturation and reaction kinetic affect histamine recycling from carcinine?
Bernhard T. Hovemann, Silvia Aust, Florian Brüsselbach, Stefanie Pütz

T4-4B Altered expression of peripheral blood cytokines in migraineurs.
Anne-Katrin Puschmann, Nurcan Üceyler, Felix Mattern, Claudia Sommer

T4-5B Effects of serotonergic compounds in Retinal Spreading Depression
Michaela Sieber, Wolfgang Hanke, Vera Maura Fernandes de Lima

T4-6B A comparison of the secretion of BDNF from hippocampal cultures induced by high-potassium depolarisation and backpropagating action potentials
Gopal Pramanik, Tanja Brigadski, Volkmar Leßmann

T4-1C The impact of chronic and acute administration of IGF-I on activity-dependent release of BDNF
Petra Lichtenecker, Tanja Brigadski, Volkmar Leßmann

T4-2C siRNA mediated knockdown of BDNF in CA1 pyramidal neurons of mouse hippocampal slice cultures
Julia Daniel, Volkmar Leßmann, Tanja Brigadski

T4-3C Stimulation of $P2Y_1$ receptors in the rat prefrontal cortex impairs sensory information processing
Holger Koch, Heike Franke, Thomas Krügel, Ute Krügel

T4-4C Involvement of P2Y receptors in signal transduction pathways in development and growth
Marcus Grohmann, Claudia Heine, Heike Franke

T4-5C Effects of High- and Low-Frequency rTMS on the Inhibitory Systems in Adult Rat Cortex
Alia Benali, Selcen Aydin-Abidin, Annika Mix, Jörn Trippe, Ulf T. Eysel , Klaus Funke

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T5: G Protein-linked and other Receptors

T5-1A Modulation of neuronal excitability through $GABA_B$ receptor-mediated tonic inhibition in the rat medial prefrontal cortex *in vitro*
Ying Wang, Kay Thurley, Florian B Neubauer, Hans-Rudolf Lüscher

T5-2A Pituitary adenylate cyclase-activating polypeptide (PACAP) selective receptor (PAC1R) expression during the earthworm embryogenesis
Akos Boros, Peter Engelmann, Ildiko Somogyi, Andrea Lubics, Dora Reglodi, Edit Pollak, Laszlo Molnar

T5-3A Identification of a neuropeptide S responsive circuitry connecting endopiriform cortex and amygdala
Susanne Meis, Jorge Ricardo Bergado-Acosta, Oliver Stork, Thomas Munsch

T5-4A Analysis of the gustatory receptor mediated signaling cascade in *Drosophila melanogaster*
Nico Bredendiek, Günter Gisselmann, Hanns Hatt, Eva M. Neuhaus

T5-1B Analysis of RNA editing of the mouse $5\text{-}HT_{2C}$ receptor by Real-time PCR
Axel Heinrich Meyer, Daniela Schul, Ana-Lucia Jongen-Relo, Alfred Hahn

T5-2B Carbachol promotes cortical differentiation in rat
Corinna Colovic, Silke Patz, Petra Wahle

T5-3B Dual modulatory role of GABA(B) receptors in hipoocampal parvalbumin-expressing cells
Akos Kulik, Daniel Althof, Anna Gross, Ryuichi Shigemoto, Bernhard Bettler, Michael Frotscher, Imre Vida

T5-4B Differential Expression of inhibitory neurotransmitter receptor subunits in the thalamocortical system
Simon Call, Hans-Christian Pape, Thomas Budde

T5-1C Molecular and pharmacological characterization of cockroach biogenic amine receptors
Britta Tropnmann, Arnd Baumann, Wolfgang Blenau

T5-2C Functional characterization of a novel splice variant of the human orexin type-2 receptor
Cordula Knopp, Annika Kühl, Kathrin Theil, Andreas Dendorfer, Jan Wenzel, Heinrich Terlau, Peter Dominiak, Olaf Jöhren

T5-3C Serotonin receptors 5-HT1A and 5-HT7, that activate different signalling pathways, form heterooligomers
Ute Renner, Andrew Woehler, Erwin Neher, Diethelm W. Richter, Evgeni Ponimaskin

T5-4C A possible role for TRPC channels in synaptic signals of olfactory bulb granule cells
Veronica Egger, Olga Stroh

Poster Topic

T6: Ligand-gated, Voltage-dependent Ion Channels, and Transporters

T6-11A Cell-autonomous homeostatic regulation in muscle cells of the fruit fly drosophila melanogaster.
Mario Wanischeck, Uwe Rose

T6-1B Nanomolar ambient ATP decelerates $P2X_3$ receptor kinetics
Ronald Jabs, Alexander Grote, Michael Hans, Zsolt Boldogkoi, Andreas Zimmer, Christian Steinhäuser

T6-2B The role of sodium channel availability in determining action potential conduction velocity in unmyelinated axons
Roberto De Col, Karl Messlinger, Richard Carr

T6-3B Functional interaction between T-type Ca^{2+} and A-type K^+ currents in intralaminar thalamocortical relay neurons
Thomas Budde, Tilman Broicher, Hans-Christian Pape

T6-4B A TTX resistant sodium current in presubicular neurons
Desdemona Fricker, Celine Dinocourt, Emmanuel Eugene, Ivan Cohen, John Wood, Richard Miles

T6-5B Modulation of the Ca^{2+}-conductance of Nicotinic Acetylcholine Receptors by the endogenous protein Lypd6
Marco Morsch, Martin Darvas, Ildikó Rácz , Andreas Zimmer, Seifollah Ahmadi, Dieter Swandulla

T6-6B A novel family of proteins that control trafficking and gating of AMPA receptors
Nadine Harmel, Jochen Schwenk, Gerd Zolles, Uwe Schulte, Peter Jonas, Bernd Fakler, Nikolaj Klöcker

T6-7B Contribution of A-type potassium current I_{SA} in b-AP and EPSP attenuation in hippocampal CA1 pyramidal neurons
Daniel Minge, Robert Bähring

T6-8B The chloride channel ClC-2 regulates neuronal excitability
Ilka Rinke, Valentin Stein

T6-9B Mechanisms of mGluR1-mediated synaptic signaling in central neurons
Horst-Alfred Henning, Jana Hartmann, Arthur Konnerth

T6-10B TASK-3-like currents in the medial amygdala of mice and rats
Tina Melanie Dobler, Erhard Wischmeyer, Maruschka Weber

T6-11B Species Differences in Reactivation of Diaphragm Muscle Force Generation after Organophosphate Poisoning
Thomas Seeger, Sascha Gonder, Franz Worek, Horst Thiermann

T6-1C Glycine receptor subunits in the murine cochlea: expression, localization and developmental regulation
Julia Dlugaiczyk, Stefanie Buerbank, Bernhard Schick, Kristina Becker, Jutta Engel, Cord-Michael Becker, Marlies Knipper

T6-2C Functional expression of purinergic P2X receptors in the rat suprachiasmatic nuclei.

Anirban Bhattacharya, Vojtech Vavra, Irena Svobodova, Hana Zemkova

T6-3C Role of GlyR alpha2 subunit for KCC2 expression
Stefanie Birgitta Schumacher, Daniel Quinones, Andrea Schlicksupp, Jochen Kuhse, Joachim Kirsch

T6-4C IDENTIFICATION OF INTERACTIONS OF THE NOVEL TROUT *SHAKER* a-SUBUNIT TSHA3 WITH OTHER *SHAKER* SUBUNITS
Regina Herrling, Gunnar Jeserich

T6-5C Chloride imaging in trigeminal sensory neurons of mice
Debbie Radtke, Jennifer Spehr, Hanns Hatt

T6-6C Characterisation of GABA Induced Responses of Trigeminal Sensory Neurons
Nicole Schöbel, Annika Cichy, Jennifer Spehr, Hanns Hatt

T6-7C Fragrant dioxane derivatives, a new class of positive modulators of $GABA_A$ receptors with selectivity for receptors containing $ß_1$ subunits
Günter Gisselmann, Olga A. Sergeeva, Andrea Kragler, Anja Poppek, Wiebke Fleischer, Olaf Kletke, Hanns Hatt

T6-8C Clinically used antidepressants augment cellular excitability by inhibiting tandem-pore domain potassium channels
Michaela Brigitte Eckert, Frank Döring, Erhard Wischmeyer

T6-9C Taurine is a full agonist at native and recombinant GABAA receptors.
Olaf Kletke, Günter Gisselmann, Hanns Hatt, Helmut L. Haas, Olga A. Sergeeva

T6-10C Expression and role of Ca2+ channel alpha2delta subunits in the peripheral auditory system of mice
Antonella Pirone, Annalisa Zuccotti, Christoph Franz, Lukas Ruttiger, Marlies Knipper, Jutta Engel

T6-11C T-type Calcium Current is upregulated by zinc in the hippocampus
Felix Benninger, Dana Ekstein, Albert Becker, Yoel Yaari

Poster Topic

T7: Synaptic Transmission, Pre- and Postsynaptic organization

T7-1A Large Dense Core Vesicle Exocytosis in Mouse Chromaffin Cells is regulated by Munc13s and Baiap3
Yong Shin, Jeong-Seop Rhee, Iris Augustin, Wolf J. Jockusch, Nils Brose, Sonja M. Wojcik

T7-2A Aberrant function and structure of photoreceptor ribbon synapses in the absence of Complexins 3 and 4
Kerstin Reim, Hanna Regus-Leidig, Josef Ammermüller, Johann Helmut Brandstätter, Nils Brose

T7-3A The proteome of the presynaptic active zone: from docked synaptic vesicles to adhesion molecules and maxi-channels
Walter Volknandt, Marco Morciano, Tobias Beckhaus, Michael Karas, Herbert Zimmermann

T7-4A Characterization of SV31, a novel synaptic vesicle membrane protein and putative transporter
Joern Barth, Jacqueline Burré, Tobias Beckhaus, Michael Karas, Herbert Zimmermann, Walter Volknandt

T7-5A Syndapin I deficiency leads to profound changes in dynamin localisation, synaptic vesicle morphology and recycling at presynaptic terminals.
Dennis Koch, Anne Stellmacher, Yaroslav Tsytsyura , Nataliya Glyvuk , Isabella Spiwoks-Becker, Tariq Ahmed, Rashmi Ahuja , Markus Moser , Susann Schüler, Anke Müller , Alexander Diesler , Rainer Spessert , Tobias Boeckers , Dirk Montag , Detlef Balschun , Reinhard Faessler , Jürgen Klingauf , Michael M. Kessels , Britta Qualmann

T7-6A Rapid time-course of back-propagating action potentials in dendritic spines of cortical neurons
Knut Holthoff, Arthur Konnerth, Dejan Zecevic

T7-7A Effects of nicotine on memory-related hippocampal network oscillations in the adult rat in vitro
Agustin Liotta, Gürsel Caliskan, Rizwan ul Haq, Christoph J. Behrens, Uwe Heinemann

T7-8A Characerization of the synaptic role and localization of the family of Liprins-alpha
Magdalena Zürner, Tobias Mittelstaedt, Susanne Schoch

T7-9A Activity dependent shift in GABAergic action in identified neurons of the immature neocortex
Werner Kilb, Katharina Achilles, Heiko J. Luhmann

T7-10A Activity-independent recruitment of functional AMPAR at neurexin/neuroligin contacts<
Martin Heine, Olivier Thoumine, Magali Mondin, Béatrice Tessier, Grégory Giannone, Daniel Choquet

T7-11A Neurobeachin: A novel regulator of functional receptors
Ramya Nair, Manfred Kilimann, Nils Brose, Jeong Seop Rhee

T7-12A Spine neck plasticity controls depolarization in the spine head
Åsa Grunditz, Niklaus Holbro, Lei Tian, Yi Zuo, Thomas G. Oertner

T7-13A The mechanism of reliable synaptic transmission in layer 4 of the visual cortex
Chao-Hua Huang, Takeshi Sakaba

T7-14A Impact of presynaptic voltage transients on postsynaptic spike timing at a graded synapse in the fly motion vision system
Ulrich Beckers, Martin Egelhaaf, Rafael Kurtz

T7-15A Multiquantal Release Underlies the Distribution of Synaptic Efficacies in the Neocortex
Alex Loebel, Gilad Silberberg, Daniela Helbig, Henry Markram, Misha Tsodyks, Magnus JE Richardson

T7-16A Identification and functional characterization of *Drosophila* SRPK3, a Ser/Thr kinase required for proper distribution of the active zone protein Bruchpilot in larvae
Alice Bloch, Mandy Jauch, Vanessa Nieratschker, Sonja Dippacher, Esther Asan, Erich Buchner

T7-17A Synaptic activation of group II metabotropic glutamate receptors (mGluRs) in the dentate gyrus of the rat
Vanessa Rupprecht, Nevzat Güler, Dirk Dietrich

T7-18A Ambient GABA concentration in layer I of the postnatal cortex
Sergei Kirischuk, Olga Myakhar, Knut Kirmse, Anton Dvorzhak

T7-19A Loss of Neuroligin2 disrupts GABA receptor integrity and leads to functional deficits in the mouse retina
Mrinalini Hoon, Gabriele Bauer, Nils Brose, Tobias Moser, Björn Falkenburger, Frederique Varoqueaux

T7-1B Active Zone scaffolding protein SYD-2 regulates Kinesin-3 activity
Sailaja Mandalapu, Christina Thiede, Stefan Lakaemper, Oliver Wagner, Barbara Koehler, Kang Shen, Dieter klopfenstein

T7-2B SNARE-dependence of the exocytosis at the inner hair cell ribbon synapse
Régis Nouvian, Anna Bulankina, Thomas Binz, Tobias Moser

T7-3B N-cadherin controls vesicle clustering during early synapse maturation
Kurt Gottmann, Adriana Stan

T7-4B The LEM-domain protein MAN1 is required for presynaptic function at the *Drosophila* neuromuscular junction
Annika Weyhersmüller, Nicole Wagner, Stefan Hallermann, Robert J. Kittel, Stephan J. Sigrist, Christos Samakovlis, Manfred Heckmann

T7-5B NR2B-containing receptors activated by ERK enhance presynaptic glutamate release in epileptic mice
Christine Gebhardt, Clive DaCosta, Axel Behrens, Oliver Kann

T7-6B Different input patterns to stellate versus pyramidal cells in the Entorhinal Cortex.
Prateep S Beed, Michael H K Bendels, Friedrich W Johenning, Christian Leibold, Dietmar Schmitz

T7-7B Optical recording of single synaptic vesicle fusion
Raunak Sinha, Bettina Görner, Jürgen Klingauf

T7-8B Deletion of either one of the NO receptors alters excitatory synaptic neurotransmission in the hippocampal CA1 field
Angela Neitz, Evanthia Mergia, Ulf Eysel, Doris Koesling, Thomas Mittmann

T7-9B All Munc13 isoforms are expressed and differentially distributed in the mouse retina.
Benjamin. H. Cooper, Maike Hemmerlein, Josef Ammermüller, Johann Helmut Brandstätter, Varoqueaux Frédérique

T7-10B Sequential Binding of Synaptobrevin to SNARE Partners Drives Priming and Fusion in Calcium-Mediated Neurotransmitter Release
, Jakob Balslev Sørensen

T7-11B Dendrite arborization and synapse maturation DASM1 - involvement in synaptic transmission
Matthias H Traut, Archana Mishra, Rüdiger Klein, Valentin Stein

T7-12B Cold-stable microtubule and associated proteins in the brain of hibernating hamsters
Tanja Treutlein, Torsten Bullmann, Max Holzer, Thomas Arendt

T7-13B Local vs. global temporal integration of excitatory input via activity-dependent dendritic spike attenuation.
Stefan Remy, Heinz Beck

T7-14B Role of the 65-kDa isoform of glutamic acid decarboxylase in GABAergic synaptic transmission in the lateral amygdala
Marc Vieler, Linda Paulukat, Maren Lange, Kay Jüngling, Hans-Christian Pape

T7-15B Modulation of synaptic plasticity: functional characterization of the effect of extracellular matrixcomponents on hippocampus neurons
Ainhara Aguado, Martin Pyka, Maren Geissler, Christian Horst Wetzel, Andreas Faissner, Hanns Hatt

T7-16B Bruchpilot controls the temporal precision of neurotransmitter release
Robert J Kittel, Stefan Hallermann, Dmitrij Liaschenko, Stephan J. Sigrist, Manfred Heckmann

T7-1C Novel interaction partners of AKAP79/150 in the synapse
Xenia Gorny, Marina Mikhaylova, Björn Schott, Michael Kreutz, Constanze Seidenbecher

T7-2C Identification and characterization of proteins of the *Drosophila* nervous system.
Partho Halder, Alois Hofbauer, Erich Buchner

T7-3C Excitatory actions of GABA and tyramine in the cockroach salivary gland complex.
Cathleen Rotte, Bernd Walz

T7-4C Synaptic depression at individual synapses of pyramidal neurons is governed by spine microanatomy
Niklaus Holbro, Asa Grunditz-Müller, Thomas G. Oertner

T7-5C Translaminar connectivity in superficial layers of the neocortex
Christian Wozny, Stephen R. Williams

T7-6C Stimulus induced translocation of alpha Ca^{+2}/Calmodulin Dependent Kinase II (a-CaMKII) to the electrical synapse protein connexin 36 (Cx36)
S. Serra Akinturk, Georg Zoidl, Rolf Dermietzel

T7-7C Central glutamatergic transmission is controlled by lysophosphatidic acid
Olga Kieselmann, Arne Battefeld, Bhumika Singh, Junken Aoki, Jerold Chun, Rosemarie Grantyn, Robert Nitsch, Ulf Strauss, Anja U. Bräuer

T7-8C Quantitative and qualitative modulation of synapsins by SAP47 in *Drosophila*
Tulip Nuwal, Sonja Racic, Natalja Funk, Erich Buchner

T7-9C NCAM ubiquitination is mediated by the SCF ubiquitin ligase HOS (betaTrCP2)
Simone Diestel, Daniel Schaefer, Harold Cremer, Brigitte Schmitz

T7-10C Analysis of the secretory apparatus of Trichoplax adhaerens
Maike Hartisch, Pawel Burkhard, Michael Eitel, Bernd Schierwater, Dirk Fasshauer, Frédérique Varoqueaux

T7-11C Towards a Mechanistic Model of Signaling Events Underlying Inhibitory Synapse Assembly
Tolga Soykan, Alexandros Poulopoulos, Kirsten Harvey, Celine Fuchs, Nils Brose, Frederique Varoqueaux

T7-12C Presynaptic mitochondria modulate the functional properties of individual Schaffer collateral boutons
Tobias Rose, Thomas G. Oertner

T7-13C Is CK2ß-deficiency in muscle fibers the cause for mitochondrial myopathies ?
Luca Simeone, Steffen W. Schubert, Dieter Heuss, Said Hashemolhosseini

T7-14C Fenestration of the calyx of Held during development occurs sequentially along the tonotopic axis and is influenced by afferent activity
Marc Christopher Ford, Benedikt Grothe, Achim Klug

T7-15C Small GTPases Alter Synaptic Plasticity and Function
Ruth Bartels, Claudia Miech

T7-16C An RNAi knockdown approach to elucidate the role of Mover – a vertebrate-specific presynaptic protein specifically associated with synaptic vesicles
Thomas Dresbach, Thomas Kremer, Christoph Körber, Christian Kempf, Ralph Nawrotzki, Thomas Kuner, Joachim Kirsch, Nina Wittenmayer

Poster Topic

T8: Synaptic Plasticity, LTP, LTD

T8-1A Spatial range of GABAergic synaptic plasticity in hippocampal slice cultures
Anne Schuemann, Tobias Bonhoeffer, Corette J Wierenga

T8-2A TRPV1 stimulation suppresses LTP in mice lateral amygdala
Carsten Zschenderlein, Doris Albrecht

T8-3A Glutamate receptor mobility is linked to learning and is dependent on n-cofilin mediated actin filament dynamics
Marco Rust

T8-4A Protein Synthesis and Degradation Regulate Activity-dependent Presynaptic Structural Plasticity
Imke Helling, Tobias Bonhoeffer, U. Valentin Nägerl

T8-5A Transient metabolic failure induced by short-term hypoxia results in a reversible suppression of memory consolidation-related hippocampal network oscillations in vitro
Marlene Sofie Jarosch, Christoph J. Behrens, Oliver Kann, Uwe Heinemann

T8-6A Trans-fissural propagation of stimulus-induced gamma network oscillations between the dentate gyrus and area CA1 in the hippocampus of adult rat in vitro
Christoph J. Behrens, Jakub Otahal, Tamar Dugladze, Tengis Gloveli, Anne Boehlen, Uwe Heinemann

T8-7A A role of testosterone in hippocampal dentate gyrus'synaptic plasticity and spatial learning in male rats
Kristina Schulz, Julietta U. Frey, Volker Korz

T8-8A Hippocampal CA1-LTP and reinforcement of an early-LTP by stimulation of the ventral tegmental area in freely moving rats
Thomas Scherf, Julietta U. Frey, Sabine Frey

T8-9A Depotentiation of early-LTP in the hippocampal CA1 region in freely moving rats by mild swim stress
Naira B. Yeritsyan, Hadir Hassan, Volker Korz, Sabine Frey, Julietta U. Frey

T8-10A Short-term plasticity at the embryonic *Drosophila* neuromuscular junction
Stefan Hallermann, Robert J. Kittel, Hartmut Schmidt, Stephan J. Sigrist, Jens Eilers, Manfred Heckmann

T8-11A Role of the actin network for synaptic tagging and late-LTP in hippocampal CA1 neurons
Binu Ramachandran, Sajikumar Sreedharan, Julietta Uta Frey

T8-12A Dopaminergic blockade within the nucleus accumbens core impairs hippocampal dentate gyrus long-term potentiation and spatial learning
Heena Tabassum, Volker Korz, Julietta Uta Frey

T8-1B Demonstration of mammalian ependymin-related proteins (MERPs) in the cerebellum, hippocampus and neocortical areas of the adult mouse brain by in situ-hybridisation and immunohistochemical staining.
David Hinchliffe, Sandra Schneider, Rupert Schmidt

T8-2B The role of BDNF during lesion induced facilitation of LTP in the visual cortex
Thomas Mittmann, Sarah Breiter, Silke Patz, Ismail Abidin, Ulf T. Eysel, Petra Wahle

T8-3B Influence of Nucleus Accumbens Core or Shell Stimulation on Early Long-term Potentiation in the Dentate Gyrus of freely moving rats
John Kudolo, Jorge A. Bergado, Julietta U. Frey

T8-4B Expression of new CPEB1 and CPEB2 splice isoforms in hippocampal neurons
Sada Lakshmi Turimella, Vamshidhar Reddy Vangoor, Peter Bedner, Lech Kaczmarczyk, Gerald Seifert, Christian Steinhäuser, Martin Theis

T8-5B Neuromodulatory effects of norepinephrine on stimulus-induced sharp wave-ripple complexes (SPW-Rs) in the adult rat hippocampus *in vitro*
Rizwa ul Haq, Agustin Liotta, Marlene Jarosch, Uwe Heinemann, Christoph J. Behrens

T8-6B Enhanced cortical plasticity of horizontal connections in the vicinity of focal laser lesions in the visual cortex
Barbara Imbrosci, Thomas Mittmann, Ulf T. Eysel

T8-7B Dendritic compartimentalization determines synaptic plasticity in sensory and associatve spines of the anterior piriform cortex
Friedrich W. Johenning, Prateep Beed, Michael Bendels, Dietmar Schmitz

T8-8B Protein synthesis and prolonged (late)-LTP in a hippocampal CA1 "two-input-in-vivo-model" as the precondition for "synaptic tagging"-experiments in the intact rat
Hadir Hassan, Julietta U. Frey

T8-9B Role of NogoA in regulating activity-dependent synaptic plasticity in the mature mouse hippocampus
Andrea Delekate, Marta Zagrebelsky, Martin E. Schwab, Martin Korte

T8-10B Formation, secretion and redistribution of the glycoprotein Ependymin and its functional localisation in an ultra-structural study of goldfish brain
Florian Kreul, Rupert Schmidt

T8-11B Learning head-centered representations by temporal invariance learning.
Sebastian Thomas Philipp, Frank Michler, Frank Bremmer, Thomas Wachtler

T8-12B Analysis of Dendritic Spine Plasticity with 2-Photon Glutamate Uncaging and 2-Photon Imaging
Volker Scheuss, Daniel Meyer, Tobias Bonhoeffer

T8-1C Interaction between short-term facilitation and depression at the calyx of Held
Juan D Goutman, Martin Müller, Ralf Schneggenburger

T8-2C NRG1-ErbB4 signaling modulates synaptic function in the mature cortex
Maike Gummert, Amit Agarwal, Konstantin Radyushkin, Susann Boretius, Alicja Stradomska, Irina Trembak, Elke Fuchs, Swen Hülsmann, Jens Frahm, Carmen Birchmeier, Hannah Monyer, Hannelore Ehrenreich, Weiqi Zhang, Klaus-Armin Nave, Markus Schwab

T8-3C Genetically Encoded Calcium Indicators as a Tool to Dissect Nuclear Calcium Transients Induced by different LTP Stimulation Paradigms in Acute Hippocampal Slices
H. Eckehard Freitag, Frank Hofmann, C. Peter Bengtson, Jan-Marek Weislogel, Hilmar Bading

T8-4C Role of TrkB.T1 and p75 neurotrophin receptors in shaping neuronal morphology of hippocampal neurons
Janina Huch, Kristin Michaelsen, Marta Zagrebelsky, Martin Korte

T8-5C Critical experimental conditions for spike time dependent plasticity in hippocampal slices
Elke Edelmann, Volkmar Leßmann

T8-6C When less is more: Impact of short term plasticity on spike sequence processing
Hinrich Kielblock, Marc Timme

T8-7C Magnetic Stimulation Induces Long-term Potentiation in Rat Hippocampal Slices
Tursonjan Tokay, Norman Holl, Timo Kirschstein, Volker Zschorlich, Rüdiger Köhling

T8-8C ERK-phosphorylation decides whether Jacob is a mediator of NMDA-receptor induced plasticity or cell death
Anna Karpova, Marina Mikhaylova, Yulia Vakhitova, Christina Spilker, Karl-Heinz Smalla, Werner Zuschratter, Thilo Kähne, Tobias M. Böckers, Eckart D. Gundelfinger, Michael R. Kreutz

T8-9C High resolution recording of organotypic brain slices with multi-transistor array
Christoph Hermann, Peter Fromherz

T8-10C Low-frequency stimulation of the temporoammonic pathway induces heterosynaptic disinhibition in the subiculum
Pawel Fidzinski, Matthias Wawra, Uwe Heinemann, Joachim Behr

T8-11C Role of the 5-HT4 Receptor in Morphogenic Signalling in Neurons
Fritz Kobe, Evgeni Ponimaskin, Diethelm W Richter

T8-12C Synapse-specific and compartment-specific excitation of dentate gyrus basket cells.
Marlene Bartos, S. Sambandan

Poster Topic

T9: Glia, Glia-Neuron Interactions

T9-1A Recognition, presence and survival of locust central nervous glia in situ and in vitro
Daniela Gocht, Simone Wagner, Ralf Heinrich

T9-2A Autocrine cell volume regulation of retinal glial cells: Involvement of voltage-gated calcium and sodium channels
Regina Linnertz, Peter Wiedemann, Andreas Bringmann, Antje Wurm, Thomas Pannicke, Andreas Reichenbach

T9-3A Spatial Expression of the Glutamate Transporters GLAST and GLT-1 during Postnatal Development of the Mouse Hippocampus
Alexandra E. Rduch, Christine R. Rose, Karl W. Kafitz

T9-4A Synaptically-induced intracellular sodium signals in hippocampal astrocytes *in situ*
Julia Langer, Christine R. Rose

T9-5A Ammonia inhibits mGluR-mediated calcium signaling in hippocampal astrocytes and neurons *in situ*.
Tanja Steiner, Tony Kelly, Christine R. Rose

T9-6A Characterization of synaptically-evoked calcium transients in different subtypes of hippocampal astrocytes
Silke Doris Meier, Corinna Walz, Christine Rosemarie Rose

T9-7A GABA transport-mediated calcium signaling in olfactory bulb astrocytes
Michael Doengi, Philippe Coulon, Hans-Christian Pape, Joachim W. Deitmer, Christian Lohr

T9-8A Vesicular release of glutamate and ATP along axons accounts for neuron-glia communication in the mouse olfactory bulb
Anne Rieger, Daniela Hirnet, Joachim W. Deitmer, Christian Lohr

T9-9A The 473 epitope influences axon growth and survival of cultured embryonic motoneurons
Stefan Wiese, Rebecca Conrad, Andreas Faissner, Alice Klausmeyer

T9-10A Deletion of aquaporin-4 induces osmotic swelling in retinal Müller cells
Thomas Pannicke, Antje Wurm, Ianors Iandiev, Gerald Seifert, Christian Steinhäuser, Peter Wiedemann, Andreas Reichenbach, Andreas Bringmann

T9-11A Acute osmotic swelling of retinal glial (Müller) cells evoked by glutamine - implications for hepatic retinopathy
Anett Karl, Andreas Bringmann, Andreas Reichenbach

T9-12A Post mortem activity of microglia in the mouse spinal cord
Eike D. Schomburg, Payam Dibaj, Heinz Steffens, Fabien Nadrigny, Frank Kirchhoff

T9-13A Transport and metabolism of fluorescent glucose in cerebellar slices
Patrick Jakoby, Raphael Courjaret, Anitsi Loaiza, Christian Lohr, L. Felipe Barros, Joachim W. Deitmer

T9-14A Nucleotide-mediated signaling in sustentacular cells in the olfactory epithelium
Ivan Manzini, Thomas Hassenklöver , Silvia Kurtanska, Stephan Junek, Ilonka Bartoszek, Detlev Schild

T9-1B MITOCHONDRIAL DIVERSITY AND CORRELATED DYm OSCILLATIONS AS REVEALED BY ONE- AND MULTIPHOTON IMAGING
Michael Müller, Vera Catharina Keil, Frank Funke

T9-2B Tenascin C Controls Oligodendrocyte Differentiation by Activation of Distinct Signalling Pathways
Tim Czopka, Alexander von Holst, Charles ffrench-Constant, Andreas Faissner

T9-3B The NAD^+/NADH redox state of astrocytes: impact on signal processing and gene expression.
Franziska Wilhelm, Jan Rillich, Ulrike Winkler, Johannes Hirrlinger

T9-4B Cell adhesion and structural plasticity of astrocytes: focus on vinculin
Ulrike Winkler, Marcello Sestu, Wolfgang H. Ziegler, Johannes Hirrlinger

T9-5B Activation of Microglia in the Retina evoked by varied Stimuli
Elke Ulbricht, Susann Uhlmann, Andreas Reichenbach, Mike Francke

T9-6B Pre-ischemic, but not post-ischemic Nogo-A deactivation aggravates neuronal injury after middle cerebral artery occlusion in mice: Implication of Rac1 and RhoA pathways
Ayman EL ALI, Ertugrul KILIC, Ulkan KILIC, Martin E. SCHWAB, Claudio L. BASSETTI, Dirk M. HERMANN

T9-7B Investigating the expression and function of CPEB proteins in astrocytes
Vamshidhar Reddy Vangoor, Sada Lakshmi Turimella, Lech Kaczmarczyk, Stefan Passlick, Amin Derouiche, Gerald Seifert, Christian Steinhäuser, Martin Theis

T9-8B Comparison of Cx43 knock-in reporter mice to investigate translational regulation of Cx43 in the central nervous system
Pavel Dublin, Peter Bedner, Joachim Degen, Lech Kaczmarczyk, Panos Theofilas, Amin Derouiche, Klaus Willecke, Christian Steinhäuser, Martin Theis

T9-9B How does proteoglycan deficiency affect the mouse brain?
Nora John, Detlef Balschun, Frank Angenstein, Heiko Niessen, Eckart D. Gundelfinger, Constanze I. Seidenbecher

T9-10B ECM and Synaptogenesis: Monitoring the Impact of Extracellular Matrix on Synapse Formation in Hippocampal Neurons
Martin Pyka, Christian Wetzel, Ainhara Aguado, Constanze Seidenbecher, Eckart Gundelfinger, Hanns Hatt, Andreas Faissner

T9-11B Astrocytes communicate with the calyx of Held synapse

Daniel Reyes-Haro, Margarethe Alwin, Jochen Mueller, Tatjyana Pivneva, Christiane Nolte, Helmut Kettenmann

T9-12B Cocultures of rodent olfactory ensheathing cells (OEC) and olfactory receptor neurons (ORN): synthesis of ciliary neurotrophic factor (CNTF) and neurite growth
Heike Bömmel, Axel Steinke, Esther Asan

T9-13B Activity-dependent currents recorded from astrocytes in the respiratory network
Christian Schnell, Yoshitaka Oku, Swen Hülsmann

T9-14B Functional ex vivo analysis of mouse microglia reveals developmental profiles in responses to Toll-like receptor (TLR) stimulation from birth to adulthood
Tommy Regen, Jörg Scheffel, Johannes Wessels, Shinichi Kohsaka, Wolfgang Brück, Denise van Rossum, Uwe-Karsten Hanisch

T9-1C Contribution of extracellular matrix (ECM) molecules to synaptogenesis and synaptic plasticity: studies in the quadruple knock out mice
Maren Geissler, Andreas Faissner

T9-2C Quantitative proteomic analysis of astrocytic secretion
Sidney Cambridge, Frank Bradke, Walter Nickel, Matthias Mann

T9-3C Functional interaction of trout myelin protein heterologously expressed in a mammalian oligodendroglial cell line
Katrin Klempahn, Gunnar Jeserich

T9-4C Impaired Adult Neurogenesis in Mice with reduced Cx43 Expression
Anna Stahr, Jennifer Behler, Diana Freitag, Madlen Guenther, Otto W. Witte, Christiane Frahm

T9-5C Activin A enhances NO release from microglial cells stimulated with Toll-like receptor agonists
Sandra Ebert, Sandra Ribes, Roland Nau, Uwe Michel

T9-6C Activated complement products C3a and C5a stimulate phagocytosis of *Escherichia coli* DH5a by murine microglial cells
Sandra Ribes, Sandra Ebert, Tommy Regen, Nina Adam, Uwe-Karsten Hanisch, Roland Nau

T9-7C Molecular composition of perineuronal nets
Gilbert Werner Walter Franken

T9-8C Glia-neuron interaction during hippocampal epileptiform activity
Claudia Böhm, Ulrich Paul Froriep, Ute Häussler, Ulrich Egert

T9-9C Effects of tyrphostin AG126 on Toll-like receptor (TLR)-activated microglia
Christiane Menzfeld, Konstantin Neumann, Denise Tischner, Holger M. Reichardt, Jürgen Wienands, Wolfgang Brück, Shlomo Rotshenker, Uwe K. Hanisch

T9-10C No evidence for spiking properties in NG2 glia of the mouse cerebellar white matter.
Karim LE MEUR, Anja Scheller , Khalad Karram , Jacqueline Trotter , Frank Kirchhoff

T9-11C Long-term, multi-cellular, time-lapse imaging analysis of spinal cord injury in vivo
Fabien Nadrigny, Heinz Steffens, Payam Dibaj, Anja Scheller, Eike D Schomburg, Frank Kirchhoff

T9-12C AQP4, Nestin and GFAP expression in striatal and midbrain mouse astrocytes *in vitro*
Britta Wachter, Eva Küppers

T9-13C Temporally controlled ablation of AMPA-type glutamate receptors in Bergmann glia
Aiman Samir Saab, Stephanie Rudolph, Petra G. Hirrlinger, Anja Scheller, Maria E. Rubio, Frank Kirchhoff

T9-14C Microglial contribution to neurodegeneration in the SOD1 (G93A) mouse model for ALS – a 2P-LSM study *in vivo*
Payam Dibaj, Heinz Steffens, Jana Zschüntzsch, Fabien Nadrigny, Eike D. Schomburg, Frank Kirchhoff, Clemens Neusch

Poster Topic

T10: Aging and Developmental Disorders

T10-1A Deformation-based morphometry revealed cerebellar volume alterations in rats with cortical dysplasia.
Silvio Schmidt, Martin Metzler, Christian Gaser, Karl-Heinz Herrmann, Jürgen Reichenbach, Otto W. Witte

T10-2A Defective sorting of L1 missense mutations in the endoplasmatic reticulum
Michael K.E. Schäfer

T10-3A Is there an impact of neuronal Ras activity on Rett Syndrome?
Janine Neumann, Rolf Heumann

T10-4A Proteolytic Processing of Reelin is Altered by Epileptic Activity in Rat Hippocampal Slice Cultures
Stefanie Tinnes, Michael Frotscher, Carola A. Haas

T10-1B Metabolic and structural changes in the rat brain after transient occlusion of the anterior cerebral artery
Heike Endepols, Uwe Himmelreich, Tracy Deanne Farr, Heiko Backes, Günter Mies, Rudolf Graf

T10-2B Transcriptomic Analysis of Schizophrenia-Related Brain Regions of Neuregulin-1 Deficient Mice
Philipp Kaiser, Martin Bastmeyer, Franco Weth

T10-3B Cognitive and emotional changes in the behaviour of rats after occlusion of the anterior cerebral artery
Hanna Mertgens, Günter Mies, Rudolf Graf, Heike Endepols

T10-4B Comparison of the cadherin expression in the cerebral cortex of wild type and reeler mice
Nicole Hertel, Christoph Redies

T10-1C Function of BACE1 and Neuregulins in the developing and adult brain
Alistair Garratt

T10-2C Fragile X Mental Retardation Protein regulates the levels of select scaffold proteins and glutamate receptor subunits in postsynaptic densities
Janin Schütt, Katrin Falley, Dietmar Richter, Hans-Jürgen Kreienkamp, Stefan Kindler

T10-3C Characterisation of dyslamination in focal cortical dysplasia with layer-specific markers
Susanne Fauser, Julia Nakagawa, Susanne Huber, Josef Zentner, Carola A. Haas

T10-4C Developmental Regulation of the Serotonergic System in the Brainstem of Mecp2-Deficiency
Till Manzke, Markus Niebert, Olivier Bidon, Gabriele Flügge, Diethelm W Richter

T10-5C Developmental expression of GFAP and S-100B in Fluoxetine treated rats
Nathalie Bock, Till Manzke, Veit Roessner, Aribert Rothenberger

Poster Topic

T11: Alzheimer's, Parkinson's and other Neurodegenerative Diseases

T11-1A Reduced life span and behavioural deficits in alpha-synuclein transgenics
Sonja Mendritzki, Saskia Schmidt, Stefan Kurtenbach, Eva Neuhaus, Hermann Lübbert, Christine C. Stichel

T11-2A Parkin-knockout mice: focus on mitochondrial alterations
Saskia Schmidt, Sonja Mendritzki, Christine C. Stichel, Hermann Lübbert

T11-3A Degeneration of dendrites occurs in a mouse model of Alzheimer's disease which exhibits senile plaques but not in another one producing only intracellular Aß
Ajeet Rijal Upadhaya, K.H. Wiederhold, D. Abramowski, E. Capetillo-Zarate, H. Yamaguchi, M. Staufenbiel, D.R. Thal

T11-4A Neonatal brainstem is prone to the generation of spreading depression during severe hypoxia
Frank Funke, Miriam Kron, Mathias Dutschmann, Michael Müller

T11-5A Impairment of cognitive and behavioural performance after temporary reelin knockdown in the mPFC of juvenile or adult rats
Jan Brosda, Michael Koch

T11-6A Intracellular Aß correlates with neuron loss in Alzheimer's disease
Ditte Zerlang Christensen, Sophie Luise Kraus, Johann-Christian Antonius Flohr, Marie Caroline Cotel, Oliver Wirths, Thomas A Bayer

T11-7A Gene expression analysis of axonal outgrowth factors in a neonate model of Parkinson's disease
Marie-Christin Pauly, Anna Papazoglou, Christina Hackl, Tobias Piroth, Guido Nikkhah

T11-8A Dopamine-dependent dyskinesia after grafting of serotonin neurons in relation to the proportion of grafted dopamine cells
Joanna Garcia, Thomas Carlsson, Guido Nikkhah, Christian Winkler

T11-9A DIAZOXIDE INCREASES THE NUMBER OF MITOCHONDRIA IN NEURITES AND CHANGES MITOCHONDRIAL TRAFFICKING
Regina Jakob, Ian J. Reynolds

T11-10A Nucleation-dependent aggregation of Aß is required for neuronal cell death
Marlen Schumann, Raik Rönicke, Klaus G Reymann

T11-11A Death-associated protein-kinase is activated in oxygen-glucose-deprivation induced cell death in organotypic hippocampal slice culture
Corinna Klette, Maria Straßburger, Ulrich H Schröder, Regine Schneider-Stock, Klaus G

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T11-12A ENHANCED HYPOXIA SENSITIVITY IN HIPPOCAMPAL SLICES FROM A MOUSE MODEL OF RETT SYNDROME
Marc Fischer, Julia Reuter, Florian J Gerich, Belinda Hildebrandt, Sonja Hägele, Dörthe Katschinski, Michael Müller

T11-13A Repetitive sensory stimulation training in stroke
Tobias Kalisch, Hubert R. Dinse, Julia Bohland, Matthias Kraemer, Elsche Freund, Elisabeth Beeser, Volker Hömberg, Klaus Martin Stephan

T11-14A Gene expression changes in brain and testis of Atxn3 ko mice
Ina Schmitt, Hassan Khazneh, Bernd O. Evert, Peter Breuer, Thomas Klockgether, Ullrich Wüllner

T11-15A Spectrally resolved recordings of the intrinsic optical signal in rat hippocampal slices during severe hypoxia
Maria Mané, Michael Müller

T11-16A Autoantibodies and circulating immune complexes in the plasma of Alzheimer's disease patients
Andrea marcello, Oliver Wirths, Thomas Bayer

T11-17A The influence of pellet density on the graft-induced functional recovery in a skilled paw-reaching test in the rodent unilateral 6-OHDA Parkinson's disease model
Karina Kohn Cordeiro, Anita Papazoglou, Wei Jiang, Octavia Diaconu, Fabian Büchele, Màté Döbrössy, Guido Nikkhah

T11-18A The type of amyloid b-protein (Ab) generation determines the phenotype of Ab-pathology in different mouse models of Alzheimer's disease.
Dietmar Rudolf Thal, Karl-Heinz Wiederhold, Ajeet Rijal Upadhaya, Dorothee Abramowski, Estibaliz Capetillo-Zarate, Haruyasu Yamaguchi, Matthias Staufenbiel

T11-19A L-glutamine induces apoptosis in microglia
Nina Svoboda, Hubert H. Kerschbaum

T11-20A Application of Parkinsonian toxins in the mouse retina
Gunnar Paul Harald Dietz, Florian Nagel, Mathias Bähr

T11-21A TDP-43 in ALS and FTD, a toxic gain-of-function?
Aaron Voigt, Till Marquardt, Jörg B. Schulz

T11-22A Environmental enrichment improves motor abilities but fails to rescue memory functions and neurogenesis in the APP/PS1KI mouse model of Alzheimer's disease
Marie-Caroline Cotel, Thomas A. Bayer, Oliver Wirths

T11-1B GDAP1, a protein mutated in hereditary polyneuropathy Charcot-Marie-Tooth disease 4A, protects from oxidative stress
Rebecca Noack, Svenja Frede, Axel Methner

T11-2B Hippocampal ß-amyloid plaques in triple transgenic mice revealed with a novel, fluorescent acetylcholinesterase inhibitor delivered from nanoparticles

Wolfgang Härtig, Johannes Kacza, Bernd-Reiner Paulke, Jens Grosche, Anke Hoffmann, Paul W. Elsinghorst, Michael Gütschow

T11-3B Impaired K^+-channel activity attenuates cyanide-induced hyperpolarization of CA1 pyramidal neurons in Mecp2-deficient mice
Miriam Kron, Michael Müller

T11-4B Micro-transplantation approach in a quinolinic acid induced rodent model of Huntington's disease
Wei Jiang, Máté Döbrössy, Anna Papazoglou, Fabian Büchele, Guido Nikkhah

T11-5B Epileptic seizure-induced changes in fear behaviour and neurophysiological activity in amygdaloid circuits
Jörg Lesting, Matthias Geiger, Thomas Seidenbecher, Hans-Christian Pape

T11-6B Generation of a neuron-specific nonviral gene transfer system *in vivo* – a possible therapeutical approach for neurodegenerative disorders
Susanne Rohn, Thomas Arendt, Uwe Ueberham

T11-7B Implicit memory and dopaminergic basal ganglia processes: a new rat model
Moritz Thede Eckart, Moriah C. Huelse-Matia, Rebecca S. McDonald, Rainer KW Schwarting

T11-8B Validating the use of BAC-GFP animals as tissue donors in HD graft studies
Máté Dániel Döbrössy, Nari Janghra, Stephen Dunnett, Guido Nikkhah

T11-9B Early detection of a behavioral phenotype in rats transgenic for Huntington's disease
Kerstin Alexandra Raber, Yvonne Kristin Urbach, Michael Stephan, Michael Bonin, Huu Phuc Nguyen, Stephan von Horsten

T11-10B Characterization of a transgenic rat model for spinocerebellar ataxia type 17 using comprehensive classical and automated phenotyping
Yvonne Kristin Urbach, Kerstin Alexandra Raber, Lothar Haeberle, Huu Phuc Nguyen, Olaf Riess, Holm Graessner, Peter Bauer, Hanna Regus-Leidig, Johann Helmut Brandstatter, Stephan von Horsten

T11-11B Altered phosphorylation but absence of neurodegeneration and no spine loss in a mouse model of tau hyperphosphorylation
Karolin Selle, Kirsten Oesterwind, Julia Jordan, Christian Schultz, Lars Lewejohann, Norbert Sachser, Lidia Bakota, Monika Hundelt, Roland Brandt

T11-12B The BAG protein family: Modulators of huntingtin toxicity, aggregation and localisation
Jan Liman, Nancy Dust, Sonja Hoffend, Kamila Sroka, Mathias Baehr, Pawel Kermer

T11-13B CK2 Dependent Phosphorylation Determines Cellular Distribution and Toxicity of Ataxin-3
Thorsten Müller, Bernd O Evert, Peter Breuer, Thomas Klockgether, Ullrich Wüllner

T11-14B Proteomics of the striatum, olfactory bulb and substantia nigra of 6-OHDA hemi-lesioned rats
Grit Lessner, Stefan Jean-Pierre Haas, Andreas Wree, Michael Kreutzer, Stefan Mikkat, Michael Glocker, Oliver Schmitt

T11-15B Membrane lipid modification by PUFAs promotes a-synuclein aggregate formation after oxidative stress in OLN oligodendroglial cells

Michael Riedel, Michael Wille, Christiane Richter-Landsberg

T11-16B Nuclear Aggregation of Polyglutamine-expanded Ataxin 3: Toxic Fragments Escape The Cytoplasmic Quality Control
Peter Breuer, Bernd O. Evert, Ina Schmidt, Ullrich Wüllner

T11-17B A DROSOPHILA MODEL FOR PARKINSONISM
Wright Jacob, Stefanie Pütz, Bernhard Hovemann, Rolf Heumann

T11-18B The role of microglial CPEB proteins in Temporal Lobe Epilepsy (TLE)
Lech Kaczmarczyk, Sada Turimella, Vamshidhar Vangoor, Patrick Wunderlich, Gerald Seifert, Jochen Walter, Christian Steinhäuser, Martin Theis

T11-19B Summary of electrophysiological and neurobehavioural experiments made wth 3-nitropropionic acid on rats, carried out in our laboratory
Andrea Szabó, Anita Lukács, András Papp

T11-20B Alterations in the dopaminergic system of mice with an alpha synuclein A30P point-mutation in the endogenous genomic locus
Florian Nagel, Mario Plaas , Eero Vasar , Sulev Koks, Edgar Kramer

T11-21B High-frequency stimulation of subthalamic nucleus silences excitatory synaptic transmission onto dopaminergic neurons in the substantia nigra pars compacta
Katja Lammert, Frank Steigerwald, Barbara E. Nixdorf-Bergweiler, Jens Volkmann, Christian Alzheimer, Fang Zheng

T11-1C Developmentof Anti-HLA antibodies after intrastriatal transplantation of human neuronal foetal cells in Huntington disease patients
Simone Krebs, Tobias Piroth, Talib Omer, Guido Nikkhah

T11-2C Ataxin-3 interacting transcription factors and implications for disease pathogenesis
Julieta P. Araújo, T. Klockgether, U. Wüllner, B. O. Evert

T11-3C On the integration of parahippocampal networks in epileptiform activity
Ulrich Paul Froriep, Ute Häussler, Claudia Böhm, Carola Anneliese Haas, Ulrich Egert

T11-4C Altered synaptic plasticity in dorsomedial striatum after status epilepticus
Josef Avshalomov, Timo Kirschstein, Rüdiger Köhling

T11-5C Gene therapy tools targeting the central nervous system by viral gene transfer
Pia Glöckner, James Uney, Thomas Arendt, Uwe Ueberham

T11-6C Two-strep grafting – a new method to enhance cell survival and study graft development in a rat model of Parkinson's disease
Anna Papazoglou, Fabian Buechele, Wei Jiang, Guido Nikkhah

T11-7C Potential role of the transcriptional co-activator PGC-1a in amyotrophic lateral sclerosis (ALS) - mRNA and protein expression studies in post mortem tissue of ALS patients and in the G93A transgenic ALS mouse model
Susanne Petri, Alexander Sarlette, Klaus Krampfl, Reinhard Dengler

T11-8C PKG inhibition protects photoreceptors in two mouse models for Retinitis Pigmentosa

Francois Paquet-Durand, Stefanie Hauck, Theo van Veen, Marius Ueffing, Per Ekström

T11-9C Muscarinic modulation of synaptic transmission and spontaneous activity in area CA1 of hippocampal slices from $Ca_V2.3$-deficient mice, lacking E-/R-type voltage-gated Ca^{2+} channels, and control animals.
Hanna C. Scheiblich, Ralf Müller, Anke Brockhaus-Dumke, Jürgen Hescheler, Marco Weiergräber, Toni Schneider, Peter Igelmund

T11-10C Spinocerebellar ataxia 2: cellular and molecular action of ataxin-2
Claudia Schob, Stefan Kindler

T11-11C The potential of aminoglycoside mediated gene based therapy of Usher syndrome 1C in the retina
Tobias Goldmann, Annie Rebibo-Sabbah, Nora Overlack, Igor Nudelman, Valery Belakhov, Timor Baasov, Tamar Ben-Yosef, Uwe Wolfrum, Kerstin Nagel-Wolfrum

T11-12C Comparative analysis of the influence of Refsum disease-associated branched chain fatty acids, pristanic acid and phytanic acid, on cell physiology in neural cells in culture
Sabine Rönicke, Stefan Kahlert , Georg Reiser

T11-13C Role of CPEBs in development and progression of temporal lobe epilepsy
Martin Theis, Peter Bedner, Kerstin Hüttmann, Vamshidhar Vangoor, Stefan Paßlick, Lech Kaczmarczyk, Eric Kandel, Christian Steinhäuser

T11-14C JNK proteins at adult rat brain mitochondria: dynamic changes of isoform presence and activity following ischemia
Thomas Herdegen, Yi Zhao, Ruwen Boehm

T11-15C Prevention of non-native disulphide bridges formation in tau protein without the use of reducing agent
Gabriela Krajciova, Rostislav Skrabana, Peter Filipcik, Michal Novak

T11-16C BAG1 mediated neuroprotection in *in vivo* and *in vitro* models of Parkinson's disease
Christoph Peter Dohm, Anja Baumann, Marlena Schnieder, Jan Liman, John C. Reed, Mathias Bähr, Pawel Kermer

T11-17C Molecular pathology of the motoneuron disease Spinal Muscular Atrophy
Nölle Anna, Jeroen van Bergeijk, Peter Claus

T11-18C Pharmacological modification of ATP- dependent microglial activation in the disease model of ALS
Jana Zschüntzsch, Swen Hülsmann, Christian Schnell, Payam Dibaj, Clemens Neusch

T11-19C Selective drug resistance in immature rat temporal cortex
Abdul Wahab, Klaus Albus, Uwe Heinemann

T11-20C Innate-adaptive immune cross-talk in a mouse model of Parkinson's disease
Candan Depboylu, Jean-Pierre Ghobril, Günter Höglinger

T11-21C Perineuronal nets are largely unaffected in Alzheimer model Tg2576 mice
Markus Morawski, Sanja Pavlica, Gudrun Seeger, Jens Grosche, Elena Kouznetsova, Reinhard Schliebs, Gert Brückner, Thomas Arendt

T11-22C Biochemical and genetic analysis of Parkinsons disease-associated proteins, molecular transporters, and stress response proteins in *C. elegans* models of manganism
Richard Nass, J. LEVORA, R. SETTIVARI

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T12: Neuroimmunology, Inflammation, and Neuroprotection

T12-1A Temporal expression of markers for revascularization in the injured rat spinal cord.
Marie-Françoise Ritz, Bertha Gutierrez, Oliver Hausmann, Ursula Graumann

T12-2A ischemic preconditioning attenuateS mitochondrial apoptosis Induced by global brain ischemia.
Peter Racay, Maria Chomova, Zuzana Tatarkova, Peter Kaplan, Jozef Hatok, Dusan Dobrota

T12-3A Expression of two-pore domain potassium channel TASK2 is altered in T lymphocte subsets of multiple sclerosis patients
Stefan Bittner, Alexander M. Herrmann, Max-Philipp Stenner, Kerstin Göbel, Patrick Meuth, Thomas Budde, Heinz Wiendl, Sven G. Meuth

T12-4A Neuroprotective effects of the survival promoting peptide Y-P30
Jenny Schneeberg, Monika Riek-Burchardt, Holger Braun, Peter Landgraf, Michael R. Kreutz, Klaus G. Reymann

T12-5A Novel ligands of the mitochondrial Translocator Protein (TSPO) as neuroprotective agents
Jehuda Arieh Veenman, Inbal Maniv, Alex Shterenberg, Evgeny Levin, Svetlana Leschiner, Einav Hadad-Tsoglin, Bishnu Dutta, Ilan Marek, Moshe Gavish

T12-6A Retinoic acid affects the expression of the pro-inflammatory cytokine IL-1ß in astrocyte primary cultures.
Philipp J. Imholz, Sabien van Neerven, Tommy Regen, Uwe-Karsten Hanisch, Jörg Mey

T12-7A CD8+ lymphocyte-mediated injury of CNS neurons: relevance of granzyme B and perforin for acute electrophysiological consequences and long-term neurotoxicity
Ole Jan Simon, Sven Günther Meuth, Alexander Michael Herrmann, Stefan Bittner, Peter Friedl, Thomas Budde, Thomas Hünig, Manfred Heckmann, Heinz Wiendl

T12-8A A novo specific 5-HT_{2B}-receptorantagonist for the prophylactic treatment of migraine
Daniel Segelcke, Michael Andriske, Xinran Zhu, Beate Schmitz, Andreas Popp, Frank Paris, Hermann Lübbert

T12-9A Collateral damage of CNS neurons during an acute oligodendrocyte-directed attack by $CD8^+$ and $CD4^+$ T cells
Kerstin Göbel, Nico Melzer, Alexander Herrmann, Chi Wang Ip, Thomas Hünig, Sven G. Meuth, Heinz Wiendl

T12-1B HUNTINGTON'S DISEASE RELATED MITOCHONDRIAL TOXINS AFFECT THE IMMUNOLOGICAL PROFILE OF MICROGLIAL CELLS TOWARDS A REDUCED ALTERNATIVE ACTIVATION
Annette Isabel Ferger, Irma Merdian, Albert Christian Ludolph, Anke Witting

T12-2B Retinoic acid reduces inflammatory chemokine production by astrocytes *in vitro*
Jörg Mey, Sabien van Neerven, Tommy Regen, Uwe-Karsten Hanisch

T12-3B A novel class of immunosuppressive compounds ameliorates experimental autoimmune neuritis.
Gerd Meyer zu Hörste, Anne Mausberg, Bianca Wolff, Tatjana Males, Hans-Peter Hartung, Carsten Korth, Bernd C. Kieseier

T12-4B Is the voltage-dependent anion channel 1 (VDAC-1) involved in Ha-Ras-mediated neuronal protection?
Sebastian Neumann, Konstantin Kuteykin-Teplyakov, Rolf Heumann

T12-5B Specific knock-down of RhoA, ROCK2 and LIMK1 promotes neurite outgrowth and axonal regeneration
Jan Christoph Koch, Uwe Michel, Johanna Knöferle, Lars Tönges, Mathias Bähr, Paul Lingor

T12-6B Anti-inflammatory but no neuroprotective effect of adjuvant glycerol in experimental meningitis
Cornelia Blaser, Angela Buehlmann, Kevin Oberson, Stephen Leib

T12-7B Altered cytokine expression patterns in patients with chronic musculoskeletal pain
Saskia Hahnenkamp, Nurcan Üçeyler, Claudia Sommer

T12-8B Pro-inflammatory cytokine expression following transient retinal ischemia/reperfusion in the rat eye. Modulation by simvastatin.
Franziska Walther, Christian Schmeer, Otto W. Witte, Stefan Isenmann

T12-9B MS-like cerebral inflammatory pathology in mice: A new experimental model in MS research
Angelika Escher, Stefan Nessler, Patrick Vollmar, Doron Merkler, Susann Boretius, Wolfgang Brück, Christine Stadelmann

T12-10B Neuro- and gliotoxicity of engineered nanoparticles
Susanne Bastian, Maria Iwe, Roland Holke, Tobias Meißner, Volkmar Richter, Annegret Potthoff, Armin Springer, Michael Gelinsky, Wolfgang Pompe, Hrissanthi Ikonomidou

T12-1C Status epilepticus: Expression of matrix metalloproteinases MMP-9 and MMP-2 in the developing rat brain
Yvonne Hoehna, Ortrud Uckermann, Marlen Habel, Tomasz Górkiewicz, Maciej Gawlak, Grzegorz M. Wilczynski, Leszek Kaczmarek, Chrysanthy Ikonomidou

T12-2C Toll-like receptor 4/MyD88 pathway mediates the microglial proinflammatory response to thrombin-associated plasma-derived protein complexes
Jörg Scheffel, Denise van Rossum, Jonathan R. Weinstein, Hassan Dihazi, Tommy Regen, Jens Kopatz, Wolfgang Brück, Helmut Kettenmann, Marco Prinz, Thomas Möller, Uwe-Karsten Hanisch

T12-3C Delayed Erythropoietin administration promotes neuronal survival and axonal sprouting with an increase in the motor recovery after mild focal cerebral ischemia in mice
Raluca Vig, Ülkan Kilic, Ertugrul Kilic, Max Gassman, Dirk M. Hermann

T12-4C Y-P30 or how does the maternal immune system participates in building up the embryonic brain?

Chloé Michel, Peter Landgraf, Ana Claudia Zenclussen, Petra Wahle, Michael Rudolf Kreutz

T12-5C Increased Inwardly Rectifying Potassium Conductance and Kir2 Channel Expression in Dentate Gyrus Granule Cells in Temporal Lobe Epilepsy
Michael Stegen, Christina C. Young, Martin Müller, Rüdiger W. Veh, Josef Bischofberger, Carola A. Haas, Jakob Wolfart

T12-6C Increased leak conductance in dentate gyrus granule cells of temporal lobe epilepsy patients with Ammon's horn sclerosis
Christina C. Young, Michael Stegen, Carola A. Haas, Josef Zentner, Jakob Wolfart

T12-7C Sustained oligodendroglial recruitment after repetitive cortical inflammatory demyelination
Enrique Garea Rodriguez, Mario Kreutzfeldt, Wolfgang Brück, Christine Stadelmann, Doron Merkler

T12-8C Cerebral peroxisome proliferator-activated receptors gamma (PPARγ) and the regulation of interleukin-1ß and interleukin-1 receptor antagonist expression after focal cerebral ischemia in rats
Juraj Culman, Torben Glatz, Ivonne Stöck, Peter Gohlke, Thomas Herdegen, Yi Zhao

T12-9C ROLE OF DIFFERENT CTL-EFFECTOR MOLECULES IN DAMAGING THE NEURO-AXONAL UNIT IN VIVO
Mario Kreutzfeldt, Doron Merkler

T12-10C Ageing effect of Trem2 expression after MCAO in mice
Matthias W. Sieber, Robert Zuender, Ralf A. Claus, Otto W. Witte, Christiane Frahm

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T13: Cognitive, Emotional, Behavioral State Disorders and Addiction

T13-1A Hemispheric differences, diurnal and stress-induced changes in the morphology of pyramidal neurons in the rat prelimbic cortex
Gabriele Flügge, Claudia Perez-Cruz, Boldizsar Czeh, Maria Simon, Eberhard Fuchs

T13-2A Chronic Restraint Stress Impairs Endocannabinoid Mediated Suppression of GABA Release in the Hippocampus of Rat
Wen Hu, Mingyue Zhang, Boldizsar Czeh, Weiqi Zhang, Gabriele Flügge

T13-3A Activities of the intracellular signaling protein Ras in differentiated neurons correlate with antidepressant-like behavior in mice
Oliver Leske, Zoe Bichler, Rolf Heumann

T13-4A Differential effects of lesions of the anterior cingulate cortex or lesions of the orbitofrontal cortex on extinction, spontaneous recovery and reinstatement of an avoidance response
Maria Imelda Noblejas, Wolfram Wetzel, Frank W. Ohl

T13-5A AMPA receptor subunit 1 (GluR-A) knockout mice model the glutamate hypothesis of depression
Miriam Annika Vogt, Sabine Chourbaji, Fabio Fumagalli, Reinhard Sohr, Angelisa Frasca, Christiane Brandwein, Heide Hörtnagl, Marco Andrea Riva, Rolf Sprengel, Peter Gass

T13-6A Behavioural and metabolic effects of chronic Cannabidiol and [3-(3-carbamoylphenyl)phenyl] N-cyclohexylcarbamate (URB 597) administration in adult Lister hooded rats (*Rattus norvegicus*)
Cathrin Jöpen, Franziska Pahlisch, Heike Endepols, F. Markus Leweke

T13-7A Extracellular cortical serotonin and depression-related behaviour in the forced swim test are influenced by interleukin-2
Britta D. Karrenbauer, Christian C. Müller, Rainer K.W. Schwarting, Rainer Spanagel, Jo P. Huston, Cornelius R. Pawlak

T13-1B Initial Sensitivity to Cocaine's Stimulant Effects Predicts Distinct Peptide Changes in the Medial Prefrontal Cortex
Elena V. Romanova, Jessica J. Stanis, Ji Eun Lee, Neil L. Kelleher, Joshua M. Gulley, Jonathan V. Sweedler

T13-2B Measuring basal and complex behaviors of rats in automated social home cage systems using IntelliCage for rat technology
Thomas Appl , Elisabetta Vannoni, Frank Buschmann, Yvonne Urbach, Kerstin Raber, Hans-Peter Lipp, Stephan von Hörsten

T13-3B Response-contingent changes in dopamine D_1 receptors in the rat prefrontal cortex during cocaine self-administration and its withdrawal
Malgorzata Filip, Przemyslaw Adamczyk, Lucyna Antkiewicz-Michaluk, Edmund Przegalinski

T13-4B Altered affective behavior in a model of multiple sclerosis: impact of neurotrophic factors
Isabella Peruga, Georg Juckel, Ralf Gold, Ralf A. Linker

T13-5B Adult female Wistar rats derived from three different breeders vary in behavior and epileptogenesis in the kindling model of temporal lobe epilepsy
Christoph Lindemann, Kathrin Töllner, Manuela Gernert

T13-6B Lithium modifies the architecture of the dentate gyrus by affecting Cajal-Retzius cells in hippocampal slice cultures
Joël Jarowyj, Michael Frotscher, Eckart Förster

T13-7B Cognitive function and emotional behaviour in the rat 6-hydroxydopamine Parkinson model
Andrea Bowe, Sabrina Winter, Joachim K Krauss, Kerstin Schwabe

T13-1C Involvement of the endocannabinoid system in differences in emotional behavior and reward sensitivity in three different rat strains
Theresa Brand, Rainer Spanagel, Miriam Schneider

T13-2C Neurosciences, Ethics, and Society
Saskia K. Nagel

T13-3C Individual anxiety-like trait behaviour affects social interaction behaviour in adult rats
Peggy Schneider

T13-4C Behavioural and neurobiological changes in reward sensitivity while pubertal development in rats
Chris Maria Friemel, Rainer Spanagel, Miriam Schneider

T13-5C Methylphenidate treatment and stress differentially modify gene expression of immediate early genes in the DAT knockout mouse, a mouse model for ADHD
Angelika G. Schmitt, F. Scott Hall , Maria T. G. Perona, Gabriela Ortega, Miryame Hofmann, Carola Gagel, I. Sora, G. R. Uhl, Klaus-Peter Lesch, Manfred Gerlach, Edna Grünblatt

T13-6C Proteomic approach to synapse proteins putatively involved in the synaptic pathology of schizophrenia
Karl-Heinz Smalla, Marina Mikhaylova, Jale Sahin, Hans-Gert Bernstein, Bernhard Bogerts, Andrea Schmitt, Roel van der Schors, August B Smit, Ka Wan Li, Eckart D Gundelfinger, Michael R Kreutz

T13-7C Effects of chronic Cannabidiol and [3-(3-carbamoylphenyl)phenyl] N-cyclohexylcarbamate (URB 597) administration in adult Lister hooded rats (*Rattus norvegicus*) on endocannabinoids and related lipids in different brain regions
Franziska Pahlisch, Cathrin Jöpen, Heike Endepols, F. Markus Leweke

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T14: Vision: Invertebrates

T14-1A Do descending neurons of the Locust *Schistocerca gregaria* respond to polarized light?
Ulrike Träger, Uwe Homberg

T14-2A Enhanced sensitivity to stimulus discontinuities by adaptation of a fly visual motion-sensitive neuron
Rafael Kurtz, Hanno Gerd Meyer, Martin Egelhaaf, Roland Kern

T14-3A Testing Image Matching in Honeybees using Computer Simulations of Landmark Manipulation Experiments
Wolfgang Stürzl, Laura Dittmar, Norbert Boeddeker, Martin Egelhaaf

T14-4A Representation of object motion by tangential cells of blowfly
Pei Liang, Jochen Heitwerth, Roland Kern, Martin Egelhaaf

T14-5A Transformation of receptive field structure and ocular dominance between different stages of the polarization vision pathway in the brain of the locust
Basil el Jundi, Stanley Heinze, Keram Pfeiffer, Uwe Homberg

T14-6A Dendritic integration of local motion signals in motion-sensitive neurons of the fly
Christian Spalthoff, Rafael Kurtz

T14-7A Exploring landmark cues in honeybee navigation
Laura Dittmar, Wolfgang Stürzl, Norbert Boeddeker, Martin Egelhaaf

T14-8A Synchronization of the wing beat cycle of the desert locust *Schistocerca gregaria* by periodic light flashes
Fabian Schmeling, Uwe Homberg, Gert Stange

T14-9A How the structure of homing behaviour shapes the responses of motion sensitive neurons in honeybees
Norbert Böddeker, Laura Dittmar, Wolfgang Stürzl, Martin Egelhaaf

T14-10A Synaptic Plasticity in Visual Pathways in the Brain of the Desert Ant *Cataglyphis fortis*
Sara Mae Stieb, Thomas Sebastian Muenz, Ruediger Wehner, Wolfgang Rössler

T14-1B Local and Global Visual Motion Sensitivity in Two Descending Neurons of the Fly
Adrian Wertz, Johannes Plett, Jürgen Haag, Alexander Borst

T14-2B HS-cells in the visual system of *Drosophila melanogaster* respond selectively to large-field horizontal motion conveyed via the L1 and L2 lamina pathways.
Bettina Schnell, Shamprasad Varija Raghu, Alexander Borst , Dierk F. Reiff

T14-3B Different receptive fields in axon terminals and dendrites underlie robust population coding in blowfly visual interneurons
Yishai Michael Elyada, Juergen Haag, Alexander Borst

T14-4B Relating neuronal to behavioural performance: Variability of optomotor responses in the blowfly
Ronny Rosner, Anne-Kathrin Warzecha

T14-5B A comparative study of dipteran flight styles and their impact on vision
Bart R. H. Geurten, Elke Braun, Roland Kern, Martin Egelhaaf

T14-6B Behavioural disambiguation of the responses of motion sensitive neurons
Jens Peter Lindemann, Pei Liang, Martin Egelhaaf

T14-7B Multimodal sensory integration in a fly motoneuron
Juergen Haag, Adrian Wertz, Alexander Borst

T14-8B Light dependent Translocation of the *Drosophila* TRPL Ion Channel to an intracellular Storage Compartment is accomplished by vesicular Transport
Claudia Oberegelsbacher, Armin Huber

T14-9B When eyes are *dimmed*: genetic conversion of *Drosophila* photoreceptor synaptic terminals to neuroendocrine terminals
Ian A. Meinertzhagen, Yoshitaka Hamanaka, Dongkook Park, Paul H. Taghert

T14-10B Plasticity of the intrinsic visuo-motor representation for flight in *Drosophila*
Fritz O, Lehmann, Thomas Hesselberg, Nicole Heymann

T14-1C Staring at the Sun - Outdoor Performance of Blowfly Photoreceptors
Anne-Kathrin Warzecha, Jan Grewe, Matti Weckström, Martin Egelhaaf

T14-2C Segmentation of Honeybee Flight Trajectories into Prototypical Movements for Analysing Navigation Behaviour
Elke Braun, Laura Dittmar, Bart Geurten, Martin Egelhaaf

T14-3C Convergence of compound eye and ocellar signals in Lobula Plate Tangential Cells of the blowfly, *Calliphora vicina*
Matthew M Parsons, Holger G Krapp, Simon B Laughlin

T14-4C Modulation of visual information processing in blowfly lobula plate tangential cells by an octopamine agonist
Kit D. Longden, Holger G. Krapp

T14-5C Analysis of the visual motion detection pathway in *Drosophila* with RicinA induced cell ablation.
Alexander Attinger, Jing Shi, Dierk Reiff, Axel Borst, Steven N. Fry

T14-6C Effects of active head movement on near-range tactile sensing and far-range vision
Volker Dürr, Jan M. Ache, André F. Krause

T14-7C New eyes on visual habituation in locust: an experiment description language for integrative neuroscience
Thomas A Nielsen, Henrik Nilsson, Tom Matheson

T14-8C A robotic platform to study closed-loop optomotor control in the blowfly
Naveed Ejaz, Kristopher Peterson, Holger G Krapp

T14-9C Reverse engineering speed control in the fruit fly *Drosophila Melanogaster*
Vasco Medici, Steven N Fry

Poster Topic

T15: Vision: Retina and Subcortical Pathways

T15-1A ERG recordings in two phyllostomid bats, *Glossophaga soricina* and *Carollia perspicillata*: light adaptation and action spectra>
Brigitte Müller, Gabriel Knop, Leo Peichl, Josef Ammermüller

T15-2A Effects of presynaptic mutations on a postsynaptic Cacna1s calcium channel co-localized with mGluR6 at mouse photoreceptor ribbon synapses
Susanne tom Dieck, Marion Maw, Johann Helmut Brandstätter, Dana Specht

T15-3A Epithelial sodium channels (ENaCs) in the retina and their possible involvement in the pathogenesis of glaucoma
Christine Schlegel, Christian Schön, Bettina Krüger, Ralf Enz, Johann Helmut Brandstätter

T15-4A A novel type of interplexiform amacrine cell in the mouse retina
Karin Dedek, Tobias Breuninger, Luis Pérez de Sevilla Müller, Stephan Maxeiner, Klaus Willecke, Thomas Euler, Reto Weiler

T15-5A Pericentrin, a centrosomal protein, identified at the basal-body complex in mammalian photoreceptor cells
Andreas Gießl, Johann Helmut Brandstätter

T15-6A Munc13 knock-in mice define segregated neurotransmitter release sites in the retina
Maike Hemmerlein, Frédérique Varoqueaux, Ben Cooper, Nils Brose, Johann Helmut Brandstätter

T15-7A A special kind of reflecting layer: The *Tapetum lucidum* of the elephant nose fish (*Gnathonemus petersii*).
Janina Gentsch, Elke Ulbricht, Felix Makarov, Jens Grosche, Andreas Reichenbach, Mike Francke

T15-8A Protocadherin ß16 at AMPA and kainate receptor containing synapses of specific neurons in the outer plexiform layer of adult primate retina
Christian Puller, Silke Haverkamp

T15-9A Horizontal cell sprouting and the formation of ectopic synapses in the outer retina of mutant mice lacking functional rods and cones
Silke Haverkamp, Isabella Spiwoks-Becker, Stylianos Michalakis, Martin Biel

T15-10A Electrophysiological Characterization of the Neurons in the Tectum Opticum of the Goldfish Regarding "Color" and "Motion"
Morna Gruber, Konstantin Behrend, Christa Neumeyer

T15-11A Presynaptic cytomatrix proteins at the photoreceptor ribbon synapse

Dana Specht, Jenny Atorf, Jan Kremers, Toshihisa Ohtsuka, Marion Maw, Johann Helmut Brandstätter, Susanne tom Dieck

T15-12A Retina Optics I: Visualization of light propagation through the vertebrate retina
Silke Agte, Sabrina Matthias, Kristian Franze, Martin Gryga, Leo Peichl, Thomas Cremer, Moritz Kreysing, Jochen Guck, Josef Käs, Andreas Reichenbach

T15-13A Retina Optics II. Nuclear architecture of rod photoreceptors adapts to vision in the evolution of mammals
Leo Peichl, Irina Solovei, Moritz Kreysing, Christian Lanctôt, Süleyman Kösem, Jochen Guck, Andreas Reichenbach, Boris Joffe, Thomas Cremer

T15-14A Retina Optics III: Living Optical Elements in the Vertebrate Retina
Jochen Guck, Moritz Kreysing, Kristian Franze, Leo Peichl, Irina Solovei, Thomas Cremer, Boris Joffe, Andreas Reichenbach

T15-15A Retina Optics IV. Nuclear Architecture of Rod Photoreceptors in Postnatal Development
Martin Gryga, Silke Agte, Boris Joffe, Thomas Cremer, Leo Peichl, Jochen Guck, Irina Solovei, Andreas Reichenbach

T15-1B Darkness-induced effects on rod ribbon synapses in Bassoon mutant mice
Isabella Spiwoks-Becker, Roman Lamberti, Rainer Spessert, Johann Helmut Brandstätter, Susanne tom Dieck

T15-2B Chromatic pathways in the mouse retina
Tobias Breuninger, Christian Puller, Silke Haverkamp, Thomas Euler

T15-3B Contrast-Dependent Temporal Resolution
Verena Marie Vergin, Carlos Mora-Ferrer

T15-4B Experiments on Spatial Depth Perception in Goldfish
Birte Frech, Anja Seegmüller, Christa Neumeyer

T15-5B optic flow generation and processing in free flight neuroethological insights from the zebra finch
Dennis Eckmeier, Roland Kern, Martin Egelhaaf, Hans Joachim Bischof

T15-6B Electrical Stimulation of The Human Retina With a Wireless Intraocular Retinal Prosthesis
Susanne Klauke, Michael Goertz, Stefan Rein, Dirk Hoehl, Uwe Thomas, Reinhard Eckhorn, Frank Bremmer, Thomas Wachtler, EPIRET3 Group

T15-7B Norrin is an angiogenic factor that protects against vascular degeneration in retinopathy of prematurity
Andreas Ohlmann, Roswitha Seitz, David Seitz, Barbara M. Braunger, Michael R. Bösl, Ernst R. Tamm

T15-8B Anatomy and Physiology of the tectum opticum in the weakly electric fish *Gnathonemus petersii*
Roland Pusch, Gerhard von der Emde, Björn Karpestam, Hans-Joachim Wagner, Jacob Engelmann

T15-9B Usher Protein MyosinVIIa Expression in the Zebrafish Retina

Corinne Hodel, Martina Heidemann, Oliver Biehlmaier, Matthias Gesemann, Stephan C F Neuhauss

T15-10B Optic nerve transection and crush lesion increases cell proliferation in the adult rat retina
Stefanie, G. Wohl, Christian W. Schmeer, Otto W. Witte, Stefan Isenmann

T15-11B Impaired Energy Metabolism Leads to Reduced Vision in the Zebrafish *noir* Mutant
Colette Michèle Maurer, Helia Berrit Schönthaler, Stephan C.F. Neuhauss

T15-12B Delayed ribbon precursor sphere formation during photoreceptor synaptogenesis in the absence of Bassoon
Hanna Regus-Leidig, Susanne tom Dieck, Johann Helmut Brandstaetter

T15-13B Hypothyroidism induces changes in adult mouse cone opsin expression and electroretinogram
Josef Ammermueller, Anika Glaschke, Leo Peichl, Martin Glösmann

T15-14B Role of Metabotropic Glutamate Receptors in the Zebrafish Retina
Marion Haug, Ying-Yu Huang, Stephan C. F. Neuhauss

T15-15B Characterization of Shepherd´s Crook neurons in the chicken optic tectum
Oguzhan Angay, Ulrike Kretzinger, Harald Luksch, Stefan Weigel

T15-1C Subcompartmental distribution of Cx45 on bipolar cells in the mouse retina
Gerrit Hilgen, Karin Dedek, Julia von Maltzahn, Klaus Willecke, Reto Weiler

T15-2C Electrical image of the nerve fibre layer of the rabbit retina
Günther Zeck, Armin Lambacher, Peter Fromherz

T15-3C Retinal cone opsin expression differs between wildtype and albino deer mice
Patrick Arbogast, Leo Peichl, Martin Glösmann

T15-4C Morphological and functional consequences of inducible ablation of retinal horizontal cells in living mice
Ulrike Janssen-Bienhold, Stephan Sonntag, Konrad Schultz, Kerstin Wellershaus, Klaus Willecke, Reto Weiler

T15-5C Light evoked current responses of bipolar cells in a retina with uncoupled horizontal cells
Mario Pieper, Karin Dedek, Reto Weiler

T15-6C Effects of temperature on retinal ganglion cell responses
Malte Thorsten Ahlers, Josef Ammermüller

T15-7C Direction-selective retinal ganglion cells in pigmented and albinotic rats
Martin Krause, Sascha Helduser, Dominik Hollatz, Klaus-Peter Hoffmann

T15-8C Monkey retinal ganglion cells retain the potential to switch into a strong regenerative state and regenerate axons *in vitro*.<
Katrin Schlich, Karin Rose, Solon Thanos

T15-9C Spatio-temporal characteristics of identified wide-field amacrine cells (WFAs) in the mouse retina: global contrast-detecting interneurons?

Gabriel Christian Knop, Karin Dedek, Reto Weiler

T15-10C Hue, Saturation, and Brightness Values Derived from the Model of Neuronal Color Coding and Elementary Color Sensations in Man
Werner Georg Karl Backhaus

T15-11C Retina-Chip Contact Probed by Thermal Noise
Ralf Zeitler, Günther Zeck, Peter Fromherz

T15-12C Expression of pannexin2 in the mouse retina
Katharina Schmidt

T15-13C A criterion for visual performance: Measuring head movement during the optokinetic reflex
Friedrich Kretschmer, Malte Ahlers, Daniel Meinhart, Immanuel Landgraf, Jutta Kretzberg, Josef Ammermüller

T15-14C Chromatic and Achromatic Temporal Resolution
Carlos Mora-Ferrer

Poster Topic

T16: Vision: Striate and Extrastriate Cortex, Eye Movement and Visuomotor Processing

T16-1A Pre-saccadic remapping of the motion after-effect
Ulrich Biber, Uwe J. Ilg

T16-2A Reorganisation plasticity in the visual cortex of the adult rat after optic nerve crush - a single cell resolution metabolic mapping study
Tamar Macharadze, Tim Wanger, Monika Marunde, Henning Scheich, Eckart Gundelfinger, Michael Kreutz, Jürgen Goldschmidt

T16-3A Transsynaptic retrograde labelling in the oculomotor system in rodent using tetanus toxin fragment C and Pseudorabies virus: opportunities and limitations
Christina Schulze, Markus Rothermel, Karoline Lienbacher, Thomas Curie, Barbara G. Klupp, Thomas C. Mettenleiter, Claudia Distler, Hanns Hatt, Klaus-Peter Hoffmann, Anja Horn

T16-4A Diminished plasticity of visual function and sensory maps after cortical stroke in mice
Franziska Greifzu, Silvio Schmidt, Karl-Friedrich Schmidt, Otto W. Witte, Siegrid Loewel

T16-5A 'SPARSIFICATION' OF NEURONAL ACTIVITY IN THE VISUAL CORTEX AT EYE OPENING
Nathalie Louise Rochefort, Olga Garaschuk, Ruxandra-Iulia Milos, Madoka Narushima, Nima Marandi, Bruno Pichler, Yury Kovalchuk, Arthur Konnerth

T16-6A How high is the spatial resolution of the human EEG?
Matthias Deliano, Ivonne Kallmeyer, Frank W. Ohl

T16-7A The main sequence of human Optokinetic Nystagmus
Andre Kaminiarz, Kerstin Königs, Frank Bremmer

T16-8A In vivo dendritic calcium signaling in layer 2/3 neurons of mouse visual cortex
Hongbo Jia, Nathalie Louise Rochefort, Yury Kovalchuk, Arthur Konnerth

T16-9A Two-photon imaging of orientation and direction selective neurons in the developing mouse visual cortex
Madoka Narushima, Nathalie Louise Rochefort, Nima Marandi, Bruno Pichler, Yury Kovalchuk, Arthur Konnerth

T16-10A Ocular dominance plasticity in adult visual cortex is limited by the immune receptor PirB
Miriam Mann, Maja Djurisic, Tobias Bonhoeffer, Carla J. Shatz, Mark Huebener*

T16-11A Characterizing the visual system of ageing mice
Konrad Lehmann, Karl-Friedrich Schmidt, Siegrid Löwel

T16-12A Changing functional organization during initial development of orientation maps in ferret visual cortex
Marcus Leinweber, Jonathan C. S. Leong, Thomas D. Mrsic-Flogel, Tobias Bonhoeffer, Mark Hübener

T16-13A Organization of the human MT+ complex as revealed by functional MRI and intraoperative electrical stimulation
Hubertus G. T. Becker, Alireza Gharabaghi, Thomas Haarmeier

T16-15A Spike Sorting Errors: Statistical Differences of Cortical 'Single Unit' and 'Single Neuron' Activity.
Martin Paul Nawrot

T16-1B Effects of feature-directed attention on the representation of speed and color changes of superimposed objects
Detlef Wegener, Maike K. Aurich, Friederike Ehn, F. Orlando Galashan, Andreas K. Kreiter

T16-2B A multi-electrode array for chronic recordings in monkey area V1 allowing for bidirectional movement of electrodes and fast electrode exchange.
Fingal Orlando Galashan, Anneke Meyer, Andreas K. Kreiter, Detlef Wegener

T16-3B Simvastatin improves spatial vision in mice following acute retinal ischemia /reperfusion
Katja Krempler, Christian Schmeer, Stefan Isenmann, Otto W. Witte, Siegrid Löwel

T16-4B Proprioception in the extraocular eye muscles of different species
Karoline Lienbacher, Michael Mustari, Bernhard Hess, Nina Peisker, Anja Horn

T16-5B Neuronal correlates of luminance change in the pigeon brain – a population code of the visual Wulst<
Benedict Ng, Onur Güntürkün, Dirk Jancke

T16-6B Measuring optokinetic response in adult zebrafish and medaka
Kaspar Müller, Stephan C.F. Neuhauss

T16-7B Analysis of temporal flash patterns in the visual system of the European starling (*Sturnus vulgaris*)
Arne Feinkohl, Georg M. Klump

T16-8B Stronger activation of the medial superior temporal area in migraineurs with aura compared to patients without aura
Andrea Antal, Katharina Saller, Carmen Morawetz, Juergen Baduewig, Walter Paulus, Peter Dechent

T16-9B Receptive field shifts in macaque primary visual cortex induced by saccade adaptation
Steffen Klingenhoefer, Markus Wittenberg, Thomas Wachtler, Frank Bremmer

T16-10B Human Ocular Following Response to Sampled Motion
Kim Joris Boström, Anne-Kathrin Warzecha

T16-11B Pigeons being spoilt for choice: a study on hemispheric dominance
Nadja Freund, Katja Brodmann, Martina Manns, Onur Güntürkün

T16-12B The onset of cortical activity after visual stimulation can be identified with multifocal visual evoked potentials (mfVEPs)
Thomas Meigen, Mathias Krämer

T16-13B Vision and visual cortical maps in mice with a photoreceptor synaptopathy: Bassoon mutant mice have reduced but robust visual capabilities
Bianka Götze, Karl-Friedrich Schmidt, Konrad Lehmann, Wilko Detlev Altrock, Eckart Dieter Gundelfinger, Siegrid Löwel

T16-1C Impact of oxytocin on cells of the primary visual cortex in albino and pigmented rats
Christina Charlotte Segerling, Klaus-Peter Hoffmann

T16-2C Gaze allocation during natural behavior in the real world
Bernard Marius Hart, Johannes Vockeroth, Frank Schumann, Klaus Bartl, Erich Schneider, Peter König, Wolfgang Einhäuser

T16-3C Stimulus evoked neuronal synchrony is reduced under saccadic viewing conditions
Markus Wittenberg, Frank Bremmer, Thomas Wachtler

T16-4C Spatio-temporal topography of saccadic suppression
Jonas Knöll, Jens Beyer, Frank Bremmer

T16-5C Single cell responses to instantaneous speed-changes of various positive and negative amplitudes in macaque area MT
Andreas Traschütz, Hanna C. Rempel, F. Orlando Galashan, Andreas K. Kreiter, Detlef Wegener

T16-6C FUNCTIONAL ARCHITECTURE OF SUPERFICIAL HORIZONTAL CONNECTIONS.
Elisha Ruesch, Kevan A. C. Martin, Stefan Roth

T16-7C How do prior expectations shape contour integration?
Marc Schipper, Udo A. Ernst, Manfred Fahle

T16-8C Attentional alteration of direction tuning of neurons in macaque area MT to two spatially separated motions
Vladislav Kozyrev, Anja Lochte, Mohammad Reza Daliri, Stefan Treue

T16-9C Visual evoked activity in V1 of anesthetized rats: from gratings to natural images
Sébastien Roux, Dymphie Suchanek, Ad Aertsen, Clemens Boucsein

T16-10C Stimulus and task-related gamma oscillations in monkey V1 induced by local and global contours
Bruss Lima, Wolf Singer, Sergio Neuenschwander

T16-11C Task-dependent viewing behavior in school children
Cornell Schreiber, Torsten Betz, Niklas Wilming, Tim Christian Kietzmann, Peter König

T16-12C Gamma oscillations in the visual Wulst of the owl: a comparative study
Sergio Neuenschwander, Lucas Pinto, Bruss Lima, Jerome Baron

Poster Topic

T17: Auditory Mechanoreceptors, Vestibular, Cochlea, Lateral Line and Active Sensing

T17-13A The role of the $Ca_v1.2$ channel in the cochlea
Annalisa Zuccotti, Wibke Singer, Hyun-Soon Geisler, Karin Rohbock, Oktar Gologlu, Hans Gerd Nothwang, Marlies Knipper

T17-14A Responses of brainstem lateral line neurons in goldfish, *Carassius auratus*, to different water flow directions and velocities
Silke Künzel, Horst Bleckmann, Joachim Mogdans

T17-1B Microtubule-associated proteins shape mechanoreceptors in Drosophila
Susanne Bechstedt, Thomas Müller-Reichert, Jörg T. Albert, Xin Liang, Jonathon Howard, Thomas Effertz, Martin C. Göpfert

T17-2B Auditory Pattern recognition by brain neurons of the grasshopper *Chorthippus biguttulus*
Olaf Kutzki, Bernhard Ronacher

T17-3B Novel Findings in Inner Hair Cells of Hypothyroid Rodents Showing Exocytosis in the Absence of Otoferlin
Christoph Franz, Stephanie Kuhn, Niels Brandt, Jutta Engel, Nikolaus Blin, Marlies Knipper

T17-4B Contralateral sound alters the f2-f1 distortion product otoacoustic emission – Impact of primary tone level and frequency specificity
Heike Althen, Anna Wittekindt, Cornelius Abel, Bernhard Gaese, Manfred Kössl

T17-5B Vibration sensitivity of leg scolopidial organs in Mantophasmatodea
Monika J. B. Eberhard, Dirk Lang, Harald Wolf

T17-6B Factors improving and impairing song pattern recognition in a grasshopper
Stefanie Krämer, Bernhard Ronacher

T17-7B Characteristics of the receptor current in locust auditory receptor cells
Karin Fisch, Andreas Herz, Jan Benda

T17-8B Mechanical feedback amplification in Drosophila hearing requires a specific subset of NompC-expressing, sound-sensitive mechanosensory cells
Thomas Effertz, Azusa Kamikouchi, Martin C. Göpfert

T17-9B The virtual ear: Deducing transducer function in the *Drosophila* ear
Qianhao Lu, Björn Nadrowski, Martin Göpfert

T17-10B Presynaptic Processing in Auditory Afferents of Bushcrickets
Berthold Hedwig, Tom Baden

T17-11B Spatial acuity of active electrolocation of objects in complex scenes by the weakly electric fish, Gnathonemus petersii
Katharina Behr, Gerhard von der Emde

T17-12B Using *Drosophila* to trace candidate deafness genes
Pingkalai Rajeswaran, Susanne Bechstedt, Björn Nadrowski, Jonathan Howard, Martin C Göpfert

T17-13B Relating filter properties of auditory transduction chains to biophysical mechanism.
Jörg Henninger, Simon Lu, Martin C. Göpfert, Jan Benda

T17-14B Insertion -Force and -Depth of Laser Fibers into a Cochlea Model
Sven Balster, Gentiana I. Wenzel, Kaiyin Zhang, Hubert H. Lim, Wolfgang Ertmer, Thomas Lenarz, Günter Reuter

T17-1C Sound induced vibration pattern on the tympanal membranes of the bushcricket *Mecopoda elongata*
Manuela Nowotny, Doreen Möckel, Melanie Weber, Manfred Kössl

T17-2C Sound Localization in Lizards: How a Pressure-Gradient Receiver may Function
Christine Voßen, Leo van Hemmen

T17-3C How crickets determine the direction of a flow field.
Andreas Norbert Vollmayr, Jan-Philipp Diepenbrock, Jan-Moritz P. Franosch, J. Leo van Hemmen

T17-4C Coding of stimulus amplitude by electrosensitive neurons in a weakly electric fish
Michael Georg Metzen, Jacob Engelmann, Gerhard von der Emde

T17-5C Physiological and anatomical evidence for sensory integration in the electrosensory lateral line lobe of *Gnathonemus petersii*
Jacob Engelmann, Sylvia Fechner, Kirsty Grant, Gerhard von der Emde

T17-6C Can Optoacoustic Stimulation replace OHC Amplification in the Cochlea?
Günter Reuter, Sven Balster, Hubert Lim, Kain Zhang, Wolfgang Ertmer, Thomas Lenarz, Gentiana Wenzel

T17-7C Neurophylogeny of the Ensifera Auditory Systems
Reinhard Lakes-Harlan, Johannes Strauß

T17-8C Differential dynamic processing of semicircular canal signals in separate subpopulations of frog extraocular motoneurons
Tobias Kohl, Sandra Pfanzelt, Christian Rössert, Hans Straka

T17-9C Modeling the origin of functional heterogeneity among auditory nerve fibers
Nikolai Mikhailovich Chapochnikov, Thomas Frank, Nicola Strenzke, Andreas Neef, Darina Khimich, Alexander Egner, Fred Wolf, Tobias Moser

T17-10C Intensity Invariance Emerging in a Feed Forward Network
Ulrike Ziehm, K. Jannis Hildebrandt, Jan Benda

T17-11C Neurophysiology and neuroanatomy of the lateral line system in *Xenopus laevis*
Zhivko Zhivkov, Francisco Branoner, Claudia Schuldt, Ulrike Ziehm, Oliver Behrend

T17-12C Coding at high precision in the velocity-regime: Application of information theory to lateral-line detection
Julie Goulet, Jacob Engelmann, Boris P. Chagnaud, Nicola Jung, Björne Scholze, Horst Bleckmann, J. Leo van Hemmen

T17-13C Dimensional Changes of tectorial membrane due to mutations in TRß and the tectorins.

Andreas Breß, Ulrike Zimmermann, Marlies Knipper, Nikolaus Blin, Markus Pfister

T17-14C Two modes of information processing in the electrosensory system of the paddlefish, *Polyodon spathula*
Leonie Pothmann, Lon A. Wilkens, Michael H. Hoffmann

Poster Topic

T18: Auditory System: Subcortical and Cortical Processing

T18-1A Delay-sensitive neurons of the auditory cortex in the Phyllostomid bat, *Carollia perspicillata*
Cornelia Hagemann, Karl-Heinz Esser, Manfred Kössl

T18-2A Distribution of Na^{+}/Ca^{2+} exchangers NCX and NCKX in the developing rat auditory brainstem
Sandra Grill, Eckhard Friauf

T18-3A Distribution and kinetics of synaptic AMPA receptors in adult MSO neurons
Kiri Anne Couchman, Benedikt Grothe, Felix Felmy

T18-4A Virtual space technique as a measure to investigate the role of the barn owl's facial ruff
Laura Hausmann, Hermann Wagner

T18-5A Adaptation in the auditory midbrain of the barn owl (*Tyto alba*) as demonstrated by a two-stimulus paradigm
Martin Singheiser, Hermann Wagner

T18-6A Estrous cycle-dependent plasticity of auditory cortical activation in mice
Christina Schmid, Günter Ehret

T18-7A Auditory motion perception following unilateral cortectomy
Jörg Lewald, Sören Peters, Michael C. Corballis, Markus Hausmann

T18-8A Potassium channel expression in the avian auditory brainstem – comparison of in vivo and in vitro development
Thomas Künzel, Marcus J. Wirth, Harald Luksch, Hermann Wagner, Jörg Mey

T18-9A Time course gene expression profiling identifies candidate genes for maturation and function of the rat superior olivary complex
Heike Ehmann, Christian Salzig, Patrick Lang, Eckhard Friauf, Hans Gerd Nothwang

T18-10A Electrophysiological recordings from the primary auditory cortex of the unanesthetized and anesthetized house mouse (*Mus musculus*)
Bettina Joachimsthaler, Günter Ehret, Simone Kurt

T18-11A General properties and synaptic input-output functions of neurons in the dorsal lateral lemniscus of Mongolian gerbil
Felix Felmy, Elisabeth MM Meyer, Benedikt Grothe

T18-12A Parallel electrophysiological and behavioral analysis of layer-specific electrical microstimulation in primary auditory cortex - implications for the subcortical-loop hypothesis
Max Happel, Marcus Jeschke, Juliane Handschuh, Matthias Deliano, Frank W. Ohl

T18-13A TRAUMA-INDUCED ALTERATION OF BDNF AND ARG3.1/ARC EXPRESSION IN THE AUDITORY SYSTEM
Wibke Singer, Lukas Rüttiger, Annalisa Zuccotti, Rama Panford-Walsh, Mirko Jaumann, Karin Rohbock, Marlies Knipper

T18-14A Effect of spoken and sung syllables on brain activity
Nicole Behne, Henning Scheich, André Brechmann

T18-1B Processing of intonation in spoken language
Christin Kohrs, Nicole Behne, Henning Scheich, André Brechmann

T18-2B Binaural Interactions in Congenital Deafness
Peter Hubka, Jochen Tillein, Dorrit Schiemann, Sylvia Heid, Emilie Syed, Rainer Hartmann, Andreas K. Engel, Andrej Kral

T18-3B Analysis and Simulation of the Neurophonic Potential in the Laminar Nucleus of the Barn Owl
Paula Tuulia Kuokkanen, Nico Lautemann, Hermann Wagner, Richard Kempter

T18-4B Chloride Homeostasis in the Avian Auditory Brainstem
Marcus Joseph Wirth, Thomas Gensch, Hermann Wagner

T18-5B Complexins are required for auditory synaptic transmission beyond the hair cell
Nicola Strenzke, Darina Khimich, Cornelia Kopp-Scheinpflug, Kerstin Reim, Soham Chanda, Anna Bulankina, Matthew Xu-Friedman, Niels Brose, Tobias Moser

T18-6B Acoustic startle response in the wild-type and domesticated Mongolian gerbil
Bernhard Gaese, Manuela Nowotny, Peter K.D. Pilz

T18-7B Missing cochlea activity leads to anatomical changes and delayed development of NMDA receptor-mediated transmission in the superior olivary complex
Jan Hirtz, Britta Müller, Eckhard Friauf, Stefan Löhrke

T18-8B Functional implications of the cochlear nucleus in dolphins
Pascal Malkemper, Stefan Huggenberger, Helmut H. A. Oelschläger

T18-9B Effects of endogenous shifting of auditory attention in rats
Jonas Ché Imam, Wolfger von der Behrens, Bernhard Gaese

T18-10B Detection of auditory evoked potentials and mismatch negativity-like responses in the awake and unrestrained rat
Fabienne Jung, Tetsuya Kumagai, Marc Tittgemeyer, Heike Endepols, Rudolf Graf

T18-11B Signal detection in modulated maskers with different envelope shapes: a study of masking release in the mouse
Georg Klump, Derik Behrens

T18-12B Intracranial Local Field Potentials can be estimated from auditory brainstem function by Artificial Neural Network simulations
Mirko Jaumann, Lukas Rüttiger, Martin Bogdan, Marlies Knipper

T18-13B Cricket brain neurons – song pattern recognition and control of walking
Maja Zorovich, Berthold Hedwig

T18-14B The BAEP audiogram of the lesser spear-nosed bat *Phyllostomus discolor*
Arne Liebau, Karl-Heinz Esser

T18-1C Cells and Kinases: How to protect the Ear from Noise
Lukas Rüttiger, Masahiro Matsumoto, Juliane Dettling, Robert Feil, Marlies Knipper

T18-2C Temporal response properties in the receptive fields of mouse auditory midbrain neurons
Günter Ehret, Marina Egorova

T18-3C Collision-like interaction of acoustic and electric stimulation in gerbil (*Meriones unguiculatus*) auditory cortex A1
Achim Engelhorn, Matthias Deliano, Frank W. Ohl

T18-4C Effects of bilateral lesioning of the medial nucleus of the trapezoid body on behavioural sensitivity to interaural time differences
Andrea Lingner, Michael Pecka, Benedikt Grothe

T18-5C Amplitude modulation coding in the mammalian auditory midbrain in the presence and in the absence of noise
Leila Khouri, Nicholas A. Lesica, Ida Siveke, Benedikt Grothe

T18-6C Representation of complex communication sounds in secondary fields of the mouse auditory cortex
Anja Luise Dorrn, Marcus Jeschke, Günter Ehret, Simone Kurt

T18-7C Investigation of neural circuits in the auditory brainstem via light-sensitive ion-channels
Christian Porres, Otto Albrecht, Benedikt Grothe, Achim Klug

T18-8C Developmental changes of $GABA_B$ receptor function in the medial superior olive
Benjamin Haßfurth, Benedikt Grothe, Ursula Koch

T18-9C Distribution of int-2/FGF3 mRNA expression in distinct neuronal populations of the adult mouse brain.
Adelheid Kresse, Tomas Hökfelt

T18-10C Tone lateralization is affected by both linguistic roles and physical properties
Lan SHUAI

T18-11C P300 and reaction time as measure of hearing effort of cochlear implant users and normal hearing listeners during sound discrimination in noise
Peter Igelmund, Hartmut Meister, Anke Brockhaus-Dumke, Dirk Fürstenberg, Hasso von Wedel, Martin Walger

T18-12C Call frequency control by neurons in the vocal motor nucleus of Greater Horseshoe Bats
Steffen Hage, Khota Kobayasi, Walter Metzner

T18-13C Modelling transmission at the bushy cell synapse in complexin-deficient mice
Andreas Neef, Nicola Strenzke, Cornelia Kopp-Scheinpflug, Soham Chanda, Matthew A. Xu-

Friedman, Tobias Moser

T18-14C Multi-Electrode Recordings of Delay Lines and Neuro-phonic Potential in the Auditory Coincidence Detector Circuit of Birds
Nico Lautemann

Poster Topic

T19: Chemical Senses: Olfaction, Taste, Others

T19-1A DIFFERENT FRUIT ODORS PRODUCE WIDELY DIVERGENT DYNAMIC RESPONSES IN DROSOPHILA ANTENNAL OLFACTORY RECEPTOR NEURONS

Julia Schuckel, Päivi Torkkeli, Andrew S. French

T19-2A Neuronal correlates of pattern recognition in a social insect
Andreas Simon Brandstaetter, Wolfgang Rössler, Christoph Johannes Kleineidam

T19-3A Correlating social organization and neuroanatomical characters in leaf-cutting ants
Christina Kelber, Flavio Roces, Wolfgang Rössler, Christoph Johannes Kleineidam

T19-4A Multi-unit recordings in the dual olfactory pathway of the honeybee
Martin F. Brill, Christoph J. Kleineidam, Wolfgang Rössler

T19-5A Adult neurogenesis in the olfactory system of homing pigeons supports the impact of olfactory cues for navigation
Martina Manns, Kathrin Goisser, Mareike Inkemann, Nina Patzke, Onur Güntürkün

T19-6A Confocal life-cell imaging in the microvillous layer of the sensory epithelium using an intact whole-organ preparation of the mouse vomeronasal organ
Daniela Fluegge, Marc Spehr

T19-7A Pectine neuropils of the scorpion - serotonine immunoreactivity and similarities to insect and crustacean olfactory lobes
Harald Wolf, Steffen Harzsch

T19-8A Characterization of responses to TAAR-specific amines in the olfactory system
Sebastian Gliem, Detlev Schild, Ivan Manzini

T19-9A DoOR - a Database of Odorant Responses in *Drosophila melanogaster*
Shouwen Ma, Martin Strauch, Daniel Münch, C Giovanni Galizia

T19-10A Novel techniques for the exploration of the honeybee antennal lobe.
Julia Rein, Martin Strauch, Giovanni Galizia

T19-11A Daytime-dependent effects of cAMP and octopamine on the pheromone transduction of the hawkmoth *Manduca sexta*
Christian Flecke, Monika Stengl

T19-12A Investigating the contributions of Ga_o and Ga_q to information processing in *Drosophila* olfactory sensory neurons

Michael Thoma, Birgit Rapp, Natalya Katanayeva, Vladimir Katanaev, C. Giovanni Galizia

T19-13A Calmodulin is Important for Pheromone Adaptation in Vomeronasal Sensory Neurons
Jennifer Spehr, Silke Hagendorf, Jan Weiss, Marc Spehr, Trese Leinders-Zufall, Frank Zufall

T19-14A Smells like nurse bee spirit?
Thomas S. Muenz, Christina Zube, Wolfgang Rössler

T19-15A Imaging Olfactory Learning in the Mushroom Body Lobes of the Honeybee
Melanie Hähnel, Randolf Menzel

T19-16A Biological activity and composition of cuticular lipid extracts of the cricket *Gryllus bimaculatus.*
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T19-17A The stimulatory heterotrimeric G-protein Ga_s is involved in olfactory signal transduction in *Drosophila*
Ying Deng, Günter Gisselmann, Hanns Hatt, Eva M. Neuhaus

T19-18A HF Magnetic Field Disrupts Magnetic Orientation in Zebra Finches
Joe Voss, Nina Keary, Tim Ruploh, Peter Thalau, Wolfgang Wiltschko, Hans-Joachim Bischof

T19-19A A dual olfactory pathway in honeybee antennal lobes: Innervation pattern of local neurons.
Katja Sabine Kroker, Anneke Meyer, C. Giovanni Galizia, Wolfgang Rössler, Christoph Johannes Kleineidam

T19-20A Processing of imperfect odor mixtures in the honeybee antennal lobe
Jacob Stierle, Paul Szyszka, Cyrille Girardin, C. Giovanni Galizia

T19-21A Imaging Temporal Odor Representation in the KENYON CELLS of honeybees
Anja Froese, Randolf Menzel

T19-22A Cannabinoids modulate odor sensitivity in the olfactory epithelium
Esther Breunig, Ivan Manzini , Detlev Schild , Dirk Czesnik

T19-23A Dis- and Reassembling Complex Natural Blends by Linked Gas-Chromatography - Optical Imaging Techniques in *Drosophila melanogaster*
Marco Schubert, Silke Sachse, Bill S. Hansson

T19-1B Peripheral and central olfactory processing of sex pheromone in an insect model
David Jarriault, Antoine Chaffiol, Louise Couton, Shereen Elbanna, Jean-Pierre Rospars, Sylvia Anton

T19-2B Neuronal representations of olfactory and visual associative learning in the honeybee (*apis mellifera*)
Ina Klinke, Randolf Menzel

T19-3B Experience modulates pheromone sensitivity in moths
Sebastian Antonio Minoli, Peter Anderson, Niels Skals, Frederic Marion-Poll, Violaine Colson, Sylvia Anton

T19-4B 3D Standard Brain of the Red Flour Beetle *Tribolium castaneum:* A Tool to Study Sex Dimorphism, Adult Plasticity and RNAi
Holger Vitt, Stefan Dippel, Brigitte Goetz, David Dreyer, Wolf Huetteroth, Joachim Schachtner

T19-5B Homeostatic Plasticity in Basal Vomeronasal Neurons: Activity-Dependent Expression of Ether-à-Go-Go Related Gene Potassium Channels
Silke Hagendorf, Corinna Engelhardt, Daniela Fluegge, Marc Spehr

T19-6B Modulation of firing activity of olfactory receptor neurons by background odorants
Janez Prešern, Virginie Party, Didier Rochat, Andrej Blejec, Christophe Hanot, Michel Renou

T19-7B Signaling Protein Distribution in Olfactory Sensory Neurons
Stefan Kurtenbach, Hanns Hatt, Eva Maria Neuhaus

T19-8B Characterization of ion channels involved in olfactory transduction in the antenna of the hawkmoth *Manduca sexta.*
Jonas Benzler, Frauke Ackermann, Thomas Gudermann, Alan Nighorn, Monika Stengl

T19-9B Neural basis of mating-dependent olfactory plasticity in a male moth
Romina Barrozo, David Jarriault, Christophe Gadenne, Sylvia Anton

T19-10B ß-arrestin2 mediated desensitization of mammalian odorant receptors
Sebastian Rasche, Anastasia Mashukova, Hanns Hatt, Eva M. Neuhaus

T19-11B Functional characterization of the scaffolding protein, Multiple PDZ Domain Protein 1, MUPP1, in olfactory signal transduction
Sabrina Baumgart, Ruth Catherine Dooley, Hanns Hatt, Eva Maria Neuhaus

T19-12B Brain structure of *Scutigera coleoptrata* (Myriapoda: Chilopoda): New insights into the evolution of mandibulate olfactory centers
Andy Sombke, Steffen Harzsch, Bill S. Hansson

T19-13B How to change while remaining the same – effects of learning on odor-evoked activity patterns in the antennal lobe
Michael Schmuker, Marcel Weidert, Randolf Menzel

T19-14B Learning in a parasitoid's brain - a closer investigation of the brain structure of Cotesia plutellae
Helga Groll, Guy M Poppy, Philip L Newland

T19-15B Homing in pigeons (Columba livia) induce ZENK activation in piriform cortex
Nina Patzke, Martina Manns, Onur Güntürkün, Paolo Ioalè, Anna Gagliardo

T19-16B The honeybee's mushroom bodies extrinsic neurons - their role in associative and non-associative learning
Ravit Hadar, Randolf Menzel

T19-17B A mass spectrometric approach to determine neuropeptides from defined brain regions of *Apis mellifera*
Anna Boehm, Susanne Neupert, Joerg Kahnt, Reiner Hedderich, Reinhardt Predel, Joachim Schachtner

T19-18B Intensity coding in the ant antennal lobe
Christina Zube, Wolfgang Rössler

T19-19B Odorant receptors of *Manduca sexta*
Ewald Große-Wilde, Forstner Maike, Jürgen Krieger, Bill S. Hansson, Dieter Wicher

T19-20B 2-Photon functional imaging of single olfactory neurons in the <*Drosophila* brain
Antonia Strutz, Bill S. Hansson, Silke Sachse

T19-21B The antennal lobes in basal hexapods: characterizing the ancestral insect olfactory system
Christine Mißbach, Steffen Harzsch, Bill S. Hansson

T19-22B Molecular basis of olfactory specialization in *Drosophila melanogaster* siblings
Sofia Lavista Llanos, Marcus C. Stensmyr, Bill S. Hansson

T19-23B In vivo imaging of odor-evoked chloride responses in the *Drosophila* brain
Veit Grabe, Bill S. Hansson, Silke Sachse

T19-24B First Order Blend Processing in the Moth Antennal Lobe
Linda S. Kuebler, Shannon O. Olsson, Bill S. Hansson

T19-1C Mixture interactions on the antenna of *Drosophila melanogaster*
Daniel Münch, Benjamin Schmeichel, Sheree Pfeiffer, Ana F. Silbering, C. Giovanni Galizia

T19-2C Uptake of odorant binding proteins in the olfactory epithelium
Heiko Brose, Jörg Strotmann, Heinz Breer

T19-3C Relating olfactory perception to physiology in Drosophila
Thomas Niewalda, Thomas Völler, Julia Ehmer, André Fiala, Bertram Gerber

T19-4C Candidate pheromone receptors of the two silkmoth species *Antheraea pernyi* and *Antheraea polyphemus*
Maike Forstner, Heinz Breer, Jürgen Krieger

T19-5C Molecular elements of pheromone reception IN MOTHs
Jürgen Krieger, Maike Forstner, Ewald Große-Wilde, Thomas Gohl, Elisabeth Bouche, Inga Gondesen, Heinz Breer

T19-6C Taste signaling elements in the gastrointestinal tract
Nicole Hass, Karin Schwarzenbacher, Heinz Breer

T19-7C Odour concentration learning in Drosophila larvae
Dushyant Mishra, Yi-chun Chen, Bertram Gerber

T19-8C Outgrowing olfactory axons contain the Reelin receptor VLDLR and navigate through the Reelin-rich cribriform mesenchyme
Carina Schnaufer, Heinz Breer, Joerg Fleischer

T19-9C Promotor-motifs governing the spatial expression pattern of olfactory receptors
Jörg Strotmann, Yong-Quan Zhang, Heinz Breer

T19-10C Grueneberg ganglion – a dual sensory organ?
Katharina Mamasuew, Heinz Breer, Joerg Fleischer

T19-11C *Drosophila* olfaction: What makes a good odor what makes a good blend?
Markus Knaden, Kathrin Steck, Bill S. Hansson

T19-12C Signaling elements in the Grueneberg ganglion
Joerg Fleischer, Katharina Mamasuew, Heinz Breer

T19-13C ODOR PREFERENCE AND SPECIALIZATION IN FRUIT FLIES
Ana Beramendi , Markus Knaden, Bill Hansson

T19-14C Olfactory coding in moths: Evolution *versus* life history
Sonja Bisch-Knaden, Marco Schubert, Celine Heinl, Silke Sachse, Bill S. Hansson

T19-15C Synaptic Input and Intrinsic Membrane Properties as Potential Mechanism for Sparsening Cockroach Kenyon Cells
Heike Demmer, Peter Kloppenburg

T19-16C Expression of the adiponectin receptor 1 in the olfactory mucosa of mice
Maria-Isabell Burry, Nicole Hass, Henriette Haub, Rebecca Stevens, Karin Schwarzenbacher, Heinz Breer

T19-17C Plasticity of microcircuits in the insect nervous system
Claudia Groh, Nancy Butcher, Andrea Nuschke, Ian Meinertzhagen, Wolfgang Roessler

T19-18C Physiological and morphological features of local interneurons in the antennal lobe of *Periplaneta americana*
Debora Fusca, Andreas Husch, Peter Kloppenburg

T19-19C G alpha protien subtypes in the zebrafish chemosensory systems
Yuichiro Oka, Sigrun I. Korsching

T19-20C Positive selection and the birth of an olfactory receptor clade in teleosts
Ashiq Hussain, Luis R Saraiva, Sigrun I Korsching

T19-21C Characterization of Transient Potassium Currents in Identified Olfactory Interneurons of *Periplaneta Americana*
Lars Paeger, Peter Kloppenburg

T19-22C Verifying and searching ligands for genetically labeled glomeruli
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T19-23C Background odors specifically change odor discrimination time and accuracy
Nico Schneider, Nilufar Shahshahani, Hartwig Spors

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T20: Somatosensation: Touch, Temperature, Proprioception, Nociception

T20-1A Dendritic activity in layer 5 pyramidal cells in the somatosensory cortex in vitro during upstates
Thomas Berger

T20-2A Infrared sensing in ants
Markus Ruchty, Linda Sara Kübler, Flavio Roces, Christoph Johannes Kleineidam

T20-3A Anatomical and neurochemical organization of the sensory system of the earthworm *Lumbricus terrestris*
Gabor Kiszler, Eszter Varhalmi, Gergely Berta, Edit Pollak, Laszlo Molnar

T20-4A Brain Centres involved in the Fruit Flies' Orientation in a Humidity Gradient
Bianca Zaepf, Roland Strauss

T20-5A A targeted induction of mitochondrial dysfunction in the peripheral somatosensory system
Ben Novak, Christine C. Stichel, Hermann Lübbert

T20-6A Mechanically induced regional changes in free intracellular Ca^{2+}, and the effect of intracellular Ca^{2+} on mechanotransduction in spider sensory neurons
Ulli Höger, Shannon Meisner, Päivi H. Torkkeli , Andrew S. French

T20-7A Antinociceptive effects of the selective COX-2-inhibitors celecoxib and lumiracoxib assessed by functional MRI (BOLD) in rats
Anna-Maria Pamberg, Kay Brune, Andreas Hess

T20-8A Nowhere to Go? Fruit Flies in a Bilaterally Increasing Temperature Gradient
Christian Berg, Roland Strauss

T20-9A Superior sensory, motor and cognitive performance in elderly subjects with long-year dancing activities
Jan-Christoph Kattenstroth, Izabela Kolankowska, Tobias Kalisch, Hubert R. Dinse

T20-1B Dependency of the negative BOLD signal on stimulus intensity in the human somatosensory cortex
Katharina Schaefer, Henrik BW Larsson

T20-2B Age related alterations of response properties of cortical somatosensory neurons after presentation of train stimuli – influence of age on temporal processing.

Marianne David, Hubert R. Dinse

T20-3B Differential effects of nitric oxide on the responsiveness of tactile hairs
Hansjürgen Schuppe, Philip L. Newland

T20-4B ENCODING OF HIGH-FREQUENCY WHISKER VIBRATIONS IN THE RAT'S BARREL CORTEX: AWAKE VERSUS ANESTHETIZED PREPARATION.
Christiane Vahle-Hinz, Maik C Stüttgen, Tobias AS Ewert, Andreas K Engel, Cornelius Schwarz

T20-5B Subacute exposure of rats to cadmium oxide nanoparticles: electrophysiological and general toxicological effects
László Nagymajtényi, Leila Sárközi, András Papp, Tünde Vezér

T20-6B Direct activation of transient receptor potential V1 by nickel ions
Matthias Lübbert, Debbie Radtke, Hanns Hatt, Christian Wetzel

T20-7B Neurophysiology of tactile shape recognition in the somatosensory cortex of the Etruscan shrew
Claudia Roth-Alpermann, Michael Brecht

T20-8B Modulation of corticomuscular synchronization during isometric compensation of dynamic forces with different levels of predictability
Xi Wang, José Raúl Naranjo, Wolfgang Omlor, Frank Huethe, Christoph Maurer, Jürgen Schulte-Mönting, Rumyana Kristeva

T20-1C Functional grouping of descending interneurons that mediate antennal mechanosensory information to motor networks
Sandra Westmark, Volker Dürr

T20-2C Martinotti interneurons control dendritic encoding of tactile stimuli
Matthew Evan Larkum, Enrique Perez-Garci, Walter Senn, Thomas Nevian, Tobias Bock, Masanori Murayama

T20-3C Changes in cortical protein expression specifically related to improved learning following transcranial magnetic theta burst stimulation of rats
Annika Mix

T20-4C Processing of proprioceptive inputs in the locust: quantitative analysis of the responses of spiking local interneurones in the metathoracic ganglion.
Andrés Vidal-Gadea, XingJian Jing, Yashuhiro Kondoh, David Simpson, Philip Newland

T20-5C Cytoarchitectonic mapping and quantitative anatomy of the Etruscan shrew cortex
Robert Konrad Naumann, Farzana Anjum, Claudia Roth-Alpermann, Michael Brecht

T20-6C Differing effects of GABA and glutamate on spider *(Cupiennius salei)* mechanoreceptors
Keram Pfeiffer, Ulli Höger, Andrew S. French, Päivi H. Torkkeli

T20-7C Induced plastic changes of tactile perception and somatosensory cortex excitability depend on stimulation frequency and temporal pattern.
Mario Gatica Tossi, Hubert Dinse

T20-8C Impact of thalamus on cortical state change in mouse barrel cortex during whisking
James Poulet, Carl Petersen

T20-9C Mechanosensory Feedback in *Drosophila*

Jan Bartussek, Elena Shchekinova, Henri Saleh, Chauncey Graetzel, Joe Howard, Martin Zapotocky, Steven Fry

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T21: Motor Systems

T21-1A Investigating the effects of proprioceptive feedback on a central pattern generator with a real-time computer model
Florian Michael Diehl, Nelly Daur, Wolfgang Stein

T21-2A Influence of movement signals from the Femur Tibia-joint in front-, middle- and hindleg of the stick insect during forward and backward walking
Katja Hellekes, Ansgar Büschges

T21-3A Expression of FoxO transcription factors in peripheral nerves undergoing Wallerian degeneration *in vivo* and *in vitro*
Heike Siebert, Bettina Franzen, Brigitte Maruschak, Wolfgang Brück

T21-4A Mechanisms in the Control of Walking Speed in the Stick Insect
Matthias Gruhn, Géraldine von Uckermann, Sandra Westmark, Anne Wosnitza, Ansgar Büschges , Anke Borgmann

T21-5A Muscle Activity of Antagonistic Leg Muscles in the Turning Stick Insect
Philipp Rosenbaum, Lyuba Zehl, Ansgar Büschges, Matthias Gruhn

T21-6A Morphology of motor neurons innervating labral muscles of *Locusta migratoria*
Abid Mahmood Alvi, Peter Bräunig

T21-7A Targeting Modules in the Gap-Climbing Control of *Drosophila melanogaster*
Tilman Triphan, Roland Strauss

T21-8A Coordinated locomotion via a gradient of synaptic strength
Carmen Ramona Smarandache, Brian Mulloney

T21-9A Modulation of corticomuscular synchronization by different frequencies of dynamic force output
Jose Raul Naranjo, Xi Wang, Wolfgang Omlor, Frank Huethe, Christoph Maurer, Jürgen Schulte-Mönting, Rumyana Kristeva

T21-1B Sonic hedgehog regulates Cadherin-20 expression by motor neurons during spinal cord development
Jiankai Luo, Min Jeong Ju, Juntang Lin, Xin Yan, Markus Markus, Eihard Mix, Arndt Rolfs, Christoph Redies

T21-2B The Preoptic Area in Anuran Amphibians
Silke Maier, Stefan Huggenberger, Wolfgang Walkowiak

T21-3B Stick insect tarsi – surface structures and muscle recruitment in posture control
Philipp Bußhardt, Harald Wolf, Stanislav Gorb

T21-4B A Tracing Study of Mesothoracic Leg Motoneurons and DUM Neurons in the stick insect *Carausius morosus*
Jens Goldammer, Joachim Schmidt

T21-5B Audio-vocal integration within the Medulla oblongata of anurans
Stefan Huggenberger, Wolfgang Walkowiak

T21-6B Revealing Excitable Subcortical Networks by Microstimulation-fMRI of the Deep Cerebellar Nuclei
Fahad Sultan, Mark Augath, Yusuke Murayama, Salah Hamodeh, Peter Thier, Nikos K Logothetis

T21-7B Identification of genes mediating the dorsal/ventral choice of sensory and motor axons in the limb
Georg Luxenhofer, Elisa Bianchi, Andrea B. Huber

T21-8B Analysis of the intersegmental sensory influences in the stick insect walking system
Anke Borgmann, Katja Hellekes, Ansgar Büschges

T21-9B Temporal patterning of a vocal pacemaker circuit in fish
Boris P. Chagnaud, Andrew H. Bass, Robert Baker

T21-10B High frequency random noise stimulation modulates levels of cortical excitability in the human motor cortex
Leila Chaieb, Daniella Terney, Vera Moliadze, Andrea Antal, Walter Paulus

T21-11B Physiological characterisation of a simply-innervated insect muscle
Anthony Joseph Clare, Gregory Sutton, Malcolm Burrows, Tom Matheson

T21-1C Temporal synchrony as critical factor for facilitation and interference of action recognition by self-generated movements
Andrea Christensen, Winfried Ilg, Hans-Otto Karnath, Martin A. Giese

T21-2C Role of an inhibitory motor neurone in aimed limb movements
Delphine Calas, Anthony Joseph Clare, Tom Matheson

T21-3C Measuring and modelling biomechanical parameters using individual stick insect extensor tibiae muscles
Christoph Guschlbauer, Marcus Blümel, Scott L. Hooper, Ansgar Büschges

T21-4C Cholinergic currents in identified leg motoneurons isolated from stick insects.
Eugenio Eduardo Oliveira, Vincent L. Salgado, Peter Kloppenburg, Joachim Schmidt

T21-5C Gain modulation of reach related neurons in the parietal reach region and dorsal premotor cortex of monkeys.
Christian Klaes, Stephanie Westendorff , Alexander Gail

T21-6C Subcellular localizations of intraflagellar transport (IFT) molecules indicate differential ciliary and novel non-ciliary functions in retinal neurons

Tina Sedmak, Martin Latz, Gregory J. Pazour, Uwe Wolfrum

T21-7C Recruitment pattern of motoneurons and interneurons in the spinal locomotor network of adult zebrafish
Jens Peter Gabriel, Abdeljabbar El Manira

T21-8C Arm Stiffness and Movement Control
Katja Fiedler, J. Michael Herrmann

T21-9C Tyramine and octopamine, a transmitter pair of specific action in insects.
Hans-Joachim Pflüger, Natalia L. Kononenko, Ricardo Vierk, Carsten Duch

T21-10C Dopaminergic Signaling in the Central Complex Promotes Sound Production
Michael Kunst, Christian Krug, Ralf Heinrich

T21-11C Grasp context representation in macaque parietal area AIP
Hans Scherberger, M. Baumann, M.-C. Fluet

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T22: Homeostatic and Neuroendocrine Systems, Stress Response

T22-1A Mating behavior of female *Chorthippus biguttulus* and its modulation by juvenile hormone
Andrea Wirmer, Melanie Faustmann, Ralf Heinrich

T22-2A Pituitary adenylate cyclase-activating polypeptide (PACAP) modulate the activity of coelomocytes during the regeneration of the ventral nerve cord ganglia in the earthworms
Ildiko Somogy, Eszter Varhalmi, Peter Engelmann, Balazs Opper, Akos Boros, Jozsef Nemeth, Andrea Lubics, Dora Reglodi, Edit Pollak, Laszlo Molnar

T22-3A Is the medial neurosecretory brain region of the earthworm the anatomical correlates of the pars intercerebralis?
Laszlo Molnar, Edit Pollak, Akos Boros, Zsofia Herbert

T22-4A Expression of small heat shock proteins in the rat brain
Britta Bartelt-Kirbach, Nikola Golenhofen

T22-5A Paternal care is critically involved in the development of Corticotropin Releasing Factor (CRF)-expressing neurons in the rodent orbitofrontal cortex, amygdala and hippocampus
, Carina Helmeke, Katharina Braun

T22-1B Peptide quantification in direct mass spectrometric tissue profiling: a case study in prohormone convertase 2-deficient *Drosophila*
Christian Wegener, Jeanne Rhea, Michael Bender, Jörg Kahnt

T22-2B LOW DOSE HEXABROMOCYCLODODECANE SUPRESS THYROID HORMONE RECEPTOR- MEDIATED TRANSCRIPTION.
kingsley ibhazehiebo, Toshiharu Iwasaki, Shimokawa Noriaki, Marina Londono, Noriyuki Koibuchi

T22-3B Individual cortisol profile associates with performance in academic examinations
Belinda Angela Pletzer, Guilherme Wood, Hans-Christoph Nuerk, Hubert H. Kerschbaum

T22-4B ELUCIDATION OF MELATONIN METABOLIC PATHWAY INVOLVEMENT ON AGING OF *ACHETA DOMESTICUS* IN INSECT LINES SELECTED FOR FAST- AND SLOW-DEVELOPMENT
Jadwiga Bembenek, Jacek Czeslaw Francikowski

T22-5B ELUCIDATION OF MELATONIN METABOLIC PATHWAY INVOLVEMENT ON AGING OF *ACHETA DOMESTICUS* IN INSECT LINES SELECTED FOR FAST- AND SLOW-DEVELOPMENT
Jacek Czeslaw Francikowski, Jadwiga Bembenek

T22-1C The role of noradrenaline within the perirhinal cortex in stress-induced potentiation of the startle

response.
Brigitte Schulz-Klaus, Peter Pilz, Andreas von Ameln-Mayerhofer

T22-2C Insulin in the brain promotes locomotor activity in lean
Tina Sartorius, Anita M. Hennige, Otto Tschritter, Hubert Preissl, Sabine Hopp, Konstantinos Kantartzis, Günther Silbernagel, Andreas Fritsche, Peter Ruth, Hans-Ulrich Häring

T22-3C Gender- and genotype-dependent differences in stress reactions in mice deficient for the serotonin transporter
Sarah Louise Nietzer, Sissi Jakob, Gabriela Ortega, Claudia Kriegebaum, Lise Gutknecht, Klaus - Peter Lesch, Angelika Schmitt

T22-4C The phylogeny of blattopteran insects: neuropeptides as a new character set
Bastian Fromm, Steffen Roth, Susanne Neupert, Reinhard Predel

T22-5C Acute stress induces increased oxytocin expression in brain regions important for emotional regulation only in male wildtype mice
Claudia Brigitte Kriegebaum, Sarah L. Nietzer, Sissi Jakob, Gabriela Ortega, Lise Gutknecht, Klaus-Peter Lesch, Angelika G. Schmitt

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T23: Neural Networks and Rhythm Generators

T23-1A Protocadherin7: Isoform Specific Function and Signaling Pathway
Kenichi Yoshida

T23-2A Tonic activation of presynaptic NMDA receptors during seizure development in the amygdala
Stéphanie Anne Graebenitz, Jörg Lesting, Thomas Seidenbecher, Hans-Christian Pape

T23-3A Dendritic architecture of pyramidal neurons in the rat infralimbic cortex affected by diurnal activity and stress
Boldizsár Czéh, Claudia Perez-Cruz, Mária Simon, Gabriele Flügge, Eberhard Fuchs

T23-4A Functional role of intralaminar thalamic neurons during spike and wave discharges in a genetic rat model of absence epilepsy
Christoph Johannes Mittag, Ali Gorji, Thomas Seidenbecher, Hans-Christian Pape

T23-5A An uncommon gain control – amplification instead of inhibition
Tim Ostrowski, Andreas Stumpner

T23-6A Dysfunction of thalamic adenylyl cyclases in an animal model of human absence epilepsy
Petra Ehling, Tatyana Kanyshkova, Arnd Baumann, Hans-Christian Pape, Thomas Budde

T23-7A Monoamines block kainate- and carbachol-induced gamma oscillations but augment stimulus-induced gamma oscillations in rat hippocampus in vitro
Anna Wojtowicz, Leander van den Boom, Arnab Chakrabarty, Nicola Maggio, Rizwan ul Haq, Christoph Behrens, Uwe Heinemann

T23-8A Analysing the central pattern generator for cricket stridulation
Stefan Schöneich, Berthold Hedwig

T23-9A A study of neuropeptidergic circadian coupling pathways in the cockroach *Leucophaea maderae*
Monika Stengl, Sandra Söhler, Thomas Reischig

T23-10A Alteration of brain states with high phase-coherence and transient states indicate the intermittency information processing in brain dynamics
abdelhafid Zeghbib, Antje Fillbrandt, Frank Ohl

T23-11A Self-organized criticality of developing artificial neuronal networks and dissociated cell cultures
Christian Tetzlaff, Samora Okujeni, Ulrich Egert, Florentin Wörgötter, Markus Butz

T23-12A State-dependent patterns of spatiotemporal coupling in rat visual cortex

Tim Wanger, Kentaroh Takagaki, Michael T. Lippert, Frank W. Ohl

T23-13A Multi-scale modelling of cortical population bursts
Bartosz Telenczuk, Andreas Herz, Gabriel Curio

T23-14A Identification and characterization of circadian clock molecules in the circadian pacemaker network of the cockroach *Leucophaea maderae*
Achim Werckenthin, Christian Derst, Monika Stengl

T23-15A Layering of the dentate gyrus is crucial for homogeneous hilar mossy cell input
Janina Kowalski, Markus Geuting, Alexander Drakew, Carola A. Haas, Shanting Zhao, Michael Frotscher, Imre Vida

T23-1B Independence of Functional Neuronal Network Architecture from Cholinergic and GABAergic Modulation: no Escape from the Small World
Kai Gansel, Wolf Singer

T23-2B Combining Experiments with a Percolation Model to Study Connectivity in Neural Cultures
Jordi Soriano-Fradera, Maria Rodriguez-Martinez, Or Cohen, Ann Keselman, Tsvi Tlusty, Elisha Moses

T23-3B Trial-to-trial variability of interaction dynamics between auditory and visual cortex during asynchronous audiovisual stimulation
Antje Fillbrandt, Hafid Zeghbib, Frank W Ohl

T23-4B Insect neuronal cell culture on multi electrode arrays
Katrin Göbbels, Volker Buck, André van Ooyen, Uwe Schnakenberg, Andreas Offenhäusser, Peter Bräunig

T23-5B Local field potential oscillations are reduced at high-beta and high-gamma frequencies in basal ganglia regions in an animal model of epilepsy
Stefanie Honndorf, Jason Chiang, Saskia Kücker, Manuela Gernert

T23-6B Pigment-dispersing hormone-immunoreactive neurons in the optic lobe of the marbled crayfish appear to be homologous to insect circadian pacemaker neurons
Abud Jose Farca Luna, Thomas Reischig, Ralf Heinrich

T23-7B Motor patterns during the initiation of different walking directions in stick insects
Denise Düsterhus, Josef Schmitz

T23-8B Perisomatic inhibition mediated by axo-axonic cells in CA3 area of the hippocampus
Tamar Dugladze, Hannah Monyer, Uwe Heinemann, Tengis Gloveli

T23-9B Electrophysiological characterization of circadian pacemaker candidates of the cockroach *Leucophaea maderae in-vivo* and at the level of single cells *in-vitro*
Nico Werner Funk, Janis Sebastian Brusius, Steffi Krannich, Monika Stengl

T23-10B MOUSE CENTRAL AND PERIPHERAL CIRCADIAN CLOCKS ARE ENTRAINED BY THE PHOTOPERIOD
Serhiy Sosniyenko, Daniela Parkanova, Martin Sladek, Helena Illnerova, Alena Sumova

T23-11B Frequency processing by the identified vibratory interneurons in a non-hearing Ensifera

(*Troglophilus neglectus*; Rhaphidophoridae) and its behavioural correlates
Nataša Stritih

T23-12B Monoaminergic innervation of NPY-immunoreactive neurons in the rat amygdala: a neuroanatomical study
Maria Roswitha Bonn, Esther Silke Asan

T23-13B The accessory medulla in the optic lobes of butterflies: Towards the solution of a homology problem in a putative circadian pacemaker neuropil
Thomas Reischig

T23-14B Protein kinase C dependent connectivity and activity dynamics in developing cortical networks
Samora Okujeni, Steffen Kandler, Oliver Weihberger, Ulrich Egert

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Pathophysiological Basis of Spreading Depolarisations, a Continuous Spectrum from Anoxic Depolarisation to Spreading Depression

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Spreading depression (SD) has first been described in 1944 by the Brazilian physiologist Leão as a cortical wave of ECoG suppression propagating with a velocity of 3-5 mm/min. The phenomenon simultaneously affects populations of brain cells. Main characteristics are not only a suppression of spontaneous electrical activity but also slow negative potential changes mostly measured as negative alterations of the direct current potential, major redistributions of ions at cellular membranes, and increases in tissue lactate. SD is not restricted to the cerebral cortex but a general phenomenon in grey matter of the central nervous system and has also been observed in subcortical structures, e.g. basal ganglia. It can be elicited by such diverse stimuli as mechanical puncture, high extracellular potassium or glutamate or by electrical high-frequency pulses. Multiple but not fully conclusive attempts have been made to explain mechanisms of SD initiation and SD propagation. In physiological conditions, SD is normally coupled with a hyperaemic vascular response that compensates for the energy needed to reinstall ion homeostasis at cell membranes.

Experimental studies and recent human studies provide evidence that SD occurs spontaneously in pathophysiological conditions such as trauma and hemorrhagic and ischemic stroke. Subsequent to induction of brain injuries, waves of depolarisation arise over prolonged periods. They are most frequent in zones surrounding infarcts, denominated then often as waves of peri-infarct depolarisation (PID). PIDs may be present as slow potential changes even in the absence of spontaneous electrical activity and have similar characteristics as SDs regarding for example disturbance of membrane ion homeostasis. Recent imaging studies of dynamic perfusional changes show that in peri-infarct regions, coupled blood flow alterations may be missing or even hypoemic so that compensatory effects are not achieved. The results using dynamic real-time imaging shed some more light on the dispersion of SD or PID waves. Both waves may propagate either in a radial fashion from core areas of injuries outwards into peripheral regions. They may, however, propagate also multiple times in a circular fashion around the injured tissue hitting thereby multiple times border zones of lesions. Resulting stepwise progressive deterioration includes both cerebral perfusion and metabolism and may finally lead to an inability of the tissue to repolarise and, in consequence, to terminal anoxic depolarisation and tissue death.

The Co-operative Study of Brain injury Depolarisations ("COSBID"): the impact of the tsunamis

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Introduction

Following the first (to our knowledge) fully validated demonstration and report of spread of depression of EEG amplitude in the injured human brain in 2002, - occurrence in humans of Leao's spreading depression - a group of clinicians and basic neuroscientists gathered to discuss the finding, and formed the Co-operative Study of Brain Injury Depolarisations ("COSBID", www.cosbid.org). On the basis of several experimental papers indicating that repeated peri-lesion depolarisations enlarge a cerebral cortical infarct (rather than simply resulting from it), the group identified testing of the (null) hypothesis "that depolarisations are not associated with worse clinical outcome" as its primary goal. Four disease states were identified for study in which emergency craniotomy is often required: (1) traumatic brain injury (TBI), (2) subarachnoid haemorrhage from ruptured intracranial aneurysm (aSAH), (3) malignant hemisphere stroke (resulting in intractable brain oedema)(MHS), and (4) spontaneous intracerebral haemotoma (ICH). A critical feature of all these conditions is the high incidence of delayed neurological deterioration in the 2-9 (depending on diagnosis) days after the acute event, usually resulting in worse outcome.

In addition to addressing the primary question, members of the group are conducting a number of important subsidiary studies capable of providing information on mechanisms whereby recurrent depolarisations might increase extent or severity of brain injury.

Strategy and Methods

The intial main study currently in progress seeks to power a study to address the primary hypothesis, and, with research ethics approval in the contributing centres, recruits patients typically in the age range 16-70 who require emergency craniotomy. Data on clinical features that covary closely with outcome are collected, together with information on aggregate duration of depolarisations, and outcome at 6 months post-trauma (extended Glasgow Outcome Scale). Impact of depolarisations on outcome in each disease group will be tested using a proportional odds model, and the 4 result sets assembled as a meta-analysis (Forest plot).

Monitoring of EEG activity is recorded at the brain surface (electrocorticography, ECoG) using a linear 6-electrode strip placed on the brain prior to closure of the surgical wound. ECoG is recorded continuously typically for 5-7 days following surgery: during this phase most patients are nursed in a high dependency or intensive care unit and are usually sedated.

Current results

In these groups of seriously ill patients, incidence of depolarisations is:-

aSAH: 70-90%

MHS: 100% (in patients where the recording strip is placed on vulnerable rather than infarcted cortex)

TBI: 50-60%

ICH: 55%

Members of the COSBID group have identified important pathophysiologcal events accompanying depolarisations in these patients, for example transient, and sometimes cumulative, depletion of brain extracellular glucose (monitored with rapid-sapling microdialysis), together with factors such as pyrexia, and systemic arterial hypotension that are associated with increased frequency of depolarisations. (Following experimental middle cerebral artery occlusion there is a robust inverse relationship between plasma glucose and frequency of peri-infarct depolarisations).

Conclusion

Initial comparison of outcome with depolarisation load in the first 84 patients with complete data indicates a strong adverse effect on outcome ($p=0.005$, Spearman rank correlation) (courtesy of Prof Gordon Murray, Univ. Edinburgh). Following this striking result, we now require to demonstrate that the effect is independent of known covariates such as initial severity of injury, age, and occurrence of secondary insults such as hypotension.

Spreading depression of high-frequency neuronal activity and low-frequency vascular fluctuations correlate in the human brain during normal and inverse neurovascular coupling

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Extensive experimental work has established that a 'Tsunami'-like process, termed cortical spreading depolarisation (CSD), causes and maintains the cytotoxic edema in the grey matter of the brain. On the cellular level, this process is ignited when passive influx of sodium and calcium across the neuronal membranes exceeds ATP-dependent sodium and calcium pump activity followed by water influx. Dependent on the capacity to recruit additional pump activity, CSD is locally reversible or not. The by far largest among all changes of the direct (steady) current (DC) potential in the brain reflects this cellular process in the extracellular space and allows the experimenter to follow the spread of the cytotoxic edema in the tissue at the typical rate of ~3mm/min. Thus, a short-lasting intracortical negative DC potential change of ~20mV for 1 or 2 minutes indicates a fully reversible cytotoxic edema while progressively longer-lasting negative DC potential changes reflect the local protraction of the cytotoxic edema that eventually result in either necrosis or apoptosis.

Experimentally, CSD induces tone alterations of resistance vessels causing transient hyperperfusion (normal neurovascular coupling) in healthy tissue or hypoperfusion (inverse neurovascular coupling) in tissue at risk for progressive damage. Due to these complex vascular signatures, CSD cannnot be identified to date by functional imaging methods alone. Here we show that a spreading depression of vascular low frequency fluctuations is an identifier of CSDs. We performed a prospective study in thirteen patients with aneurysmal subarachnoid hemorrhage using subdural opto-electrodes for simultaneous laser-Doppler flowmetry and direct current-electrocorticography. Either no perfusion change, hypo- or hyperperfusion occurred in response to CSD and was always accompanied by a spreading depression of low-frequency vascular fluctuations ($f<0.1$Hz) that correlated with the spreading depression of high-frequency neuronal activity. This novel 'functional marker' offers the option to determine progressive ischemic damage in patients non-invasively. Thus it might be possible in the future to non-invasively differentiate whether a patient is in the phase of progressive ischemic damage or already in the phase of repair and regeneration that require opposing treatment strategies.

The Relation between Spreading Depolarisations and Outcome after Acute Neuronal Injury in the Human Brain<

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Purpose: To test if the occurrence of spreading depolarisations in the acutely injured human brain is uncorrelated to six month outcome (null hypothesis).

Background: Periinfarct depolarisations (PID) and cortical spreading depression (CSD) are waves of massive depolarisation that spreads over the cortical surface at a rate of 2-3 mm per minute. It has been shown in animals that restoration of the membrane potential after the severe depolarisation is a highly energy demanding process, often accompanied by a marked rise of cerebral blood flow. If perfusion is restricted, blood flow may be unchanged or even undergo a prolonged severe reduction which may promote expansion of an ischemic lesion. We have previously reported that CSD is a common phenomenon in acute brain injury following brain trauma (Strong 2002), subarachnoid haemorrhage (SAH) (Dreier 2006) and severe ischemic stroke (Dohmen 2008).

Methods: Patients operated for acute brain injury had a subdural strip with 6 platinum electrodes placed close to the main lesion. Electrocorticogram was recorded for up to 10 days. The recordings were analyzed on Chart software according to predefined criteria (Fabricius 2006). The total duration of depressed cortical activity after PID or CSD was quantified. After six months, patients clinical state was quantified using the extended Glascow outcome scale (eGOS).

Results: Repeated episodes of CSD (up to 90) were recorded in 53% of patients with traumatic brain injury, 55% of patients with intracerebral haemorrhage, 72 % of patient with SAH, and 81% of patients with severe ischemic stroke. A Spearman rank correlation showed an association of 6 month eGOS with the total duration of depressed cortical activity after depolarisations (P=0.005). Outcome varied considerably with diagnosis: In severe ischemic stroke patients recovery of cortical activity after CSD was very prolonged or absent, and outcome was severe, vegetative or fatal. In comparison, 75% of four patients with intracerebral haemorrhage and multiple CSDs had a moderate or good outcome. In these patients recovery of cortical activity after CSD was fast (4-9 minutes).

Discussion: The association of outcome with depressed cortical activity does not demonstrate if the depolarisations are the cause or just a marker for bad outcome. Animal data suggests that these depolarisations may be a key factor for the expansion of the ischemic penumbra. To address this issue, a covariant analysis is in progress to show if the depressions are independent predictors of outcome.

Conclusion: Spreading depolarisations are related to long term outcome in patients with acute brain injury.

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Spreading Depolarisations Occur in Human Ischemic Stroke with High Incidence

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Objective: Spreading Depolarisations i.e. Cortical spreading depression (CSD) and peri-infarct depolarisation (PID) have been shown in various experimental models of stroke to cause secondary neuronal damage and infarct expansion. For decades it has been questioned whether Spreading Depolarisations occur in human ischemic stroke. Here, we describe CSD and PID in patients with malignant middle cerebral artery (MCA) infarction detected by subdural electrocorticography (ECoG).
Methods: Centres of the Co-operative Study of Brain Injury Depolarisations (COSBID) recruited 16 patients with large MCA infarction. During surgery for decompressive hemicraniectomy, an electrode strip was placed on the peri-infarct region, from which 4 ECoG channels were acquired.
Results: A total of 1638 h was recorded; mean monitoring time per patient was 109.2 h. A total of 127 CSD and 42 PID events were observed. In CSD, a stereotyped slow potential change spreading between adjacent channels was accompanied by transient depression of ECoG activity. In PID, a slow potential change spread between neighbouring channels despite already-established suppression of ECoG activity. CSD or PID was observed in all but 2 patients. In these two patients, the electrode strip had been placed over infarcted tissue and accordingly, no local ECoG or recurrent transient depolarisation activity occurred throughout the observation period. Our results suggest that terminological classification of spreading depolarisations as either CSD or PID depends on time and location of the recorded event and thus largely on definition criteria. The present classification of events as either CSD or PID may therefore be an artificial dichotomy, whereas the terms may in fact represent two extremes of a single continuum. Most Spreading Depolarisations appeared repetitively in clusters showing a periodicity between 30 -120 min. Repetitive PID were associated with infarct growth. Considering the results of experimental focal ischemia where periodical cycling of spreading depolarisations around an ischemic lesion lead to infarct growth we suggest such deleterious cycling of spreading depolarisations to occur also in human ischemia reflected by the periodical occurrence of spreading depolarisations in our study. Interpretation: Spreading Depolarisations occured spontaneously with high frequency in this study of patients with malignant MCA infarction. This suggests that the large volume of experimental studies of occlusive stroke that implicate spreading depolarisations in its pathophysiology can be translated, with appropriate caution, to patients and their treatment.

Spreading depression in migraine.

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The characteristic development of sensory disturbances during migraine auras suggests that the underlying mechanism is a disturbance of the cerebral cortex, probably the cortical spreading depression of Leao. This viewpoint is supported by the finding of unique changes of brain blood flow during attacks of migraine with aura, which have been replicated in animals exposed to single episodes of spreading depression. Spreading depression is a transient depression of electrical activity that moves across the cerebral cortex at a rate of 3-5 mm/min. The depression is associated with a dramatic failure of brain ion homeostasis and efflux of excitatory amino acids from nerve cells. Diffusion of potassium and glutamate in the extracellular space from the affected regions to the surrounding tissue is probably the mechanism by which the depression propagates through the grey matter. Recent experiments have shown that spreading depression in a variety of species including man is dependent on activation of a single receptor, the NMDA-receptor, one of the three subtypes of glutamate receptors. On this background, new drugs have been developed which are now undergoing clinical trials as anti-migraine medication. The combined experimental and clinical studies point to fruitful areas in which to look for migraine treatments of the future, and provides a framework within which important aspects of the migraine attack can be modelled.

The role of dendritic processing in the retina

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The retina is a sophisticated image processor that extracts and encodes important features from the observed scene before this information is sent to the brain. Moreover, the retina underlies very unique spatial constraints: To prevent 'information gaps' in the visual field each of the different feature-extracting microcircuits needs to be replicated at every retinal position. At the same time, the retinal tissue needs to be thin to allow light passing through the retinal layers to reach the photoreceptors. Thus, tight packing of neuronal hardware is required. One solution that benefits such tight packing is to implement complex computations already at the level of single neurons, for instance in a neuron's dendrites.

Using a combination of electrophysiological techniques and two-photon Ca^{2+} imaging of light stimulus-evoked activity we study dendritic processing in the retinal circuitry that detects the direction of image motion. The computation of motion direction is performed presynaptically to the direction-selective (DS) ganglion cells by starburst amacrine cells (SAC). In response to moving stimuli, SACs generate directionally-tuned Ca^{2+} signals in their dendrites (*Euler et al., 2002, Nature 418:845-852*) as the result of a "dendrite-autonomous" computation (*Hausselt et al., 2007, PLoS Biol. 5(7), e185*). Here, we took a closer look at the subcellular distribution of SAC Ca^{2+} signals and at the possibility that internal Ca^{2+} stores are involved.

In agreement with earlier observations, light-evoked Ca^{2+} signals were most prominent in varicosities. These are mainly located in the distal third of the dendrites, which correspond to the SAC's synaptic output region. In comparison, Ca^{2+} signals induced by somatically applied voltage steps covered more dendritic area including inter-varicosity regions. About 45% of all varicosities were found to be active ('hotspots'), that is, they displayed light-evoked Ca^{2+} signals. The majority of these 'hotspots' (~ 48%) were direction-selective (DS) with a preference for centrifugal motion. The remaining 'hotspots' were either non-DS (~ 33%) or even had a preference for centripetal motion (~ 19%). While active varicosities tended to be located more distally and silent ones more proximally, interestingly, both kinds of varicosities could also be found in close proximity on the same branchlet.

To determine if Ca^{2+} stores are involved in the generation of SAC Ca^{2+} signals we applied drugs that block or modulate different intracellular Ca^{2+} pathways. Light-evoked Ca^{2+} signals in SACs were abolished (or strongly reduced) by 2-APB, suggesting the involvement of IP_3 receptor-mediated Ca^{2+} signaling. Voltage responses in SACs were, however, also reduced, suggesting that 2-APB also affects the pathways presynaptic to SACs.

In conclusion, detailed mapping of the light stimulus-evoked Ca^{2+} signals along the dendrites of SACs revealed a surprising heterogeneity that suggests differential signal processing at the scale of tens of micrometers. In addition, our pharmacological data suggests that the light-evoked dendritic Ca^{2+} responses rely, at least in part, on internal Ca^{2+} stores.

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Approach Sensitivity in the Mammalian Retina

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It is important for animals to detect and avoid approaching objects – whether predators attacking, or obstacles in the animal's path. In animals and humans, approaching motion, such as that of looming objects, elicits a variety of behaviours, such as startle and protective motor responses. Neurons have been identified in locust, frog, and pigeon that respond selectively to approaching motion stimuli. However, the neural circuits and mechanisms underlying 'approach-sensitive' computations have yet to be pinpointed. We have combined genetic labelling of neural cell types, two-photon microscopy, electrophysiology, and theoretical modelling to describe an approach-sensitive neuron in the mammalian visual system, a ganglion cell type in the mouse retina, as well as the neural circuit that enables it to distinguish approaching from non-approaching stimuli within its receptive field. The essential building block of the circuit is an inhibitory pathway that carries signals fast enough to suppress responses to non-approaching objects. This fast inhibition is ensured by the presence of an electrical synapse. We demonstrate that this, together with other circuit properties, renders the retinal ganglion cell approach-sensitive. This work yields the first description of a mammalian approach-sensitive neuron, and it also reveals a fundamental role of electrical synapses in a neural computation. The circuit module that contains the electrical synapse is well-known for its role in night vision. Intriguingly, under the day-time conditions of our experiments, the information flow through the same circuit module is reversed, to yield a fast inhibitory pathway. This dual use of a given circuit module illustrates an evolutionary strategy for packing distinct physiological functions into a single anatomical circuit.

Temporal filtering by A-type ganglion cells in the mouse retina

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One of the primary roles of the retina is to separate the photoreceptor signal into fast and slow components. Early extracellular recordings from cat, primate and salamander ganglion cells provided the first evidence for a segregation of temporal events. “Sustained” ganglion cells responded to a step change in illumination with persistent firing or with changes in their maintained firing frequency, whereas “transient” cells produced short bursts of action potentials at the initiation and/or termination of the step. Cleland and Levick (1974) later subdivided cat ganglion cells with sustained and transient discharge patterns into “brisk” units, which responded to relatively high temporal frequencies, and “sluggish” units, which responded optimally to stimulation at lower frequencies. But how does the retina separate a single photoreceptor response into the distinct temporal components signaled by the ganglion cells? Previous studies have attributed temporal filtering largely to the bipolar cells (DeVries, 2000, 2006). However, the segregation of temporal information continues at the level of the ganglion cells. In this study we combine patch clamp recordings from A-type ganglion cells in the isolated mouse retina with pharmacological blockade of different voltage-gated channels in order to dissect their effects on temporal filtering. We show that different voltage-gated sodium channels, T-type calcium channels and HCN channels all act as high-pass filters, irrespective of their contribution to the membrane time constant.

Reading out a Correlated Population Code

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Throughout the brain, information is represented by large populations of neurons, yet little is known about the population code. A key issue is the pattern and strength of correlations among neurons. We used the retina as a model system to study how correlations effect a neural code. We presented the retina with a set of 36 different shapes, recorded light responses from 162 ganglion cells, and asked how well the brain could discriminate one shape from all the rest. Decoders that took into account correlation could reach zero sampled errors in a many-hour physiology experiment, while decoders that ignored correlation performed much worse, in some cases having error rates >100-fold higher. This effect required groups of 100+ ganglion cells. Decoders that approximated the correlation structure in the population by matching all pairwise correlations (maximum entropy model) were quite successful, especially for a spike latency code. These results show that both temporal information and correlation among cells can dramatically effect the fidelity of a population code.

Spike rates and temporal structure of retinal ganglion cell responses encode different stimulus properties

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The central nervous system depends on spikes of retinal ganglion cells (RGC) as the only source of information about the visual environment. Since natural stimuli are multidimensional (e.g. light intensity, spatial and temporal contrast, velocity of moving stimuli), several stimulus properties must be encoded simultaneously by the RGC responses.

In this study, we analyzed RGC encoding of velocities and velocity changes of moving stimuli based on multi-electrode recordings from the isolated turtle retina. The stimulus consisted of a dot pattern moving at one of 9 different velocities in one dimension. Every 500ms, the velocity changed abruptly to a new value [1].
Combining spike responses of multiple individual RGC, we used the method of metric based clustering [2,3] to calculate the classification performances for

1. stimulus velocity (9 different combinations of speed and direction)
2. velocity transitions (72 different combinations of velocities before and after the most recent transition).

Our main finding, which was also supported using different analysis methods, is that velocities and velocity transitions (i.e. instantaneous accelerations) are encoded by different RGC response properties. Constant velocities can be estimated best based on the number of spikes in long integration time windows. The spike rate of RGC responses encodes the constant velocity during the entire stimulus duration of 500ms. In contrast, estimation of velocity transitions is improved by using the temporal structure of RGC responses on time scales of 30-100ms. Most information about the stimulus change is present in the first response segment following the transition.

The classification performance of both constant velocities and velocity transitions improves with the number of cells used for stimulus estimation. The best classification results are obtained for cell ensembles containing both and direction-selective and symmetrically tuned RGC, in particular if the knowledge of which spike was fired by which cell is used for classification ($k>0$ in fig.1). However, the ensemble mean classification performance is lower than the sum of the classification percentages of the individual cells. Therefore, a certain degree of redundancy in stimulus encoding must be assumed, even for different RGC types.

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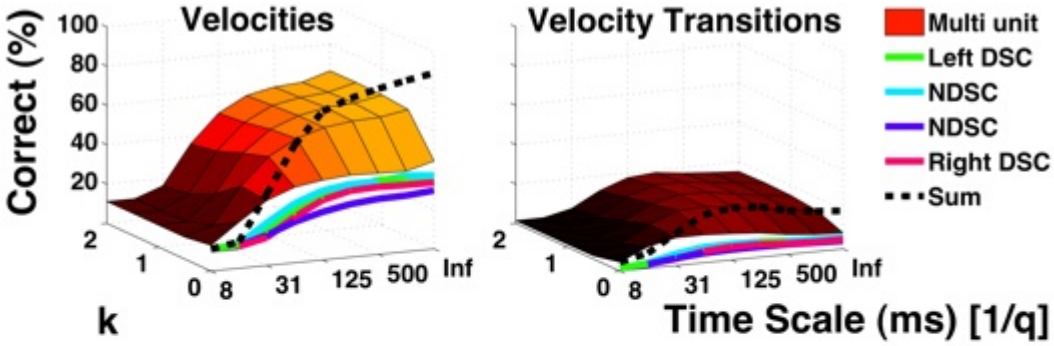

Mean classification performance for velocities and velocity transitions using non-, left and right direction-selective cells (NDSC, Left DSC and Right DSC, respectively). Solid lines: Classification performance of individual RGC based on [2]. Dashed line: Sum of individual classification performances. Coloured surface: Classification performance combining different RGC based on [3]. Axes: 1/q indicates the time scale (precision) of spike firing (Inf: spike firing rate without time precision). For the multi-neuron analysis, k reflects the importance of knowledge of cellular origin of each spike (k=0: not important, k=2: very important). Chance levels: Velocities=11.11%, Velocity transitions=1.39%.

What is the goal of neural image processing in the retina?

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Vision scientists since Helmholtz have argued that human visual perception is best understood as an inference process that seeks to explain the physical causes of the retinal image. Photographers know very well that the task of image acquisition itself is intrinsically tied to this process of image interpretation. For a similar reason, it is important to our understanding of the retina that we can say how the generation of nerve impulses is shaped by the task of image interpretation. The redundancy reduction hypothesis put forth by Barlow is an attempt to turn this kind of thoughts into mathematical models that can offer a computational interpretation of neural response properties observed experimentally. While many studies have investigated how neural filter properties may be shaped by the spatial statistics of natural images, the temporal properties of the retinal sampling process have mostly been ignored. Here, we study the spatio-temporal statistics of temporal sequences of images that are obtained when a static scene is dynamically sampled with saccadic gaze shifts and fixational eye movements. We present new analytic results which explain the effect of the dynamic sampling process on the spatio-temporal correlation function of the visual input. Based on these results, we will speculate about possible implications for neural image coding in the retina.

Microglia engraftment in the postnatal brain: the questions shape the answers

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Microglia are crucially important myeloid cells in the central nervous system (CNS) and constitute the first immunological barrier against pathogens and environmental insults. The factors controlling microglia recruitment from the blood remain elusive and the direct circulating microglia precursor has not yet been identified /in vivo/. Using a panel of bone marrow chimeric and adoptive transfer experiments, we provide evidence that circulating Ly-6C^hi * *CCR2^+ monocytes were preferentially recruited to the lesioned brain and differentiated into microglia. Importantly, microglia engraftment in CNS pathologies, which are not associated with overt blood-brain barrier disruption, required previous conditioning of brain, e.g. by direct tissue irradiation. Our results identify Ly-6C^hi CCR2^+ monocytes as direct precursors of microglia in the adult brain, and establish the importance of local factors in the adult CNS for microglia engraftment.

Chemokines in neuron-microglia signaling

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Microglia are the sentinels of the CNS. Accordingly, microglia activity is aimed to protect and to restore and only in case of un-controlled or impaired microglia function these cells may have detrimental effects. The control of microglia activity is thus an important issue to understand.

For a long time neurons mainly have been regarded as targets of (over)activated microglia, with little control of microglia function. There is, however, now accumulative evidence that neurons express so called "On" and "Off" signals to inform microglia about their status and are thus capable of influencing microglia activity.

The family of chemokines are versatile signals specialized to control cell-cell interactions. Neurons express a variety of chemokines in a temporarily and spatially regulated manner and microglia respond to these messengers via the appropriate receptors. Here I would like to discuss the function of two neuronal chemokines, CCL21 and CX3CL1. CCL21 is specifically expressed in endangered neurons and belongs therefore to the class of neuronal "On" signals. Our recent data indicate that neuronal CCL21 is sorted in large-dense core vesicles, transported into neuronal axons and released in a regulated manner. Since microglia activity in various models of neuronal damage is changed in animals with disturbed CCL21 signaling, we suggest a role of CCL21 in directed neuron-microglia communication.

CX3CL1 is a chemokine that is constitutively expressed in neurons and can therefore by regarded as an "Off" signal. CX3CL1 signaling downregulates various microglia responses, thereby dampening potential microglia neurotoxicity. CX3CL1 is found in brain in two forms, membrane bound and soluble. Earlier studies concerning CX3CL1 have focused on its soluble form. However, since neurons express high amounts of CX3CL1 in their membrane we studied the effects of membrane-bound CX3CL1 on microglia activity. I here will present findings which indicate that also membrane-bound CX3CL1 dampens microglia pro-inflammatory action. We therefore suggest that CX3CL1 is a neuronal regulator of microglia activity when both cell types are in close contact to each other.

Thus chemokines enable neurons to control the activity of microglia in various ways, making them important messengers in neuron-microglia communication.

Effects of neuropeptides on microglia under pathophysiologic conditions

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Various neuropeptides have wide variety of physiological functions but little is known about their function in microglia. Expression of some neuropeptides is up-regulated in many brain regions following nerve injury and in the basal forebrain of patients with neurodegenerative diseases such as Alzheimer's disease. The rise in endogenous neuropeptides observed either after injury or in various disease sites is reported to be an adaptive response by the activation of receptors and the following signal transduction. Receptors for some neuropeptides are reported to be expressed in microglia. Therefore, we analyzed the effects of several neuropeptides on microglia using primary cultured rodent microglia. Motility of microglia under the control of temperature (37°C) and gas (10% CO_2/90% air) was monitored with time lapse video microscopy system. **Chemotaxis was tested using a 48-well microchemotaxis Boyden chamber. We observed that micoglial migration was enhanced by galanin, substance P, vasopressin, somatostatin, endothelin, as well as bradykinin. As for the mechanism on increased** microglial migration, we found pharmacologically that signal transduction induced by neuropeptides were different from that induced by ATP. In most cases, activation of Ca^{2+}-dependent K^+ channels was important, suggesting that Ca^{2+} increase and subsequent activation of K^+ channels is required. Using *in vivo* lesion models and pharmacological injection to the brain, it is shown that neuropeptides-dependent microglial accumulation is also dependent on the activation of Ca^{2+}-dependent K^+ channels. Our results may help to understand the functional importance of interplay between neuropeptides and microglia in neuronal injury or inflammation in the brain.

Neurotransmitter control microglial functions

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Microglia, the brain-specific macrophages are the major immunocompetent cells in the CNS and play an important role in mediating responses to pathological events, such as trauma, infection or ischemia. In the normal brain, these cells are ramified and were termed ´resting´ microglia. Recent studies indicate that they are not at all resting, but continuously survey their surrounding space with their processes. We have addressed the question whether microglial cells have the potential to detect neuronal activity by expressing neurotransmitter receptors. We found that microglial cells express dopaminergic, adrenergic and purinergic receptors. Activation of these receptors modulates the executive functions of microglial cells such as the release of cytokines or cell migration. One receptor family prominently expressed by microglial cells are receptors for nucleosides such as ATP, or ADP and for nucleotides such as or adenosine. ATP can be released either in the normal brain by astrocyte activity and in pathology from damaged cells. Specific ectoenzymes control the degradation of ATP into derivatives such as adenosine. Microglial cells express these enzymes such as cd39 which converts ATP into AMP. We found that deletion of cd39 affects microglial migration in culture and in an in vivo mouse model of middle cerebral artery occlusion resulted in larger ischemic brain lesions and a decreased density of microglial cells in the prenumbra comparing to controls. Thus microglial cells are part of the signalling network mediated by neurotransmitters and since these receptors control microglial functions this is a new potential route how these signalling substances may influences the outcome of pathological events in the brain.

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Microglial Cells as Sensors and Modulators of Brain Pathology

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Microglia represent the first line of defence in the CNS. They dynamically immunosurvey the neuropil, and they serve important maintenance functions for the neurons and other glia. Acute injury to the CNS leads to prompt activation of microglia, which in the case of ischemic stroke are supplemented by large numbers of bone marrow-derived macrophages as well as granulocytes. In comparison, the pathology that occurs in Alzheimer's disease results in a chronic, low-grade microglial-macrophage response. Our observations of the cell population kinetics and the cytokine-response of microglia-macrophages after acute neural injury and in chronic Alzheimer's-like pathology will be reported. The presentation will also contain new data on the apparent selectivity in cytokine response by different subsets of microglia and macrophages in ischemic stroke, pointing to a hitherto unrecognized functional diversity of these cells. Finally, I will present new data suggesting that microglial- not leukocyte-derived TNF has a neuroprotective role after ischemic stroke in mice, pointing to a key role of microglia in determining the survival of endangered neurons in cerebral ischemia.

Microglia: how big are they really?

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While microglia, the brain's intrinsic macrophages, have a rather detrimental role in a mouse model of multiple sclerosis (MS) (Heppner et al., 2005), they appear to have a beneficial effect on clearing infectious prions (Falsig et al., 2008). These data demonstrate the diversity of microglial function within various disorders of the central nervous system (CNS) and highlight the microglial compartment as potential therapeutic target in inflammatory CNS disorders. Since peripheral macrophages entering the brain were reported to influence the pathogenesis of Alzheimer's disease (AD) (see Jucker and Heppner, 2008), we studied the role of intrinsic, resident microglial cells in AD. To this effect, CD11b-HSVTK (TK) transgenic mice allowing ablation of microglia upon treatment with Ganciclovir (GCV) (Heppner et al., 2005) were crossed to APPPS1 transgenic mice, a model of Alzheimer's disease (AD) exhibiting rapid and early onset of amyloid pathology (Radde et al., 2006). Firstly, we tested whether amyloid plaques develop in the absence of microglia by applying GCV intracerebrally via osmotic minipumps to young APPPS1-TK mice prior to amyloid deposition. Secondly, the impact of microglia on amyloid plaque maintenance was studied by applying GCV to aged amyloid-bearing APPPS1-TK mice. Our experiments in line with other studies demonstrate that microglial function differs considerably within various CNS diseases: while in autoimmune inflammatory conditions such as MS microglial activation appears to be a harmful event, in other pathological settings microglia may rather have a beneficial effect. Thus, manipulation of microglia as a putative therapeutic strategy is of relevance to certain CNS diseases.

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Molecular mechanisms of dopamine neurodegeneration: *C. elegans* as a novel pharmacogenetic model for Parkinson's disease and manganism

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Parkinson's disease (PD) is a progressive neurodegenerative disorder largely characterized by the loss of dopaminergic neurons. Although the etiology of the disease is unknown, it is believed to be multifactorial, with both genetic and environmental contributions that result in oxidative damage and mitochondrial dysfunction. We have established novel *C. elegans* models for Parkinson's disease that now allow for the evaluation of PD-associated orthologues and other proteins in their role in dopamine (DA) neuron vulnerability in response to endogenous and exogenous insults, and provides opportunities to identify and characterize novel PD therapeutic targets and leads. Here we describe our model system, as well as the expression and biochemical investigations of WT and mutant PD- and DA neuron-associated proteins, and their effects on mitochondria function and DA neurons under basal conditions and following exposure to neurotoxins. qPCR gene expression studies of PD- and DA neuron-associated proteins show significant changes in expression of most of the familial PD genes as well as the dopamine transporter (DAT) and vesicular monoamine transporter (VMAT) following exposure to the neurotoxins. Microarray studies in both ΔDAT and WT worms at different ages following exposure to PD-associated toxins also indicate significant changes in genes involved in axonal growth, synaptic transmission, oxidative stress, and complex I of mitochondria. Biochemical analysis show that mutations within some of the PD-associated genes in *C. elegans* affect mitochondrial membrane potential, respiration capacity, and dopamine and glutathione concentrations. Mutations within several PD- and DA neuron-associated proteins increase the vulnerability of DA neurons to mitochondria-targeted neurotoxins, and anti-oxidant flavonoids that may increase mitochondrial function significantly protect against DA neurodegeneration. These investigations, as well as our preliminary results from genetic screens to identify modifiers of DA neuron vulnerability will be presented. Overall these studies suggest that mutations within DA neuron- and PD-associated genes result in increases in oxidative stress and mitochondria dysfunction, and further validates *C. elegans* as a powerful genetic model to explore PD.

Dopamine modulation of cognitive functions in rodents: Implications for psychiatry

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In a range of species discrete frontal cortico-basal ganglia circuits have been implicated in various cognitive and executive functions such as decision making, working memory or action-outcome learning. For example, the anterior cingulate cortex (ACC) and the nucleus accumbens are key components of a circuit that is critically involved in effort-based decision making, i.e. in encoding whether or not an action is worth performing in view of the expected benefit and the cost of performing the action.Our recent studies demonstrate that after an intra-ACC blockade of dopamine (DA) D1 receptors rats no longer selected the high cost -high reward option in a cost-benefit T-maze task, if having the choice between climbing a barrier to obtain a large reward in one arm or to run for a low reward into the other arm with no barrier present[1]. Thus, an impaired prefrontal DA neurotransmission biases behaviour to low effort - low reward response options. Various psychiatric conditions such as depression are characterized by the presence of energy-related dysfunctions, e.g., psychomotor slowing or anergia, and difficulties in decision making. Animal models as used here may, therefore, provide a better understanding of the neural and neurochemical substrates underlying these decision making problems. Furthermore, we demonstrated that the level of D1 receptor activity in the ACC influences effort-based decision making, i.e. too little and too much DA D1 activity can impair performance[2]. Thus, the inverted U-shaped principle of D1 receptor function known to mediate working memory[3] also applies to effort-based decision making. This observation implies that both dopamine agonists such L-DOPA or cocaine and dopamine antagonists such as numerous antipsychotic drugs could impair effort-based decision making in humans. Remarkably, prefrontal DA D1, but not D2, receptors mediate effort-based decision making[1] and working memory[3], while both prefrontal DA D1 and D2 receptors act in a cooperative manner to facilitate distinct forms of behavioral flexibility[3,4]. Therefore, mesocortical DA modulation of different types of cognitive and executive functions appear to be mediated by dissociable patterns of prefrontal DA receptor activity.

The capacity of humans and animals to accurately evaluate the causal effectiveness of their actions is crucial to adapt to changing environments. This capacity relies on the ability to learn associations between actions and outcomes and to detect changes in the causal effectiveness of actions. These cognitive processes are governed by a frontal cortico-basal circuit that involves the prelimbic subregion of the prefrontal cortex and the dorsal striatum. Our recent rodent studies provide support to the notion that DA signaling in a particular subregion of the dorsal striatum, the posterior dorsomedial striatum (pDMS), is critically involved in action-outcome learning. Rats subjected to a pDMS DA depletion responded inflexible as they were insensitive to changes of action-outcome relationships[5]. Thus, the rat's ability to detect changes in the causal efficacy of actions depends on DA signaling in the pDMS. Notably, several psychiatric disorders are characterized by behaviours that are insensitive to their detrimental consequences, e.g., the inability of addicts to modify the instrumental act of drug intake despite knowledge of the negative consequences of this behaviour. Our animal data point to the possibility that a striatal DA dysfunction could play a role in such impairments of goal-directed action in humans.Supported by the DFG.

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Can methylphenidate “normalize” inattentiveness, brain hypofunction and synaptic wiring? Functional imaging and neuroanatomical analysis in a novel animal model for ADHD

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We have previously shown that early stressful experience can induce behavioural changes such as increased locomotor activity (hyperactivity) and reduced responses to familiar conspecific vocalizations (attention deficit). These behavioural changes are paralleled by altered dopaminergic functions and disturbed development of synaptic connectivity, particularly in the prefrontal anterior cingulate cortex (ACd). Since these changes are reminiscent of symptoms of the Attention-Deficit Hyperactivity Disorder (ADHD), we studied the effects of acute and chronic methylphenidate (MP) treatment on behavior, brain activity, and brain morphology.

Juvenile degus (*Octodon degus*) were stressed by one hour daily parental separation from postnatal day (PND) 1 to 21. On PND 22 degu pups were tested for hyperactivity in a classical open field tests and attention deficit in a modified version of the open field test, where the pups were exposed to familiar conspecific vocalisations. To analyze if the behavioral alterations were paralleled by changes of brain activity we applied the 2-fluoro-2-deoxyglucose (2-FDG) technique for functional brain imaging. Additionally, dendritic spine densities and dendritic length of pyramidal neurons in the ACd were quantified using the Golgi-Cox staining technique.

We found that the observed behavioral disturbances, hyperactivity and inattentiveness, were paralleled by a reduced metabolic activity of a number of prefrontal, sensory and mesolimbic brain areas. Acute treatment with low doses of MP (1mg/kg) lead to a partly normalization of the behavioral deficits. Moreover, in the stressed animals acute MP treatment also lead to a ‘normalized’ metabolic activity in prefrontal brain areas but not in sensory areas such as the auditory cortex.. On the morphological level we were able to show that subchronic prepubertal treatment with 1mg/kg MP restored synaptic density in the ACd of hyperactive animals.

Our results show that early stress experience in degus leads to behavioral and brain functional deficits that are similar to symptoms of ADHD in humans. These deficits can be partly normalized or restored by treatment with methylphenidate, indicating an involvement of the dopaminergic system. This is supported by a parallel neurochemical study in this animal model, where functional dopaminergic dysfunctions could be demonstrated in the ACd and Nucleus Accumbens. In summary, our findings reveal that early separation stress in degus may provide a suitable novel animal model to study the brain functional deficits underlying behavioral disorders such as ADHD.

Neurobiological and clinical aspects of dopamine dysfunction in human ADHD

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Abnormalities in reward processing with reduced ventral striatum activation during gain anticipation have been found in adolescents and adults with attention deficit hyperactivity disorder (ADHD) using functional magnetic resonance imaging (fMRI) within a motivational paradigm (‘monetary incentive delay task’). However, groups of ADHD patients in previous studies were mixed regarding ADHD subtype, gender and, in part, drug treatment status.

This study sought to compare ventral striatum and prefrontal activations during reward anticipation and feedback in homogenous groups of adults with ADHD subtypes and healthy controls.

The abovementioned fMRI paradigm was used. Twelve drug-naïve male adults with ADHD combined type, twelve drug-naïve male adults with ADHD predominantly inattentive type, and twelve adult healthy control persons were included. Groups were matched for age, gender, and intelligence quotient.

>Compared to healthy controls, ADHD subgroups showed significantly less ventral striatum activation during reward anticipation ($p<0.01$). Moreover, activation patterns in the basal nuclei and cortical regions differed significantly between ADHD subtypes, and combined type patients displayed increased ventral striatum activation compared to predominantly inattentive patients ($p<0.01$). Contrary to our expectations, during gain feedback the predominantly inattentive patients displayed significantly increased activations in multiple cortical and subcortical areas when compared with combined type subjects. Compared with both ADHD groups, the healthy controls, however, showed increased activations in prefrontal regions during gain feedback, which was not consistent with the results of a recent study that has shown increased prefrontal activations in adults with ADHD under that condition.

This is the first study to reveal differences in reward processing between ADHD subtypes and to show significant abnormalities of both subtypes compared to healthy controls, using an fMRI motivational paradigm.

Animal models of schizophrenia - focus on the dopamine hypothesis

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The dopamine hypothesis of schizophrenia is one of the most influential theoretical constructs in schizophrenia research. Based on clinical and experimental pharmacological data, as well as on neuroimaging data, it states that many symptoms of schizophrenia are due to a hyperfunctional mesostriatal and/or a hypofunctional mesocortical dopamine system.
Animal models of neuropsychiatric diseases play an essential role in experimental neurology and biological psychiatry. They strive to provide a causal relationship between an equivalent of a symptom and an experimentally controlled treatment.
According to the diverse symptoms of schizophrenia, a large number of different animal model approaches have been published in the past. However, one simple behavioural paradigm stands out providing a particularly high translational value: Prepulse inhibition (PPI) of the acoustic startle response. PPI is the reduction in startle magnitude that occurs when the startling noise pulse is shortly preceded by a weak acoustic or tactile prepulse, and is based on a sensorimotor gating mechanism in the brainstem. PPI has been shown already thirty years ago to be impaired in schizophrenic patients and is reduced in laboratory animals by stimulation of striatal and/or blockade of prefrontocortical dopamine receptors. We will show data from two different approaches: First, adult rats that have sustained neonatal lesions of the medial prefrontal cortex show an enhanced sensitivity of neurons in the nucleus accumbens to the inhibitory effect of the direct dopamine receptor agonist apomorphine, and a more pronounced PPI-deficit after systemic apomorphine treatment. Second, apomorphine reduces PPI only at interstimulus intervals of 100-1000 ms and enhances the orienting response towards the prepulse alone. These animal model data shed some light on the nature of PPI-deficits in schizophrenia patients.

Reward prediction error and its dysfunction in psychiatric disorders: role of dopaminergic mechanisms

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It has been suggested that phasic alterations in dopamine firing encode a reward prediction error, for example when reward arrives surprisingly or when a conditioned stimulus that predicts reward appears suddenly. Computational models can simulate this neuronal behavior and suggest that dopamine firing constitutes an error of reward prediction that drives learning and thus goal-directed behavior. In humans, it has been shown that phasic activation of the ventral striatum, a core area of the brain reward system that is strongly innervated by dopamine, can be interpreted as encoding such an error of reward prediction. Paradigms in functional imaging used conditioned stimuli that predict monetary reward to activate a phasic response in the ventral striatum. Dopaminergic modulation of this phasic response is indirectly suggested by the effects of genetic variation on dopamine metabolism (e.g. genotype of the COMT). In schizophrenia, it was suggested that irregular or chaotic firing of dopaminergic neurons interferes with such a prediction error and attributes incentive salients to otherwise irrelevant stimuli, and indeed functional imaging showed that unmedicated schizophrenics display a reduced activation of the ventral striatum when confronted with stimuli that indicate potential reward. Other neuropsychiatric disorders such as alcohol dependence are known to reduce dopamine D2 receptor availability also in the ventral striatum, and this hypofunction of the dopamine system has been shown to be associated with reduced activation of the ventral striatum during reward anticipation. Current research shows that functional imaging can not only assess the effects of (already learned) conditioned stimuli, but it can also be used to image the learning process, for example in probabilistic reversal learning tasks. First data of alterations in these learning paradigms in schizophrenic patients will be presented.

Prof. Dr. Andreas Heinz

From anatomical description to genetic manipulation of brain circuitry

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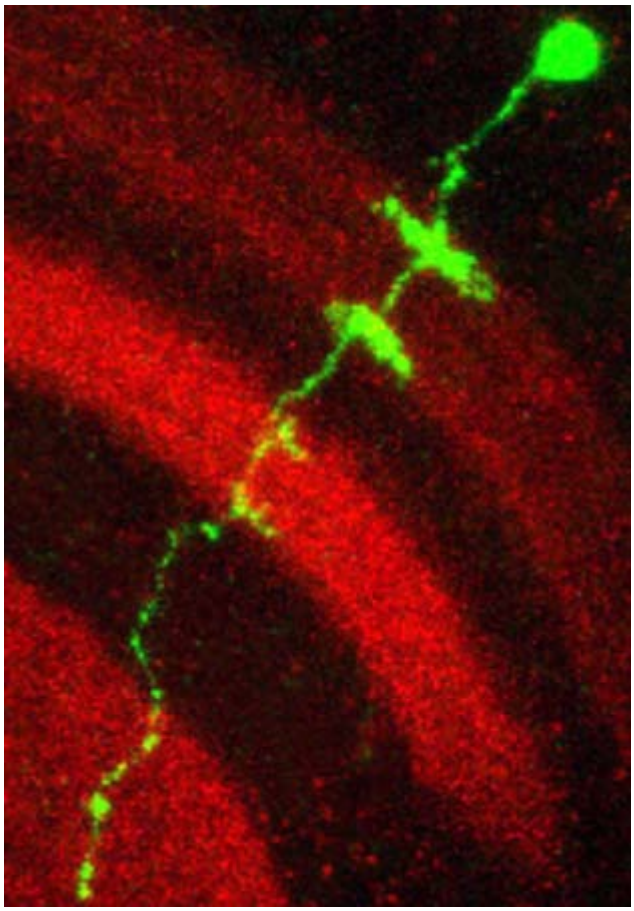
The sophisticated behaviour of a fly requires a sophisticated brain. The brain of Drosophila is an object of intensive studies due to its accessibility to genetics. While classical histological methods like silver stainings and Golgi impregnations have been used to describe the main neuronal types and structures in the fly brain, modern genetic tools allow for the manipulation of individual cell types during development as well as during adult life. Taking the visual system of Drosophila as an example, this talk will illustrate the potential inherent in the merging of neuroanatomy with genetics.



Visual processing in the *Drosophila* brain: a combined optophysiological, electrophysiological and genetic approach.

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The detection of moving objects, their direction of motion and the analysis of optic flow are fundamental to the survival of animals with eyes. Nonetheless, it is still not clear how the brain computes visual motion information. Small scale neural networks in the *Drosophila* visual system represent an ideal system to address the fundamental rules of visual motion processing. This notion is based on genetic amenability, a crystalline-like organization of the neural lattice and the presentation of defined visual stimuli to the fly's eye (Goetz, 1964) that can similarly be fed into a well established computational model (Reichardt, 1961). However, this approach was so far hampered by difficulties in recording from *Drosophila* neurons in the intact animal during visual stimulation. Here we report a combined physiological and genetic approach that aims to unravel the cellular mechanisms of visual motion detection in *Drosophila*.

In a first step we have recently shown (Joesch et al., 2008) that the electric response characteristics of Lobula Plate Tangential Cells (LPTCs) are indicative of Reichardt-like computations (Reichardt, 1961). Now we combine whole cell patch clamp recording from LPTCs to genetic manipulation of the presynaptic motion detection circuitry. Direct recording from visual interneurons presynaptic to LPTCs and LPTC-compartments is performed by combining the expression of the genetically encoded calcium indicator TN-XXL (Mank et al., 2008) with multi-photon microscopy and precisely timed visual stimulus presentation. Using this approach we aim to identify neurons and small neuronal circuitries that accomplish elementary visual motion detection.

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Investigation of selective visual attention in *Drosophila* during tethered flight

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Selective visual attention (SVA) is revealed by an animal responding to only one visual stimulus, while suppressing any responses to other simultaneously presented visual stimuli. During tethered flight at the torque meter *Drosophila* displays SVA, if in the two lateral halves of the visual fieldtwo equally strong motion stimuli are concurrently presented[1]. The fly's yaw torque tends to alternately follow the motion on one or the other side.

Apparently, flies tend to shift their SVA together with their intended flight direction, regardless of whether the turning is elicited by an external motion stimulus (see above), or whether the turning manoeuvre (yaw torque) is spontaneously generated. This becomes obvious from an experiment in which the fly is conditioned to prefer one of two stationary visual patterns presented in the right and left frontal quadrants of the visual field. During spontaneous yaw torque towards one side the fly is heated, during torque towards the other side heat is switched off. Between training and test, the positions of the two visual patterns are exchanged. The fly preferentially turns towards the pattern it had previously seen on the "safe" side, which, however, now is located in the previously "punished" quadrant[2]. Note, that throughout the experiment the fly is immobilized and flying stationarily, and that the patterns are presented at stable retinal positions.

SVA is an active process. We plan to investigate in which regions of the central brain this process can be influenced by the genetic manipulation of neurons. Moreover, we have designed several new paradigms in which the flies' SVA can be guided through presentation of additional external stimuli.

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Segregated neural pathways for *Drosophila* gravity sensing and hearing

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The fruit fly *Drosophila melanogaster* responds to gravity and sound. When being startled, the flies tend to walk up against the earth's gravitational field, which is known as negative gravitaxis. When exposed to male courtship songs, in turn, females reduce locomotion whereas males start chasing each other. Previous studies had indicated that these sound- and gravity-evoked behaviours might both rely on Johnston's organ (JO), which monitors stimulus-induced movements of the distal part of the antenna. However, which of the fly's ca. 480 JO neurons respond to these movements and are required for gravity and sound sensing had remained unclear. To assess stimulus-evoked neural activities in the fly's JO, we developed a live fly preparation that allows to monitor intracellular calcium signals in JO neurons through the cuticle: deflecting and vibrating the fly's antenna maximally activated distinct clusters of JO neurons that target different regions in the brain. Deflection-sensitive neurons responded if the antenna is pushed back or forth, depending on their position in the organ. Silencing these neurons impaired gravitactic behaviour. Vibration-sensitive JO neurons were subdivided into low-frequency neurons required for the detection of loud courtship songs and high-frequency neurons that seemed specialized for sensitive hearing. Hence, while the fly's abilities to sense gravity and sound share one sensory organ, gravitactic and acoustic information may be segregated at the very first stage of neural processing. Identification of higher-order neurons revealed tight commissural connections in the pathways downstream of sound-sensitive JO neurons and the descending tracts downstream of gravity-sensitive JO neurons, each of which parallels the architecture of the cochlear and vestibular pathways in our brains.

The Molecular Biology of Drosophila Olfaction

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The olfactory systems of vertebrates and invertebrates display remarkable similarities in their neuroanatomical organisation and neurophysiological sensory coding properties. However, while all known vertebrate olfactory and pheromone receptors belong to the G protein-coupled receptor superfamily, insects have evolved a novel family of polytopic transmembrane proteins to mediate odour detection. To identify molecules that act with these receptors, we have performed a bioinformatics screen, based upon the assumptions that such molecules will display the same insect-specific conservation and restricted tissue expression as insect odorant receptors. This screen yielded all previously identified classes of olfactory molecules as well as a large number of novel genes that we find expressed in diverse populations of chemosensory neurons. We will describe our current analysis of some of these molecules, which reveals insights into the molecular mechanisms of odour detection by odorant receptors and new olfactory sensory pathways.

Neural circuits underlying olfactory processing in *Drosophila*

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Odors are recognized by primary olfactory sensory neurons (OSNs), which are located on the antennae and the maxillary palps. OSNs express odorant receptor genes (62 defined members) which encode the odorant receptors (ORs). The OSNs send their axons to the antennal lobe, which consists of olfactory glomeruli. Each OSN expresses a single OR gene, and all OSNs expressing the same OR converge onto a common glomerulus. A glomerulus receives not only the input from OSNs, but contains a highly ordered synaptic organization including synaptic microcircuits between OSNs, local interneurons and projection neurons.

It has been shown that inhibitory interactions play an important role in odor coding on all these processing levels. However, the necessary tools to study the former processes in imaging studies have so far been lacking. In order to visualize inhibitory responses, we used a fluorescent protein, namely Clomeleon, that functions as an indicator for chloride ions — the main mediator of synaptic inhibitions in mature neurons. Using the standard GAL4-UAS system in *Drosophila melanogaster*, we genetically expressed Clomeleon in subpopulations of olfactory neurons to measure and characterize neuronal inhibitions at different processing levels in the *Drosophila* olfactory system.

Neural circuits underlying olfactory learning and memory in *Drosophila*

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Insects can form positive and negative associative memories of an odour depending on how the odour is delivered: If it is paired with a reward or punishment, odour memory will be positive or negative, respectively. To understand the differentiation at the level of neuronal circuits, we decided to block the activity of specific groups of neurons in the *Drosophila* brainand to contrast its effect on positive and negative odour memories. Although the positive and negative memories drive opposite responses to the odour (i.e., approach and avoidance), the Kenyon cells of the mushroom bodies (MBs)are commonly required for both types of memories in *Drosophila*. By blocking the particular sets of Kenyon cells with shi^{ts1}, we found that the degrees of requirement of these cells are different in positive and negative memories. Toward explaining this differential requirement of the MB, we systematically analyzed the anatomy and the behavioural functions of GAL4 drivers labelling MB-extrinsic neurons. The effects of these drivers on these opposing memories are categorized into common, differential, or no requirement.

Dissecting neuronal networks underlying ethanol induced behaviors - Serotonin regulates ethanol tolerance in *Drosophila*

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Serotonin (5HT) has an essential role in the development of ethanol tolerance and alcohol dependence. However, it remains elusive how 5HT exerts its function in theses processes. Here we show that increased serotonin signaling impairs the development of ethanol tolerance. The serotonin transporter (SERT) is a key regulator for serotonin signaling. The *Drosophila* SERT (dSERT) is exclusively expressed in all serotonergic neurons throughout the adult brain. By immunolabeling we detected differences in the localization of dSERT and 5HT in adult serotonergic neurons. In addition alterations of SERT function with pharmacological and genetic tools result in reduced ethanol tolerance. However, neither general increase nor decease of 5HT levels affects ethanol tolerance. These finding suggests that local differences in 5HT concentration rather than absolute 5HT levels are important to mediate behavioral changes. Furthermore we identified a subset of serotonergic neurons in which Serotonin signaling has to be regulated properly for tolerance development. Our data identify a role for serotonin in the modulation of ethanol tolerance in *Drosophila melanogaster* and suggest that proper regulation of serotonin signaling during the development of ethanol tolerance is conserved between insects and higher vertebrates.

Neurogenesis from glial cells: new views on stem cells and repair in the brain

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The findings that glial cells act as neural stem cells or neuronal progenitors in various contexts, has prompted us to examine the molecular mechanisms that regulate neurogenesis from glial cells and are absent in most glial cells in the adult mammalian brain parenchyma. I will discuss some of these molecular mechanisms as well as our transcriptome analysis providing more general insights into the identity of adult neural stem cells and their differences from parenchymal astrocytes. I will discuss pathways that are not active in 'normal' parenchymal astrocytes, and become activated after injury. This is relevant to our recent discovery that a subset of reactive astrocytes after brain injury actually resume proliferation and even stem cell properties. These exciting findings provide novel approaches towards repair of neurons after brain injury. Towards this end I will also discuss factors that are active in astrocytes of the neurogenic niches in the adult mouse brain. The activation of factors instructing stem cell fate and neurogenesis and the inhibition of anti-neurogenic factors finally allows to instruct neurogenesis from glial cells in vitro and in vivo after brain injury. Taken together, our work on the mechanisms regulating neurogenesis from glial cells has demonstrated that even adult astroglial cells in the injury site of an adult mammalian brain can be reverted towards neurogenesis and may serve to repair neurons after injury. I will also present latest data on the re-activation of neurogenesis from astrocytes of human patients in vitro. Thus, glial cells reacting to brain injury provide a novel source of stem cell, local to the injury side, whose properties we still need to understand better in order to utilize these cells for repair.

Molecular control of neurogenesis in the adult forebrain

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In the postnatal vertebrate subventricular zone (SVZ) new neurons are permanently generated from local stem cell populations and migrate via the rostral migratory stream (RMS) to the olfactory bulb (OB) where they differentiate into granule cells and periglomerular interneurons. Over the past years this system attracted considerable attention. First, the demonstration that new neurons can be generated and integrated into the existing circuitry fundamentally changed our view of brain development and function. Second, the discovery of comparable neural stem cells and progenitors in humans raised hope for the use of adult neurogenesis for brain repair either by transplantation of adult derived progenitors or via the activation and recruitment of the intrinsic neurogenetic pool. In addition, based on its particular experimental advantages, the postnatal SVZ-RMS-OB system became an important model to study the molecular and cellular mechanisms that underlie the regulation of forebrain neurogenesis.
We performed systematic comprehensive gene expression analyses of defined neuronal cell populations isolated from the postnatal and adult SVZ and OB. These analyses provided new general insights into the proliferation, migration and differentiation of neurons in the forebrain and allowed the description of regulatory cascades. In addition, candidate factors to be implicated in specific steps of the neurogenic process have been identified and functionally analyzed. For example, the proteoglycan Agrin, which is essential for the induction/assembly of the neuromuscular junction, is strongly expressed in adult neuronal precursors in the RMS. Using a transplantation approach we found that Agrin is necessary for synaptogenesis of new neurons within the existing olfactory circuitry. The function of other factors that determine neuronal fate or regulate subsequent steps in the differentiation process will be discussed.

The instructive role of extracellular matrix molecules in the neural stem cell niche

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Several studies in the last decade confirmed the occurrence of neurogenesis in discrete areas of adult mammalian CNS overturning the long held dogma, that the adult brain cannot generate new neurons. The subventricular zone of the lateral ventricles and the dentate gyrus of the hippocampus are the main neuroproliferative regions of adult brain that contain either adult stem or progenitor cells. Furthermore, evidence shows that the basal rate of adult neural stem/progenitor cells within these discrete areas of the adult brain is modulated by several physiological and pathological conditions, such as ischemic and traumatic injuries, indicating the potential to repair the damaged brain. CNS lesions have been reported to provide an attractive source for various transplanted stem cell populations fostering the idea of possible neural repair, but little is known about an involvement of endogenous neural stem/progenitor cells in these processes. Nevertheless, the awakening of endogenous stem/progenitor cells is foreseen as an attractive alternative strategy. The present investigation was designed to study the stimulation of endogenous stem/progenitor cells in non-neurogenic regions of the adult brain after focal laser lesions in the visual cortex of young rats, because in this lesion paradigm enhanced synaptic plasticity in the penumbra region has been observed. It remained open, if neurogenesis could have contributed to these findings, but our current observations supported this hypothesis. Within 72 hrs after lesion, we recorded an ipsilateral upregulation of proliferative cells in the cortical layers I and VI of the visual cortex as well as in white matter areas. In the same time window, we found a strong ipsilateral upregulation of cells that are immunoreactive for the immature neural stem/progenitor cell markers nestin, vimentin and the 473HD-epitope, which is found on neural stem/progenitor cells during development and in the adult neurogenic brain regions. At the 5th day after lesion, these cells formed a more-or-less continuous pathway that extended from the inferior part of the temporal horn of the lateral ventricle, along the corpus callosum to the lesion area. Because 473HD-immunoreactivity was associated with BrdU, vimentin and nestin but not with GFAP or microglial markers in vivo, we evaluated the phenotypes of 473HD-expressing cells in vitro. Importantly, we were able to isolate and expand stem/progenitor cells from the lesion area that gave rise to self-renewing, multipotent neurospheres. The number of neurosphere-forming cells from the contralateral side was three-fold lower and the latter spheres were not self-renewing and generated only astrocytes. These data demonstrate that adult progenitors present in the injured side of the visual cortex have distinct properties and damaged environmental cues may instruct endogenous progenitors to proliferate and differentiate into specific cell types. Details of such specificity and the underlying mechanisms still remain poorly understood. Thus, we identified a rapidly appearing progenitor population with neural stem cell properties in vitro that may be induced to form neurons de novo after defined laser-lesion in the rat visual cortex. However, in context with above evidence, our observations suggest that the latent regenerative potential of the adult CNS may be much greater than previously thought.

Molecular control of cell fate specification in the cerebral cortex

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During the development of the mammalian neocortex, neuronal progenitors located in the ventricular and subventricular zones of the dorsal telencephalon give rise to multiple projection neurons that are arranged in six cortical layers in the mature brain. Neurons within each layer are generated at similar times and share similar morphology and patterns of connectivity. The molecular determinants of the fate of these cells are still elusive.

In recent years our research was focused on identification and characterization of genes that control cell fate specification in the cerebral cortex. One of the genes we identified, Satb2 is crucial for postmitotic specification of callosaly projecting upper layer cortical neurons. Another transcription factor we identified, Sip1 was shown to be the cause of Mowat-Wilson syndrome in humans. In the hippocampus Sip1 controls non-canonical Wnt signaling by suppressing Sfrp1 gene expression. Inactivation of Sip1 in the hippocampus induces Sfrp1 activation, that in turns leads to inactivation of Wnt/JNK signaling, elevated cell death and subsequent degeneration of hippocampal formation. In the neocortex Sip1 inactivation induces premature and excessive production of upper layer neurons at the expense of deep layer neurons. Furthermore, it causes precocious generation of glial cells at late corticogenesis. Molecular basis of Sip1 and Satb2 action will be discussed.

TRANSCRIPTIONAL CONTROL OF NEURONAL MIGRATION IN THE MOUSE BRAIN

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The generation of neurons by neural stem cells is a complex process involving the tight coordination of multiple cellular activities, including cell cycle exit, specification of particular neuronal fates, initiation of neuronal differentiation and migration. Proneural genes play a central role in the regulation of this process of neurogenesis. Their activity is restricted to defined regions of the developing nervous system where they promote the acquisition of both generic and region-specific properties of neurons. In the embryonic forebrain, the proneural gene Neurogenin2 (Ngn2) is expressed in the dorsal telencephalon and promotes the generation of cortical projection neurons, while Mash1 is expressed in the ventral telencephalon and is required for the generation of cortical interneurons.

The mechanisms underlying the functions of proneural transcription factors in neurogenesis, and particularly their contribution to the regional specification of neurons, are poorly understood. We have begun to identify the transcriptional targets and regulatory pathways activated by proneural proteins in the embryonic telencephalon. In particular, we have found that the small GTP-binding proteins Rnd play important roles in the regulation of neuronal migration downstream of proneural proteins. Rnd2 is a direct transcriptional target of Ngn2 in the dorsal telencephalon and it promotes the migration of cortical projection neurons. Rnd3 is directly regulated by Mash1 in the ventral telencephalon as well as in cortical progenitors. These proteins therefore participate to distinct programmes of migration activated by proneural factors at different stages of migration and/or in different neuronal populations. We are currently investigating the mechanisms involved in regulation of migration by Rnd2 and Rnd3.

The developmental genetic basis of cortical interneuron diversity: the role of Nkx2-1 target genes.

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My laboratory is interested in how developmental genetic events result in the generation of specific classes of cortical interneurons. Moreover, we wish to determine the logic by which these subtypes integrate into the cortex. We are currently using a variety of genetic tools for fate mapping and manipulating the activity of newborn neurons during the early postnatal period. In this lecture, I will discuss how gene expression within progenitors and postmitotic interneurons interfaces with spontaneous neuronal activity. Our results suggest that a combination of genetic programming and environmental cues determines both their intrinsic physiological properties and their integration into neuronal circuits.

Skin-derived progenitors pre-differentiated into Schwann cells for spinal cord repair

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Cellular transplantation is increasingly considered as a treatment option for spinal cord injury (SCI) although the optimal type and source of cell as well as mode of transplantation is still to be determined. We have previously shown that Schwann cells generated from skin-derived precursors (SKP-SC) following transplantation into the contused spinal cord of rats bridged the consution sites, myelinated spared axons in the bridge as well as the host tissue rim and enhanced locomotor recovery (Biernaskie et al. 2007 Journal of Neuroscience, 27:9545-59).

More recently, we have hypothesized that SKP-SC promote plasticity/regeneration of rubrospinal axons and enhance functional recovery after incomplete cervical spinal cord injury. SKPs from green fluorescent protein (GFP) expressing neonatal rats were differentiated into Schwann cells *in vitro* and acutely transplanted into a (left) cervical (C4/5) dorsolateral funiculus lesion of young adult GFP-negative rats. Behaviorally, rats transplanted with SKP-SC showed improved balance between the left forelimb and the other limbs during walking on a CATWALK (footprint analysis) and increased use of the left forelimb during vertical exploration in a cylinder test. Electrophysiological stimulation of the red nucleus revealed lower thresholds necessary to evoke an electromyographic response in left forelimb muscles of SKP-SC transplanted rats, indicating increased rubrospinal efficacy. This increased rubrospinal efficacy was corroborated by enhanced branching of rubrospinal axons within the gray matter rostral to the lesion; however there was no evidence for regeneration or sparing of rubrospinal axons across the lesion site. Only a fraction of the acutely transplanted SKP-SC survived at 10 weeks after injury. However, these cells integrated into the ventral wall of the lesion site, myelinated axons, reduced astrogliosis and tended to reduce the size of the disrupted host cytoarchitecture.

In the light of the possible use of SKP-SC in autotransplantation paradigms we tested their integration into the contused spinal cord (epicenter) at 5 weeks after the injury. This would roughly reflect the time required to harvest skin and grow up sufficient SKP-SC. Transplantations were well tolerated with only a transient decline in motor performance. At six weeks post transplantation, SKP-SC were found to survive and integrate into the host injury sites and in conjunction with large cohorts of endogenous Schwann cells these bridged parts of the contusion sites and were accompanied by numerous host axons. Po staining for peripheral myelin revealed extensive myelination in these bridges as well as in the spared host spinal cord rims. Immunostaining for GFAP indicated a stronger reaction of the host astroglia to the invading endogenous Schwann cells than to SKP-SC. These observations indicate that SKP-SC have beneficial properties distinct from Schwann cells and that these cells which are readily available from skin may be successfully used in auto-transplantations.

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MODULATION OF INTRINSIC AND EXTRINSIC FACTORS FOR AXONAL REGENERATION AFTER SPINAL CORD INJURY.

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Multiple mechanisms, both intrinsic and extrinsic to injured neurons, have been shown to limit axonal regeneration after spinal cord injury (SCI). Thus, targeting a single mechanism seems unlikely to elicit axonal regeneration over extended distances.
In previous studies, we have demonstrated that gradients of neurotrophin-3 established by lentiviral gene transfer allows for sensory axons to extend for short distances across spinal cord lesions filled with a cellular graft. More recently, we have shown that conditioning lesions to enhance cellular programs for axonal regeneration in combination with NT-3 gene transfer further increases the number and distance of axons extending beyond the lesion site. Importantly, these "combinatorial treatments" are still effective at chronic stages of SCI and result in similar genetic programs that support axonal regeneration. Thus, conditioning lesions initiated before and after SCI are equally effective. Importantly, regenerating dorsal column sensory axons can not only bridge across an acute cervical lesion site but also re-innervate their original target nucleus (nucleus gracilis) to form new synapses. This response is highly dependent on chemotropic guidance by neurotrophic factors to the target nucleus. Current experiments are investigating the mechanisms underlying the enhanced regenerative response after combinatorial treatments to augment regeneration of spinal and supraspinal projections after SCI.

Therapeutic concepts to overcome regeneration barriers in spinal cord injury

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There are numerous molecular pathways that prevent repair in the adult mammalian CNS. However, these mechanisms divide into two principal categories: (a) those that affect the intrinsic ability of neurons to promote a repair response after damage and (b) mechanisms in the environment surrounding neurons that block those regenerative attempts. Here I will focus primarily on the environmental mechanisms, in particular on those associated with meningeal fibroblasts and astrocytes contributing to fibrotic wound healing scar as a regeneration barrier.

As in other tissues lesion scarring is also common to the mammalian nervous system. In traumatic brain and spinal cord injury (SCI) the lesion scar is comprised of a fibrous scar in the lesion core and an astroglial scar in the surrounding parenchyma. Recently, we have demonstrated that the collagenous basement membrane-rich fibrous scar is a major impediment for axon regeneration in lesioned CNS, presumably acting as a physiological barrier due to binding and accumulation of axon-growth inhibitory molecules. In order to overcome the extracellular regeneration barrier we have developed novel pharmacological and immunochemical treatments to suppress collagenous scarring in the lesion zone by local application of either potent iron chelators and cyclic AMP or specific antibodies directed to collagen IV which resulted in suppression of fibrous scarring and, in consequence, long-distance regenerative growth of injured axons in different animal models of brain and spinal cord injury. Axons regenerated through both grey and white matter and developed terminal arborizations in grey matter regions. In contrast to controls, injured animals receiving this treatment showed significant functional recovery in the open field, in the horizontal ladder and in CatWalk locomotor tasks. In addition to axon growth promoting effects, the proprietary pharmacological treatment showed a strong neuroprotective influence that retrogradely rescued, e.g., primary cortical (layer V) motoneurons projecting into the descending corticostpinal tract that normally die (30%) after thoracic axotomy. Local and long-distance neuroprotective functions of the treatment are indicated by significantly reduced protein oxidation and lipid peroxidation in lesioned spinal cord as well as by specific changes in the gene expression profile in sensorimotor cortex including down-regulation of pro-apoptotic genes as well as up-regulation of anti-apoptotic genes.

Transient local inhibition of fibrous scarring is a unique treatment strategy for traumatic CNS injury with a high clinical impact. This treatment is complementary to and seems to be compatible with most, if not all, therapeutic strategies known so far.

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Enhancing injury induced plasticity following spinal cord injury in rats

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Spinal cord injury (SCI) is a devastating event, that results in the loss of motor and sensory functions of the body innervated by the spinal cord below the injury site. Recovery following SCI is limited because severed axons of the central nervous system (CNS) fail to regenerate spontaneously. Additionally, therapeutic strategies aimed at promoting significant regeneration are currently unavailable. Nevertheless, depending on the severity of the injury, some recovery of sensory and motor function will occur over the weeks following the injury both in patients and animal models of SCI. The mechanisms of this recovery may include axonal sprouting, synaptic rearrangements and changes of cellular properties in spared neuronal circuits within the entire CNS, often referred to as plasticity. A successful approach to further promote both, this naturally occurring “repair mechanism” and also functional recovery following SCI is intensive rehabilitative training. Training promotes significant recovery by up-regulating growth associated factors, changes in cortical maps, and increasing collateral sprouting. These mechanism can be further enhanced by administrating neurotrophic factors such as BDNF. When applied to the cell body of lesioned corticospinal axons BDNF can promote the rewiring of lesioned axons via spared propriospinal interneurons, thereby circumventing the need for long distance regeneration. Thus, promoting injury induced plasticity is a promising avenue in treating injuries to the central nervous system.

Spinal Cord Injury Assessment and Treatment

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After a spinal cord injury (SCI) of the cat or rat, neuronal centers below the level of lesion exhibit plasticity than can be exploited by specific training paradigms. In individuals with complete or incomplete SCI, human spinal locomotor centers can be activated and modulated by locomotor training (facilitating stepping movements of the legs using body weight support on a treadmill to provide appropriate sensory cues) (for review see Dietz 2002,2003). Individuals with incomplete SCI benefit from locomotor training such that they improve their ability to walk over ground. Load- or hip joint-related afferent input seems to be of crucial importance for both the generation of a locomotor pattern and the effectiveness of the training (Dietz et al. 2002). However, it may be a critical combination of afferent signals that is needed to generate a locomotor pattern after severe SCI. Mobility of individuals after a SCI can be improved by taking advantage of the plasticity of the central nervous system and can be maintained with persistent locomotor activity. In the future, if regeneration approaches can successfully be applied in human SCI, even individuals with complete SCI may recover walking ability with locomotor training (Curt et al. 2004). During the past few years, several approaches to spinal cord repair have been successfully established in animal models. For their use in clinical trials of SCI in human beings, specific difficulties that affect the success of clinical trials have to be recognised (Dietz and Curt 2006). First, transection of the spinal cord is commonly applied in animal models, whereas contusion, which generally leads to injury in two to three segments, represents the typical injury mechanism in human beings. Second, the quadrupedal organisation of locomotion in animals and the more complex autonomic functions in human beings, challenge translation of animal behaviour into recovery from SCI in people. Third, the extensive damage of motor neurons and roots associated with spinal cord contusion is little addressed in current translation studies. Fourth, there is increasing evidence for a degradation of neuronal function below the level of the lesion in chronic complete SCI. This degradation might have a considerable impact in chronic SCI subjects for a regeneration-inducing treatment. Therefore, it’s relevance needs to be investigated.

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Spinal cord injury induced immune depression syndrome (SCI-IDS)

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Infections are among the leading causes of death in spinal cord injured patients and are associated with hampered wound healing, prolonged hospitalization and impaired neurological recovery. Here, we have analyzed fluctuations of immune cell populations following human spinal cord injury (SCI) and in an experimental rat model of SCI by FACS analysis from acute until chronic stages. In humans, a rapid and drastic decrease of $CD14^+$ monocytes (<50% of control level), $CD3^+$ T-lymphocytes (<20%, $p<0.0001$) and $CD19^+$ B-lymphocytes (<30%, $p=0.0009$) and MHC class II $(HLA\text{-}DR)^+$ cells (< 30%, $p<0.0001$) is evident within 24 hours after spinal cord injury reaching minimum levels within the first week compared to controls. $CD15^+$ granulocytes were the only leukocyte subpopulation not decreasing after SCI. Experimental SCI of rats not receiving methylprednisolone induced - likewise – depletion of $ED9^+$ monocytes (<65%), $CD3^+$ T-lymphocytes (< 30 %, $p=0.0066$), $CD45\ RA^+$ B-lymphocytes (< 45%, $p<0.0001$), MHC class II (< 40%, $p=0.0003$) and $OX\text{-}62^+$ dendritic cells (< 55%, $p=0.0052$) within the first week after SCI. HIS 48^+ granulocytes remained on levels similar to sham operated controls. A contributing, worsening effect of high dose methylprednislone cannot be excluded with this pilot study. We demonstrate that spinal cord injury is associated with an early onset of immune suppression and secondary immune deficiency (SCI-IDS). Identification of patients suffering spinal cord injury as immune compromised is a clinically relevant, yet widely underappreciated finding.

Behavioral report of single-neuron stimulation in the somatosensory thalamocortical system

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We detect sensory stimuli by perceiving the activity modulation in some subset of the billions of neurons in our brains. Conventional thought has been that sensory detection involves the joint activity of large groups of neurons distributed across areas. Recent findings, however, suggest that cortical processing is mediated by fewer action potentials (APs) than previously thought. Interestingly, electrical stimulation of even a single sensory cortical neuron can elicit sensations. We found that initiating short trains of APs in a single neuron in the rat barrel somatosensory cortex can lead to behavioral responses in a detection task (Houweling & Brecht, 2008). Stimulation effects varied greatly between cells. Whereas stimulation of putative excitatory neurons led to weak biases towards responding, stimulation of putative inhibitory neurons led to more variable and stronger sensory effects. These results demonstrate that single neuron activity can cause sensations, suggesting a much sparser cortical code for sensation than previously anticipated.We recently extended this approach to the rat ventral posterior medial(VPM) nucleus of the thalamus to quantify the sensory impact of individual thalamic neurons (Voigt, Brecht & Houweling, 2008). In contrast with our single-cell stimulation experiments in barrel cortex, we found that stimulation of single thalamic neurons does not lead to a behaviorally reportable effect. These results are surprising given the relatively small number of VPM neurons and previous observations that single neurons in other parts of the vibrissal system do have an impact on perception or motor output. Our findings therefore suggest that neural representations in whisker thalamus are more distributed than in barrel cortex.

Cortical feed-forward networks for binding different streams of sensory information

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The cortex is an architectural masterpiece. It combines horizontal layers with vertical columns, anatomical organization with functionality. Layers contain different cell types that form different connections with each other, whereas columns are defined by the features of the information they process, like brightness of a spot in the receptive field. Over the last 50 years, the idea of the 'functional column' has provided dominant influence on our understanding of cortical circuits. However, recent findings have put this arrangement of computational units into question. The finer scale structure of micro-columns and sub-networks become more and more the focus of investigation. Yet, the formation and structure of these cortical assemblies is still largely unknown. I will present evidence that these sub-networks form micro-circuits that are tuned for binding different streams of sensory information. Further, I will show that different learning rules exist for bottom-up or top-down pathways. The different temporal windows for plasticity induction at synapses conducting sensory or context information might lead to the assembly of highly specific cortical circuits tuned for integration of different sensory features. The development of a novel 3D network imaging technique allows now to record from visually identified neuronal networks in the intact brain during sensory stimulation. This technique has been used to study the interaction of cortical sub-networks in the supragranular layers of rat visual cortex. Neuronal Networks can be identified that specifically encode simple gratings or more complex stimuli like plaid patterns and natural scenes. We found specific clusters of neurons responding to artificial stimuli while the same neurons formed different clusters in response to natural stimuli. Together, fine-scale local networks of neurons might play an important role in sensory stimulus representation and computation in the cortex.

Mapping the Matrix: From Synapses to Circuits in Neocortex.

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Every thought, every idea, every memory, every decision, and every action we have to make, arises from the activity of neurons in our brains. The results of some of this activity surround us: household objects, books, technology and art. Of all brain structures, the neocortex, which forms over 80% of the volume of the human brain is, arguably, the most critical to what makes us human. This is a paradox, because the basic local architecture of the neocortex in all mammals, from mouse to man, appears to be very similar and is determined by the laminar distribution of relatively few types of excitatory and inhibitory neurons organized according to common principles of connectivity. These local circuits are organized in a framework of a six-layered columnar architecture, in which neurons with functional properties in common lie in discrete layers and in vertical slabs or columns.

The uniformity of its construction suggests that the neocortex provides circuits that are optimized for a class of cortical 'algorithm' that can be implemented for the full range of demands of behaviour, including perception, cognition, and action. A number of models indicate the forms of general computation that could be carried out in a uniform cortical architecture. Typically these models address a single principle of operation in a small group of neuron, while in others a more detailed model is imposed on the columnar architecture of cortex. Experimental results in alert behaving primates, together with theoretical studies, suggest that cognitive operations proceed very rapidly across different cortical areas by feedforward categorization and feedback modulation, with slower refinement by lateral local interactions. These new ideas point to a direction of theoretical advance that requires explorations beyond the local cortical column to consider the entire cortical ‘sheet’ as a single network. We are now specifying the local lateral connections and the long distance connections in the cortex, because we believe these will provide important insights into the implementation of the rich repertoire of behaviours that we primates possess.

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Computational power of recurrent neural networks: The role of spikes, network structure, and dynamics

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Computer simulations of cortical microcircuit models based on detailed connectivity data from Thomson and others have shown that the laminar structure of cortical microcircuits has a strong influence on the transmission and fusion of information within the dynamical system. Specific computational advantages of such data-based lamina-specific circuit models over randomly connected models were exhibited.
These findings in circuits of spiking neurons stand in contrast to the experience with circuits of analog neurons (Echo State Networks) where no dependency of computational performance on network structure has been observed.

We investigate this apparent dichotomy in terms of the connectivity of circuit nodes in circuits composed of analog neurons and binary neurons (threshold gates). Our analyses based amongst others on the Lyapunov exponent reveal that the phase transition between ordered and chaotic network behavior of binary circuits qualitatively differs from the one in analog circuits. This explains the observed decreased computational performance of binary circuits of high connectivity. Furthermore, a novel mean-field predictor for computational performance is introduced and shown to accurately predict the numerically obtained results.

The effect of degree distribution on the dynamics of sparsely connected neuronal networks

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Most work on sparsely connected networks of spiking neurons has made use of the assumption that there is a fixed probability of connection between any two neurons. This results in a Binomial degree distribution which is sharply peaked about the most likely value for large enough numbers of inputs. This, in turn, ensures that all neurons receive, on average, the same amount of input, allowing for powerful meanfield techniques to be applied. However, much recent electrophysiological work indicates that local cortical circuits are not consistent with the assumption described above. I will discuss how one might relax this assumption and allow for much broader degree distributions. For the case of the incoming connections, the effect of degree distribution can be accounted for in a modified rate equation in which the degree is treated as a continuous, pseudo-spatial variable. This allows one to calculate fixed point solutions and their stability using standard techniques. I will show how varying the degree distirbution shifts stability boundaries of various cortical states relative to the Binomial case, and compare analytical results to full network simulations. I will also discuss how the degree distribution for outgoing connections affects correlations in the output of the neurons.

Propagation of synchronous activity in circuits without specific feed-forward anatomy

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Patterns of precisely timed spikes and synchronization in the millisecond range have been experimentally observed in different neuronal systems. Despite the current debate about the role of precisely timed spikes for neural computation it is still unclear under which conditions and how they could emerge in neural circuits. Here we present the first study of recurrent spiking neural networks with supra-additive dendritic interactions [see, e.g., Schiller and coworkers, J. Neuroscience, 2003, Nature Neuroscience 2004]. We show that supra-additive dendritic interactions, as recently uncovered in experiments, enable the propagation of synchronous activity in recurrent networks - a possible cause for spike patterns. Interestingly, this occurs already in recurrent networks that are purely randomly connected. These findings indicate that even networks without any specific non-random connectivity features may generate patterns of precisely timed spikes. In particular, super-imposed feed-forward structures in `synfire-chains' may not be required for temporally coordinating and propagating spiking activity across network if nonlinear dendritic features of the neurons' are present. As such our study presents an alternative viable mechanism for the occurrence of patterns of precisely timed spikes in recurrent networks and adds a novel perspective towards understanding the dynamics of networks with nonlinear interactions in general.

Role of lipids in neuroprotection and neurodegeneration in Alzheimer's disease amyloidosis

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Alzheimer's disease (AD) is the major neurodegenerative disorder. While still incurable, the understanding of the molecular and cellular etiology of the disease is advancing at a rapid pace. Simple overproduction of a small peptide, the amyloid beta peptide 42 (Aß42), is sufficient to cause amyloid plaques build-up and cognitive deficits in mice and AD in humans. Ab production is very sensitive to changes in the lipid composition of cellular membranes. Cholesterol has received special attention, because it has an especially strong influence on Aß production and epidemiological studies show a clear correlation between high cholesterol and increased AD risk whereas some cholesterol lowering drugs correlate with a reduced risk.

The Aß-peptides are produced during degradation of a larger precursor protein (APP). For long it has been assumed that Ab peptides are intermediates of APP degradation with no specific biological function.

We found that physiological Aß levels in brain (and other organs) function as potent signaling molecules that alter the activity of lipid metabolic enzymes and that APP processing is involved in the regulation of lipid metabolic pathways. Theses pathways involve feed-back regulation, where the lipids which are targeted by APP processing themselves influence A production. As expected from a physiological regulatory cycles, APP processing typically responds already to small changes in membrane composition, but extreme (non-physiological) alterations in lipid composition are required to abolish Aß generation.

Thus far we identified 5 lipid classes (sterols, sphingolipids, plasmalogens and gangliosides and some n-3 FA) involved in Aß regulation, but approx. 96% of all cellular lipids remain unaltered by APP knock-out, suggesting that the influence of Aß or APP on lipid homeostasis is rather specific. This is also illustrated by the molecular fine-tuning which separates cholesterol from sphingomyelin/ceramide homeostasis. The protease involved in the final step of Aß generation (γ-secretase) predominantly generates Aß40. This peptide, which is <u>not</u> involved in AD, reduces cellular HMGR activity and hence cholesterol *de-novo* synthesis. Aß42, a minor γ-secretase product, not only causes AD but also very efficiently increases the activity of sphingomyelin degrading enzymes. However, within the physiological concentration range, neither Aß species has any influence on the respective other pathway. Feed-back regulation apparently has less specificity, as e.g. cholesterol equally increases all Aß peptides.

We have also studied fatty acids and the role of fatty acids in various lipids in respect to Aß generation. While most had no or marginal effects on amyloid metabolism some significantly increase or decreased Aß generation. Especially interesting are omega-3 polyunsaturated fatty acids, especially DHA and EPA, because of favorable pharmacokinetics and the ability to decrease Ab generation. Several groups including ours report that DHA supplementation to the diet reduces amyloid accumulation/neurodegeneration in mice. Like statins these fatty acids are anti-inflammatory and vascular benefits have been reported. Importantly, DHA is the main antioxidant in the human brain.

These results add to a rising number of results, which strongly point towards a significant impact of lipid metabolism in Alzheimer's disease. The future challenges will be to decipher the molecular pathways which link the neurodegeneration in Alzheimer's disease with lipids and based on that to optimize the therapeutic approach.

Spine alterations in Alzheimer's disease models

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Dendritic spines are segregate compartments on the dendritic shaft and have been proposed as important sites of neuronal contacts. Recent data indicate that spines are highly dynamic structures and that spine shape correlates with the strength of synaptic transmission. Several mental disorders including Alzheimer's disease are associated with spine pathology suggesting that spine alterations play a central role in mental deficits. Using mice transgenic for familial mutations in the APP gene ($APPP_{SDL}$), it was shown on Golgi stained coronal brain sections that spine density is reduced on hippocampal and neocortical pyramidal cells, due to the APP background. To evaluate spine alterations in more detail, an *ex vivo* model of organotypic hippocampal slice cultures was established. For visualization of individual neurons virus-mediated expression of fluorescent constructs was performed. Spine number and morphology were determined by high resolution confocal imaging in combination with algorithm-based image analysis. A strong decrease in spine number was observed from slices that have been prepared from APP_{SDL} transgenic mice compared to control mice. The decrease of spine number could be prevented with the non-transition state gamma-secretase inhibitor N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT) indicating a role of Ab in mediating spine loss. Furthermore, spine lengths were reduced and an increased fraction of stubby spines on the expense of mushroom-type spine was observed. Spine loss and morphological changes were increased in "aged" slice cultures. The *ex vivo* approach together with two photon microscopy allows to follow alterations of individual spines in living neurons.

The results indicate that the spine pathology of APP transgenic mice is recapitulated in organotypic hippocampal slices and can be visualized by virus mediated expression of fluorescent markers. The data show that Aß affects spine density and morphology. Loss of spines in combination with changes in spine type may contribute to the mental deficits observed in AD patients.

Molecular determinants of neural plasticity in the adult visual cortex

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The action of visual experience on visual cortical circuits is maximal during a critical period of postnatal development. The long-term stabilization of the circuit changes induced by experience-dependent plasticity are likely mediated by signalling cascades regulating experience-dependent gene transcription. We studied the pathways linking experience-dependent activation of ERK to CREB-mediated gene expression in vivo. In juvenile mice visual stimulation that activates CREB-mediated gene transcription, also induced ERK-dependent MSK phosphorylation and histone H3 phosphoacetylation, an epigenetic mechanism of activation of gene transcription. In adult animals, ERK and MSK were still inducible, however visual stimulation induced weak CREB-mediated gene expression and H3 post-translational modifications. An intracellular signalling cascade that controls experience-dependent transcription during the critical period is downregulated at transcriptional level in the adult. Additional mechanisms might be involved in regulating plastiicyt of the audlt cortex. These includes regulation of the extracellular matrix. Thus, different molecules belonging to different cellular functions might be at the basis of experience-dependent in the juvenile and adult cortex.

Intrinsic growth potential, regulatory molecules and experience: the complex interplay regulating neuronal plasticity

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Neuronal connections are shaped during development, but may require to be modified in response to interactions with the external world and the internal milieu (physiological plasticity) , or as a consequence of pathological events (compensatory plasticity). Such remodelling process involves the strict regulation of the extension and retraction of neuronal processes, most notably axons.
Over the last decade, by focussing on the rodent cerebellum as an experimental model, we investigated how the interaction between intrinsic neuronal properties, extrinsic environmental signals, and experience regulate the extent of axon modifications in both the intact and lesioned brain. In this talk, we will summarize our findings showing the crucial contribution of cell-autonomous mechanisms in the control of axon growth, and the role of inhibitory environmental cues (NogoA, Chondroitinsulphate proteoglycans) in the restriction of growth protein expression (c-Jun and GAP-43) and axon remodelling. Further, we will discuss recent data suggesting that, in turn, during physiological and compensatory plasticity, mechanism are spontaneously triggered that actively modulate the expression/activity level of growth-inhibitory extracellular signals, particularly those forming perineuronal nets.
These data highlight that, for a better rewiring, therapeutic interventions in the injured CNS should combine pharmacological manipulations of growth regulatory molecules and experience-derived stimuli favouring plastic modifications.

Synaptic plasticity and cell cycle activation in neurons are alternative effector pathways. The role of proliferation control for plasticity and neurodegeneration

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Higher cerebral functions are based upon a dynamic organization of neuronal networks. To form synaptic connections and to continuously re-shape them in a process of ongoing structural adaptation, neurons must permanently withdraw from the cell cycle. In other words, synaptic plasticity can only occur on the expense of the ability to proliferate. This mechanism of synaptic plasticity, i.e. of structural stabilization and labilization underlaying a life-long synaptic remodelling are largely based on external morphoregulatory cues and internal signaling pathways that non-neuronal cells have phylogenetically acquired to sense their relationship to the local neighbourhood and to control after development is completed proliferation and differentiation in the process of tissue repair and regeneration. Here, we put forward the hypothesis that differentiated neurons after having withdrawn from the cell cycle are able to use those molecular mechanisms primarily developed to control proliferation alternatively to control synaptic plasticity. The existence of these alternative effector pathways within a neuron, puts it on the risk to erroneously convert signals derived from plastic synaptic changes into positional cues that will activate the cell cycle. This cell cycle activation potentially links synaptic plasticity to cell death. Preventing cell cycle activation by locking neurons in a differentiated but still highly plastic phenotype will, thus, be crucial to prevent neurodegeneration.

NOVEL THERAPEUTIC APPROACHES CONSTITUTING MULTIMODAL NEUROPROTECTIVE AND NEURORESTORATIVE DRUGS FOR ALZHEIMER'S DISEASE

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Alzheimer's disorders (AD), and Parkinson's disease (PD) are initiated by cascade of neurotoxic events, that includes oxidative stress, brain iron dysregulation, glutamate excitotoxicity, inflammatory process, neurotxic processing of APP misfolding of proteins Ab peptide and a-synuclein. Significant percentage of AD subjects also suffer from extrapyramidal symptoms (Lewy Body disease) and depression and PD with dementia and dpression.These subjects are benefiting from drugs developed to act on a single molecular target. Such drugs have limited symptomatic activities and current pharmacological approaches are highly limited in their ability to modify the course of the disease, offering incomplete and transient benefit to patients. However, the new therapeutic strategies for neurodegenerative diseases, such as AD, PD, Huntington disease and ALS (amyotrophic lateral sclerosis) are those in which drug candidates are designed expressly to act on multiple neuronal and biochemical targets involved in the neurodegenerative process and neurotransmission. Monoamine oxidase (MAO) B activity, iron, and glutamate excitotoxicity increase in ageing brain AD and PD. They are thought to contribute to oxidative stress dependent neuronal death. The iron deposition in AD hippocampus and substantia nigra of PD , can induce oxidative stress via interaction with hydrogen peroxide produced by MAO-B and other oxidative processes to promote the Fenton chemistry and generate the neurotoxic reactive hydroxyl radical. Furthermore such radical cause aggregation of iron responsive amyloid precursor protein (APP) and a-synuclein to highly toxic aggregates that inhbit both mitochondrial function and ubiquitin-proteasome system (UPS). Thus we have developed molecular entities that combine two or more of cholinesterase inhibition, brain selective MAO inhibition, iron chelation, inhibitors of glutamate release, anti apoptotic-neurorescue and neurorestorative activities. These iron chelator drugs are also inhibitors of iron dependent prolył4-hydroxylase, which inactivates of hypoxia inducing factor (HIF) via ubiquitination .They increased release of neuroprotective and neurotrophic erythropoietin and cause differentiation of neurons. This has been attributed to prevention of entry in to cell cycle as a consequence of Cyclin D inhibition. Two of such multimodal compounds presently under development are ladostigil, a cholinesterase inhibitor derivative of rasagiline (azilect) and iron chelator-MAO B inhibitor M30 series. These drugs possess iron chelating-radical scavenging, brain selective MAO-A and B inhibitory activity, acetylcholine and butyrylcholinesterase inhibitory moieties. Animal behavioral and neurophramacological studies have shown their anti Alzheimer, anti Parkinson and anti depressant activities. Both drugs also have neuroprotective and neurorestorative activities in neuronal cell cultures and in vivo. These properties indicate that multimodal drugs rather than "magic bullets" might serve as an ideal drug for treatment of PD and AD, for which they are being developed.

Novel mechanisms for cognitive dysfunction and loss of synaptic plasticity provoked by stress: corticotropin releasing hormone (CRH) and dendritic spines

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Chronic stress causes dendritic regression and loss of dendritic spines in hippocampal neurons, that is accompanied by deficits in synaptic plasticity and memory. However, the responsible mechanisms remain unresolved. Here we found that within hours of the onset of stress, the density of dendritic spines declined in vulnerable dendritic domains. This rapid, stress-induced spine loss was abolished by blocking the receptor (CRFR1) of corticotropin-releasing hormone (CRH), a hippocampal neuropeptide released during stress. Exposure to CRH provoked spine loss and dendritic regression in hippocampal organotypic cultures, and selective blockade of the CRFR1 receptor had the opposite effect. Live, time-lapse imaging revealed that CRH reduced spine density by altering dendritic spine dynamics: the peptide selectively and reversibly accelerated spine retraction, and this mechanism involved destabilization of spine F-actin. In addition, mice lacking the CRFR1 receptor had augmented spine density. These findings support a mechanistic role for CRH-CRFR1 signaling in stress-evoked spine loss and dendritic remodeling.

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Stress hormones modulate AMPAR mobility and facilitate synaptic plasticity

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Humans and rodent animals retain memories for stressful events very well. The *facilitated* retention of these memories is normally very useful: the organism can appraise and if necessary avoid similar negative situations in future. Stress activates the hypothalamo-pituitary-adrenal (HPA) axis and autonomic nervous system which in rodents results in enhanced levels of corticosterone and (nor)adrenalin (NE), in addition to other stress hormones. Corticosterone and NE have been reported to facilitate learning and memory processes, but exactly how these hormones exert their effects on learning is not understood.

It is known that learning critically depends on glutamate receptors. AMPA receptors within the hippocampus mainly comprise GluR1/2 or GluR2/3 heteromers, and their trafficking is thought to play an important role in activity dependent plasticity and hippocampal learning processes. We find that stress hormones selectively increase surface expression of the AMPAR subunits in primary hippocampal cultures. Corticosteroid hormones act relatively slow, while activation of beta-receptor agonists acts relatively fast to raise the number of synaptic AMPARs. It will be discussed that the time scale at which these hormones act (NE rapidly, glucocorticoids slowly) and the different types of AMPA receptors that they may target could be relevant for stress-induced facilitation of learning and memory.

Stress and corticosteroids modulate the use of multiple memory systems in mice and man

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Stress is known to affect the strength of learning and memory. Here, we used tasks for humans and mice to demonstrate that stress and glucocorticoid hormones act not only within a given memory system, but control a switch between different memory systems. Tasks allowed caudate-based stimulus-response and hippocampus-based spatial learning, until a change in setup revealed the used strategy. Healthy young men and women were trained in a 3D model of a room to find a win-card out of four which could be identified via the relation between multiple distal cues (spatial strategy) or a single proximal cue (stimulus-response strategy). The used strategy was inferred from a test trial in which the proximal cue was relocated. Psychosocial stress 30 minutes prior to learning favored the use of a stimulus-response strategy. C57BL6/J mice had to find an exit hole on a circular hole board in a fixed location flagged by a proximal stimulus. Relocation of the proximal stimulus in a test trial revealed the applied strategy. All naïve mice used a spatial strategy. While injection stress (vehicle; s.c.), restraint stress and corticosterone (250 µg/kg; s.c.) given 30 minutes before the task resulted in shifts to the stimulus-response strategy, administration of an antagonist of the mineralocorticoid receptor (MR; RU28318;50mg/kg; s.c.), either alone or in combination with restraint stress or corticosterone, reduced the use of the stimulus-response strategy. Next to strategies, stress and corticosterone affected the number of hole visits, latencies and distance to exit hole. We conclude that stress affects not only the performance within a memory system, but also coordinates the use of spatial and stimulus-response learning. Glucocorticoids appear to be key players in both modes of action.

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Early life stress shapes limbic and prefrontal synaptic networks and affects cognitive function in adolescence and adulthood

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Stress is known to be a potent modulator of learning and memory processes. The impact of stress is frequently regarded as deleterious to cognitive function. However, a growing body of evidence delineates a much more sophisticated relationship between stress and cognitive function. In the present study in rats we tested the impact of stress on an associative learning task using the two-way active avoidance (TWA) paradigm, since it is known to be very stressful to the trained animals. We have previously shown that learning in this paradigm is strongly age-dependent, with pre-weaning rats performing less well compared to adults. The aim of our study was two-fold: *i.*) to test the hypothesis that the inferior learning success in juveniles is caused by their disability to cope with the stress-component (foot shock, unfamiliar environment, separation from siblings) of the learning environment, which may correlate with a persisting over-activation of the hypothalamus-pituitary- adrenal (HPA)- axis. This was tested by blocking corticosteroid synthesis by systemic application of metyrapone, i.e., mimicking a reduced stress response during training. Learning performance as well as the dendritic and synaptic complexity in learning-related brain regions was quantified. *ii.*) to test whether early life stress induced by repeated maternal separation during the first two weeks of life impairs TWA learning.

Our results revealed that: *i.*) TWA training is stressful to both pre-weaning and adult rats, indicated by a dramatic increase in plasma corticosterone and ACTH levels as well as in ultrasonic stress vocalization. No indication for HPA- axis hyperfunction during training was observed in the juveniles, and metyrapone treatment did not improve juvenile´s performance. Thus, the impaired learning performance cannot be explained by excessive stress hormone release. *ii.*) in contrast to our prediction, repeated maternal separation improved avoidance learning in pre-weaning and adults, indicating that mild stress-“training” during earl life can facilitate memory formation. *Supported by SFB 779.*

Stress and cognition: The role of novel synaptic cell adhesion molecules

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Stress exerts profound effects on brain structure and cognition, with excessive stress resulting in important and lasting alterations in memory function. Moreover, stress is a key risk factor for different diseases that affect memory performance and has been shown to exacerbate memory decline during aging. Pharmacological or genetic modulation of receptor systems mediating the effects of stress interfere with memory acquisition, consolidation and retrieval. Specifically, memory performance is affected in animal models with an alteration of stress system function or in animals exposed to chronic stress situations.

Although the role of stress during various stages of development on cognition is well established, the molecular mechanism underlying stress actions on learning and memory is presently unclear. One possibility is the involvement of novel cell adhesion molecules (nCAMs), which are expressed in a brain region- and synapse-specific manner. To address the function of nCAMs in stress-induced cognitive deficits we employ different models of chronic stress during three different developmental stages: the early postnatal period, adolescence and young adulthood. We can show that the persistent cognitive deficits induced by chronic stress are accompanied by specific alterations of nCAM expression levels. Further, we demonstrate that hippocampus-dependent, stress-induced memory impairment is dependent on corticotropin-releasing hormone signalling, as both, the cognitive deficits and changes in nCAM expression levels, are absent in corticotropin-releasing hormone receptor 1 knockout mice. Taken together, we provide evidence for a direct involvement of specific nCAMs in mediating stress-induced effects on cognition.

Coding of celestial *E*-vector orientations in the central complex of the desert locust

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Many insects use a celestial compass for spatial orientation and navigation. Several studies showed that insects strongly rely on the polarization pattern of the blue sky as a guiding cue (e.g., Wehner and Müller, 2006, Proc Natl Acad Sci 103, 12575) but the position of the sun and the chromatic gradient in the sky offer useful information, too. To elucidate the neuronal mechanisms underlying celestial compass orientation, we have analyzed the polarization vision system in a favorable insect species, the desert locust *Schistocerca gregaria.*

Polarotactic behaviour of the locust is mediated, as in other insects, through photoreceptors in a specialized dorsal rim area of the compound eye. Polarization vision pathways, identified through dye injections and intracellular recordings, include dorsal rim areas in the lamina and medulla, the anterior lobe of the lobula complex, the anterior optic tubercle, and the central complex. Many neurons of the central complex were sensitive to dorsally presented polarized light and, generally, showed polarization opponency with characteristic differences in tuning width and background firing properties between cell types. Most cell types received binocular polarized light input with similar prefered *E*-vector orientation when selectively stimulated through the right and the left eye. Receptive fields were generally directed towards the zenith and subtended an area of 90° or more, often extending symmetrically to both sides. Single-cell recordings revealed a compass-like linear map of *E*-vector tunings in the columns of the protocerebral bridge, a subcompartment of the central complex (Heinze & Homberg 2007, Science 315:995). The receptive field organization and compass-like arrangement of *E*-vector tunings suggest that the central complex computes and codes for azimuthal directions. This may be used for azimuth-independent recognition of objects in space as well as for azimuth coding during long-range navigation and path integration. Supported by DFG grant HO 950/16-2.

Central nervous control of grasshopper sound production: neurons, chemical messengers and the flow of information the central complex.

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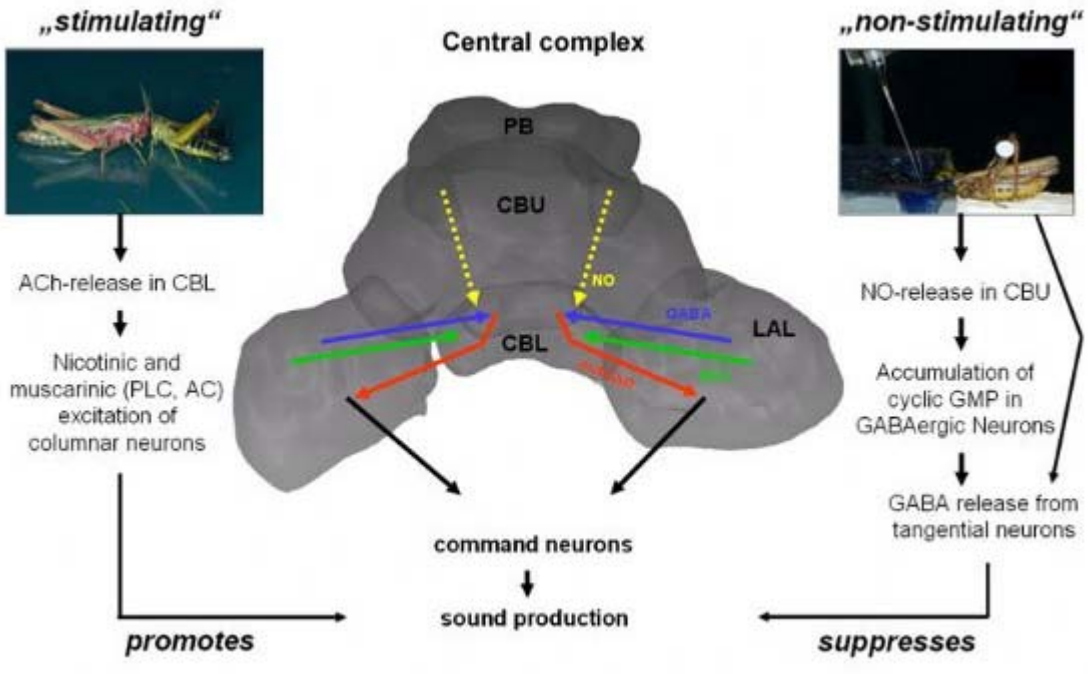
The central complex in the protocerebrum of acoustically communicating grasshoppers coordinates the type, intensity and timing of sound signals used for mate attraction, courtship and rivalry. Sound production depends on the balance of fast and slow excitation and inhibition in central complex neuropils and various transmitters, modulators and intracellular signalling pathways that promote or suppress sound production have been identified by pharmacological stimulation and confirmed by anatomical studies. Two of these signaling pathways have been associated with particular behavioral situations. Hearing and recognizing the conspecific song activates cholinergic projections to the central complex leading to both nicotinic excitation of yet unknown targets and muscarinic excitation of columnar neurons, mediated by phospholipase C and adenylyl cyclase-initiated intracellular signaling pathways. Expression muscarinic ACh receptors in the central complex is limited to a subset of columnar neurons with their cell bodies located in the pars intercerebralis, which are thought to contact pre-motor elements in the lateral accessory lobes. In contrast, a different set of pars intercerbralis neurons with columnar projections in the upper division of the central body and tangential neurons with cell bodies in the ventro-median protocerebrum contain the enzyme nitric oxide synthase and accumulate citrulline in situations that are unfavorable for sound production. Since liberation of nitric oxide in the central body inhibits sound production via soluble guanylyl cyclase activation and cyclic GMP production in the central body lower division, these citrulline-accumulating central complex neurons may translate inappropriate behavioral situations into nitric oxide-mediated suppression of sound production. By applying multiple antibodies directed against components of signaling pathways that contribute to the control of grasshopper sound production to the central complex and conducting physiological studies on pre-identified central complex neurons in primary cell culture, we are currently attempting to identify the points of convergence of different signals in order to trace the flow of information within the central complex.



DEALING WITH UNPREDICTABLE BARRIERS IN NATURAL TERRAIN: ROLES OF BRAIN AND LOCAL CONTROL CENTERS

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The ability of animals to negotiate unpredicted barriers in natural terrain is biologically remarkable and makes them attractive models for robotic design. Animals evaluate objects in their path using sensors on their head then use that information to formulate commands that alter behavior. In order to understand this process in insects, we employ a range of behavioral and neurobiological studies directed at both thoracic local control circuits and brain centers.
Behavioral studies indicate that cockroaches use antennae to investigate objects in their path. If the antennae contact a shelf from the top, the insect will climb over, while contact from the bottom will cause it to tunnel under the barrier. Antennal contact of a wall generates turning movements. Other sensors such as vision also affect decisions. Insects that have experienced bilateral lesion of circumoesophageal connectives, that disconnect the brain, deal with barriers in a less controlled manner. These animals crash into walls, and then force themselves around or over them by brute force. Lesions within the brain also generate abnormal behaviors if they involve the central complex (CC).

Details of turning can be observed in cockroaches tethered over a lightly oiled glass plate. Pushing on one antenna generates turning movements that switch from symmetrical left-right leg movements to asymmetrical actions. In particular, the leg on the inside of the turn changes from rearward extension during stance to lateral extension during swing. Associated with this alteration is an increase in distal motor activity, reduction of proximal activity and changes in relative timing of joint extension. These changes could be evoked through alteration of a limited number of critical local reflexes that place the leg in a different mechanical state and lead to further changes.

Our behavioral observations indicate that mechanical stimulation of antennae provides much of the necessary information for evaluating barriers and lesion studies implement the CC. We, therefore, investigated responses of CC units to mechanical stimulation of antennae. We inserted 16єhannel extracellular probes into the CC of restrained cockroaches. Using cluster cutting software, we can isolate 5-20 units in most experiments. We stimulate each antenna by activating a servo motor attached to capillary tubes containing each antenna. By lining up the activity in each unit with the onset of stimulation, we identify antenna sensitive units and begin to determine various response properties. Over half of 242 units in the ellipsoid and fan-shaped body responded to antenna stimulation. Velocity is encoded in most of these units and about one third of them are biased to one antenna. Many units are also sensitive to visual cues. Additional recordings using wire bundles in the CC of tethered insects and intracellular recordings in restrained animals complement the multi-channel recording studies.

Our studies suggest that a large population of neurons within the CC processes information on barriers and ambient conditions, then formulates appropriate commands. These commands descend to local control centers where they may act by altering a few critical reflexes, leading to a new mechanical state. The initial changes in leg movements start a cascade of reflex adjustments ultimately pushing control to a final new stable state.

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Embyronic development of the central complex in the grasshopper.

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Characteristic of the central complex of the insect midbrain is a modular neuroarchitecture, exemplified in the grasshopper by columnar fibre bundles, among them the so-called w, x, y and z tracts. These bilaterally symmetrical fibre bundles represent the stereotypic projection of axons from clusters of neurons in the pars intercerebralis to the central body and then to identified commissures across the brain midline. Despite extensive analyses of this neuroarchitecture in adults, little is known about its ontogeny in any insect. The central complex of the grasshopper brain is essentially a protocerebral structure, and we have identified a subset of four neuroblasts in each protocerebral hemisphere as being the progenitor cells for four bilateral clusters of neurons whose axons we show project via discrete tracts (w, x, y, z)into the region where the central complex will form. Intracellular dye injection associated with lineage analyses reveal the clonal mechanism of tract formation. The oldest (first born) cell from each cluster acts as a pioneer in that it projects the first axon laterally into the respective initial tract. Later born cells from each cluster project axons onto the pathway established by the pioneer, leading to the axons within a column being organized according to age, a feature we term temporal topology. Each pioneer axon subsequently turns medially and enters the primary brain commissure where it fasciculates with the growth cones of identified pioneers of this pre-existing commissural fascicle. The pioneer axons of each tract do not fasciculate with one other prior to their entry into the commissure, so that the tracts appear to be established independently during early embryogenesis, thus prefiguring the modular w, x, y, z columns of the adult central complex.

Modules of Behavioral Control in the Central Complex of *Drosophila*

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In order to analyze principles and neuronal circuits underlying behavioral control, we study oriented walking and climbing in the fly *Drosophila* as a model system. With the help of neuroanatomical and behavioral mutant lines we were able to define and localize modules of higher behavioral control in particular neuropilar regions of the central complex, modules that are at the basis of the fly's autonomy and high maneuverability.

The *tay bridge*^{1} (*tay*^{1}) mutant was originally isolated on the basis of reduced walking speed and activity. In addition, *tay*^{1} is defective in the compensation of rotatory stimuli during walking and histologically, *tay*^{1} causes a mid-sagittal constriction of the protocerebral bridge, a constituent of the central complex. To associate the behavioral phenotypes with the anatomical defect in the protocerebral bridge, we cloned the gene and used different driver lines to express the *tay* cDNA in various neuronal subpopulations of the central brain in *tay*^{1}-mutant flies. These experiments showed an association of the aberrant walking speed and activity with the structural defect in the protocerebral bridge. In contrast, the compensation of rotatory stimuli during walking was rescued without a restoration of the protocerebral bridge. The results of our differential rescue approach are supported by neuronal silencing experiments using conditional tetanus toxin expression in the same subset of neurons. These findings show for the first time that the walking speed and activity is controlled by different substructures of the central brain than the compensatory locomotion for rotatory stimuli.

In a study of climbing behavior, mutant lines defined a module for decision making, which could be functionally separated from control modules for distinct adaptations in the execution of climbing.

In a visual orientation paradigm flies approach an object which becomes invisible during approach. Wild-type flies are nevertheless pursuing the chosen course for several seconds. In order to discern between a mere control of straight walking and the involvement of an orientation memory, we systematically lured the flies out of their straight path to the vanished object. As soon as the perturbance disappears as well, wild-type flies turn back towards the direction of the firstly presented, still invisible object with a high probability. The outcome implies the existence of an orientation memory, where - at least for a short time – the position of the visual object or the path to it is stored and can be retrieved. Whenever the neurotoxin tetanus toxin was conditionally expressed exclusively in various subsets of ellipsoid-body ring neurons via the GAL4/GAL80 system, flies took a random decision. The ellipsoid body is another of the four neuropils of the central complex. In the presence of continuously visible objects the orientation behavior of those flies is inconspicuous. Thus, functional modules for acute visual orientation and for a spatial orientation memory can be separated. The described orientation memory depends on *ignorant* encoding for an S6KII kinase.

Mid-Line Neuropils in Arthropods: Divergent Evolution from an Ancestral Groundplan

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Comparative studies across the Tetraconata (Crustacea + Hexapoda) demonstrate the presence of one or more unpaired midline neuropils that are connected to a characteristic assembly of lateralized satellite regions. Studies on insects demonstrate that mid-line neuropils (central bodies) play crucial roles in the control of complex motor actions by multijoint appendages. Comparisons across insects and crustaceans reveal that in any taxon variation of the modular groundplan organization of the central body reflects that taxon's motor repertoire. Although midline neuropils of chelicerates and "myriapods" appear very different from those of the Tetraconata, certain fundamental aspects of their organization are nevertheless shared, suggesting that within the Arthropoda there has been divergent evolution of neuronal organization of midline neuropils. Apart from raising fundamental questions about the possible function of midline neuropils in chelicerates, current neuroanatomical data provides further support for a clade that unites the Chelicerata and Myriapoda as sister groups.

How do synapses form in the CNS? Role of astrocyte-secreted extracellular matrix proteins in regulation of synapse formation.

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Synapses are asymmetric cellular adhesions that are critical for nervous system development and function, but the mechanisms that induce their formation are not well understood. Thrombospondin (TSP) is a large oligomeric astrocyte-secreted extracellular matrix protein that is sufficient to induce synapse formation in the central nervous system and is necessary for astrocyte-enhanced synaptogenesis *in vitro*. In this study we identify the thrombospondin receptor involved in synapse formation as the calcium channel subunit a2d1, which is also the receptor for the anti-epileptic and analgesic drug gabapentin. We show that a2d1 interacts with the epidermal growth factor-like repeats common to all thrombospondins. a2d1 overexpression increases synaptogenesis *in vitro* and *in vivo* and a2d1 is required for thrombospondin and astrocyte-induced synapse formation *in vitro*. We found that gabapentin is a potent inhibitor of excitatory CNS synapse formation *in vitro* and *in vivo*. These findings identify a2d1 as a novel signaling receptor that induces synapse formation and suggest that gabapentin may mediate its therapeutic function by blocking new synapse formation.

Perineuronal nets structure the perisynaptic extracellular space

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Brain synapses are wrapped by a dense meshwork of extracellular matrix (ECM), which consists of glycoproteins and proteglycans of glial as well as neuronal origin This specific ECM is known since 100 years as perineuronal nets (PNN) and is thought to be critical for the development as well as for the function of brain synapses. Here, we tested the hypothesis whether the PNN can act as diffusion barrier for AMPA-type glutamate receptors on the cell surface. Using single particle tracking and fluorescence recovery after photobleaching we found that this net-like appearing ECM forms surface compartments, which act as lateral diffusion barriers for AMPA-type glutamate receptors. Removal of the ECM using the hyaluronic acid digesting enzyme hyaluronidase increased extrasynaptic receptor diffusion and the exchange of synaptic AMPA receptors via lateral diffusion. Using whole-cell patch-clamp recording we measured an increased paired-pulse ratio as a functional consequence of ECM removal. These results suggest that surface compartments formed by the ECM hinder lateral diffusion of AMPA receptors and thereby may modulate short-term synaptic plasticity.

Neurotrypsin-mediated proteolysis of agrin at CNS synapses - a key to cognitive functions?

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The synaptic serine protease neurotrypsin is considered essential for cognitive function, because its deficiency in humans results in severe mental retardation. At present, the only known proteolytic substrate of neurotrypsin is the proteoglycan agrin (Reif et al., FASEB J. 21: 3468-3478, 2007). Neurotrypsin cleaves agrin at two homologous, highly conserved sites, resulting in a 90-kDa fragment (agrin-90) confined by the two cleavage sites, and a 22-kDa fragment (agrin-22) consisting of the C-terminal laminin G domain. Stimulation of neural activity causes exocytosis of neurotrypsin from presynaptic nerve endings and local cleavage of agrin at the synapse (Frischknecht et al., J. Neurosci., 28: 1568-1579, 2008; Stephan et al., FASEB J., 22:1861-1873, 2008). We recently found that neurotrypsin-dependent agrin cleavage is responsible for the transient increase of dendritic filopodia in hippocampal CA1 neurons observed after induction of long-term potentiation (LTP). LTP-associated filopodia promotion required neurotrypsin; it was abolished in neurotrypsin-deficient mice, although LTP was intact. We then studied whether the neurotrypsin-dependent increase of dendritic filopodia was mediated by the cleavage of agrin. We found that application of the recombinant C-terminal 22-kDa fragment (agrin-22) to neurotrypsin-deficient hippocampal slices completely rescued the lost activity-dependent increase in filopodia number. No effect on filopodia was found with the neurotrypsin-dependent 90-kDa fragment (agrin-90) or when agrin-22 was presented in conjunction with agrin-90 (agrin-110).

Our results further indicate that neurotrypsin-mediated agrin cleavage represents a novel molecular mechanism of synaptic coincidence detection. Coincident activity of the pre- and postsynaptic cells has been considered as a cellular mechanism resulting in strengthened synaptic connections and thus to underlie memory and learning ever since the famous Hebb postulate. Detailed analyses revealed that presynaptic exocytosis of neurotrypsin depended on action potentials and activation of presynaptic calcium channels, whereas NMDA receptor-mediated postsynaptic signaling was required for neurotrypsin-dependent agrin cleavage. Therefore, we concluded that presynaptic activity results in the exocytosis of inactive neurotrypsin. Only if pre- and postsynaptic activities coincide is externalized neurotrypsin activated and rendered capable of cleaving agrin. Cleavage of agrin and release of its C-terminal 22-kD fragment in turn induces dendritic filopodia. Because dendritic filopodia represent nascent synapses, the neurotrypsin-dependent cleavage of agrin at the synapse may be instrumental for a Hebbian organization and reorganization of synaptic circuits in the CNS.

Regulation of Synaptogenesis and Synaptic Activity by Chondroitinsulfate Proteoglycans

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Synapses represent specialized cell-cell contact sites between nerve cells. These structures mediate the rapid and efficient transmission of signals between neurons and are surrounded by glial cells. Former investigations have shown that astrocytes and astrocyte-derived extracellular matrix (ECM) components are important for formation, maintenance and function of synapses in the CNS. In order to study the effects of glial-derived ECM on synaptogenesis, we have established an in-vitro co-culture system for E18 rat hippocampal neurons and various glial cell types. Neurons were cultured without direct contact to various glial cells for a period of three weeks. Starting with 10 days in culture, the concordant expression of pre- and postsynaptic proteins could be documented. Moreover, the colocalization of bassoon and proSAP1 indicated the formation of structurally intact synapses. A technique was developed that permitts the semi-automated quantitative determination of the number of synaptic puncta per neuron. Using this method, significant differences of the efficacy of cell types for and the effects of defined treatments on synaptogenesis could be documented. Thus, primary rat or mouse astrocytes proved the most efficient cell type in fostering synaptogenesis.

Our experimental approach is based on the concept of the tripartite synape that consists of a pre-synapse, a postsynaptic membrane and closely apposed astrocytes as a third element. We have shown that astrocytes support the survival of embryonic hippocampal neurons and the formation of structurally intact synapses, as documented by the co-localisation of bassoon- and proSAP1-positive puncta. The development of synapses was paralleled by the emergence of perineuronal net (PNN) like structures that contained the 473HD-epitope and tenascin-C. In order to probe for an influence of glial-derived extracellular matrix on synaptogenesis, the co-cultures were treated with enzymes that degrade the ECM structure. The effects of the treatments on the number of synaptic puncta in the transwell co-culture assay system was evaluated.

To investigate the functional role of glial ECM molecules (glycoproteins and chondroitin sulfate proteoglycans) on synaptic transmission, whole-cell voltage-clamp recordings of cultured primary rat hippocampal neurons were performed. Cells obtained from E18 rats were plated on a feeder layer of astrocytes and were used for electrophysiological recordings between 8 and 10 days in vitro. Untreated cultures served as control and were compared with cultures treated either with hyaluronidase (HD) or other enzymes to remove ECM components. Crucial parameters investigated in that approach were the amplitudes of pharmacologically isolated excitatory miniature postsynaptic currents (mEPSCs). In addition to mEPSCs, amplitudes of mIPSCs were also investigated. The detailed signalling pathway that links the ECM molecules to functional properties of neurotransmitter receptors is of special interest in further experiments.

Using a gene trap library, it could be shown that chondroitinase ABC treatment increases the nucleotide exchange factor vav3 in neural cells. These aspects will be discussed in the presentation.

Regulation of hippocampal synaptic plasticity by extracellular matrix molecules

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Conspicuous aggregates of extracellular matrix (ECM) molecules in the hippocampus appear as so-called perineuronal nets, which surround cell bodies and proximal dendrites of fast-spiking interneurons in a mesh-like structure embedding synaptic contacts and astrocytic processes. The ECM of perineuronal nets is enriched in chondroitin sulfate proteoglycans, hyaluronic acid and the glycoprotein tenascin-R. Experiments in dissociated hippocampal neurons revealed that formation of perineuronal nets requires spiking of neurons and Ca^{2+} influx via GluR2-lacking AMPA receptors and L-type voltage-dependent Ca^{2+} channels (L-VDCC)[1]. Removal of perineuronal nets with chondroitinase ABC, an enzyme digesting chondroitine sulfates, does not affect the number and distribution of perisomatic GABAergic contacts but results in an increase in excitability of perisomatic interneurons and impairment of long-term potentiation (LTP) in CA3-CA1 synapses. Mice deficient in tenascin-R also show impaired LTP in CA3-CA1 connections, accompanied by elevated levels of excitatory synaptic transmission and reduced levels of perisomatic inhibitory currents mediated by $GABA_A$ receptors. These changes in inhibition causes metaplastic shift in the threshold for induction of LTP in tenascin-R mutants, which can be reverted by pharmacological treatments transiently increasing the levels of GABAergic inhibition[2]. Tenascin-R-deficient mice also show retarded progression of kindling and a number of morphological abnormalities. Particularly, the number of S100 expressing astrocytes in the dentate gyrus is enhanced by tenascin-R deficiency and correlates negatively with the kindling rate[3].
Apart from perineuronal nets, many ECM components, for instance, tenascin-C, show diffuse expression throughout the extrasynaptic space. Strikingly, deficiency in tenascin-C leads to impairment in L-VDCC-dependent LTP and long-term depression[4]. There is increasing evidence that also other ECM molecules may regulate synaptic plasticity via L-VDCCs. Thus, ECM of perineuronal nets and widely distributed perisynaptic ECM regulate synaptic plasticity via diverse mechanisms, involving modulation of GABAergic inhibition and postsynaptic Ca^{2+} channels.

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Extracellular matrix modification, plasticity and rehabilitation

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Both axon regeneration and plasticity are inhibited by molecules and structures in the extracellular matrix. The role of chondroitin sulphate proteoglycans (CSPGs) in restricting CNS plasticity at the end of critical periods was discovered through the demonstation that treatment with chondroitinase, which removes glycosaminoglycan (GAG) chains from chondroitin sulphate proteoglycans (CSPGs), leads to a rapid recovery of function after injury and to reactivation of ocular dominance plasticity. The structure responsible for the restriction of [lasticity in the adult CNS are probably perineuronal nets (PNNs) which are present around may neuronal cell bodies and dendrites. These contain inhibitory CSPGs, hyaluronan, link protein and tenascin-R. The components of PNNs are produced either by the neurones themselves or by surrounding glial cells. All neurones with PNNs express both a hyaluronan synthase enzyme and a link protein, and these are probably the key components that trigger the formation of the structures. A link protein knockout animal lacks normal PNNs on its dendrites. The GAGs within PNNs have a different sulphation pattern to those in the general CNS matrix, giving them high affinity for binding molecules which may affect plastic behaviour in neurons such as BDNF and semaphorin-3, which are concentrated around synapses by PNNs. Making the brain or spinal cord plastic by itself promotes some recovery of function after injury. However recovery can be greatly increased by combining rehabilitation with chondroitinase to drive plasticity to produce appropriate circuitry. The amount of new circuitry that the spinal cord can make may be limited, because increased recovery in some functions due to rehabilitation is associated with decreased functional recovery in other behaviours.

Schizophrenia-related symptoms and mitochondrial dysfunction in G72/G30 transgenic mice

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Genetic studies have implicated the evolutionary novel, primates-specific gene locus G72/G30 in schizophrenia, bipolar- and panic-disorders. It encodes for a protein LG72 whose function has been controversially discussed as putative regulator of the peroxisomal enzyme D-amino-acid-oxidase (DAO), or as a mitochondrial protein, which promotes robust mitochondrial fragmentation in mammalian cell lines including human and rat primary neurons. Because of this conserved function we here have generated “humanized” BAC transgenic mice (G72Tg) expressing alternatively spliced G72 and G30 transcripts, and the LG72 protein. G72 expression is prominent in granular cells of the cerebellum, the hippocampus, the cortex and the olfactory bulb. Most strikingly, G72Tg mice displayed deficits in sensorimotor gating which could be reversed with haloperidol, increased sensitivity to PCP, motor-coordination deficits, increase compulsive behaviors and deficits in smell identification. These results demonstrate that expression of the human G72/G30 gene locus in mice produces behavioral phenotypes that are relevant to psychiatric disorders.

Mouse mutants of Neuregulin-1 and their relevance for schizophrenia

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The human Neuregulin-1 (NRG1) gene, which encodes a family of neuronal growth factors, has been identified as a susceptibility gene for schizophrenia (SZ). ErbB4, a transmembrane tyrosine kinase, is the predominant NRG1 receptor in neurons of the central nervous system (CNS). Multiple functions of NRG1-ErbB4 signaling have been suggested, including a role in neuronal migration and synaptic plasticity. However, in vivo data for the adult brain are lacking, due to the embryonic lethality of NRG1 and ErbB4 null mutations in mice. Here, we have generated a set of conditional mouse mutants and transgenic mice to study the effect of altered levels of NRG1-ErbB4 signaling on synaptic plasticity and behavior in adult mice. Conditional null mutants lacking NRG1 or ErbB4 in cortical projection neurons beginning at postnatal stages (using a CamKII-Cre driver line) develop normally and exhibit no obvious defects in cortical development. Moreover, cortical expression levels of AMPA and NMDA receptors were unaltered in the absence of NRG1 or ErbB4. However, behavioral and electrophysiological analysis of projection neuron-specific NRG1 mutants at older stages revealed impaired synaptic plasticity, learning and memory. Unexpectedly, the corresponding ErbB4 mutants were largely unaffected. Subsequent expression analysis demonstrated that ErbB4 mRNA is predominantly expressed in parvalbumin expressing interneurons, but absent from calbindin and calretinin expressing cells. In contrast, ErbB4 expression was rarely detected in pyramidal neurons. Using a parvalbumin-Cre driver line, we have generated mice lacking selectively ErbB4 in interneurons, which are currently being analysed. We suggest that NRG1-ErbB4 signaling in the mature brain modulates synaptic functions, most likely acting through an interneuronally expressed ErbB4 receptor. Impaired synaptic plasticity as a consequence of chronic alterations in the level of NRG1-ErbB4 signaling might contribute to the pathophysiology of SZ.

Role for Reelin in neurologic and psychiatric disease

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The extracellular matrix protein Reelin is known to control the radial migration of cortical neurons during development. However, Reelin is also expressed in the mature brain, suggesting additional functions. Indeed, Reelin was found to play a role in synaptic plasticity (Beffert et al., 2005), and Reelin expression was found decreased in neurologic and psychiatric diseases such as epilepsy (Haas et al., 2002) and schizophrenia (Impagnatiello et al., 1998). In epilepsy, Reelin deficiency was recently described to cause granule cell dispersion, a characteristic loss of granule cell lamination associated with Ammon's horn sclerosis. Antibody blockade of Reelin function induced granule cell dispersion in adult, naïve wildtype animals at sites of antibody infusion (Heinrich et al., 2006). These latter findings in particular point to a role for Reelin in stabilizing cortical structure in the mature brain (Förster et al., 2006).

How does Reelin stabilize cortical architecture? In order to address this question, we have begun to study the effects of Reelin signaling on cytoskeletal proteins such as cofilin. Recent findings will be presented which have shown that Reelin signaling leads to serine3 phosphorylation of cofilin, an actin-depolymerizing protein that promotes the disassembly of F-actin. Phosphorylation of cofilin renders it unable to depolymerize F-actin, thereby stabilizing the cytoskeleton. The Reelin receptors ApoER2 and VLDLR, the adapter protein Disabled-1 (dab1), Src family kinases (SFKs), and PI3K are involved in cofilin phosphorylation. Phosphorylation of cofilin takes place in the leading processes of migrating neurons as they approach the Reelin-containing marginal zone of the cortex. Neuronal processes are stable on Reelin-coated stripes but grow on control stripes by forming lamellipodia. These findings in a stripe choice assay as well as real-time microscopy studies suggest that Reelin stabilizes the cytoskeleton of neuronal processes, thereby anchoring them to the marginal zone which appears to be required for directional neuronal migration during cortical development. We hypothesize that Reelin-induced stabilization of the cytoskeleton may also be involved in the maintenance of cortical architecture during adulthood. Conversely, decreased Reelin expression in neurologic and psychiatric diseases may result in structural reorganization such as granule cell dispersion in epilepsy.

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The MK-801 model mimicks drug-induced psychotic illnes

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The psychotomimetic effects of N-methyl-D-aspartate (NMDA) receptor antagonists such as phencyclidine (PCP) in healthy humans and their ability to exacerbate psychotic symptoms in schizophrenic patients have promoted a view of schizophrenia as being related to altered glutamatergic neurotransmission. This prompted us and others to develop animal models for psychosis based on a glutamatergic approach. Pharmacological induction of a state of impaired glutamatergic neurotransmission based on chronic, low-dose application of MK-801, a highly selective noncompetitive NMDA antagonist, revealed marked parallels between schizophrenia and our animal model. MK-801 altered the expression of NR1 splice variants and NR2 subunits of the NMDA receptor in a pattern partially resembling the alterations detected in schizophrenia. Ultrastructurally, the number of gamma-aminobutyric-acid (GABA)ergic parvalbuminpositive interneurons was relatively decreased, a finding which again parallels observations in post mortem brain from schizophrenic patients. As a functional consequence, local inhibition of pyramidal cells which is largely mediated by recurrent axon collaterals, originating from GABAergic interneurons, was altered. Not unexpectedly, these animals showed cognitive deficits resembling findings in schizophrenic humans. These convergent lines of evidence suggest that our approach has a significant potential of serving as a model of the pathobiology of several aspects of psychosis and consequently could contribute to the development of new therapeutic strategies.

The hypoxic rat model for obstetric complications in schizophrenia

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Introduction:
Hypoxia has been discussed as a possible factor of obstetric complications in the pathophysiology of schizophrenia. The present study investigated the effects of mild chronic neonatal hypoxia in rats as an animal model of schizophrenia.

Methods:
(1) After chronic neonatal hypoxia between postnatal days (PD) 4-8, half of the pups were fostered by normally treated nurse animals to control for possible maternal effects, and (2) tested on PD 36, 86, 120 and 150 using three different behavioural tests: prepulse inhibition (PPI), social interaction and recognition, and motor activity in an open field. (3) Before the PD 150 test, 50% of the animals had been chronically treated with the antipsychotic drug clozapine (45 mg/kg/day). (4) At PD 155, different brain regions have been used for expression profiling of synaptic genes on cDNA microarrays ("glutamate chip") with qRT-PCR confirmation.

Results:
Rats exposed to hypoxia exhibited deficits in locomotor activity on PD 86, 120, and 150, as well as a PPI deficits on PD 120 and 150, but not before. Chronic treatment with clozapine reversed hypoxia-induced PPI deficits, but not the decreased locomotor activity. In a second experiment, where clozapine was chronically administered before PD 120, development of the PPI deficit in the animals exposed to hypoxia was prevented. Several presynaptic genes such as SNAP-25, syntaxin 1A, neurexin, neuropeptid Y and complexin I were downregulated and subunits of the NMDA receptor were upregulated by hypoxia. These differential gene regulations could be partially compensated for by clozapine treatment.

Discussion:
The time course of hypoxia-induced PPI deficits and their reversal by clozapine supports the validity of our animal model and the hypothesis that hypoxia as an obstetric complication is an important factor in the pathophysiology of schizophrenia. Differential gene expression in cortical and subcortical brain regions as well as correlations to deficits of PPI support the view of an involvement of synapse-associated gene products and glutamatergic and GABAergic neurotransmission in the pathophysiology of behavioural deficits occurring as delayed responses to neonatal hypoxia in adulthood.

Oscillatory activity in hippocampal networks in vitro: role of perisomatic-targeting interneurons

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Gamma frequency oscillations are characterized by intense synaptic excitation of hippocampal GABAergic interneurons despite relatively low frequency discharge of pyramidal cell somata. We describe direct patch-clamp recordings from the pyramidal cell axons of the hippocampal CA3 area during gamma frequency oscillations showing that distal axonal compartments fire action potentials at high frequency, which was not detected by intrasomatic recordings. We further investigated the role of synaptic inhibition mediated by perisomatic-targeting axo-axonic and basket cells in this dichotomy. During kainate-induced gamma rhythms axo-axonic cells showed very high frequency firing that was not correlated with the field. This behavior was in stark contrast to that of basket and other inhibitory cells. In addition, dual patch-clamp recordings demonstrated that intrasomatic activation of axo-axonic cells, to generate outputs at this very high frequency, significantly suppressed antidromic invasion of action potentials into pyramidal cell somata. This suppression was not seen with intrasomatic activation of basket cells. Axonal action potential generation may therefore serve to maintain sufficient excitatory input to interneurons to generate a population gamma rhythm while axo-axonic inhibition reduces their influence on somato-dendritic principal cell excitability.

Multiple gamma rhythm-generating microcircuits in entorhinal cortex

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Local circuits in medial entorhinal cortex (mEC) and hippocampus generate gamma frequency population rhythms independently. Temporal interaction between these areas at gamma frequencies is implicated in memory – a phenomenon linked to activity of NMDA-subtype glutamate receptors. While blockade of NMDA receptors does not affect frequency of gamma rhythms in hippocampus, it exposes a second, lower frequency (25 – 35 Hz) gamma rhythm in mEC. In experiment and model, NMDA receptor-dependent mEC gamma rhythms were mediated by basket interneurons, but NMDA receptor-independent gamma rhythms were mediated by a novel interneuron subtype – the goblet cell. This cell was distinct from basket cells in morphology, intrinsic membrane properties and synaptic inputs. The two different gamma frequencies matched the different intrinsic frequencies in hippocampal areas CA3 and CA1, suggesting that NMDA receptor activation may control the nature of temporal interactions between mEC and hippocampus, thus influencing the pathway for information transfer between the two regions.

Period concatenation underlies interaction between gamma and beta rhythms in neocortex.

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The neocortex generates rhythmic electrical activity over a large frequency range, and can simultaneously express different rhythms. These rhythms can also undergo transitions to other frequencies. We describe recordings from in vitro preparations of rat somatosensory cortex showing that two frequencies (gamma-40 Hz and beta2 - 25 Hz), co-expressed in superficial and deep cortical laminae with low temporal interaction, can combine to generate a third frequency (beta 1- 15 hz) with strong temporal interaction among laminae. The process occurs via period concatenation, with basic rhythm-generating microcircuits underlying gamma and beta2 rhythms forming the building blocks of the beta1 rhythm by a process of addition. The mean ratio of both sets of adjacent frequency components was the same, approximately the golden mean, with implications for interactions of the rhythms.

Cellular mechanisms of transient assembly formation by hippocampal principal cells

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The hippocampal formation expresses a variety of rhythmic activity patterns which are related to behavioural states. These network oscillations may organize the activity of selected neurons to bind them into transient assemblies which can represent external cues and give rise to memory formation or -consolidation, respectively.

We have analyzed fast (200 Hz) "ripple" oscillations which occur on top of spontaneous propagating sharp waves (sharp wave-ripple complexes, SPW-R) in mouse hippocampal slices. Simultaneous recordings of the local field potential together with membrane potential of individual CA1 pyramidal cells revealed that only a fraction of pyramidal cells generates action potentials during SPW-R. Non-participating cells were consistently inhibited during SPW-R. They did not fire action potentials during the field events even upon somatic depolarization by current injection, pointing towards a strong signal-to-background separation for members of the oscillating assembly.

Participating cells showed strongly phase-coupled action potentials during ripples, mostly as single spikes during a fraction of all SPW-R. Surprisingly, these ripple-coupled action potentials of participators had an aberrant waveform. The depolarizing phase rose steeply from a GABA-A-receptor-mediated somatic hyperpolarization. Threshold was ~10 mV more negative than for "normal" action potentials outside ripples. Moreover, a pronounced after-depolarizaiton became visible which was largely absent in action potentials outside ripples. Ripple-coupled action potentials survived hyperpolariaztions of the soma by up to -30 mV through negative current injection and, in some cases, showed notches during the rising phase. Upon hyperpolarization, we also observed some partial spikes or spikelets. Together, these data point towards an ectopic origin of ripple-associated action potentials in the axon.

Pharmacological experiments indicate that activation of GABA-A-receptors is critically involved in the generation of ripple-coupled action potentials. Application of diazepam increased the incidence of spikes while gabazine, locally applied to stratum oriens, blocked their generation. The positive modulation by benzodiazepines was absent in slices from mice with targeted mutation of the benzodiazepine-site in alpha2-subunits, indicative of a role for axo-axonic synapses in spike generation.

Together, our findings indicate that cells participating in sharp wave-associated ripple oscillations generate action potentials by a non-conventional mechanism involving activation of axonal GABA receptors and antidromic spike generation. The activity of cells which participate in SPW-R is strictly separated from EPSP-induced spike generation of pyramidal cells, ensuring a clear distinction of members of the transient, oscillating assembly.

Ripple oscillations and reactivated cell assemblies in the hippocampus

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The hippocampus is involved in the formation of spatial and episodic memories, and hippocampal pyramidal cells fire in relation to space. Cells with similar spatially-selective firing fields (place fields) tend to fire together in subsequent sleep, suggesting that place cells encoding similar places form cell assemblies for reactivation. Such 'reactivation' is thought to be involved in the consolidation of episodic memories.

Here we examined the formation of reactivated cell assemblies by measuring changes in CA1 pyramidal cell firing-associations between sleep sessions before and after exploration. The largest increases in firing-association occurred between cells representing the most visited regions of the environment. These increases were also dependent on the number of times the cells fired together with short latencies during exploration. The largest increases were seen between cells firing <50 ms apart. Moreover, cell pairs with non-overlapping fields, which tended to fire independently, showed a reduction in association strength proportional to the amount of independent firing. We propose that reactivated cell assemblies are formed through Hebbian increases in firing-association, and are shaped by negative changes. Our results also provide evidence that reactivated patterns are determined by recent behaviour. Such experience-dependent replay may point to the recurrence of episodic-like memory traces.

Development of basket cells from slow to fast signaling devices: contribution to gamma oscillations

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Gamma frequency (30-100 Hz) oscillations in the mature cortex underlie higher cognitive functions. Fast signaling in GABAergic interneuron networks plays a key role in the generation of these oscillations. During development of the rodent brain, gamma activity appears at the end of the first postnatal week but frequency and synchrony reach adult levels only by the fourth week. However, the mechanisms underlying the maturation of gamma activity are unclear. Here I demonstrate that hippocampal basket cells (BCs), the proposed cellular substrate of gamma oscillations, undergo marked changes in their morphological, intrinsic and synaptic properties between postnatal day (P) 6-25. During maturation, action potential duration, propagation time, duration of the release period, and decay time constant of inhibitory postsynaptic currents decreases by ~30-60%. Thus, postnatal development converts BCs from slow into fast signaling devices. Computational analysis reveals that BC networks with young intrinsic and synaptic properties as well as reduced connectivity generate oscillations with moderate coherence in the lower gamma frequency range. In contrast, BC networks with mature properties and increased connectivity generate highly coherent activity in the upper gamma frequency band. Thus, late postnatal maturation of BCs enhances coherence in neuronal networks and will thereby contribute to the development of cognitive brain functions.

Effects of matrix stiffness on cell function: reciprocal responses of neurons and astrocytes

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Cortical neurons and astrocytes respond strongly to changes in matrix rigidity when cultured on flexible substrates. On soft gels, astrocytes do not spread, remain unactivated, and have disorganized F-actin and intermediate filament systems compared to the cytoskeletons of astrocytes on hard surfaces. Neurons, however, extend long neurites and polymerize actin filaments on both soft and hard gels, but extend and branch neurites more extensively as substrate stiffness is reduced. Compared to tissue culture plastic or stiff gel substrates coated with laminin, on which astrocytes overgrow neurons in mixed cultures, laminin-coated soft gels encourage attachment and growth of neurons while suppressing astrocyte growth. The stiffness of materials required for optimal neuronal growth, characterized by an elastic modulus of several hundred Pa is in the range measured for intact rat brain. The effects of substrate stiffness are evident when cells are grown on synthetic gels made from flexible rubberlike polymers or when they are embedded in natural biopolymer gels such as fibrin. Dissociated embryonic rat cortices grown on flexible fibrin gels, a biomaterial with potential use as an implant material, display a similar mechano-dependent difference in cell population. These data emphasize the potential importance of material substrate stiffness as a design feature in the next generation of biomaterials intended to promote neuronal regeneration across a lesion in the CNS while simultaneously minimizing the ingrowth of astrocytes into the lesion area.

The growth cone as active mechanosensor

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Nervous tissue consists of several different types of cells, blood vessels, and extracellular matrix. All these building blocks differ in their mechanical properties. Particularly during growth and migration, the local mechanical environment of neurons may thus change dramatically. The softness of radial glial cells, along which neurons preferentially grow, and the neuronal preference for soft substrates strongly point towards a role of mechanics in neuronal guidance. Here we show how neurons detect and avoid stiff substrates and how their mechano-responsiveness is used to guide their axons along distinct pathways.

In vitro, neurons continuously probe the mechanical properties of their environment. Growth cones visibly deform substrates with a compliance commensurate with their own. To understand the growth cones' sensing of stiff substrates, we investigated their precise temporal response to well-defined mechanical stress. Externally applied mechanical stress exceeding the threshold of ~300 pN/mm^2 caused a calcium influx through mechanosensitive, stretch-activated ion channels in the growth cone membrane that triggered neurite retraction. Subsequently, neuronal processes re-extended, thereby enabling exploration of alternative directions.

To study the physiological consequences of this mechano-responsiveness, *Xenopus* eye primordia were cultured on polyacrylamide gels of various compliances. The morphology of the outgrowing retinal ganglion cell axons dramatically depended on the mechanical properties of their substrate. If the axons grew either on soft or on stiff surfaces, they spread over a wide area to explore different directions. In contrast, if they grew on substrates of intermediate compliance, they fasciculated and grew into one common direction, resembling an optic nerve. The concerted growth along pioneering axons depended not only on the substrate's compliance but also on that of the axons themselves. Hence, neurons may actively use mechanics as previously unknown guidance cue during growth and migration. This knowledge may ultimately help in finding new implants that promote axonal regeneration in the injured nervous system.

Tension stimulates axonal elongation by 'stretch and intercalation'

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Although tension is known to be an important regulator and stimulator of axonal growth, the axonal response to tension is poorly described. Further, most work on axonal elongation is focused on the growth cone and the responses in the axon are poorly understood. We report an investigation of elongation and growth along the axon itself in two regimes of axonal growth: via growth cone advance and by experimentally applied tension. We find that elongation along the length of the axon initially occurs by viscoelastic stretching (creep) of the axon as seen by the movement of branch points; movements of 'docked' mitochondria in the axoplasm; and movement of marker beads along the axolemma. This is initially accompanied by thinning caliber of the axonal shaft and reduced density of mitochondria along the axon shaft. Over a time scale of hours, stretching is compensated by intercalated addition of mass as seen by a recovery of mitochondrial linear density along the shaft and recovery of axonal caliber. Our results indicate that the axon is capable of elongating throughout its length. This 'stretch and intercalation' model for growth events within the axon shaft were entirely similar for growth cone-mediated and 'towed' elongation. These results provide additional support for the hypothesis that tension is the principal stimulus for elongation of the axon per se and that for the trailing axon, the growth cone is primarily a 'tractor,' exerting tension to guide and elongate it.

The forces of neuronal growth

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The correct development of the central nervous system requires accurate and reliable neuronal network formation, a process accomplished by a highly dynamic structure at the tip of a growing neurite, called the growth cone. To find its proper target, each growth cone integrates chemical and mechanical signals, and converts these signals into changes of its cytoskeleton. Here, the interaction between the actin cortex and the membrane plays a key role. We investigated the dynamics and the forces of growth cone motility by quantifying leading edge movement, actin polymerization and retrograde actin flow. Subsequently, theory of viscoelastic materials was used to determine the magnitude and the spatial distribution of the internal forces that drive the retrograde flow. The calculation of these forces requires the viscoelastic material properties of the growth cone which we measured by rheology using an atomic force microscope. Furthermore, the substrate forces were determined by a deformable substrate essay, which shows that the substrate forces exerted by a growing neurite on its environment are consistent with the internal forces. The combination of the traction forces with the internal forces allows displaying the full forces that act within the growth cone, and quantifies the growth cone's steady pulling force on the neurite stump. Our analysis of neuronal growth mechanics exhibits surprisingly special properties of growth cones, suggesting that mechanics plays a fundamental role in neuronal navigation.

Tension-based morphogenesis in the nervous system: where it stands and what it means

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The nervous system is remarkable for the complexity of its shape and for the fact that some aspects of shape vary dramatically across and even within species whereas other characteristics are notably stable. What factors and forces account for these fundamental aspects of neural structure? In 1997, I proposed that mechanical tension along axons and dendrites is a major driving force underlying morphogenesis throughout the nervous system (Van Essen, Nature, 1997). For example, the sheet-like nature of structures such as the cerebral and cerebellar cortex and the retina can be explained by tension along radially oriented processes of dendrites and glial cells. The convoluted nature of cerebral cortex in many species can be explained by tension along long-distance cortico-cortical pathways.

In the ensuing decade, several indirect lines of evidence strengthen the case for tension-based morphogenesis, but it remains an attractive hypothesis rather than a proven theory. This presentation will review key evidence pertaining to this hypothesis as well as prospects for more critical experimental tests. I will also illustrate how mechanical tension may contribute to the variability of cortical folding patterns in normal individuals as well as folding abnormalities that have been identified in a number of disease conditions. Finally, the functional significance of tension-based morphogenesis as a basis for compact wiring of the nervous system will be discussed.

Viscoelastic properties of brain tissue: application to an in-vivo Alzheimer mouse model

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Introduction: Alzheimer's disease (AD) is characterized by progressive cognitive deterioration with declining activities of daily living and neuropsychiatric symptoms. It is the most common cause of dementia. It is recognized that the production and maintenance of myelin is essential for normal brain function. Age–related breakdown of myelin negatively impacts cognitive performance with the neurofibrilary tangles and amyloid plaques being the hallmarks of the disease [1]. This study aims to validate the hypothesis that AD alters the mechanical properties of the axons in the corpus callosum (CC).

Materials & Methods: As a unique tool to study those properties non–invasively, we use 3D MR–elastography (MRE) operating at 1000Hz mechanical excitation frequency with a high–field MRI system (Bruker Pharmascan 7T). Transgenic mice expressing mutant human PS1–Leu235Pro and APPswe were established at the NIH, NC3. Seven 19 to 22–month old female (25–40g) APP/PS1 (n=4) and wild–type (WT) mice (n=3) were imaged several times. The elastography data acquisition was performed for 15 axial slices in the region of the corpus callosum with an isotropic resolution of 300µm. Total acquisition time was around 2 hours. Post–processing of the mechanical wave was done either using an isotropic or a transverse isotropic mechanical model. This latter anisotropic model assumes that the viscoelastic properties of the material can be described by two complex shear moduli (with the real-part typically associated with elasticity and the imaginary-part with viscosity), one parallel to the local fibre direction (µ||, h||) and one perpendicular to it (µ^, h^) with 2 additional Euler angles (q,j) determining the local orientation of the fiber [2].

Results: In a first approach, the isotropic complex shear modulus was used. The CC appears stiffer than the rest of the brain. The viscosity also showed greater values in this region (Fig.1a-c). However, using those two parameters averaged over the CC, no significant distinction could be made between the AD group and the WT group (Fig.1d). In a second approach, the transverse isotropic model was applied to the same datasets. As expected, µ|| appears much greater than µ^. This means that the wave speed along the axon is higher than in the transverse direction. Inversely, h|| appears much lower than h^. Using only the two elasticities averaged over the regions of interest previously used with the isotropic model, a clear improvement was visible in the separation of the 2 groups (Fig.1e). Additionally, a decrease of the perpendicular viscosity was noticed in the CC of the AD mice.

Discussion: Those promising preliminary results show that Alzheimer's disease influences the mechanical properties of the white matter in the region of the Corpus Callosum. The exact origin of the measured variations is currently under investigation via immunohistology. Most likely, two phenomena occur sequentially: demyelination and neuron death. Both of them should contribute to decrease the anisotropy of the Corpus Callosum. This study also shows the need of an anisotropic viscoelastic model to properly reconstruct the complex shear modulus from the displacement data. The diagnostic information is lost when utilizing only the isotropic modulus. Longitudinal studies are currently prepared to follow the temporal evolution of the mechanical properties for each mouse individually. In a next step we will study the effect of demyelination/remyelination on mechanical properties of corpus callosum using the well-established cuprizone-challenged mice [3]. MRI scanning protocol will combine T2-weighted images, diffusion tensor imaging and elastography. Correlations between different MRI scans, clinical global motor score and histopathological data will be conducted.

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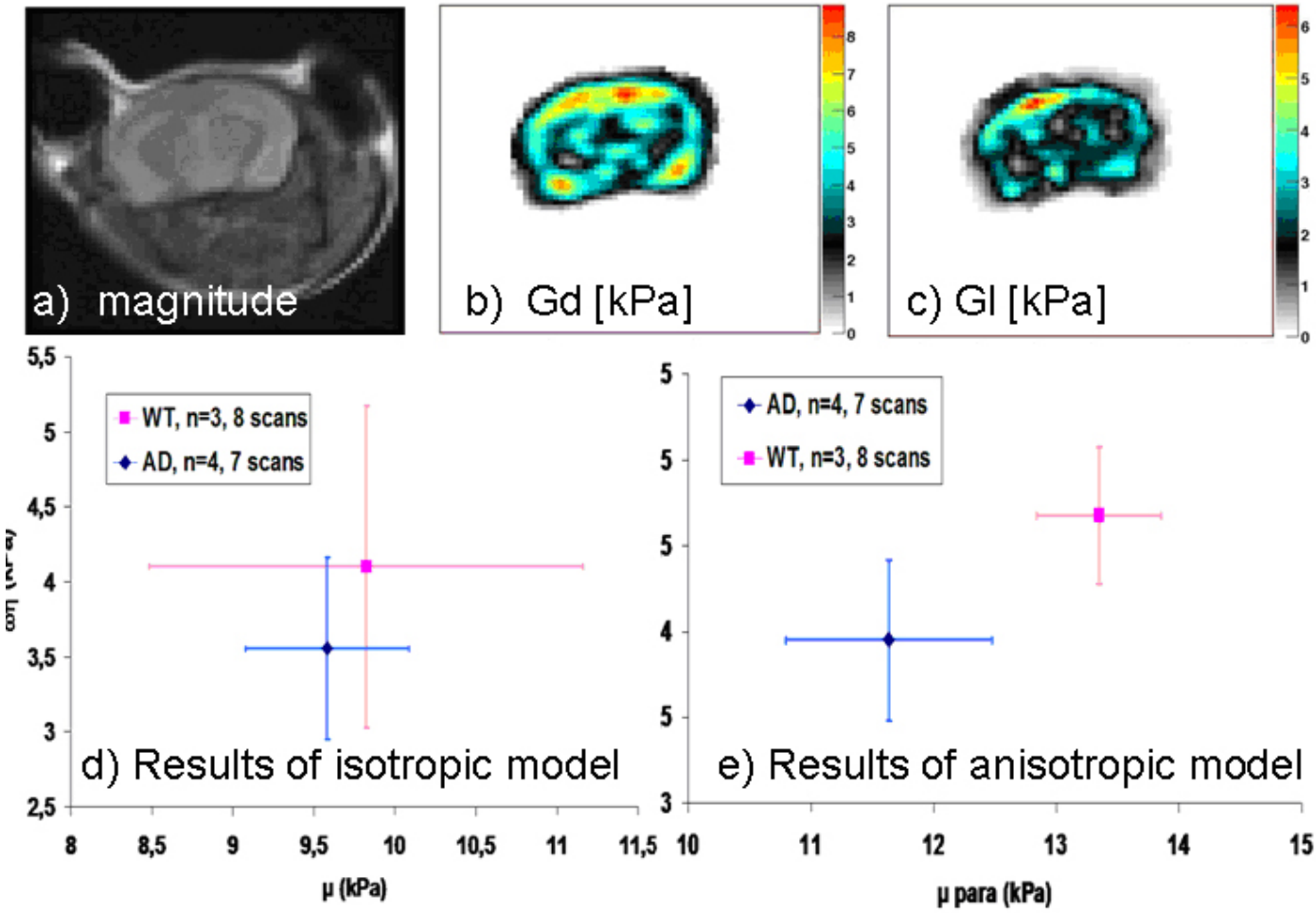

Fig.1: a) Axial oriented MR-magnitude image of mouse brain showing the region of the CC. The corresponding images of the isotropic elasticity (b) and isotropic viscosity (c) demonstrate strongly elevated values. The correlation between isotropic elasticity and viscosity does not allow the separation between the two groups (d). However, when utilizing the anisotropic mechanical model (μ|| versus μ□□, both groups differ significantly (e).

Relationships between structural and computational properties of cortical microcircuits

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Abstract: I will present a theoretical framework for analyzing the computational power of cortical microcircuit models. Within this framework one can then test whether more detailed models for cortical microcircuits (that include laminar structure, small-world property of the connection matrix, different types of dynamic synapses, different types of neurons) have more computational power than randomly connected networks with the same number of neurons and synapses, and I will report results of extensive computer tests.

Organizing Learning in Neural Circuits: the Retroaxonal Hypothesis

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Classically, neurons communicate by anterograde conduction of action potentials. However, information can also pass backward along axons, a process that is well characterized during the development of the nervous system. Recent experiments have shown that changes to a neuron's output synapses may "backpropagate" along the axon to cause changes in the same neurons inputs. Here we suggest a computational role for such "retroaxonal" signals in adult learning. We hypothesize that strengthening of a neuron's output synapses stabilizes recent changes in the same neuron's inputs. During learning, the input synapses of many neurons undergo transient changes, resulting in altered spiking activity. If this in turn promotes strengthening of output synapses, the recent synaptic changes will be stabilized; otherwise they will decay. A representation of sensory stimuli therefore evolves that is tailored to the demands of behavioral tasks. We describe experimental evidence in support of this hypothesis, and outline a candidate molecular mechanism involving the activation of CREB by retrograde neurotrophin signals.

Modifications in Motor Cortical Dynamics induced by intensive Training: the Complementarity of Spike Synchrony and Firing Rate

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The brain uses sensory, temporal and contextual information to successfully organize a goal-directed movement. All these pieces of information have to be processed, selected and assembled to appropriately organize the forthcoming movement. When providing prior information about parameters, such as movement direction, or the moment when to move, movement initiation is faster and cortical neurons selectively modulate their activity in relation to information about not only spatial parameters [1], but also about temporal parameters [2,3]. It is commonly accepted that perceptually and behaviorally relevant events are reflected in changes in firing rate in widely distributed populations of neurons. Another concept, the temporal coding hypothesis, suggests that not only changes in firing rate but also precise spike timing constitutes an important part of the representational substrate for perception and action, such as spike synchronization or other precise spatio-temporal patterns of spike occurrences among neurons organized in functional groups, commonly called cell assemblies. We have shown that the strength of precise spike synchrony among pairs of motor cortical neurons modulates in time, independent of the firing rate modulation of the participating neurons [4]. Furthermore, the timing of the modulation of both synchrony and firing rate at the level of neuronal populations suggests that synchronous neuronal activity may be preferentially involved in early preparatory and cognitive processes, including signal expectancy [4], whereas the modulation in firing rate may rather controls movement initiation and execution [5].
Here we asked the question if intensive training of the animal induces long-term modifications in the temporal structure of both synchrony and firing rate at the population level. We trained three monkeys in a delayed choice-reaction time task in which the selection of movement direction depends on correct time estimation (see [2]). The activities of simultaneously recorded single neurons in motor cortex were analyzed by using the *Unitary Event* technique [6].
Our data show (i) that the timing of the task is represented in the temporal structure of significant spike synchronization at the population level and (ii) that this temporal dynamics of synchrony is shaped during training. It becomes more structured, that is the emergence of significant synchrony becomes more localized in time during late experimental sessions than during the early ones in parallel with the improvement of the behavioral performance. (iii) Both synchrony and firing rate modulate systematically in time, albeit with a different time course. Whereas the time course of synchrony modulation was about the same in all three monkeys, the time course of firing rate modulation was different. The fact that in our data synchrony both increased and became more structured with training, whereas firing rate concurrently decreased in the same neurons, suggests that synchrony and firing rate represent different coding dimensions.

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Neural codes preceding, during and following object perception

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What does it take to consciously perceive an object? We recorded single and multi-unit activity in awake monkey primary visual cortex while the animals were engaged in a task to 'consciously' detect figures from background. Successful detection of the figure depends on: 1. the strength and synchrony of V1 neural activity preceding the onset of the visual stimulus by about 100ms, 2. whether re-entrant signals reach V1 at about 100ms after stimulus onset. Furthermore, the speed of detection depends critically on re-entrant V1 activity shortly preceding the saccade that is used to signal detection. We conclude that perceiving an object does not simply depend on a feedforward cascade of sensory to motor processing, but involves several re-entrant processing loops.

The role of oscillatory gamma activity: from working memory to consolidation

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While it has been proposed that feature binding is achieved by neuronal gamma band synchronization (30-100 Hz), the role of the gamma synchronization might extend beyond object representations. We employed a working memory paradigm in which subjects had to retain faces presented at various orientations. During the retention of faces orientations, we observed sustained gamma activity in the occipital cortex. This suggests that occipital gamma activity is important for maintenance of visual working memory. In a subsequent memory study we investigated gamma activity reflecting encoding and recall of pictures of landscapes and buildings. We found that occipital gamma activity predicted successful memory encoding. Finally, in a study on memory stabilization (consolidation) subjects learned to associate faces to specific locations on a screen. We later tested recall of the locations when only a face was presented. Stronger occipital gamma activity was observed for recall of recently but not remotely learned associations. In sum, occipital gamma activity can be induced not only by visual stimuli, but also by the recall or maintenance of memory representations. The gamma activity during long-term memory recall is possibly a consequence of a reconstructive process re-evoking the initial memory representation. Future work is required in order to elucidate the top-down mechanism responsible for evoking the occipital representations.

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Neuropeptide signalling in sea urchins

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Peptides are the largest class of signal molecules found in animals. Neuropeptides act as neurotransmitters, neurohormones or neuromodulaters and are involved in the regulation of many, if not all, of the physiological processes in Metazoa. At present more than 20000 peptides originating from animals from all classes are known (Liu et al, 2008). In contrast, our knowledge of neuropeptides from the phylum echinodermata, which includes species such as the starfish, sea cucumber and sea urchin, is very limited. Nevertheless, the neurobiology of echinodermates is of interest due to the close relationship with vertebrates.

In this study, we identified 88 peptides from 25 precursor proteins (86 peptides and 24 precursors of which are novel) from the radial nerves of the purple sea urchin using a combined bioinformatic and mass spectrometric approach. This project represents the first large scale confirmation of neuropeptides from the phylum echinodermata. Many of the identified peptides and proteins show little or no homology to known proteins. Additionally, four of the precursors were not originally annotated as gene products and were only discovered through searches of the raw genome with partial de novo sequences from tandem mass spectra. This study illustrates that automated bioinformatic methods for the discovery of neuropeptides and hormones are complicated by the lack of genomic, peptidomic and functional information from closely related species. However, combining bioinformatic and experimental discovery tools such as mass spectrometry (MS) can aid in the discovery and annotation of such genes.

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Neuropeptide discovery, processing and function in *Aplysia*

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Gastropod mollusks present exceptional opportunities to study fundamental mechanisms of neuronal system function and their role in behavior formation. The sea slug, *Aplysia californica*, has taken a leading role in this research. The *Aplysia* nervous system contains about 10,000 large neurons, many of which have unique morphological, biochemical and electrophysiological parameters that allow them to be identified animal to animal. These characteristics help to understand the cellular bases of a variety of animal behaviors including feeding, defense, and mating, as well as to elucidate fundamental mechanisms of learning and memory. The majority of *Aplysia californica* neurons are peptidergic; there are a surprisingly large variety of peptides synthesized, transported and released upon different stimulation paradigms. Here, we combine several approaches to identify signalling peptides involved in interneuronal communication.

First, using a combination of genomic and bioinformatics approaches we have identified more than 100 putative prohormones and mapped their expression in the CNS. Second, we have employed mass spectrometry and have characterized more than eight hundred peptides as products of these prohormones, many of which now have bioactivity data indicating they are indeed intercellular signaling peptides. Specifically, we integrated a number of analytical approaches including multidimensional chromatography followed by tandem MS, single cell mass spectrometry and mass spectrometric imaging. The detection of hundreds of peptides in biological samples provides a daunting challenge; answering which of the observed peptides has to be chosen for further tests to determine if they are indeed intercellular signaling molecules. We have created two integrative and interconnected approaches to aid this discovery process. The first approach adds functional information using mass spectrometry by determining peptide localization in individual identified neurons, peptide transport to terminals and constitutive or stimulated release from cells. Mass spectrometry also allows us to find posttranslational modifications such as amidation, pyroglutamation, and acetylation that can be hallmarks of bioactivity. The second approach employs in silico prediction of prohormone processing and determination of putative cleavage sites using a Web-based tool Neuropred (http://neuroproteomics.scs.uiuc.edu/neuropred.html), which produces a 95% success rate in recognition of *Aplysia* prohormone cleavage sites. The successful application of these approaches and methods has allowed the *Aplysia californic*a secretome to become one of the most completely characterized intercellular peptide signaling sets throughout Metazoa.

Evolution of peptidergic systems in insects as revealed by single cell mass spectrometry

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MALDI-TOF mass spectrometry is the method of choice to study peptidergic intercellular communication capabilities of neurons, may offer unparalleled information about co-localized neuropeptides, and therefore complements or verifies immunocytochemical findings. Here we present an overview of the methods that are used for cell identification, dissection, and subsequent mass spectrometric analysis of peptidergic neurons in insects. Such approaches were successfully used for the analysis of cockroach neurons, moth neurons, and *Drosophila* neurons with a size of 10 µm and revealed novel insights about prohormone processing. The detailed analysis of the peptidome of homologous neurosecretory neurons in distantly related insects provides insights into the conservation of neurosecretory systems in insects. Location, projection, sequences, expression of neuropeptide genes and processing of respective neuropeptide precursors each underwent a specific rate of evolution, which is discussed. Particular sequence modifications of bioactive neuropeptides, that co-evolve with their receptors, can be used for the analysis of phylogenetic relationships. Mass spectrometric methods allow for a fast screening of neuropeptides from a large number of species and, as a test case, we present a peptidomic approach to study phylogeny.

Processing of peptides in mouse brain and the evolution of neuropeptide processing enzymes

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Neuropeptides are produced from larger precursors by the selective action of a variety of enzymes, including endoproteases, carboxypeptidases, and in many cases additional enzymes required for the formation of C-terminal amide groups or other post-translational modifications. Over the past three decades, many of the peptide processing enzymes have been identified in mammals and other organisms. After reviewing the field and discussing the similarities and differences in peptide processing enzymes found in various organisms, recent work using quantitative peptidomics to study mouse brain peptide processing will be presented. Whereas peptidomics is simply the identification of the peptides present in a tissue or organism, quantitative peptidomics includes analysis of relative levels of peptides. For this, post-extraction labeling with isotopically distinct but otherwise identical reagents provides for accurate quantification of relative peptide levels. By comparing peptide levels in brain regions of mice lacking a specific peptide processing enzyme with wild type mice, it is possible to determine the functional role of this processing enzyme. Results will be presented from recent studies examining mice lacking the active form of a peptide processing carboxypeptidase (CPE) as well as studies on mice lacking prohormone convertase 2 activity. Finally, discussion will include the identification of novel mouse peptides and consideration of their evolution based on bioinformatics analysis of diverse species. The overall focus on all of these related topics will be the functional implications of the results.

Evolution of G-protein coupled neuropeptide receptors in insects

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G protein-coupled receptors (GPCRs) for neuropeptides, protein hormones and biogenic amines play a central role in the physiology of insects, because they coordinate vital processes such as development, growth, feeding, homeostasis and reproduction. During the last few years, the genomes from about two dozen insect species have been sequenced. These genomic data can be used to establish a complete inventory of neuropeptide GPCRs in these insects and, by a comparative genomics approach, to analyze the evolution of these proteins. Initially, we and other groups focused on orphan GPCRs from the fruit fly *Drosophila melanogaster* that were cloned and functionally expressed in mammalian cell lines. Thereby, many of these GPCRs could be matched with their neuropeptide ligands. When we extended our research to other insects with sequenced genomes such as mosquitoes, the honey bee *Apis mellifera*, the red flour beetle *Tribolium castaneum* or the parasitic wasp *Nasonia vitripennis*, we discovered some novel receptor/neuropeptide couples that are absent in *Drosophila*. On the other hand, some neuropeptide receptors previously identified in the fruit fly are not present in other insects. This shows that while most of these receptor/neuropeptide systems are probably essential and therefore well conserved, some can easily duplicate or even disappear during insect evolution. These more variable endocrine systems might be important for these animals to adapt to different life styles or special ecological niches.

Signalling and trafficking pathways involved in autophagosome formation

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Autophagy is a highly conserved lysosomal degradation process which is crucial for cell health and cell survival. In mammalian cells, autophagy is initiated by many different events, including developmental cues, neurodegeneration, infection and immune responses, and stresses, either from extracellular (starvation) and intracellular (unfolded protein response) sources. Autophagosomes form from a double-membrane vesicle, called an isolation membrane or phagophore. This double membrane expands and encloses cytosolic components, including organelles. The sequestered content is then targeted for degradation mediated by lysosomal proteases the nascent autophagosome acquired via fusion with endosome and lysosomes.

Our interests lie in understanding the source of the isolation membrane, and how it expands to sequester cysosolic components using a molecular cell biological approach and cell models undergoing starvation-induced autophagy. We focus our experiments on understanding the role of several key mammalian orthologues of the yeast Atg (Autophagy related) proteins involved in the early stages of autophagosome formation including ULK1, a serine threonine kinase, mAtg9, a multi-spanning membrane protein, WIPI-1 and 2, Ptd Ins 3P binding proteins, and Beclin1, a component of the class III Vps 34 kinase complex. We are studying the role of these proteins in the formation of the autophagosome, in particular focusing on identify new effectors, to gain a further understanding of the early signalling and trafficking events leading to a productive autophagy response.

Autophagy Dysfunction and Neurodegeneration in Alzheimer's Disease and other Late-Age Onset Neurodegenerative Diseases

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Primary lysosomal dysfunction in congenital "lysosomal storage" disorders is well known to cause severe neurodegenerative phenotypes associated with accumulations of lysosomes and autophagic vacuoles. Recently, the number of recognized inherited adult-onset neurodegenerative diseases that are caused by proteins that regulate protein sorting and degradation within the endocytic and autophagic pathways has grown considerably. I will briefly discuss examples of neurodegenerative diseases across the lifespan characterized by prominent autophagic -lysosomal dysfunction, which may share mechanisms of neurodegeneration related to degradative failure and lysosomal destabilization. I will highlight Alzheimer's disease (AD) as a disease within this group and discuss how the genes and other risk factors promoting this disease contribute to progressive lysosomal system dysfunction and neuronal cell death. Our recent studies indicate that neuronal macroautophagy is constitutively active and that clearance of autophagic substrates is exceptionally efficient in healthy neurons. Impaired autophagosome clearance rather than autophagy induction most likely accounts for the extensive autophagic-lysosomal pathology observed in sporadic AD. We have also observed that apoptosis is a form of neuronal cell death in PS/APP mice modeling AD-like neurodegeneration. Pyknotic neurons in adult PS/APP mice exhibited aging-dependent apoptotic changes, including DNA fragmentation, caspase-3 activation, and caspase-cleaved alpha-spectrin generation and ultrastructural changes, identical to those of developmental neuronal apoptosis in wild-type mice. In affected neurons, activated caspase-3 and caspase-3-cleaved spectrin are abundant in autophagic vacuoles, accumulating in dystrophic neurites of PS/APP mice similar to AD brains. Administration of the cysteine protease inhibitor, leupeptin, promotes accumulation of autophagic vacuoles containing activated caspase-3 in axons of PS/APP mice and, to a lesser extent, in those of wild-type mice, implying that this pro-apoptotic factor is degraded by autophagy. Leupeptin-induced autophagic impairment increases the number of apoptotic neurons in PS/APP mice. These findings suggest cross talk between autophagy and apoptosis, which influences neuronal survival in AD-related neurodegeneration. This research was supported by the National Institute on Aging.

The role of autophagy in protein metabolism: starvation adaptation, egg-to-embryo transition and intracellular clearance

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Autophagy is the primary means for the degradation of cytoplasmic constituents in the lysosome. When autophagy is induced, a portion of cytoplasm is sequestered by autophagosomes, and then delivered to lysosomes to be degraded. To monitor autophagy *in vivo*, we have generated a transgenic mouse model in which autophagosomes are labeled with GFP-LC3, a mammalian homolog of yeast Atg8. Using this autophagy-indicator mouse model, we have observed that autophagy is induced in various tissues following food withdrawal and during the early neonatal periods. Autophagy-defective Atg5-/- (KO) mice exhibit severe nutrient insufficiency soon after birth, suggesting that autophagic degradation of self-proteins is critically important to tide over starvation.

We also discovered that Autophagy is essential for preimplantation development of mouse embryos. It is known that maternal proteins are rapidly degraded in fertilized eggs and new proteins encoded by the zygotic genome are synthesized. We found that the level of autophagy was low in unfertilized oocytes; however, autophagy was upregulated shortly after fertilization. Analysis of oocyte-specific Atg5 knockout mice revealed that autophagy-defective embryos failed to develop beyond the 4- and 8-cell stages. Protein synthesis rate reduced in these autophagy-deficient embryos, suggesting that degradation of maternal proteins by autophagy is critical to produce necessary amino acids during preimplantation development in mammals.

Although such dramatic induction is the characteristic feature of autophagy, autophagy occurs constitutively at low levels under normal conditions. Histological examination of Atg5KO newborns revealed that ubiquitin-positive aggregates are accumulated in hepatocytes and neurons. To further understand the role of the basal autophagy, we generated neural cell-specific Atg5 KO mice. Although those mice survived the neonatal starvation period, they developed progressive deficits in motor function and neurodegeneration. Ubiquitin-positive aggregates massively accumulated in large neurons of various neural tissues. These results suggest that induced autophagy is important for amino acid production, whereas basal autophagy is important for intracellular quality control under normal conditions.

Lysosomes, autophagy and CNS disorders

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The lysosome is the cell´s main digestive compartment into which many types of macromolecules are delivered for degradation. Lysosomes can be involved in various cellular processes such as cholesterol homeostasis, autophagy, repair of the plasma membrane, bone remodelling, defence against pathogens, cell death and signaling. More than 50 acid hydrolases have been identified which are involved in the ordered degradation of a variety of proteins, lipids, carbohydrates and nucleic acids. Functional deficiencies of several lysosomal proteins give rise to lysosomal storage disorders which in many cases involve pathologies of the CNS.

The presentation aims to give an overview about the central role of this compartment in mediating autophagic degradation pathways. I will also try to highlight pathologies of the CNS which are caused by an impaired function of the lysosomal system.

Transgenic and virus-based mouse models for Alzheimer's disease: new views on an old problem ...

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Accumulation of amyloid peptides is the earliest molecular defect in Alzheimer's disease (AD), due to increased production or diminished clearance. Integral to the pathology of AD - and its definition - is protein tau pathology, due to over-phosphorylation, conformational changes and aggregation. The hypothesis that amyloid pathology precedes and induces tau pathology in AD is experimentally supported in our transgenic models, further demonstrating that GSK3 is an essential kinase in the underlying mechanisms.

We generated two bigenic mouse models: (i) APP.V717I x Tau.P301L mice (biAT) with combined amyloid and tau-pathology, and (ii) GSK3ß x Tau.P301L mice (biGT) with dramatic tauopathy. Comparative analysis, including the parental APPV717I and Tau-P301L strains, yields important data on pivotal roles of GSK3 in AD pathology. BiAT mice present with progressive, synergistic amyloid and tau pathology in CA1/2 and cortex, similar in most aspects to pathology in AD patients. In biGT mice, the neuronal co-expression of Tau.P301L and GSK3ß yields a dramatic forebrain tauopathy in older biGT mice, with tangles in most forebrain neurons. Remarkably, no major neuron-loss is observed, demonstrating that tangles are not neurotoxic per se! Moreover, in contrast to the parental tau-P301L mice that die before age 1 year, biAT mice and biGT mice survive longer, resp. to 15-18 months and 18-24 months, in close correlation with their reduced brainstem tauopathy (Terwel et al, Am J Pathol, 2008).

The close parallel between biAT and biGT mice comprises (i) similar pathological phospho-epitopes of protein tau, (ii) similar aggravation of tauopathy, with neurofibrillary tangles in hippocampus and cortex; (iii) similar defects in behavior and cognition, (iv) similar prolonged survival correlated with reduced brainstem pathology. Last but not least, both GSK3a/ß isozymes are activated in the parental APP.V717I amyloid mice already at young age (4-8 months) when they exhibit already defects in cognition and LTP, but only intracellular amyloid.

We developed also novel in vivo paradigms for AD pathology based on adeno-associated virus (AAV) mediated expression of APP and/or protein Tau, delivered into mouse brain by stereotactic injection in hippocampus. Robust expression of human APP and human Tau is evident in hippocampus and cortex as early as 1.5 weeks post-injection of the respective vectors. AAV-APP vectors gave rise to accumulation of amyloid peptides intracellularly and of diffuse Aß deposits, without neurodegeneration. In contrast, AAV-Tau vectors resulted in dramatic neurodegeneration in CA2, progressing to CA1, within 3 weeks p.i. Adjacent neurons contained hyper-phosphorylation tau, but "tangled" neurons were rare. Ultrastructural analysis revealed them to undergo a necrotic type of cell-death.

The combined data corroborate the hypothesis that not amyloid but tauopathy is neurotoxic, however not due to formation of neurofibrillary tangles. Moreover, they position GSK3 as a major signaling link from amyloid to tau pathology in AD. The models offer wide pre-pathology windows to define molecular signals that act up- and downstream, or in parallel with GSK3 in activation by amyloid of molecular causes of tauopathy, and of their respective roles in the cognitive defects.

Neuronal loss, neurotrophins and cholinergic denervation in a tau transgenic model

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There are two brain lesions in the brains of patients presenting with Alzheimer's disease (AD): amyloid deposits and neurofibrillary tangles. Although the amyloid side has been widely explored, the mechanisms underlying Tau toxicity are unknown. Several potentially damaging pathways have been proposed such as Aß toxicity, altered axonal transport, neurotrophins availability… It is not yet clear to what extent these or other "environmental" stimuli, separately or in combination, contribute to the development of Tau pathology. NGF (Nerve Growth Factor) and BDNF (Brain Derived Neurotrophic Factor) and their respective receptors (TrkA and TrkB) are key actors in neuronal survival, synaptic plasticity and cognitive function. Neurotrophins pathways are likely to be altered in AD and linked to hippocampal dysfunction, cognitive decline and neuronal death.

To better understand neuronal loss related to Tau pathology, we developed a Tau transgenic model, THY-Tau22, which recapitulates the early aggregation of Tau in the hippocampal formation.

THY-Tau22 transgenic line expresses human 4-repeat tau mutated at sites G272V & P301S under a thy1.2-promoter, displaying AD-like tau pathology in the absence of any motor dysfunction. They display hyperphosphorylation of Tau protein on several AD-relevant Tau epitopes and NFT-like inclusions (Gallyas & MC1-positive), starting from 3-6 months of age in the hippocampus and accompanied by a mild astrogliosis. These mice also display deficits in hippocampal synaptic transmission and impaired behaviour characterized by an increased anxiety, delayed learning from 3 months and reduced spatial memory at 10 months. There are no signs of motor deficits or changes in motor activity over all ages investigated. This mouse model therefore displays the main features of Tau pathology and several of the pathophysiological disturbances observed during neurodegeneration.

Both AT8 and AT100 immunoreactivities were analyzed on coronal sections and basal forebrain cholinergic neurons (BFCN) were also identified in old animals. Sagittal sections were labelled with AT8 antibodies. Interestingly, the septohippocampal fibers were immunoreactive suggesting that Tau pathology occurs in BFCN innervating the hippocampus. To assess this hypothesis and the functional aspect of axonal retrograde transport of BFCN, FG, a retrograde tracer was injected in the hippocampus of both Thy-Tau22 and wild-type littermate controls. FG-labelled BFCN were then identified in medial septum and quantified. A loss of FG labelling was observed in THY-Tau22 compared control littermates suggesting an alteration of the axonal retrograde transport.

In humans, cholinergic neurons of the basal forebrain innervate different cortical regions and may be involved in memory. For instance, BFCN cholinergic inputs from medial septum (MS) to the hippocampus appear to be of critical importance in mediating mnemonic function. Conversely, hippocampal neurons produce NGF which is retrogradely transported to the basal forebrain.

Altogether, these data data suggest that NGF imbalance may occur in Tau transgenic mice as seen in AD following impairment of retrograde axonal transport.

An emerging non-mainstream therapeutic application to combat sporadic AD pathology

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Compelling evidence suggests that pyroglutamyl-modified ß-amyloid peptide species $Aß_{3(pE)-40/42}$ play a major role in Alzheimer's disease. Formation of pyroglutamic acid (pGlu) requires cyclization of an N-terminal glutamate residue rendering the modified peptide uncharged, and thus hydrophobic and degradation resistant. Therefore, $Aß_{3(pE)-42}$ exerts a high tendency of aggregation and its occurrence correlates with dementia in AD patients and neuron loss in animal models. Pyroglutamated ß-amyloid peptides have been identified in plaques of sporadic AD, down syndrome patients in the late 80ies. However, only recently it became evident, that plaques of brains of nondemented elderly mainly consists of full length $Aß_2$ while plaques of AD patients contain also high amounts of $Aß_{3(pE)-42<}$. Visualizing plaque pathology in diverse model animals using PIB also strongly correlate to the AD symptomatic severity of the particular mouse model and to $Aß_{3(pE)-42}$, but not to $Aß_{1-40/42}$,. Additionally, it has been shown *in vitro*, that $Aß_{3(pE)-42}$ aggregates about 200fold faster than $Aß_{1-40/42.}$. Moreover, the peptide unfolds also profound seeding capacity. However, molecular mechanisms leading to the $Aß_{3(pE)-42}$ species were always suspected to occur spontaneously. We discovered that Glutaminyl Cyclase (QC) is co-localized with APP and unfolds a glutamyl cyclization activity in the secretory pathway.

In vitro, in situ and *in vivo*, QC converts N3Glu-Aß to the appropriate pGlu-Aß species. Using newly generated transgenic mice we could dissect the specific toxicity of $Aß_{3(pE)-42}$ resulting in animals developing a dramatic neurodegenerative pathology already within only few weeks of age. Several studies applying QC-inhibitors have been conducted in parallel with mice of different genetic AD backgrounds.

Oral treatment of the transgenic mice and of newly generated transgenic *Drosophila* flies with QC inhibitors led, in four different studies to significant reduction of $Aß_{3(pE)-42}$ and - obviously causally related - of total the total $Aß_{1-40/42}$ and of the plaque burden in the mice. We also found in prevention studies (up to 10 month, starting at age of 4 months), reduction of neuroinflammatory markers as well as memory enhancement during fear conditioning and Morris water maze tests.

The inhibition of Glutam(in)yl Cyclase *in vivo* can be considered as a potentially causative new treatment of neurodegeneration.

In vivo imaging in neurodegenerative disease: Tracking down structural correlates of synaptic failure

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Synapse degeneration is held to be an early and critical pathophysiological event in neurodegenerative diseases; however, the mechanisms involved are not understood. We aim to analyze the kinetics of dendritic spine loss throughout disease progression in mouse models of prion diseases and Alzheimer's disease by applying long-term 2-photon *in vivo* imaging. Transgenic mice expressing yellow fluorescent protein (YFP) in neocortical pyramidal neurons were either infected with scrapie prions or crossed with transgenic animal models of AD.

We imaged dendritic tufts in the somatosensory cortex from the pre-symptomatic to the terminal phase of scrapie in the same animal over up to two months. *In vivo* 2-photon imaging revealed a linear decrease of spine density. Interestingly, only persistent spines (lifetime = 8 days) disappeared, while the density of transient spines (lifetime = 4 days) was unaffected. Prior to spine loss, dendritic varicosities emerged preferentially at sites where spines protrude from the dendrite, indicating that the location where the spine protrudes from the dendrite may be particularly vulnerable. Dendritic varicosities, which we also see in some transgenic animal models of Alzheimer's disease, may actually be the cause of spine loss. The fact that spine loss at individual dendrites can be imaged throughout the entire course of disease both in scrapie and in some transgenic animal models of AD does not support the notion that synapse loss in these conditions is a secondary consequence of nerve cell death.

Axonopathy in Alzheimer mouse models

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Deficits in axonal transport have been implicated to play a significant role in the progress of neurodegenerative diseases like Alzheimer's disease (AD). These defects can manifest as axonal swellings or spheroids, which correspond to axonal enlargements and aberrant accumulation of axonal cargoes, cytoskeletal proteins and lipids. While axonopathy and impairments in motor function represent common pathological alterations in transgenic mouse expressing mutant isoforms of the Tau protein, they have only been recently reported to play a role in Alzheimer's disease mouse models based on overexpression of the Amyloid Precursor Protein (APP). This is an interesting finding with implications for the human situation, as there is mounting evidence that motor problems occur also early in the AD process, rather than being a feature related exclusively to end-stage AD-pathology.

We analyzed different APP/PS1 transgenic mouse models and provide compelling evidence for age-dependent axonal degeneration. The most severe phenotype is present in APP/PS1KI mice, showing characteristic axonal swellings, spheroids, axonal demyelination and myelin ovoids in an age-dependent manner. Interestingly these changes are both detectable in brain and spinal cord and can be detected on the light- and electron microscopy level. By comparing APP single transgenic with hemi- and homozygous PS1 bigenic mice, a clear aggravation of the axonal phenotype was noted, which is correlated with the increase in Aß pathology. In addition, abundant accumulation of intraneuronal N-modified Aß, Thioflavin S-positive material and ubiquitin was found within the somatodendritic compartment of spinal cord motor neurons. This phenotype is corroborated by early deficits in a variety of motor tasks, including reduced rotarod performance. We conclude that the accumulation of intraneuronal Aß-amyloid peptides might induce axonal transport deficits, which eventually lead to axonal degeneration in AD.

Paradigm shift in Abeta toxicity

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The concept of the ß-amyloid cascade in Alzheimer's disease (AD) provides the basis for current therapeutic strategies in AD. However, this concept is also a matter of ongoing controversial discussions, since plaque load in AD brains, in contrast to number of tau neurofibrillary tangles does not correlate with the disease state. Extracellular plaques mainly contain ß-amyloid peptides, which are derived by two proteolytic cleavages from the larger amyloid precursor protein (APP). In addition to neuropil deposition of Aß peptides into amyloid plaques, there is increasing evidence that Aß accumulation occurs in neurons and that this represents an initial step in the disease process.

Early pathological changes, like deficits in synaptic transmission, behavioural alterations, differential glutamate responses and deficits in long-term potentiation have been reported in different APP transgenic mouse lines. In the APP/PS1KI mouse model, human mutant APP751 harbouring the Swedish and London mutations, is expressed under the control of the murine Thy-1 promoter, whereas murine PS1 with two FAD-linked mutations (PS1M233T and PS1L235P) is expressed under the control of the endogenous mouse PS1 promoter. We have previously reported that these mice harbour abundant intraneuronal Aß42 accumulation. They develop an early and robust brain and spinal cord axonal degeneration, as shown by the occurrence of axonal spheroids, together with a reduced ability to perform motor performance tasks, including balance beam, string suspension or the rotarod. Cognitive deficits, studied by the use of the Y-Maze and the T-maze continuous alternation task (T-CAT), were also evident as early as at the age of 6 months. The APP/PS1KI mice are smaller than controls and show development of a thoracolumbar kyphosis, together with an incremental loss of body weight. Between 2 and 6 months of age a significantly increased accumulation of intraneuronal Aß peptides, including N-terminal modified species like pyrGlu-Aß was detected, which correlated well with hippocampal neuron loss of 30%, synaptic dysfunction and reduced performance in working memory tasks. Interestingly, the APP/PS1KI mice exhibit a vast heterogeneity of N-truncated Aß42 peptides including N-terminally truncated and pyroglutamate modified $Aß_{3(pE)-42}$.

It is well established that only a fraction of Aß peptides in the brain of AD patients contain N-terminal aspartate ($Aß_{1D}$) which is generated by proteolytic processing of APP. N-terminally truncated and pyroglutamate modified $Aß_{3(pE)-42}$ have been previously shown by Takaomi Saido and others to be a major peptide in AD brains. Here we show that $Aß_{3(pE)-42}$ induces neurodegeneration and concomitant neurological deficits in transgenic mice. The mouse models are based on neuron specific expression of $Aß_{3E-42}$ and $Aß_{3Q-42}$, which were fused to the pre-pro-sequence of murine thyrotropin-releasing hormone. Eight weeks after birth, massive neurological impairments of the mice become apparent, which correlate with Purkinje cell degeneration. The results suggest that intraneuronal $Aß_{3(pE)-42}$ due to its high stability and aggregation propensity triggers Aß accumulation providing strong evidence that this modified Aß peptide is neurotoxic *in vivo*. In summary, these observations support further evidence for a pivotal role of intraneuronal Aß as a trigger in AD-typical neurodegeneration.

Representation in large-scale random networks of cortical neurons

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The wide range of time scales involved in neural excitability and synaptic transmission lead to ongoing change in the temporal structure of responses to recurring stimulus presentations on a trial-to-trial basis. This is probably the most severe biophysical constraint on putative primitives of stimulus representation in neuronal networks. In this presentation I analyze time and rate based representational schemes in that respect, using large-scale random networks of cortical neurons that develop *in-vitro*, on multi-electrode arrays. The capacity of different representational schemes to handle trial-to-trial variation in response patterns is examined n a range of spatial and temporal classification tasks.

State dependent I/O gain and interaction with ongoing activity in cortical networks *in vitro*

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The human brain, specifically the neocortex, receives massive sensory input, processes this information and generates output commands of astonishing precision and reliability. Although individual neurons fire reproducibly to natural stimuli, the responses of cortical networks to repeated sensory stimuli, however, vary considerably with respect to the number and distribution of the neurons involved as well as to timing and number of spikes, likely because incoming spike input interacts with ongoing activity. For example, visual responses in cat visual cortex depend on the activity at stimulus onset [1].
Our goal is to understand how neuronal networks respond to incoming stimuli, which interactions arise and how these influence their responses. We aim for predictable input/output relationships in user-defined, closed-loop interaction with neuronal networks.
As generic network models, we recorded spike activity in cortical cell cultures grown on microelectrode arrays. This enabled multi-site electrical stimulation to study the spatio-temporal processing of input patterns. Defined pharmacological modifications allow the identification of key mechanisms underlying spontaneous and induced activity. We show that stimulus/response dynamics are network-state dependent. Ongoing activity, consisting of network-wide bursting, modulates reliability, length and delay of polysynaptic responses. High bursting activity prior to stimulation resulted in short responses and large delays. Low bursting activity before stimulation led to long responses and short delays. Stimulus efficacy was modulated by 20-60 sec long periods of increased firing 3-4-fold above baseline, so called superbursts. Efficacy was maximal and responses were longest during superbursts. Responses were shortest or stimulation even failed to elicit spikes directly after superbursts.
State dependent stimulus efficacy hampers the identification of input/output relations and defined interaction with networks. Phase-coupled input, that is, stimulation during a pre-defined network-state increased response reliability and reproducibility. Gap–junction blockage with mefloquine or 2-APB, or blockage of NMDA-receptors by AP-5 suppressed superbursts and resulted in more homogenously distributed burst lengths and intervals. Pharmacologically modified network states combined with electrical stimulation allowed us to reliably predict response types by means of Echo State Networks (ESN).
Modulation of input/output gain by ongoing activity thus gives rise to state-dependent processing of external inputs. Controlled interaction with and defined modulation of ongoing activity can significantly contribute to predictable responses and an understanding of the processing and storage capabilities in neuronal networks *in vitro*.

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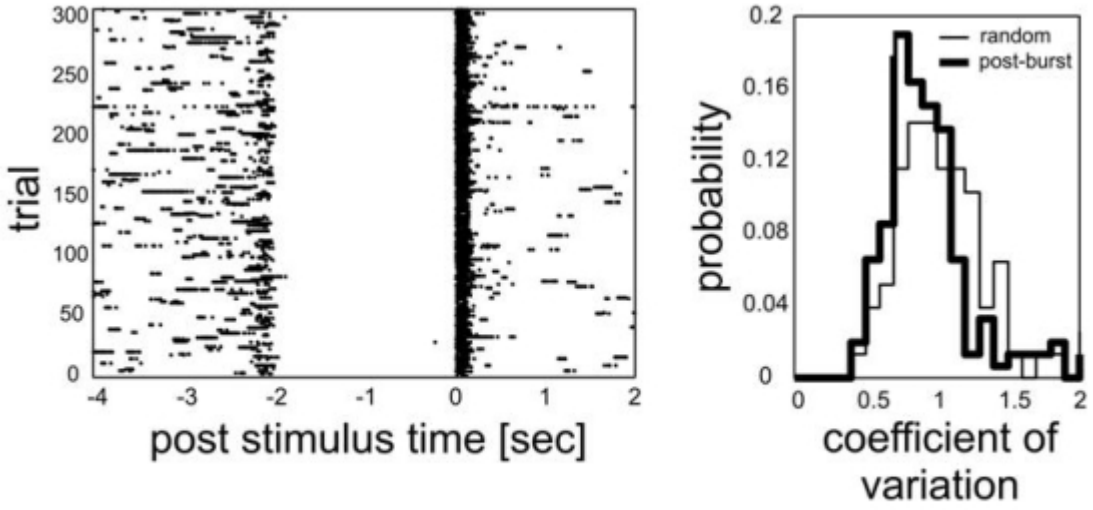

left: post-burst stimulation and responses. *right:* smaller coefficient of variation values for post-burst stimulation.

Delay Lines and the Neurophonic Potential in the Sound-Localization Circuit of Birds

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Barn owls (*Tyto alba*) are nocturnal hunters that are able to catch their prey in complete darkness by only using auditory cues. The cue used to localize the azimuthal position of a sound source is the interaural time difference (ITD). ITD is the difference of the arrival time of a sound at the two ears. A variety of specializations at different levels and a separate neural circuit, the time pathway, have evolved to achieve the high temporal resolution.
The time pathway starts in the cochlear nucleus magnocellularis (NM). The axons of NM neurons project bilaterally to nucleus laminaris (NL), making NL the first binaural stage in the time pathway. The NL neurons are narrowly tuned to sound frequency and act as coincidence detectors. Simultaneous inputs from the right and left side cause the neurons to be maximally active. Firing frequency changes periodically in dependence of an imposed phase shift between the left and right inputs. Nucleus laminaris contains both a tonotopic map and a map of ITD. The projections from the ipsi- and contralateral NM form delay lines. The ipsilateral axon collaterals contact and penetrate NL from dorsal, while the contralateral axon collaterals run on the ventral side and transverse NL from ventral to dorsal. The map of ITD results from the synapses of the axon collaterals with NL neurons at different dorso-ventral depths (barn owl) or different medio-lateral positions (chicken). In this way a time-code present in the NM collaterals is converted into a place-code in NL neurons.
The key elements and features of such a sound-localization circuit have been proposed by Jeffress in 1948. Since then a large amount of evidence has been accumulated, supporting the hypothesis that this model is realized in birds. However, the existence of delay lines in the barn owl has not yet been directly shown. To do so, we used slices of the NM-NL circuit and recorded the extracellular multi-unit activity in NL at many different positions with multielectrode arrays (MEA) while electrically stimulating the NM inputs. The simultaneous measurement of response latency at many positions in NL allows to directly demonstrate the existence of delay lines.
Latencies changed within the NL of the barn owl from medial to lateral as well as in the dorso-ventral direction. Latencies between two neighbouring electrodes (distance: 200 µm) were about 34 to 250 µs corresponding to propagation velocities between 0.8 – 5.9 m/s. Thus, our preliminary data provide the first direct demonstration of delay lines in the barn owl.
We also want to find out more about the so-called neurophonic potential which is a frequency-following potential occurring in early stages of the time pathway. The possible sources of this potential in NL are the phase-locked signals from the afferents from NM, their synapses and the postsynaptic potentials of NL neurons. Theoretical considerations suggest the NM afferents to be the most likely sources of the neurophonic potential in NL of barn owls. We want to falsify that experimentally by blocking the postsynaptic activity to separate the different sources.

CMOS-Based High-Density Microelectrode Arrays for Subcellular Resolution Recordings

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Microfabrication techniques and, in particular, microelectronics (Complementary Metal Oxide Semiconductor, CMOS) technology are very powerful tools to devise bioelectronic and multielectrode microsystems. CMOS-based, fully integrated microelectrode arrays for bidirectional communication (stimulation and recording) with electrogenic cells are presented. These complex microsystems with integrated filter and amplification stages feature a high electrode density (3150 electrodes per mm^2) as well as low noise levels (5-6 μV_{rms}) in the recorded signals and are capable of monitoring relevant electrophysiological responses of cells to electrical stimuli or to pharmacological agents with prospective applications in the fields of neuroscience or pharmascreening.

In many experiments with living tissue, it is desirable to adapt the locations of the recording sites with regard to the biological structure. The approach presented here provides a reconfigurable routing for an almost arbitrary set of electrodes to the readout channels.

Acute sagittal cerebellar slices have been used to assess the performance of the device. In these preparations, predominantly Purkinje cells are spontaneously active. The Purkinje cells are effectively disconnected from each other as the parallel fibers have been cut. The cellular electrical fields hence can be considered to be independent, which facilitates spike sorting and eases the respective waveform interpretations.

Artificial vision by multi-site electrical retina stimulation

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Introduction. Subretinal implants are designed to stimulate the retinal network in patients suffering from Retinitis Pigmentosa (RP) by converting the retinal image of visual scenes into spatiotemporal patterns of electrical stimulation. A prototypic device with an array of microelectrodes has been tested in a clinical study and visual sensations (phosphenes) were confirmed in blind volunteers (Zrenner et al., Invest Ophthalmol Vis Sci 2007;48: E-Abstract 659). We have conducted several investigations during the last decade in order to investigate the response properties of the retina to multi-site application of voltage impulses.

Methods. Retinas were explanted from the enucleated eyes from freshly hatched chicken and adult RCS rats and adhered to microelectrode arrays (MEA) with the photoreceptor side down. Stimulation was achieved by application of voltage steps and monophasic impulses (duration up to 500 µs, amplitude up to 3 V). The retinal response was measured from individual ganglion cell bodies with a glass pipette (Stett et al., Vis. Res. 40:1785–1795, 2000). In order to investigate the receptive field of ganglion cells that is sensitive to electric stimulation we used line electrodes (length 1 mm, width 10 µm, interelectrode distance10 µm) for stimulation. To identify electrically activated intraretinal signal paths we added Dopamine (1 mM) and high concentrations of Mg^{2+} (10 mM) to the bath ringer to de–couple amacrine cells and to suppress synaptic transmission.

Results. The threshold for anodal voltage steps was significantly lower than for cathodal voltage steps and lower than for anodal voltage impulses as revealed by chronaxie curves from RCS rat retina. In chicken retina, the median threshold for network activation with planar disc electrodes (diameter 10 µm) was 0.5 nC for anodal voltage impulses and 1.6 nC for cathodal impulses (Stett et al., J. Neur. Eng. 4:S7-S16, 2007). This is consistent with the results from simulations showing that anodal voltages depolarise the presynaptic terminal of bipolar cells, where as cathodal voltages hyperpolarise and inhibit the synapses. Temporal ON and OFF responses could be measured when the stimulating line electrode ran through a well–defined field surrounding the ganglion cell bodies in RCS rat retina. These receptive fields had sharp borders with a transition from no response to half–maximal response within 40 µm. The width of the corresponding spatial sensitivity profile was 190 µm (median of full width at half maximum). Administration of Dopamine led to a significant decrease of the diameter of the receptive field. This gives evidence that in the partly degenerated neuronal network of theRCS rat retina capabilities for lateral signal processing ispresent and can be activated electrically with an accuracyof less than 0,2° angle of vision.

Conclusion. Local stimulation excited spatial confined patches in intact and degenerated retinas. This is consistent with the findings in the clinical study where patients reported discrete and separated phosphenes evoked by multi-electrode stimulation. We conclude that subretinal electrical multi-electrode stimulation can providea useful range of localized phosphene perceptions in blindpatients.

Selective erasure of a fear memory

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Memories are thought to be encoded by sparsely distributed groups of neurons. However, identifying the precise neurons supporting a given memory (the memory trace) has been a long-standing challenge. We have shown previously that lateral amygdala (LA) neurons with increased CREB are preferentially activated by fear memory expression, suggesting they are selectively recruited into the memory trace. Here we used an inducible diphtheria-toxin strategy to specifically ablate these neurons. Selectively deleting neurons overexpressing CREB (but not a similar portion of random LA neurons) after learning blocked expression of that fear memory. The resulting memory loss was robust and persistent, suggesting that the memory was permanently erased. These results establish a causal link between a specific neuronal subpopulation and memory expression, thereby identifying neurons within the memory trace.

Cytoarchitectonics and connectivity of the intercalated cell masses of the rodent amygdala

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The intercalated cell masses of the amygdala are a heterogeneous population of scattered cell clusters interspersed among the various nuclei of the amygdala and are considered as a portion of the central division of the extended amygdala with similar connectivity and function. Functionally, paracapsular intercalated (Ip) neurones have been proposed to act as an inhibitory gate between the basolateral and the central nuclei of the amygdala under cortical control. Current views on the function of the amygdala assign a paramount role to Ip neurones in inhibiting conditioned fear and in the storage of extinction memory. In a previous work, we showed that GABAergic monosynaptically connected Ip neurones in the intermediate capsule display distinct short-term plastic synaptic properties depending on the frequency of stimulation of the presynaptic neurone. Important differences were also observed in the dendritic and axonal patterns of different cell pairs, which correlated with differences in the synaptic efficacy.

In order to more accurately define the axonal patterns of Ip neurones we have performed whole cell patch clamp recordings of synaptically-coupled and –uncoupled Ip neurones from acute slices of amygdala, in which only one cell has been filled with biocytin and analysed. We observed several categories of neurones diverging in the direction, spatial spread and density of their axonal projections, which might represent different categories of cell type. Moreover, we have analysed the number and type of synaptic contacts between Ip neurones. As expected the presynaptic GABAergic neurone gave rise to symmetric synapses, which were mostly established on small dendritic shafts. However, other specific domains of the plasma membrane of the postsynaptic neurone were also found innervated, such as the spine neck. We are currently evaluating how such diverse anatomical features correlate with the heterogeneous short-term synaptic plasticity observed amongst Ip cells.
Our findings based on the output of neurones belonging to different clusters and on their differential expression of neuronal markers, such as mu opioid receptors, GABA-A subnits and metabotropic glutamate receptors, suggest a functional specialization of distinct clusters, which should therefore be considered and classified separately. Understanding the diverse patterns of innervation, the selective postsynaptic domains targeted and the correlation with the physiological properties of the networks will help to shed light on the functional role of this important but poorly understood class of GABAergic neurones.

Function and plasticity of inhibitory circuits in the amydgala

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The amygdala is a key brain structure for acquisition and storage of fear memory traces. While there is compelling evidence that synaptic strength changes at excitatory inputs to principal neurons in the basolateral complex (BLA) following fear learning, little is known about alterations in other cell types of the amygdala network, or about the synaptic and cellular mechanisms of extinction. Surrounding the BLA are clusters of inhibitory neurons, the intercalated cells (ITCs), which are thought to control impulse traffic into and out of the BLA. Particularly, the activity of medial cluster ITCs may impact behavioral output, as they send inhibitory projections to the central amygdala, the main output station. Indeed, recent evidence suggests a key role of medial ITCs in expression of extinction. One possibility is that synaptic drive to ITC interneurons may be altered following fear learning and extinction. Here, we start addressing this by investigating basic properties and activity-driven changes of inputs to the medial cluster ITCs in an ex vivo approach. We use GAD-67-GFP knock-in mice to visualize and target medial ITCs for patch-clamp recordings in brain slices. Employing electrical stimulation of sensory inputs from thalamus (internal capsule) and cortex (external capsule), and from the BLA, we find that these inputs differ in pre- and postsynaptic properties, including synaptic transmission dynamics, and postsynaptic glutamate receptor composition (i.e. relative NMDA-R to AMPA-R content); parameters that may dictate susceptibility and expression mechanisms for plastic changes at these particular synapses. Furthermore, medial ITCs appear to regulate the subunit composition of AMPA-Rs (i.e. Ca^{2+}-permeability) in a cell-specific rather than input-specific manner. We are currently investigating if and which of these synaptic inputs undergo plastic changes. Our data suggest that acute changes (i.e. pairing induced long-term-potentiation) as well as behaviourally-driven changes following fear conditioning and extinction training can be induced at glutamatergic inputs from cortical afferents to the medial ITCs.

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Neural mechanisms underlying auditory discrimination during fear conditioning.

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In this project we are studying the neural mechanisms underlying fear generalization. We are using classical fear conditioning as a behavioral paradigm. During fear conditioning a previously neutral stimulus, such as a tone, is paired with an aversive stimulus, such as footshock, so that the tone and shock co-terminate. Animals quickly learn that the tone predicts shock delivery. After conditioning the tone elicits fear responses, such as freezing, where animals stop all movement except respiration. The amygdala, a structure in the temporal lobe, is crucial for the acquisition and storage of fear memories. Information about the tone reaches the amygdala, either directly from the auditory thalamus, or indirectly via the auditory cortex. Lesioning each pathway alone does not affect the acquisition of auditory fear conditioning, however lesioning both impairs this form of learning. How each pathway contributes to the acquisition of auditory fear conditioning remains unclear. It has been proposed that the direct thalamic route is faster (fewer synapses), but less accurate than the indirect cortical route. Thus, it might be expected that the cortical route to the amygdala prevents generalization of the fear response to sounds that were not paired with footshock. To test this possibility, we lesioned specifically the indirect route (by lesioning MGv, the only auditory thalamic nucleus that provides the major source of input to primary auditory cortex, and does not project to the amygdala). We then tested the fear response of rats to a tone that was paired with shock (tone+) and one that was explicitly unpaired (tone-). We found that control rats fear more the tone+ than the tone-, whereas lesioned rats showed equivalent levels of fear to both stimuli. Thus, as expected the indirect cortical pathway to the amygdala is necessary for intact discrimination of sounds that are predictive of threats from ones that are not. Next we examined the role of the direct thalamic pathway in the acquisition of this task. Surprisingly, we found that lesioning MGm (the thalamic nucleus that projects directly to the amygdala) also impaired discrimination. This finding is in contrasts with the hypothesis that this input pathway is not important for intact discrimination, but rather for enabling fast responses to sounds that are predictive of threats . Possible forms of interaction between the two pathways and how these contribute to normal auditory discrimination, in the context of fear conditioning, will be discussed.

Auditory cortex contribution in fear conditioning to complex sounds

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Specific learning-induced re-tuning of receptive fields of auditory cortex neurons is a well described phenomenon. However, the significance of this plasticity for memory formation of auditory stimuli in the context of fear conditioning paradigms is still under debate. The most challenging finding is that rodents can be conditioned to pure tones, even if their auditory cortex had previously been removed. In a series of experiments we tested the hypothesis that subcortical projections from the auditory thalamus to the amygdala are sufficient to sustain learning to simple pure tones, however that conditioning and memory to complex, temporally modulated tones would require auditory cortex function. We first established a fast, reliable learning paradigm to complex tones. In additional experiments we found that lesions to the auditory cortex before, as well as after learning, abolished conditioning to complex auditory stimuli. These findings strongly support the model of an essential role of the auditory cortex in memory formation of more natural, temporally modulated sounds. Furthermore, they are reminiscent to a phenomenon known as “blind sight”, where patients with cortical lesions in the visual cortex can subconsciously perceive the presence of a simple visual stimulus whereas conscious perception of more complex stimuli is abolished.

Neurotransmission in amygdaloid circuits related to fear memory and extinction

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Fear is a crucial component of behavior that is generated in anticipation of or in response to stimuli which threaten to perturb homeostasis. Fear-relevant associations can be learned and consolidated as part of long term memory. After learning, fear responses are modulated through processes termed safety learning and extinction. Perturbation of these mechanisms can lead to disproportional anxiety states, for instance reflecting an anxiety disorder. One model of fear learning and memory is classical ("Pavlovian") fear conditioning, in which exposure to an innocuous conditional stimulus, such as a sensory cue or a context, is associated with an unconditioned aversive stimulus. It has also proven a useful model for understanding the psychological and pathological basis of emotional disorders. Fear conditioning is critically dependent on the integrity of the amygdala, which is a convergence site for the sensory and affective information. In addition, the hippocampal formation processes multimodal information concerning contexts that define the time and place of aversive experiences. Finally, the prefrontal cortex guides fear memory extinction, most likely through formation of a novel memory trace that competes with the one initially formed during fear learning. Recent years have seen significant progress in the identification of the mechanisms and synaptic circuits governing synaptic plasticity and network activities in these circuits. In particular, influences of the prefrontal cortex on a defined GABAergic neuronal population in the amygdala, termed the intercalated paracapsular cells, have been found to be critically involved in the extinction of fear memory. Some principles and involved neurotransmitter systems will be presented.

Motor cortex activity during tool use in macaque monkeys

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The fast-conducting component of the primate corticospinal tract, which also gives rise to the cortico-motoneuronal (CM) system, is known to be active during skilled use of the hand and digits such as in precision grip. It has also been speculated that the CM system, which is most highly developed in dexterous primates, might also underpin tool use. To investigate this question we took advantage of the fact that that macaque monkeys can be trained to use a tool, manipulating a rake to retrieve out-of-reach food (Ishibashi et al., 2000). We trained two Rhesus macaques to perform both this task and a precision grip task.

Although the rake task is dominated by use of the proximal arm as the monkey reaches out with the rake and then pulls in the reward with it, detailed EMG analysis using implanted subcutaneous electrodes revealed that the intrinsic hand muscles are also active as the monkey manipulates the exact position and orientation of the rake. EMG from these muscles showed some features similar to that seen during precision grip, such a fractionation of activity across muscles acting upon different digits.

We used the Thomas multiple electrode system to record from pyramidal tract neurons (PTNs) in the M1 hand area during performance of both tasks (one experiment complete, one ongoing). In the first monkey, PTNs were identified antidromically from stimulating electrodes implanted in the medullary PT. Intracortical stimulation evoked low-threshold hand/digit movements at 73 of 113 sites at which a PTN was recorded. 42/113 PTNs had short antidromic latencies (< 1.0 ms), and some of these PTNs were identified as CM cells by spike-triggered averaging. The discharge of many of these 'fast' PTNs was as deeply modulated during the precision grip task as during the rake task, particularly showing bursts of activity during grasp and manipulation of the rake. Modulation of discharge during the rake task was quite different to that seen when the monkey was not using the rake, but simply reached for and retrieved the food reward.

These preliminary results show that fast-conducting PTNs active during independent finger movements, employed in the precision grip task, are also active during manipulation of a rake tool and are consistent with the CM system being recruited during tool use by primates.

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Reference:
Ishibashi H et al (2000). Can.J.Physiol Pharmacol, 78, 958-966.

Grasp movement planning and decoding in premotor and parietal cortex

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Hand manipulations play a crucial role for human and non-human primate behavior. Neurons in the anterior intraparietal area (AIP) and the ventral premotor area (F5) have been shown to encode planning signals for hand grasping movements. We investigated spiking and local field potential activity in these areas while animals performed a delayed grasping task, and we were able to decode grasp movement intentions from these signals in real time.

Macaque monkeys were trained to grasp an object (handle) that is positioned in one of 5 different orientations either with a power grip or a precision grip. Importantly in this task, the instruction of how to grasp the object (power vs. precision grip) as well as the object orientation in space was separated in time from movement preparation (planning) and movement execution. Results showed that individual neurons in AIP and in F5 represented the grip type and target orientation during the cue, planning, and execution phase of memory-guided hand movements. Activity was context-dependent of the instructed grip and synchronized between AIP and F5 in particular during the planning epoch. Furthermore, we permanently implanted a total of 80 electrodes in AIP and F5 to implement a brain-machine interface for hand grasping. Using these implanted electrodes and a modified version of the delayed grasping task, we were able to decode the grip type and the object orientation in real time and without the animal moving its hand with an accuracy of 91% correct (in 4 conditions) and 72% correct (in 6 conditions).

These results suggest that neural activity in AIP and F5 is crucially involved in the generation of hand grasping movements and that the parietal and premotor cortex are suitable for the real-time decoding of hand movements, e.g., as needed for the development of a neural prosthesis.

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Reach movement planning in the fronto-parietal sensorimotor network

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Movement planning does not only depend on the current sensory environment, but also on internal behavioral goals or imposed rules. We can flexibly associate the same sensory stimulus with different motor actions, e.g., reach towards an object or avoid it. How do rule-based decisions about a pending movement modify sensorimotor transformations to achieve goal-directed behavior, and how is this reflected in the sensorimotor areas of the fronto-parietal reach network?
We had shown previously that the parietal reach region encodes motor goal rather than visual sensory information during the planning phase of a movement (Gail and Andersen, 2006). But it is still unclear where and how the integration of an abstract task-rule with spatial sensory information initially occurs within the frontoparietal sensorimotor network. In neural network simulations we could demonstrate, that the motor-goal encoding in the parietal sensorimotor area PRR might actually be a consequence of 'top-down' projections from more motor-like structures and that flexible visuomotor remapping can be achieved via mechanisms similar to gain-modulation (Brozovic et al., 2007).
In current studies we test these two modeling predictions with simultaneous multi-channel electrophysiological recordings from the parietal reach region and dorsal premotor cortex of monkeys performing variations of anti-reach tasks. Results are consistent with the idea of fronto-to-parietal projections of motor-goal information, and suggest that flexible, context-specific visuomotor mapping may indeed be achieved via mechanisms equivalent to gain modulation.

1. Brozovic M*, Gail A*, Andersen RA (2007) Gain mechanisms for contextually guided visuomotor transformations. J Neurosci 27: 10588-10596.
2. Gail A, Andersen RA (2006) Neural Dynamics in monkey parietal reach region reflect context-specific sensorimotor transformations. J Neurosci 26: 9376-9384.

Role of prefrontal and anterior cingulate cortex in the control of saccadic responses

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The anterior cingulate cortex (ACC) and prefrontal cortex PFC) are proposed to play an important role in the cognitive control of action. Cognitive control consists of top-down control processes that enable us to act in a purposeful, goal-directed manner; a remarkable feat considering the inherent difficulty involved in suppressing an automatic prepotent response while facilitating an alternative voluntary behaviour. Clinical studies have shown that patients with lesions of the ACC or PFC are impaired on the anti-saccade task, a well-established test of cognitive control that requires subjects to look away from a briefly presented visual stimulus. This task requires both suppression of the visual grasp reflex, a reflexive orienting response (pro-saccade) toward the stimulus, and facilitation of a voluntary anti-saccade away from the stimulus. The ACC and PFC are closely interconnected and both areas project extensively to the superior colliculus (SC), a midbrain oculomotor structure critical for the generation of saccadic eye movements. It has been hypothesized that ACC and PFC provide direct top-down signals to the SC that modulate the activity levels of SC neurons on anti-saccade trials (Munoz and Everling, 2004).

To directly test this hypothesis, we inactivated the ACC and PFC in macaque monkeys and recorded single neuron activity in the SC. Custom-designed cryoloops were being used to reversibly deactivate neural tissue in the prinical sulcus and in the cingulate sulcus by passing chilled methanol through the lumen of stainless steel tubing. Termination of cooling returns cortical tissue to normal body temperature within 2-3 minutes, at which point neural activity in the cooled area has returned to normal. Therefore, normal behaviour can be observed both before *and* after inactivation.

While cooling either ACC, PFC, or both, the activity of single neurons in the SC was recorded while the animals perform a randomly-interleaved anti/pro-saccade task. Inactivation of the PFC or ACC lead to an increased activity of SC neuron and impaired the animal's ability to perform antisaccades and enhanced the performance of prosaccades. These results indicate that the PFC and ACC play a major role in suppressing the activity in the oculomotor system and thereby allow the successful performance of voluntary behaviour.

Munoz DP, **Everling S** (2004) Look away: The anti-saccade task and the voluntary control of eye movement. Nature Reviews Neuroscience 5: 218-228

Cortical mechanisms of spatial updating for saccades and reaching movements

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How the brain represents space and uses this information to generate actions is a central question in the neurosciences and an essential step toward an understanding of brain disorders. We performed fMRI and MEG neuroimaging experiments to analyze the time-varying aspects of spatiomotor processing in human subjects. The fMRI results show that various parietal and frontal areas encode the spatial goals of saccades and reaching movements in topographic maps. Using repetition suppression methods, we distinguished between retinal (eye-centered) and non-retinal (e.g., head-centered) spatial reference frames in these regions. This revealed a clear dominance of eye-centered coding in the maps within the parietofrontal network. The fMRI results also demonstrate a spatial updating process in parietal cortex by showing shifts of activity on the parietal map during changes of gaze. In close connection to these findings, MEG recordings of oscillatory activity show that this parietal updating is accompanied by dynamic changes in spatially-tuned neuronal synchronization in the gamma band. From the MEG experiments, we conclude that gamma band synchronization reflects a mechanism to encode the motor goals in the visuomotor processing for eye and hand movements.

Probabilistic models of sensorimotor learning

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The effortless ease with which humans move our arms, our eyes, even our lips when we speak masks the true complexity of the control processes involved. This is evident when we try to build machines to perform human control tasks. While computers can now beat grandmasters at chess, no computer can yet control a robot to manipulate a chess piece with the dexterity of a six-year-old child. I will review our recent work on how the humans learn to make skilled movements covering structural learning and generalization, how we learn the dynamics of tools and how we make decisions in the face of uncertainty.

Ectopic expression of microbial rhodopsins in inner retinal neurons for restoring vision after photoreceptor degeneration

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The severe loss of photoreceptor cells caused by congenital retinal degenerative diseases, such as retinitis pigmentosa (RP), could result in complete blindness. We explore the possibility of genetically converting inner retinal neurons into directly photosensitive cells – thus imparting light-sensitivity to retinas that lack rod and cone photoreceptor cells. Using delivery by adeno-associated viral vectors, we show that functional expression of microbial rhodopsins of channelrhodopsin-2 (ChR2), a light-sensitive cation channel from green algae, and halorhodpsin (HaloR), a light-driven chloride pump from halobacteria, can be achieved in rodent inner retinal neurons *in vivo*. We also show that expression of ChR2 and HaloR in surviving inner retinal neurons of a mouse with retinal degeneration can restore both ON and OFF light responses to the retina. Our results suggest that expression of microbial rhodopsin, such as ChR2 and HaloR, in surviving inner retinal neurons is a potential strategy for restoring vision after photoreceptor degeneration.

Restoring visual function in retinal degeneration by targeted optogenetic tools.

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Genetically encoded optical neuromodulators create an opportunity for circuit-specific intervention in neurological diseases. One of the diseases most amenable to this approach is retinal degeneration, where the loss of photoreceptors leads to complete blindness. To restore photosensitivity, we genetically targeted a light-activated cation channel, channelrhodopsin-2, to second-order neurons, ON bipolar cells, of degenerated retinas in vivo in the Pde6brd1 (also known as rd1) mouse model.
In the absence of 'classical' photoreceptors, we found that ON bipolar cells that were engineered to be photosensitive induced light-evoked spiking activity in ganglion cells. The rescue of light sensitivity was selective to the ON circuits that would naturally respond to increases in brightness. Despite degeneration of the outer retina, our intervention restored transient responses and center-surround organization of ganglion cells. The resulting signals were relayed to the visual cortex and were sufficient for the animals to successfully perform optomotor behavioral tasks.

Preliminary Results from the Argus II Epiretinal Prosthesis Feasibility Study

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The feasibility of the Argus II chronic epiretinal implant to partially restore vision to subjects blinded by photoreceptor degeneration is currently under investigation in eight clinical centers worldwide. In this presentation, we present 6 month safety and effectiveness data.

Seventeen subjects have been implanted and have reached the 6 month time point, which is the data cut-off for this presentation. All subjects had bare light perception or worse vision due to Retinitis Pigmentosa (see clinicaltrials.gov for inclusion criteria). The electronics were sutured episcleraly and then after a vitrectomy the array of 60 stimulating electrodes was inserted through the pars plana and tacked to the retina in the macular region. Video captured by a miniature camera mounted on the subject's glasses was continuously sampled and the stimulation current in each electrode was matched to the brightness at the corresponding area of the scene.

The average age of the subjects was 60+/-9 years. The median surgical time was 3 hours and 9 minutes. All subjects were able to take their Argus II system (the glasses and battery-operated video processing unit) home for use outside the clinic. As of December, 2008 the subjects have been implanted an average of 14+/-6 months. To date, there have been nearly 20 subject-years of experience with the Argus II implant.

The safety of the device has been acceptable and as expected for a new implant design. Most of the major adverse events occurred within one month of the time of surgery and all resolved by the six month endpoint. These major events included conjunctival erosion (n=5), hypotony (n=4) and endophthalmitis (n=3). There were no device failures and no explants by the six month endpoint.

All seventeen subjects were able to see phosphenes (spots of light) produced by electrical stimulation of the electrodes and stimulation thresholds were tracked over time. Clinical demonstration of the light sensitivity provided by the system could be obtained with Goldmann Perimetry. The system provided significant improvements for tasks of spatial localization, motion detection and orientation and mobility.

With six months follow-up on seventeen subjects, this is the largest study of a visual prosthesis. The results demonstrate that the Argus II system is a reliable device with a reasonable safety profile. Using the system, blind subjects are able to detect light and improve their performance on simple visual tasks.

Functional results with subretinal implants for the restitution of vision in the blind

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Since 1995 the SUBRET-Consortium has developed a subretinal, actively powered multiphotodiode array that - after thorough in vitro and in vivo testing - has been implanted into the eyes of blind patients suffering from retinitis pigmentosa. Ten patients have received subretinal implants (3 x 3 x 0.1 mm, 1500 microphotodiodes, amplifiers and electrodes 50x50µm, spaced 70µm), powered and controlled via a subdermal cable that enters the body retroauricularly and ends in a thin, transsclerally placed intraocular foil between the retinal pigment epithelium and the neuroretina. Additionally a field with 16 electrodes for direct stimulation was built into the tip of the implant. Patients were tested for 4 weeks.

Results: Electrical stimulation of rows, columns and blocks of 4 electrodes allowed some patients to clearly distinguish horizontal from vertical lines and positions and to identify simple patterns correctly, respectively. Dot alignment and direction of dot movement was properly recognized, if three neighbouring electrodes were switched on simultaneously or sequentially. Brightness perception of spots varied from scale 0 to 5 in a linear manner if voltages between 1.5 and 2.5 were applied (randomly 6 times) to a square of 4 electrodes. This corresponds to a charge increase of approximately 0.23 mC/cm^2 for each of the 5 steps. A difference in brightness between two consecutive pulses was discerned, if a difference in charge of at least 16 µC/cm^2 was applied. The subjective size of spot perception upon stimulation of a square of 4 electrodes increased from 1 to 5 mm at arms length, if the voltage was increased from 1,5 to 2,5 V.

In SLO microperimetry of the chip, single light spots down to 100 to 400 µm in diameter were detected, allowing the patient to localize a white plate on a black table cloth correctly. Spatial discrimination better than one degree of visual angle can be achieved under certain conditions in patients. The degree of spatial resolution varies in the individual patients, depending on the duration of retinal disease and the individual structural conditions of the retina at the site of implantation.

The state of the perfusion of the retina is of critical importance and careful assessment of the vascular situation and retinal thickness by OCT is mandatory for planning the optimal localization of the implant. Neuronal reorganization seems to play a minor role, given the size of retinal area stimulated by individual electrodes. Temporal resolution depends on numerous factors such as puls form of stimulation, characteristics of preceeding stimuli, as well as size of retinal area stimulated.

In summary: These investigations in patients show the narrow window for safety and efficacy of multielectrode stimulation of the retina on the one hand and the necessity of individual adaptation for feasible approaches on the the other. Nevertheless, subretinal electrical multielectrode stimulation can provide a useful range of localized brightness perceptions in blind patients within a limited range of temporal, spatial and electrical parameters.

The bystander hypothesis of retinal degeneration

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Retinitis pigmentosa constitutes a group of retinal diseases which is heterogeneous in terms of clinical symptoms, mutated genes and types of mutations. Although most of the genes causing the disease are expressed solely in rods, they lead to both rod and cone degeneration. The bystander hypothesis proposes a mechanism that may be responsible for this effect. This hypothesis suggests that gap junctions might provide a major pathway for the spread of cellular death from dying rods to genetically normal cones. If the bystander hypothesis is valid, then it may be possible to protect cones from dying by blockingthis pathway. Various substances are known to block gap-junctional communication, and could potentially open the way for a very different approach to therapeutic intervention. However, for the development of pharmacological treatments that selectively affect the junctional properties between the two types of photoreceptors, it will be essential to identify the proteins that form the gap junctions between rods and cones.

Gap junctions are composed of proteins that form channels which connect the cytoplasm of adjacent cells and allow exchange of small metabolites between coupled cells. The structural components of gap junction channels in vertebrates are connexin and, as recently identified, pannexin proteins. These gene families encode for 20 different connexins and 3 different pannexins in the mouse. Rods and cones communicate via gap junctions to ensure proper signal flow through the retina under all adaptational states. While there is substantial evidence that the channels on the cone side are composed of connexin36 (Cx36), it is not clear what protein makes up the channels on the rod side.

To identify the corresponding rod counterpart, we examined the expression of 13 connexin and 2 pannexin genes in rod photoreceptors by single cell RT-PCR. We found no satisfactory evidence for connexin expression, but pannexin2 (Panx2) was strongly expressed in mouse rod photoreceptors. Panx2 localization to photoreceptors was confirmed by fluorescent in situ hybridization and immunocytochemistry. In order to demonstrate the formation of possibly functional channels, we conducted functional expression studies with HeLa cells transfected with Panx2 and Cx36.

Our physiological data with transfected Hela cells do not support the formation of functional gap junctional channels formed by Cx36 and Panx2. We discuss the hypothesis for other gap junction-forming proteins as a counterpart to Cx36 in photoreceptors, and the alternative hypothesis that hemichannels expressed in rods release an apoptotic signal which acts through a receptor-mediated pathway in the cones.

A cGMP SIGNALING PATHWAY ESSENTIAL FOR SENSORY AXON BIFURCATION

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Axonal branching is an important mechanism that contributes to the formation of nervous system circuitry enabling the distribution and integration of information. However, the signaling cascades that underlie axonal branching *in vivo* have remained poorly understood thus far.
We have studied the central trajectories of dorsal root ganglion (DRG) axons that display at least two types of ramifications after entering the spinal cord: (1) bifurcation at the dorsal root entry zone (DREZ) and (2) interstitial branching from stem axons to generate collaterals that penetrate the grey matter.
We identified a cGMP signaling pathway comprising the natriuretic peptide receptor 2 (Npr2) - also termed GC-B - and the cGMP-dependent protein kinase I (cGKI) - also known as PKGI - critically involved in the establishment of the highly stereotyped pattern of axonal bifurcation of sensory axons at the DREZ of the spinal cord. Single axon labeling using DiI revealed that embryonic mice with an inactive receptor guanylyl cyclase Npr2 or deficient for cGKI lack the bifurcation of sensory axons at the DREZ, i.e. the ingrowing axons either turned rostrally or caudally instead. In contrast, interstitial branching of collaterals from primary stem axons remained unaffected. Also evident was a small number of axons which prematurely entered the grey matter of the spinal cord. Cross-breeding experiments of mice mutant for Npr2 or cGKI with a mouse line expressing EGFP in sensory neurons under control of the Thy-1 promoter demonstrated that the bifurcation error is maintained to mature stages. Consistently, biochemical investigations revealed the generation of cGMP in DRG upon stimulation of Npr2 and indicated several phosphorylation targets of cGKI. Furthermore, in a next step we examined the mechanism of Npr2 activation by a ligand localized in the dorsal spinal cord for which data will be presented.
At a functional level, the distorted axonal branching is accompanied by reduced synaptic input, as revealed by patch clamp recordings of second-order neurons in the dorsal horn of the spinal cord of Npr2 loss of function mutants. Hence, our data demonstrate that a cGMP signaling cascade including Npr2 and cGKI is essential for axonal bifurcation at the DREZ and influences neuronal connectivity in the dorsal spinal cord.

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Control of neurite branching by diverse Ig receptors

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We are studying the molecular mechanisms of neuronal wiring and in particular the specificity of molecular recognition processes mediated by membrane receptors. We are using a combination of genetic, molecular, biochemical and structural methods to address the question of membrane receptor diversity and specificity. We are studying how alternative splicing contributes to increasing protein diversity and how this is utilized for fine-tuning of axon/dendrite branching and synaptic target choice within the Central Nervous System (CNS). Furthermore, we are comparing the molecular basis of recognition specificity of Ig-receptors in neuronal wiring as well as immune responses.

Much of our work investigates the function of the Drosophila Ig-domain containing neuronal receptor "Dscam", a protein that is highly related to the human Down syndrome cell adhesion molecule (DSCAM). The fly Dscam gene is highly complex. Through alternative splicing, up to 38,016 protein iso¬forms can be formed. All Dscam iso¬forms share the same overall mo¬lecular architecture but differ primarily in three distinct Ig-domains (Ig2, Ig3 & Ig7). Biochemical studies have shown that Dscam receptors can bind each other in a ho-mophilic fashion. Remarkably, this homophilic binding is isoform specific, suggesting the existence of an enormous binding specificity enabling the formation of potentially 18,000 different dimers.

Genetic analysis has provided evidence that Dscam play an important role for the con-nectivity of axons as well as complex dendrite morphogenesis. During dendrite morphogene-sis the homophilic binding specificity of Dscam isoforms is required in vivo and enables a "self" or "non-self" recognition, which controls dendrite self-avoidance. Based on phenotypic analysis we propose that in multidendritic neurons direct isoform specific homophilic Dscam-Dscam interactions result in signal transduction events that lead to repulsion of dendritic branches expressing identical Dscam isoforms. A particular focus of our current work is on elucidating the signal transduction mechanisms that convert homophilic binding into repulsi-on.Dscam and the specificity of isoforms is also involved in a number of axonal wiring pro-cesses and the selection of spatially distinct synapses. For example, we found evidence that the molecular diversity of Dscam is functionally required for wiring specificity of afferent somatosensory projections in the CNS (Chen et al. 2006). We were able to show that reducti-on of the isoform diversity strongly impairs the precision of axonal branching and the choice of synaptic connections of mechanosensory neurons within the CNS. In this context we are investigating whether Dscam isoforms may play an instructive role controlling axon branch formation and connectivity.

Prohibitin might be involved in the dendritic pathology of chronic schizophrenia

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Schizophrenia is a common disorder, affecting 1% of the population, independent of regional origin or cultural background. Subtle structural alterations in the thalamus, hippocampus and cortex have been associated with schizophrenia and particularly the left dorso-lateral prefrontal cortex was reported to exhibit more prominent morphological abnormalities. Importantly, this work has emphasized a synaptic and dendritic pathology in the disease process that is not well understood. To gain better insight into the molecular basis of schizophrenia a number of proteomic screens on postmortem brain in schizophrenia have been performed using these brain regions. In addition, corresponding screens in animal models of psychosis have been published. Complementary work has been done with gene expression profiling of schizophrenic human brain and corresponding animal models. A major shortcoming of these approaches is that although they focus on region-specific differences, they detect rather global changes in brain protein composition rather than drawing attention to the organelles that are most likely affected in schizophrenia. Thus, proteomic screens of schizophrenia-related protein alterations in protein homogenates are corrupted by the fact that the majority of cells that will be looked at are glial cells and not neurons. In particular, the important processes related to the synaptic pathology of schizophrenia might have been overlooked so far. We have therefore prepared synapse-enriched preparations of human left dorso-lateral prefrontal cortex in chronic schizophrenia and rat cortex in the ketamine model of psychosis. Subsequent proteomic analysis revealed changes in the synaptic proteome in both conditions. However, there was only limited overlap between both screens questioning the reliability of the rat model. Interestingly, the only protein that was up-regulated in both screens, Prohibitin, is associated with the synapse in an NMDA-receptor dependent manner. Forced expression of Prohibitin leads to a reduction in dendrite length, complexity and width closely resembling the dendritic pathology of schizophrenia. In addition, we found an increased number of irregular spine synapses. Further studies were aimed on downstream effectors of Prohibitin. Taken together, this provides evidence for a causal role of Prohibitin in the synapto-dendritic pathology of schizophrenia.

Deconstructing synapses with Wnt antagonists

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Synapses are highly dynamic structures that are formed, remodelled and eliminated throughout life. Following the initial peak of postnatal synaptogenesis these events determine and sculpt functional neural circuits. Whilst emerging evidence is unravelling many of the key molecules involved we are still missing vital mechanistic details.

Our lab has previously reported that secreted Wnts act as target-derived signals that regulate terminal arborisation of axons and presynaptic assembly.
Loss of function studies using Wnt deficient mice combined with gain of function approaches revealed that Wnt signalling regulates synapse formation by inducing the recruitment of synaptic components to future synaptic sites. This is achieved through the activation of a canonical Wnt signalling pathway that involves LRP6, Dishevelled and inhibition Gsk3ß, a serine/threonine kinase. We have used the canonical Wnt antagonist Dickopf-1 (Dkk1) to determine the role of Wnt signalling in synapse formation and maintenance. In young neurons, Dkk1 blocks Wnt-mediated presynaptic differentiation in hippocampal neurons. Notably, Dkk1 reduces the number of functional synaptic sites in the absence of exogenous Wnt. These data confirm our hypothesis of an endogenous canonical-Wnt signal regulating synaptogenesis. Intriguingly, in mature neurons Dkk1 rapidly reduces (within 30 minutes) the number of presynaptic sites and the co-localisation of presynaptic proteins. These results are consistent with a process of presynaptic disassembly. We also find ultrastructural correlates for synaptic disassembly and shrinking. In summary, we demonstrate Wnt blockade rapidly reduces the number of synapses in mature neurons indicating Wnt signalling is not only required for the formation of nascent synapses, but also for sustained synaptic function and maintenance.

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Signaling Mechanisms in Cortical Axon Branching, Growth and Guidance

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Extracellular guidance molecules regulate axon outgrowth, branching and guidance. Using dissociated cortical cultures and live cell imaging, we found that the guidance molecule netrin-1 promotes profuse axon branching without affecting axon length. Rapid axon branching is induced by local application of netrin-1 to the axon, which evokes localized repetitive calcium transients involving CaMKII and MAPK signaling. We also found that localized calcium transients occur spontaneously in small regions of the axon. Do these localized transients regulate differential outgrowth of axons and their branches? Using fluorescence calcium imaging, we found that higher frequency calcium transients occur in rapidly extending axonal processes whereas lower frequencies occur in processes that stall or retract. Induction of localized transients by photolysis of caged calcium promotes process outgrowth simultaneous with retraction of another process of the same axon. This suggests a competitive mechanism that favors for growth processes with higher calcium activity. In contrast to the effects of netrin-1 on cortical axons, we found that bath applied Wnt5a, a morphogen known to guide cortical axon outgrowth in vivo, doubled cortical axon outgrowth over several days in vitro without increasing axon branching. Time lapse imaging in a turning assay revealed that localized gradients of Wnt5a applied to cortical axonal growth cones induced repulsive turning behaviors as well as increasing rates of axon outgrowth four fold in 1 hour. Investigation of the role of calcium signaling in these axonal behaviors showed that Wnt5a evoked repetitive calcium transients which were essential for promoting axon outgrowth and repulsive growth cone turning. Interestingly we found that axon outgrowth and repulsive growth cone turning required different calcium signaling pathways. Blocking calcium release from stores through Ip3 receptors by applying 2APB inhibited Wnt5a promoted outgrowth of cortical axons but did not affect repulsive growth cone turning. When calcium entry through TRP channels was blocked pharmacologically, however, Wnt5a induced repulsive growth cone turning as well as axon outgrowth were inhibited. Taken together these results show that axon outgrowth and branching are independently regulated and that localized calcium signaling, which can be activated by extracellular guidance cues, is one mechanism for regulating axon outgrowth, branching and guidance.

Differential Regulation of Peripheral Arborization and Central Afferent Bifurcation of Somatic Sensory Neurons by Slit/Robo Signaling

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Axon branching is a critical morphogenetic process in building functional neural circuits. To identify molecule mechanisms underlying this process, our studies focus on sensory axon projections from the dorsal root ganglion (DRG), because they develop unique and stereotypic branched structures during embryonic development. For example, their peripheral projections in the skin form exuberant axonal arbors, while their central projections bifurcate and send collaterals at the dorsal root entry zone of the spinal cord. Early studies using culture DRG neurons identified the amino-terminal fragment of Slit2 as a branching and elongation promoting factor (Wang et al, *Cell*, 96;771,1999). On the other hand, Slit signals through Robo receptors and is known to negatively regulate axon guidance and cell migration in many cell types. Here, we describe our recent studies of this signaling pathway in knockout mice and demonstrate that both positive and negative functions of Slit/Robo signaling are involved in controlling different branching patterns of sensory axon projections.

First, we found that the positive activity of Slit proteins on DRG sensory axon branching in culture can be mimicked by activation of Robo receptors using either specific antibodies or myristyolated cytoplasmic domains. This branching activity appears to be involved in developing peripheral sensory axon arbors *in vivo*, as we found a reduction in ophthalmic branches of trigeminal sensory branching in *Slit2;Slit3* double or *Slit1, 2, 3* triple mutants. A similar phenotype is observed in *Robo1; Robo2* double mutants as well, confirming that Robo is the receptor in mediating this branching activity.

Second, by studying the central projection of DRG neurons in the spinal cord, we have discovered that the same pathway is also required for proper bifurcation, another branching process important for establish functional sensory circuits in the spinal cord, but through a different cellular mechanism. In *Slit1;Slit2* or *Robo1;Robo2* double mutant mice, we have found that sensory axons penetrate the spinal cord prematurely. Interestingly, single cell analysis suggests that this defect is due to the loss of an inhibitory guidance function on one of the daughter branches during bifurcation. This conclusion is supported by the finding of a transient growth cone collapsing activity of Slit on young DRG neurons in culture. In addition, Slit/Robo signaling appears to cooperate with cGMP signaling, a pathway recent found to be required for the formation of two daughter branches. Thus, our in vivo analysis has also revealed that a simple bifurcation process is regulated by multiple signaling pathways.

Taken together, our studies have uncovered dual functions of Slit/Robo signaling in sensory axon branching. It contributes to patterning both the peripheral and central branches via distinct positive branching and negative guidance actions respectively.

New avenues to enhance translatability in episodic-like learning and memory

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Translational medicine attempts to increase predictability of preclinical outcomes for human clinic. Therefore, significant improvements in animal models, their enhanced alignment to human disease conditions, and the development of novel biomarkers and test paradigms are essential.

Towards this end, we have recently established scalp electroencephalogram (EEG) recording techniques for small rodents, especially mice that enable long-term recordings of multi-channel EEG up to several days without cable interference. These enable registration of global brain activity with particular reference to sleep, activity, but also behavioural context and systematically varied stimulus protocols. Our focus is on recognition memory as a form of episodic-like learning in mice. Moreover, superficial recording techniques allow for longitudinal studies (pre-symptomatic, symptomatic and during different stages of treatment) and examples for neurodegenerative disease models will be presented. Similar recordings are already in place for humans so that translatability can be monitored.

A second methodical improvement is the use of micro-PET (positron emission tomography). This may be used simply for detailed regional analysis of metabolism, but more importantly as a pre-clinical tool for drug metabolism and bioavailability. A more difficult and yet under-explored aspect which also hold translational value is the imaging during and after behavioural testing. Possible avenues for future work will be discussed.

mGluR8 - a new target for anxiety disorders?

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The amygdala is hyper-responsive in patients with mood and anxiety disorders. Amygdala responsivity is governed by a local neural network and its balanced glutamatergic and GABAergic transmission. The metabotropic glutamate receptor subtype 8 (mGluR8) is presynaptically located and its activation modulates glutamatergic and GABAergic transmission. Therefore, mGluR8 became a candidate for a target for anxiety disorders.

The present study shows that (a) mGluR8 is highly expressed in the primate (cynomolgus monkey) and rodent amygdala, especially in the baso-lateral subnucleus (which is important for contextual fear), (b) mGluR8 knockout mice have increased spontaneous activity of the central-medial amygdala output nucleus showing that mGluR8 critically contribute to amygdala physiology and output, and (c) mGluR8 knockout mice showed a robust attenuation of contextual fear whereas cue-induced fear was not affected. Latter was measured in the conditioned freezing paradigm in which animals have to learn to associate both a context (the test environment) as well as a discrete, highly predictive cue (tone stimulus), with a fear-inducing foot shock stimulus. In addition, mGluR8 KO mice are not altered in their response to foot shocks, in their spontaneous locomotor activity, as well as in their behavior in two different animal models of schizophrenia.

In summary, these data confirms that mGluR8 is a target for mood and anxiety disorders.

Animal models of Parkinson´s disease

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to be prepared

Molecular analysis of neural Sox protein functions

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Sox proteins are important regulators of many processes during vertebrate development. In the developing peripheral and central nervous systems, many Sox proteins are highly expressed in a temporally dynamic and often cell-type restricted manner. Some Sox proteins are essential for maintenance of stem cell pluripotency, proliferation and precursor cell survival, whereas others are crucial for cell specification and lineage progression. Over the years, it has emerged as a common concept that one cell type expresses more than one Sox protein. Among co-expressed Sox proteins, some are closely related whereas others belong to different subgroups. Closely related Sox proteins usually perform at least partially redundant functions when co-expressed. Distantly related ones, in contrast, usually regulate different processes or modulate the activity of other Sox proteins. Using select examples from the developing peripheral and central nervous systems, data will be presented on the functions of SoxC, SoxD and SoxE proteins on neuronal and glial development and their possible interactions.

Transcriptional control of autonomic neuron specification and differentiation

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The peripheral nervous system serves as an excellent model system to study the mechanisms that control the emergence of distinct neuronal subtypes from pluripotent neural crest stem cells. The development of noradrenergic sympathetic neurons is initiated by a group of transcription factors, including Phox2a/b, Hand2 and Gata2/3. These factors control, directly or indirectly, the acquisition of generic neuronal and subtype-specific properties. While previous studies identified the fate-determining role of these factors during initial stages of sympathetic neuron development, their function at later stages is not well understood. Here, we show that Phox2b and Hand2 display important functions not only in progenitor cells, but also in proliferating, immature neurons and in postmitotic, differentiated sympathetic neurons.

We provide evidence for a role of Phox2b in the control of sympathetic neuron proliferation and demonstrate increased proliferation and de-differentiation of sympathetic neurons in response to mutations in Phox2b, observed in familial and sporadic forms of neuroblastoma. Our findings indicate that these aberrant Phox2b functions may predispose to tumor-initiating events in neuroblastoma.

Using in vitro and in vivo loss-of-function approaches, we reveal an essential and selective function for the transcription factor Hand2 in the control of noradrenergic differentiation. Hand2 induces and maintains the expression of the noradrenergic marker genes tyrosine hydroxylase and dopamine-b-hydroxylase in progenitor cells and differentiated postmitotic sympathetic neurons, respectively. This result demonstrates that similar mechanisms underly the establishment and maintenance of the noradrenergic phenotype.

Molecular mechanisms of learning and memory at the synapse

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A key prerequisite for learning and memory at the single neuron level is that a nerve cell is able to individually modify its response to synaptic input in an experience-dependent fashion. Recent evidence indicates that the asymmetric localization of mRNAs within nerve cells plays an important role in this process. Certain neuronal mRNAs, e.g. encoding for MAP2 or the a-subunit of the Ca2+/CaM-dependent protein kinase II are transported to dendrites, where they are locally translated at the activated synapse. Defects in RNA localization can have severe consequences for the function of cells as documented for human diseases including fragile X mental retardation, spinal muscular atrophy, and myotonic dystrophy. In this talk, I will review a first working model of dendritic mRNA transport and local protein synapse that leads to the observed structural and functional rearrangement of a ‘potentiated’ synapse. This is thought to critically contribute to synaptic plasticity and thereby to learning and memory.

Molecular coordination of postsynaptic plasticity mechanisms

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The capability of the mature plastic brain to learn, memorize and regenerate critically relies on continuous induction, maturation and plastic modulation of synaptic contacts. Dendritic spines are the postsynaptic receptive regions of most excitatory synapses and their morphological plasticity plays a pivotal role in higher brain function. Changes in synaptic activity are accompanied by alterations in spine shape, size and number.

Major components of the spine heads, which contain the postsynaptic reception and signal transduction apparatus, are elaborate actin cytoskeletal and cytomatrix structures of the postsynaptic density (PSD). Dynamics of the postsynaptic actin cytoskeleton is the driving force behind formation and morphological changes of postsynaptic spines and plays an important role in dendritic spine reorganizations, which are the basis for postsynaptic plasticity. An understanding of postsynaptic structural and thus functional plasticity therefore ultimately requires detailed molecular insights into the tight regulation of actin dynamics in dendritic spines and in how such changes in the postsynaptic cytoskeleton are controlled by synaptic activity sensed by further components of the PSD.

Our studies focus on proteins, which interact with components of the actin polymerization machinery, induce cortical cytoskeletal reorganizations and are furthermore linked to different postsynaptic membrane receptors via association with the PSD scaffolding components ProSAPs/Shanks. Such adaptor and modulator proteins are prime candidates for a prominent role in the formation and/or plastic reorganization of postsynaptic morphology. Our studies reveal important insights into the molecular basis by which structural changes and adaptations of postsynaptic specializations are brought about and how they are coupled to synaptic transmission. In order to address these questions experimentally, we make use of gain-of-function and loss-of-function phenotypes as well as extensive biochemical interaction and reconstitution studies and analyze in molecular detail the role of an interconnection between actin cytoskeletal dynamics and specialized postsynaptic cytomatrix components. Studying the molecular mechanisms, which regulate spine morphology, is essential for an understanding of the cellular changes that underlie plasticity in the forming, adult, aging and regenerating brain.

Signaling from synapse to nucleus via Jacob

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N-methyl-D-aspartate (NMDA) receptors and Ca^{2+} can exert multiple and very divergent effects within neuronal cells such as enhancing synaptic plasticity or triggering neuronal degeneration. Recently we identified a novel morphogenetic pathway linking the activity of NMDA receptors to CREB-dependent nuclear signaling events in primary neurons. Important proteins on this pathway are the neuronal Ca^{2+}-sensor Caldendrin that is tightly associated with the postsynaptic density, its binding partner, the synapto-nuclear messenger Jacob and Importin-a, a component of the nuclear import machinery. We found that Caldendrin can bind in a Ca^{2+}-dependent manner to the central IQ-like motif of Jacob, a region that also contains a bipartite NLS. NMDA receptor stimulation results in the nuclear accumulation of Jacob that crucially requires the classical importin pathway. Caldendrin controls Jacob's extra-nuclear localization by competing with the binding of Importin-a to Jacob's nuclear localization signal in a Ca^{2+}-dependent manner. This competition requires sustained Ca^{2+}-levels, which presumably cannot be achieved by activation of extrasynaptic NMDA receptors. Extrasynaptic NMDA receptors as opposed to their synaptic counterparts trigger a CREB shut-off, and cell death. Indeed the nuclear accumulation of Jacob results in a rapid stripping of synaptic contacts and in a drastically altered morphology of the dendritic tree. Nuclear knock-down of Jacob prevents CREB shut-off after extrasynaptic NMDA receptor activation while its nuclear overexpression induces CREB shut-off without NMDA receptor stimulation. Importantly, nuclear knock-down of Jacob attenuates NMDA-induced loss of synaptic contacts, and neuronal degeneration. This defines a novel mechanism of synapse-to-nucleus communication, which links the activity of NMDA receptors to nuclear signaling events involved in modeling synapto-dendritic input and NMDA-receptor induced cellular degeneration.

The role of SRF-directed gene expression in neuronal network assembly

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SRF, a MADS box transcription factor, regulates a plethora of target genes including immediate early genes (e.g. c-fos, Egr1, Bdnf) and genes encoding actin cytoskeletal proteins (e.g. ß-Actin, Gelsolin, Vinculin). The consequences of forebrain-specific conditional SRF ablation resulting in impaired neurite outgrowth, axonal pathfinding errors and aberrant synaptic targeting, will be presented. In addition, SRF regulates via a paracrine mechanism oligodendrocyte differentiation and thereby influences myelination in the CNS. SRF's dual role in neuronal and glial cell differentiation will be discussed.

Carbachol promotes cortical differentiation in rat

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Immature neuronal networks display special synchronous activity pattern during the late embryonal and early postnatal period. They occur in retina, spinal cord, hippocampus and neocortex and have been termed calcium waves, retinal waves, giant depolarising potentials or early network oscillations. They are believed to contribute to the selforganisation of neuronal ensembles which then become modifiable by experience-dependent plasticity during the postnatal critical period. In the perinatal cortex, they can be electrically evoked and induced by certain transmitter receptor activation for instance by Carbachol (CCH) activating primarily muscarinic acetylcholine receptors.

While pharmacology and acute cellular responses are quite well characterized, less is known about the consequences on structural and neurochemical differentiation, for instance, early activity patterns may long-term modify gene expression. To enlarge on these questions we used the model system of newborn rat organotypic visual cortex cultures which can be stimulated with CCH for an early limited period of time and submitted to analysis days or weeks later. First, we found that all muscarinic receptors M1-M4 receptor mRNA´s were expressed in organotypic cultures, and they were not regulated by CCH. In response to CCH stimulation Ras and p42/44 MAPK are transiently activated, and the mRNA expression of c-fos, BDNF and NT-3 increased, suggesting activity-dependent actions. Affimetrix chip analysis revealed that early stimulation with CCH for the first 5 days in vitro did indeed cause an altered gene expression at 10 DIV. For instance, genes important for cortical development like IGF-2 and IGFBP-2, angiotensin II receptor type 1a and thrombospondin 1 as well as certain oligodendrocyte-specific genes are upregulated, and this has been confirmed with PCR. Contrary to expectations, early stimulation with CCH did neither boost the expression of presynaptic proteins like synapsin 1, synaptophysin, synaptotagmin 1 and the vesicular glutamate transporter 1, nor postsynaptic proteins like PSD-95 and NMDA-R subunit 1. Yet, CCH promoted pyramidal cell differentiation by increasing length and branching of apical dendrites in layer II/III and VI, as well as basal dendrites in layer VI. Furthermore, it promoted the growth of GABAergic interneuronal somata and dendritic complexity. In conclusion, an early activation of the young cortical neurons and the cortical network with CCH elicits long-term changes in gene expression and morphological differentiation.

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Shaping early cortical circuits by electrical activity

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No abstract

Glycans in Brain Development: The Novel Monoclonal Antibody 575 Detects a Carbohydrate Epitope on Neural Precursor Cells

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From a small number of cells that form the neural ectoderm, a highly complex central nervous system (CNS) emerges during embryogenesis. Important landmarks of embryonic neural development are cell proliferation, fate specification and cell migration. These processes are coordinated by cell intrinsic and extrinsic mechanisms. We are interested in the role of glycoconjugates for neural development. This group comprises highly glycosylated molecules including glycoproteins, glycolipids and proteoglycans. Glycoconjugates are involved in the modulation of signal transduction and cell adhesion. Remarkably, glycosylation is essential for proper function. Glycoconjugates are primarily localized on the cell surface and serve as excellent biomarkers at different stages of cellular differentiation. However, the role of their various glycan chains is only barely understood.
Here, we present a promising tool for new insights into glycobiology: The novel monoclonal antibody (mAb) 575. The mAb 575 detects a carbohydrate epitope present on high molecular weight proteins. During embryogenesis it is found on the cell surface of neural precursor cells of the embryonic cortex. Immunohistochemical analysis showed a strong correlation of the 575-epitope with established neural precursor cell markers such as Nestin and RC2. Interestingly, the number of 575-epitope expressing cells is much lower in the ganglionic eminence, the future striatum, than in the embryonic cortex. This implies a special function for the 575-carbohydrate and its carrier proteins during early cortical neurogenesis. Fluorescent activated cell sorting of embryonic cortical cells revealed that the 575-positive population consists of multipotent neural precursors with neurosphere forming capacity. Conversely, the 575-negative cell population lacks this property.

Further investigations are planned to reveal carrier proteins of the 575-epitope and to investigate their function in CNS development.

Influence of Leukaemia associated transcription factor *Af9/Mllt3* on cell specification in the cerebral cortex of the mouse

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Mutations of leukaemia associated AF9/MLLT3 are implicated in neurodevelopmental diseases such as epilepsia and ataxia. Murine Af9 is transcribed in various CNS structures including the subventricular zone (SVZ) of the cerebral cortex, hippocampus, cerebellar cortex, septum and various thalamic structures, the choroid plexus, and the midbrain/hindbrain boundary. Expression of Af9 in the neurogenic SVZ of the cerebral cortex overlaps with Svet1, Cux2, and partially with Tbr2, confining its activity to the neurogenic compartment of the SVZ. In contrast to Svet1 and Cux2 expression, Af9 transcription is not limited to upper layer neurons but is found in the entire cortical plate. Analysis of Af9 mouse mutants reveals that Af9 is implicated in the formation of the 6-layered cerebral cortex by suppressing a Tbr1-positive cell fate and controling the pool of Tbr2-positive intermediate progenitor cells. As part of an extensive network of interacting proteins involved in epigenetic DNA modification, we show that Af9 regulates gene expression in part by decreasing DNA methylation.

Regulation of neural stem cell behaviour in the developing spinal cord by extracellular matrix molecules

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During the development of the central nervous system neural stem/progenitor cells give rise to three major cells types, namely neurons, astrocytes and oligodendrocytes. The processes that lead to the generation of distinct cell lineages are highly regulated by environmental cues such as growth factors or cytokines. Recent studies in the developing forebrain have shown that also extracellular matrix (ECM) molecules including glycoproteins such as Tenascin-C (Tnc) or chondroitin sulphates are involved in neural stem cell self-renewal and differentiation. However, little is known about ECM effects on neural stem cell behaviour in the developing spinal cord. Therefore, we undertook a systematic description of the expression of Tnc and of a particular chondroitin sulphate glycosaminoglycan in the developing spinal cord. The latter is present on isoforms of the receptor protein tyrosine phosphatase beta/zeta (RPTPßζ) gene and is recognized by the monoclonal antibody 473HD. In addition to the expression analysis a correlation of the expression of these molecules with growth factor responsiveness was attempted using the neurosphere culture system. We found that during neurogenesis at E11.5 Tnc is absent from the spinal cord whereas some 473HD positive cells are scattered throughout the neuropil. At that age the isolated neural stem/progenitor cells were primarily FGF-responsive, as revealed by neurosphere formation assays. When neurogenesis has ceased and oligodendrocyte precursor cells appear in the spinal cord (E15.5), both Tnc as well as the 473HD-epitope become strongly up-regulated, in particular in the ventral horns, but also at the ventricular zone. This up-regulation is accompanied by the emergence of an EGF-responsive neural stem/progenitor cell population. A similar kinetic of growth factor responsiveness has allready been reported by Represa et al. in 2000 in the cervical spinal cord. Furthermore many Tnc and/or 473HD positive cells were also positive for radial glia cell markers such as brain lipid binding protein (BLBP) or the glutamate/aspartate transporter (GLAST). This specific expression pattern prompted us to perform functional investigations both *in vitro* via neurosphere formation assays and *in vivo* via BrdU incorporation analysis using Tnc deficient mice. Furthermore, it is possible to enrich for 473HD-positive cells using a recently established immunopanning protocol. This enables us to establish whether the 473HD-positive cells from the spinal cord possess characteristics of neural stem cells. In addition, we can enzymatically eliminate the sulphated glycosaminoglycan side chains and monitor the cell fate after their removal. Taken together this study will lead to further insights into the role of extracellular matrix molecules on neural stem/progenitor cell behaviour in the developing spinal cord.

Human Ntera-2 cells as a predictive in vitro test system for developmental neurotoxicity

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Given the significant potential of chemicals and drugs to interfere with the development of the brain, regulatory test guidelines have been adopted for the prediction and assessment of developmental neurotoxicity (U.S.EPA OPPTS 870.6300 and OECD TG 426). However, current *in vivo* test methods are laborious, costly and necessitate the use of high numbers of laboratory animals. The aim of this study was the development of standardized predictive cell-based *in vitro* assays as a possible replacement for *in vivo* tests. We employ the NTera-2 cell line, a human teratocarcinoma cell line that can be induced to differentiate into neurons (review: Paquet-Durand and Bicker, 2007).

To assess developmental neurotoxicity, we established molecular and mechanistic endpoints like *proliferation, differentiation*, and *migration*. These endpoints were tested in the presence of compounds with known developmentally toxic potential (positive test compounds): lead, methyl mercury, valproic acid (VPA), methylazoxy methanol acetate (MAM). As control substances with acute neurotoxic but not developmentally neurotoxic potential (negative test compounds), we used paracetamol and glutamate.

To test an influence on proliferation, we measured viability of proliferating NT-2 precursor cells using the Alamar Blue assay in a 96-well format at several time points during 10 days of incubation. Developmental neurotoxicity was assumed when IC50-values differed between three days of incubation (acute toxicity) and 10 days incubation (inhibition of proliferation). We found that lead, methyl mercury, and MAM inhibit proliferation, negative test compounds do not.

To assess differentiation into neurons, we examined the expression of the neuron specific marker ß-tubulin type III under treatment with test substances. Using immunofluorescence staining, we automatically evaluated expression using a fluorescence plate reader, confirmed the results by manual counting and compared the result to acute cytotoxicity determined by a viability assay. VPA and MAM specifically inhibited neuronal differentiation, whereas methyl mercury and the negative control substances did not.

Cell migration was assayed by placing spheres of differentiating NT2-cells, which form during culture under non-adherent conditions, onto an adherent substrate in the presence of test compounds. Cell migration out of these neurospheres was determined microscopically and compared to acute toxicity. Again, we found developmental neurotoxins (lead and MAM) to specifically inhibit migration, whereas IC50 values for negative test compounds but also for VPA and methyl mercury did not differ from acute toxicity values.

In summary, we could perform *in vitro* tests for developmental endpoints (proliferation, differentiation, and migration) which are predictive for most of the the effects of known developmental neurotoxins. Thus, the NTera-2 cell line offers a promising perspective towards establishing

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BMP7 release from endogenous neural precursors attenuates the tumorigenicity of glioma stem cells

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We have established previously that endogenous neural precursor cells (NPCs) are attracted to experimental brain tumors (gliomas) in large numbers and reduce tumor size. We now report that NPCs attenuate the tumorigenic potential of glioma initiating cells via BMP7 release. Using an in vivo model we show that glioma-associated PSA-NCAM-positive NPCs express BMP7, but not BMP2 or BMP4. BMP7 is the pre-dominant BMP in neuroshere cultures and is exclusively expressed by PSA-NCAM-positive NPCs, but not by differentiating or tumor cells. Moreover, NPCs constitutively release BMP7. Neurosphere conditioned media or recombinant BMP7 were applied to murine or human glioma and, BMP-receptor dependently, reduced the CD133-positive population, attenuated expansion, sphere formation and cell viability and induced markers of differentiation. Likewise, NPCs caused smad1/5/8 signalling, ID1 and ID2 expression and Olig2 down-modulation in human CD133+ glioma. Importantly, exposure of CD133+ murine glioma cells to recombinant BMP7 prior to intracerebral implantation of 100 viable cells in a mouse model significantly prolonged survival as compared to injection of untreated control cells. Overall, our results show that NPC-released BMP7 attenuates tumor initiation by CD133+ glioma cells and point out a role for BMPs as paracrine tumor suppressors in the CNS.

Evidences for glycinergic control of cortical migration

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During early development, glutamatergic neurons are generated in the ventricular zone and migrate radially through the intermediate zone to reach specific positions in the cortical plate. Postmitotic neurons show a very early expression of voltage- and ligand-gated channels and it was suggested, that these could participate in the regulation of migration [1]. While the role of glutamate and GABA receptors for migration had been clearly established [2,3], a contribution of glycine receptors, which are abundantly expressed throughout the immature cerebral cortex and mediate depolarizing responses [4], had not yet been addressed. Therefore we investigated the effect of the glycinergic system on radial migration using a BrdU-based *in-vitro* migration assay in organotypic slice cultures and patch-clamp methods.

Our experiments revealed that a blockade of glycine receptors by 1 µM strychnine impaired migration (n = 70 slices). A significant larger portion ($p = 0.0036$) of neurons failed to migrate from the ventricular zone to the upper cortical layers. The addition of 500 µM glycine did not change the migration pattern (n = 86 slices), perhaps due to rapid elimination of the added glycine from the extracellular space by cellular uptake and/or degradation. Since it has been suggested that taurine acts as an endogenous agonist of glycine and GABA receptors [5], we also investigated the effect of taurine on radial migration. These experiments revealed that both exogeneous application of 2 mM taurine (n = 38 slices) and blockade of taurine transport by 300 µM guanidinoethan sulfonate (GES, n = 83 slices) also impaired cortical migration. Under both conditions a significantly ($p = 0.041$ for taurine, $p = 0.008$ for GES) larger portion of BrdU-labelled cells failed to migrate from the ventricular zone to the upper cortical layers. These effects could not be explained by receptor desensitization, since whole-cell patch clamp experiments showed that the membrane currents induced by persisting taurine application desensitised by only 22 ± 12 % (n = 5).

In summary our results demonstrate that interference with the glycinergic system impaired radial cortical migration, which suggests that adequate activation of glycine receptors is required for the proper formation of cortical layers.

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Scratch2 in neurogenesis of the mammalian brain

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Scrt2 is a newly identified transcriptional repressor, regulated by Pax6, that is expressed in the intermediate progenitor cells (IPC) of the subventricular zone (SVZ) in the developing cortex. Scrt2 is a vertebrate specific member of the highly evolutionary conserved superfamily of snail transcription factors.These factors have been shown to play critical roles in cell migration, regulation of cell death and survival as well as in assymmetric cell division and neuronal differentiation. We start to analysed the function of Scrt2 in proliferation and differentiation of the mouse cortex with GOF and LOF-approaches and looking for cofactors in the regulation of potential target genes with an E-box motif in their regulatory sequence, that is recognized by the snail transcription factors as well as the bHLH transcription factors.

Common Cadherin-Based Adhesive Cues Behind Neuroangiogenesis?

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The development of a functional vascular system is a complex process and a primary requirement for embryogenesis. Cadherins genes are important regulators of vascular development, mediating the formation of the primitive capillary plexus and its pruning, endothelial integrity, vascular permeability, formation of the blood-brain barrier, pericyte stabilization, and tumor angiogenesis (Cavallaro *et al.*, 2006). Cadherins are a superfamily of Ca^{2+}-dependent cell adhesion molecules with more than 100 members (Redies et al., 2005). They are multifunctional transmembrane glycoproteins found in several kinds of cell-cell contact, including adherens junctions. Cadherins play a crucial role in the development of both the vascular system and the nervous system of vertebrates. They regulate a wide variety of developmental mechanisms, including cell proliferation, cell differentiation, cell-cell recognition, neurite outgrowth, synaptogenesis and angiogenesis.

Previously, we cloned eighteen novel members of the classic cadherin and d-protocadherin subgroups from the brain of ferret, an animal model suitable for cerebrovascular research. Their expression patterns were investigated by *in situ* hybridization in the developing brain of the ferret. We showed that all these cadherin molecules exhibit lamina-specific expression pattern in the developing visual cortex (Krishna-K et al., 2008). Here, we identified seven members of the cadherin superfamily (CDH4, CDH5, CDH6, CDH7, CDH11, PCDH1 and PCDH17) and an intracellular binding partner of d-protocadherins, protein phosphatase 1a, as novel markers for developing blood vessels in the ferret brain. Some of the cadherin molecules are restricted to specific brain regions or a subset of blood vessels, for example in the cortical plate. The expression levels show a peak during perinatal vascular development. Our results suggest that multiple cadherins, which are also involved in neurogenesis, are regulators of angiogenesis in developing vertebrate brain, supporting the idea of a common mechanism behind neurogenesis and angiogenesis ("neuroangiogenesis").

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Nucleotides as potential cell cycle modulators of basal stem cells in the olfactory epithelium

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As the olfactory epithelium (OE) is in direct contact with the external environment its differentiated cells, i.e., olfactory receptor neurons (ORNs) and sustentacular cell (SCs), are exposed to a variety of stress factors such as toxic substances or physical injury. A permanent cell renewal and regeneration is therefore required throughout lifetime. It is known that the olfactory stem cells, also known as basal cells (BCs), which account for the ongoing cell renewal, lie and proliferate within the basal compartment of the OE. Nevertheless, the knowledge about BCs and the control of their proliferation and differentiation is rather limited.
Nucleotides are known extracellular signaling molecules acting via cell-surface receptors termed purinergic receptors. It is known that purinergic receptors are present in the OE of larval *Xenopus laevis*. In a parallel study we could show that nucleotides initiate intracellular calcium waves in SCs. This suggests that nucleotides may function as intraepithelial signaling molecules. In other sensory epithelia, e.g. the retina, nucleotides are established modulators of cell proliferation and differentiation. Here we tested whether nucleotides exert a similar modulatory role also on BCs in the OE larval *Xenopus laevis*. We thereby used a combination of functional calcium imaging in acute OE slices and bromodeoxyuridine (BrdU) stainings to visualize dividing cells. Application of ATP evoked distinct increases in intracellular calcium concentration $[Ca^{2+}]_i$ in cells in the basal compartment of the OE. This characteristic increases in $[Ca^{2+}]_i$ could be evoked in both the presence and absence of extracellular calcium. In contrast, a depletion of the intracellular calcium stores abolished the $[Ca^{2+}]_i$ responses. Accordingly, BCs seem to express only metabotropic P2Y purinergic receptors. The determined order of potency of a variety of purinergic agonists suggests that multiple P2Y receptor subtypes are involved in the nucleotide-induced responses of BCs. Suramin, an unspecific P2 receptor antagonist, reversibly reduced ATP-induced calcium signals in BCs. Intraperitoneal injections of suramin significantly diminished the number of BrdU-labeled cells in the OE. Taken together, we collected evidence that nucleotides, most probably locally released in the OE, regulate cell renewal via activation of diverse P2Y receptor subtypes on BCs.
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A similar set of genes patterns the vertebrate neural plate and the insect head

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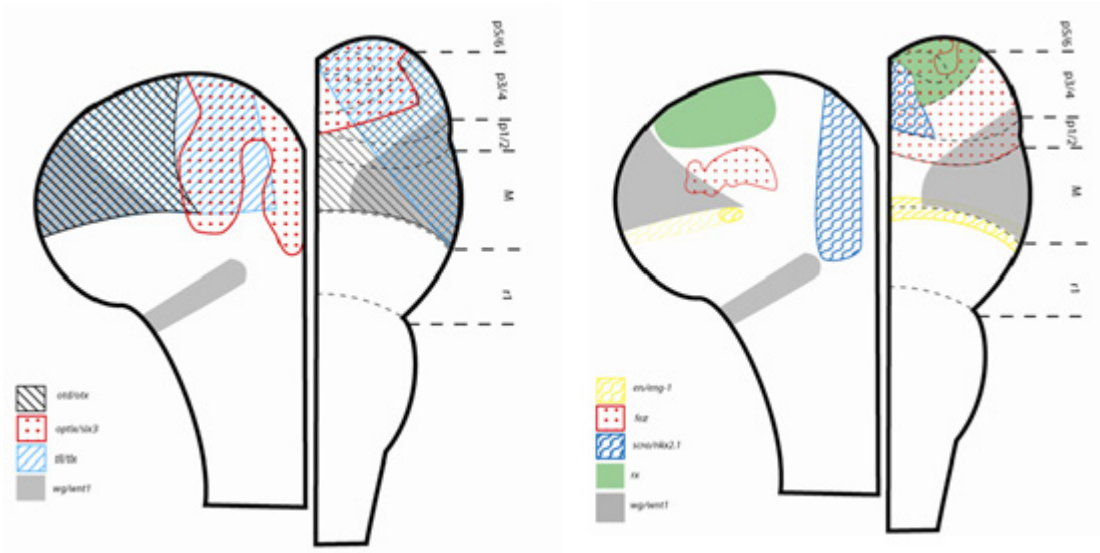
The body axis of bilaterian animals is subdivided in a posterior region that is marked by expression of Hox cluster genes and an anterior region devoid of them. Several genes are expressed in the anterior region in vertebrates and insects which has led to the conclusion that cephalization has already occurred in the last common ancestor of bilaterian animals. However, a comprehensive comparison of gene expression patterns has been lacking so far.

We have screened the genomic sequence of the red flour beetle *Tribolium castaneum* for the orthologs of 35 genes involved in vertebrate neural plate patterning. Only five of them are not found in either the *Tribolium* or *Drosophila* genomes or their orthology is unclear. For the remaining genes we determined the expression patterns and find similar expression of 24 genes. Our results suggest that the anlagen of the vertebrate fore- and midbrain correspond to the ocular and pre-ocular region of the insect head and that the ocular parasegment boundary is a signaling center corresponding to the vertebrate mid-hind-brain boundary.

RNAi mediated knockdown of some head specific genes has revealed regulatory interactions among these genes. For instance, we find repressive function of *six3* on *wnt1* which is also described for the vertebrate neural plate. For the other interactions, however, no vertebrate data is available. Our data indicate that the core of bilaterian early brain patterning is conserved.



Relation between granule cell dispersion, neurogenesis and the spread of epileptiform activity in the hippocampus

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In mesial temporal lobe epilepsy (MTLE) epileptic seizures are accompanied by hippocampoal sclerosis, characterized by loss of neurons in the CA-region and the hilus, gliosis and pronounced granule cell dispersion (GCD). We have previously shown that GCD is most likely caused by a displacement of adult neurons in TLE patients and in the intrahippocampal kainate model for MTLE in mice, since neurogenesis is lost in these areas (Heinrich et al., 2006, JNS; Fahrner et al., 2007, Exp. Neurol.). In contrast, an increase in neurogenesis has been proposed to underlie the network changes in MTLE in other epilepsy models (Scharfman et al., 2000, JNS). Here, we want to investigate whether there is a spatial relationship between the spread of epileptiform activity (EA) during status epilepticus and recurrent seizures, the extent of GCD and changes in neurogenesis in the intrahippocampal kainate (KA) model for MTLE in mice.

Therefore, we recorded EA with electrodes implanted into the dentate gyrus at several positions along the septo-temporal axis in the ipsilateral and contralateral hippocampus of KA-injected mice. We recorded the initial status epilepticus and recurrent EA for several weeks after the injection. In parallel, we investigated the extent of GCD and neurogenesis in both hippocampi along this axis by combined bromodeoxyuridin (BrdU) injections and doublecortin (DCX) staining.

We show that during status epilepticus EA could be recorded at all positions in the ipsilateral and contralateral hippocampus. Additionally, at later time points recurrent EA was not limited to the area of strongest hippocampal sclerosis surrounding the injection site, but it spread along the whole length of the hippocampus. In contrast, GCD was limited to an area surrounding the injection site. Notably, the loss of DCX-staining for newly formed granule cells was spatially correlated with GCD and recovered at distance. In the distal and contralateral hippocampus DCX-staining was even increased compared to saline-treated controls, indicating increased neurogenesis. Therefore, we assume that status epilepticus and/or recurrent EA stimulate neurogenesis in the KA mouse model, as described in other animal models for MTLE. In contrast, GCD as well as the disturbance of the neurogenic niche close to the injection site seem to have additional underlying mechanisms despite EA.

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Hypoxia Precedes Vascular Endothelial Growth Factor Up-regulation and Angiogenesis in a Mouse Model of Temporal Lobe Epilepsy

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Temporal lobe epilepsy is characterized by recurrent, focal seizures and Ammon's horn sclerosis which includes selective neuronal death and a widening of the granule cell layer, termed granule cell dispersion (GCD). These electrophysiological and neuropathological features can be mimicked by unilateral, intrahippocampal kainate injection in adult mice. Using this animal model we have previously shown that GCD results from a displacement of adult granule cells since neurogenesis in the subgranular zone is lost in the kainate-injected hippocampus. Here, we studied whether GCD is accompanied by changes of vascular endothelial growth factor (VEGF) expression, which is part of the physiological neurovascular germinal niche in the dentate gyrus. We induced GCD by intrahippocampal kainate injection and simultaneously analyzed the expression of VEGF mRNA. Coinciding with GCD formation, we found a strong induction of VEGF mRNA in astrocytes in the ipsilateral hippocampus which lasted for many weeks. In parallel, the expression of flk-1, one main VEGF receptor, was selectively up-regulated in endothelial cells in the region of GCD. These molecular changes were accompanied by severe alterations in the blood vessel pattern as revealed by immunolabeling for the endothelial cell marker CD31. Moreover, by sequential injection of kainate and bromodeoxyuridine in combination with immunolabeling for CD31 we detected proliferating and sprouting endothelial cells showing angiogenesis within the dispersed granule cell layer. Finally, pimonidazole labeling identified local hypoxia as a potential trigger for VEGF and flk-1 induction. These results show that GCD development is accompanied by angiogenesis most likely triggered by hypoxic conditions. This work was supported by the DFG (Transregio SFB TR3, SFB 780) and the German Federal Ministery of Education and Research (Grant 01GQ0420).

Sim1 is a novel regulator in the differentiation of a mouse serotonergic neuron subpopulation

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Tyrosine hydroxylase (TH) positive, midbrain dopaminergic neurons (mDA) and hindbrain serotonergic neurons (5-HT) represent clinically important ventral neuronal subpopulations. However, the transcriptional network underlying their development is not yet complete. In the present study we have investigated the impact of the bHLH/PAS domain transcription factor *single-minded (*sim) in the differentiation of mouse mDA and rostral 5-HT neurons. *In vivo*, we show that Sim1 expression co-localizes with both TH and 5-HT, and that *Sim1-/-* newborn mice have significant reduced numbers of 5-HT neurons in the dorsal raphe nucleus. In contrast, development of mDA is Sim1-independent. *In vitro*, using gain of function and loss of function approaches we show regulation of the serotonergic markers *Pet1* and *Tph2,* by *Sim1*. In contrast, *Sim1* has no influence on the expression of the early markers*Gata2* and *Mash1*. Moreover, we have identified target genes of *Sim1*, namely *Lhx8*, *RGS4*, *Brn3.2* that are upregulated in ventral hindbrain compared to ventral midbrain and are putative candidates involved in the specification of mouse rostral 5-HT neurons. Together, the results introduce Sim1 as an important regulator in the terminal specification of a subpopulation of 5-HT neurons, in the dorsal raphe nucleus, acting upstream of*Pet1* and *Tph2*. In addition, Sim1 acts to modulate serotonin release of the B7 group of 5-HT neurons via regulating RGS4. As RGS4 is only transiently up-regulated by Sim1, there likely exists is a feedback loop to maintain a proper level of RGS4 and hence the homeostasis of serotonin release. Our study underscores that subpopulation of a common neurotransmitter phenotype use distinct combinations of transcription factors to control the expression of shared properties. Moreover, the results of the present study complement the transcriptional network underlying 5-HT neuron development by introducing *Sim1*, *Lhx8*, *RGS4* and *Brn3.2* as novel determinants.

Polysialic Acid in Dopaminergic System Development

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Polysialic acid is a carbohydrate polymer, which occurs primarily as a modification of the neural cell adhesion molecule NCAM and plays a pivotal role in the developing nervous system of vertebrates. In the current study, expression and function of polysialic acid in the development of the dopaminergic system of the ventral midbrain of mice were investigated. Using immunohistochemistry, abundant expression of polysialic acid could be demonstrated in the nascent substantia nigra and ventral tegmental area of the midbrain at embryonic day 14.5 (E14.5). The presence of the two polysialyltransferases, which synthesise polysialic acid, ST8SiaII and ST8SiaIV, was studied by quantitative real-time RT-PCR. From E10.5 to birth both enzymes were found to be expressed at comparable, steadily increasing levels. Asking for a possible role of polysialylation in the developing dopaminergic system, mice lacking both polysialyltransferases where analysed. Immunohistochemistry for the dopaminergic marker tyrosine hydroxylase was performed at embryonic E14.5 and in postnatal day 30 mice and mRNA expression levels of several marker genes of dopaminergic neurons were analyzed by real-time RT-PCR. Surprisingly, no differences between wild type and mutant mice could be detected. Similarly, enzymatic removal of polysialic acid from cultured neurons of the ventral embryonic midbrain had no effect on the expression of dopaminergic marker genes. We conclude that, despite the abundant expression of polysialyltransferases and polysialic acid in the embryonic ventral midbrain, polysialylation seems to be dispensable for the correct formation of the dopaminergic system.

Sip1 controls progenitor fate and timing of production of cortical neurons and astrocytes

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The transcriptional repressor Sip1, or Smad- interacting protein 1, has been shown to play a key role in early neurodevelopment. Mowat- Wilson Syndrome in humans is associated with mutations in Sip1. In the developing mouse cortex, it is expressed mostly in the differentiating field, and weakly in the proliferative zones. We found that stage specific ablation of Sip1 leads to several defects in the development of the neocortex. Both deletion of Sip1 in cortical progenitors as well as exclusively in postmitotic cortical plate neurons, led to a reduction in the size of deep layer neuronal populations and premature production of upper layer neurons. We also observed an increase in astrocyte production, and traced its origin to increased and ectopic proliferation of progenitors committed towards an astrocytic lineage at E17.5, and premature birth of astrocytic precursors at E15.5. We also observed elevated postnatal proliferation close to the medial cortex. Our data suggest that Sip1 exerts a non- cell autonomous effect on the specification of deep layer vs upper layer neuronal fate, and neuronal vs astrocytic fate of progenitors in the germinal zone, at early and late stages of development, respectively. Comparison of gene expression profile between wildtype and Sip1 conditional knockouts enabled us to identify Neurotrophin-3 and Fgf9 as putative downstream targets of Sip1 in the cortex. Upregulation of Nt3 and Fgf9 expression in Sip1 mutant cortical plate coincides with the onset of premature upper layer neuron production and precocious astrogliogenesis, respectively. We therefore believe that Sip1 influences cortical progenitor fate via Nt3 and Fgf9 mediated signaling pathways. We are currently working towards proving this hypothesis and identifying other downstream targets of Sip1 in the cortex.

Characterization of primary neurospheres generated from mouse ventral rostral hindbrain.

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Serotonergic (5-HT) neurons of the formatio reticularis play a key role in the modulation of behaviour as anxiety, sleep and mood. Dysfunction of the serotonergic system is associated with severe neurological and psychiatric disorders as schizophrenia, autism, migraine and depression. Although of great clinical relevance, little is known about the dependency of 5-HT progenitor cells on extrinsic and intrinsic factors involved in their specification towards 5-HT neurotransmitter phenotype. Rostral 5-HT neurons are generated in rhombomeres 1-3 caudal the isthmus organizer (IO) around E11.5 in mice. Extrinsic factors, such as fibroblast growth factor 4, an early pre-patterning factor synthethised within the primitive streak, fibroblast growth factor 8 (FGF8) secreted from the IO, and the floor-plate factor sonic hedghog (Shh), direct the development of hindbrain 5-HT neurons. In addition, the development of 5-HT neurons is regulated by transcription factors, such as Nkx2.2, Lmx1b, Gata2 and Pet1. However, most members of the complex network of intrinsic and extrinsic factors are not yet identified. In order to complete the network, a primary neurospheres culture system from mouse ventral rostral hindbrain embryonic day (E) 12 has been established. Dissociated primary cells from ventral rostral hindbrain were cultured under serum-free conditions in the presence of mitogenic factors FGF2 and epithelial growth factor (EGF). BrdU incorporation and the presence of cells immunreactive for the progenitor cell marker nestin within the primary neurospheres, as well as the successful formation of secondary neurospheres proved that the generated primary neurospheres consist of proliferative-active progenitor cells. Primary neurospheres from ventral rostral hindbrain have been further characterized by PCR and immunocytochemistry for the expression of different marker genes as *oligodendrocyte marker clone 4* (*O4*), *ßIII-tubulin*, *tryptophan hydroxylase 2* (*tph2*), *glial fibrillay acidic protein* (*gfap*) and *nestin*. The results show that primary neurospheres of mouse ventral rostral hindbrain (E12) exhibit a mixed cellular composition, comprised of differentiated cells, as oligodendrocytes, neurons, and even 5-HT neurons, as well as progenitor cells. Moreover double immunocytochemistry for BrdU and ßIII-Tubulin or O4 demonstrate that primary neurospheres from ventral rostral hindbain possess the capacity of multilineage differentiation.Thus, the primary neurosphere culture system presented here exhibits main characteristics of progenitor cells: a) the ability to proliferate and b) the capacity of multilineage differentiation. It allows the enrichment of progenitor cells, to trigger them towards a specific cell fate, and therefore it appears to be a suitable tool to investigate the development of 5-HT neurons.

Characterization of neurosphere-generating cells in the developing peripheral nervous system.

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Developing peripheral ganglia contain multipotent progenitor cells that acquire a sensory neuron fate in response to Ngn1/2 or differentiate to sympathetic neurons in response to BMP4 and its downstream transcription factors (Phox2a/b, Ascl1, Hand2). Here, we address the question as to the identity of multipotent progenitors in chick and mouse PNS.

Clonal and non-clonal neurosphere cultures were established that allow to propagate selfrenewing cells from embryonic chick, quail and mouse peripheral ganglia. Here we focus on neurospheres derived from chick DRG. Neurosphere-generating cells represent about 1-2% of the ganglion cell population at E9. Self-renewal in subsequent passages (>10) increased to about 12% without evidence for cell senescence. Under differentiating conditions, 3rd passage clonal neurospheres generated cells positive for the neuronal marker TuJ1, O4-positive glial cells and melanin granula-containing melanocytes, demonstrating the multipotency of sphere-generating cells.

Notably, neurospheres were derived with similar frequency from DRG close to hatching (E18), i.e. the proportion of sphere-generating cells did not decrease after the termination of neurogenesis. This suggested that the neurosphere-generating cells might be generated by de-differentiation rather than representing bona fide tissue stem cells. Thus, we analyzed whether satellite cells can give rise to neurospheres. The O4-positive satellite cell population from E9 chick DRG, enriched by MACS-sorting to about 99% purity, displayed a significantly higher sphere-generating ability as compared to both the unsorted population and the O4-depleted cell population. Clonal spheres derived from the O4-positive population produced, similar to spheres from the O4-negative population, TuJ1-immunoreactive cells, O4-positive Schwann cells and melanin-positive melanocytes under differentiation conditions.

These results indicate that O4-positive satellite cells are reprogrammed to multipotent neural crest progenitors.

Loss of p75 affects adult neurogenesis within the adult dentate gyrus of mice

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Aside from binding to the high-affinity receptors of the trk family, all neurotrophins can bind to the low affinity receptor p75. The receptor p75 is highly expressed in the dentate gyrus (DG) of the hippocampus. Since the DG is one of the two brain areas that are known for their capacity to generate new neurons even in the adult brain (neurogenesis), we analyzed the impact of a deletion of p75 by using adult knockout mice (p75ExIV) and their control littermates. Concerning the p75ExIV mice it should be noted that these mice display a higher cholinergic hippocampal innervation.
Adult neurogenesis is a complex process that can be subdivided into different stages. We therefore used respective markers (phospho-Histon 3 (pH3), NeuroD and doublecortin (DCX)) to follow possible changes occurring at different stages of adult neurogenesis. We analyzed the rate of neurogenesis in the p75 deficient mice by using an unbiased counting rule. Moreover, we studied the morphology of the newly formed DCX-positive neurons within the DG by using 3-dimensional reconstructions.
Loss of p75 seems to affect differentiation of newly generated neurons rather than proliferation. There were no obvious alterations in the population of pH3 or NeuroD positive cells by comparing p75 deficient mice with age-matched control littermates. However, the numbers of DCX-positive neurons in the DG of p75 deficient mice were significantly increased. Moreover, the reconstruction and subsequent Scholl-analysis of DCX-positive neurons in the granular layer of the DG revealed that the DCX positive neurons of the p75 deficient mice are more complex in their morphology than those of the control littermates.
Based on our data, it can be suggested that signaling via p75 is important for proper neurogenesis in the adult DG, especially differentiation of newly formed neurons. However, whether the observed effects are directly related to the loss of p75 or may be related to the enhanced cholinergic innervation of the p75 deficient mice, or other indirect mechanisms, has to be clarified.

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Functional Analysis of Neuroblastoma Phox2b Mutations in Immature Sympathetic Neurons

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The paired homeodomain transcription factor Phox2b is essential for the development of autonomic neurons. In Phox2b-deficient mice neuron differentiation is blocked, resulting in apoptotic cell death of autonomic progenitors. Human heterozygous PHOX2B mutations account for a series of disorders of the autonomic nervous system, including neuroblastoma (NB), congenital central hypoventilation syndrome (CCHS) and Hirschsprung disease (HD). Whereas polyalanine expansion leads to CCHS, NB patients harbor either a missense or frameshift heterozygous mutation of the PHOX2B gene. PHOX2B mutations discovered in familial NB represent the first genetic predisposition to NB.
To determine the molecular basis of altered PHOX2B functions that may be involved in the formation of NB we have ectopically expressed wild-type and NB Phox2b variants in proliferating, immature sympathetic neurons from E7 chick embryos and analysed effects on differentiation, survival and proliferation. Phox2b and NB variants display differential and selective effects on the expression of characteristic marker genes in sympathetic neurons. The NB Phox2b variant $Phox2b^{nt463}$ leads to de-differentiation, resulting in a significantly decreased TH, DBH and trkA expression. $Phox2b^{nt463}$ seems to have acquired dominant-negative (dn) functions, as its effects are mimicked by a $Phox2b^{dn}$ variant composed of the Phox2b homeodomain fused to the engrailed repressor domain. The de-differentiation effects elicited by $Phox2b^{dn}$ and $Phox2b^{nt463}$ are accompanied by increased cell death, also observed in response to $Phox2b^{nt421}$, whereas $Phox2b^{wt}$ reduced apoptotic cell death. Notably, proliferation of immature sympathetic neurons is strongly reduced by overexpression of $Phox2b^{wt}$. This antiproliferative effect, mediated by $p27^{kip1}$, is lost in all missense and frameshift Phox2b mutants analysed.

These results indicate that different NB mutations lead to loss- and gain-of-function Phox2b variants, affecting differentiation, apoptosis and proliferation of immature sympathetic neurons. We propose that the loss of antiproliferative functions and/or the acquisition of de-differentiating functions of Phox2b variants may predispose immature sympathetic neurons to NB.

Analysis of neural stem cell identity in the larval brain of *Tribolium castaneum*

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In *Drosophila* much is known about the mechanisms that are involved in selecting the cells of the neuroectoderm that develop into neuroblasts (NBs), the neuronal stem cells. After selection of NB-fate the cells delaminate and divide asymmetrically - generating ganglion mother cells, which are the precursors for neurons and/or glial cells of the CNS. Trunk-NBs acquire different identities depending on their position in the neuroectoderm and their time of delamination, but only little is known about the mechanisms selecting the identity of brain NBs. The signals that make NBs different from each other must have acted before delamination, since afterwards they develop autonomously. We want to understand how brain neuroblasts acquire different identities and which parts of the brain develop from them.

In *Drosophila* four NBs are known to form the mushroom bodies (MB) of the brain. The genes *Dm-eyless*, *Dm-twin of eyless* and *Dm-dachshund* play a very important role in their formation. We identified putative MB-NBs in *Tribolium castaneum*. Further on, we showed by RNAi-experiments, that *Tc´optix/six3* is required for shaping the respective expression domains of these MB-genes.

Furthermore we are focussing on NB that arise within the anteriormost region that we found to correspond to the vertebrate forebrain, namely NBs that are *Tc-six3* and *Tc-rx* positive. In polychaetes and vertebrates these genes are involved in forming neurosecretory systems and in arthropods in formation of central structures in the brain (i.e. central complex in *Drosophila*). However, *Drosophila* larvae lack a morphologically distinguishable central complex while *Tribolium castaneum*, represents a more conserved situation of brain patterning. We have generated brain imaging lines, where reporter genes are driven by regulatory regions of *Tc-elav* (neural cells) and *Tc-repo* (glial cells) and an artificial *Pax6* sensor. In addition, we are generating beetles with reporter genes controlled by regulatory region of *Tc-six3* or *Tc-rx*, respectively. These strains will enable us to functionally analyse the mechanisms required to determine the identity of the respective NBs.

The bHLH transcription factor Hand2 is essential for the maintenance of noradrenergic properties in differentiated sympathetic neurons

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The basic helix-loop-helix transcription factor *Hand2* is essential for the proliferation and noradrenergic differentiation of sympathetic neuron precursors during development. Here we address the function of *Hand2* in postmitotic, differentiated sympathetic neurons. Knockdown of endogenous *Hand2* in cultured E12 chick sympathetic neurons by siRNA results in a strong, about 60% decrease in the expression of the noradrenergic marker genes dopamine-b-hydroxylase (DBH) and tyrosine hydroxylase (TH). In contrast, expression of the pan-neuronal genes TuJ1, HuC and SCG10 was not affected. To analyze the in vivo role of *Hand2* in differentiated sympathetic neurons we used mice harboring a conditional *Hand2*-null allele and excised the gene by expression of Cre recombinase under the control of the DBH Promoter. Mouse embryos homozygous for *Hand2* gene deletion showed decreased sympathetic neuron number and TH expression was strongly reduced in the residual neuron population. The in vitro *Hand2* knockdown also enhances the CNTF-induced expression of the cholinergic marker genes choline vesicular acetylcholine transporter (VAChT) and cholineacetyltransferase (ChAT). Taken together, these findings demonstrate that the *Hand2* transcription factor plays a key role in maintaining noradrenergic properties in differentiated neurons.

Haloperidol and atypical neuroleptic drugs increase glutamate sensitivity in neurally differentiated NTera2 cells as revealed by calcium imaging

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Schizophrenia is a devastating neuropsychiatric disorder of the prefrontal cortex, etiology of which has long been thought to include primarily a malfunctioning of the brains dopaminergic system. Major support for this hypothesis has come from the fact that the only effective treatment consists in the application of so called neuroleptic drugs which are known to block dopamine receptor functioning. However, in recent years it has become more and more clear that also changes in other neurotransmitter systems, such as glutamate and GABA seem to be altered in the schizophrenic brain. A major drawback for many studies on effects of neuroleptic drugs on brain functions is that they were performed in rodents or rodent derived cell culture models that might react in a manner different from human brain cells. In order to circumvent this problem we used here NTera2/D1 cells, a cell line of human origin that can be differentiated by retinoic acid into neurons and astrocytes. Neurally differentiated NTera2 cells were treated for 2 weeks with the classical neuroleptic drug Haloperidol (HAL, 100nmol/l), as well as with the atypical neuroleptic drugs Clozapine (CLOZ, 100nmol/l), Olanzapine (OLA, 10nmol/l) and Risperidone (RIS, 100nmol/l) and were then submitted to acute application of 100µmol/l of the neurotransmitters glutamate (Glu), dopamine (Dop), serotonin (Ser) and gamma aminobutyric acid (GABA). Acute neurotransmitter elicited changes in intracellular calcium concentration (F/F_0) were then monitored by fluorescent imaging of the calcium sensitive dye Fluo-3. It turned out that in untreated control cultures responses to either of the four transmitters could be detected in different cellular subsets including neurons as well as glial cells. Thus, whereas GABA excited calcium transients in cells with a clear neuronal morphology, Glu was able to do so both in neurons and glial cells, and Dop and Ser were active primarily in glial cells. To our great surprise, long term treatment with all four neuroleptic drugs lead to significant changes in neurotransmitter sensitivity only for Glu, whereas sensitivities to other neurotransmitters, i.e. Dop, were not changed at all. In conclusion our study is the first one to reveal significant changes in Glu signalling by neuroleptic drug treatment in a human derived neural cell culture model. Therefore neurally differentiated NTera2 cells could provide an easy to handle model system for the study of cellular mechanisms underlying the effects of present and future neuroleptic drugs in neurons and glial cells of a human origin *in vitro*.

Reelin-induced phosphorylation of cofilin in neuronal processes is required for the directional migration of neurons

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Reelin, an extracellular matrix protein secreted by Cajal-Retzius cells in the marginal zone of the cortex, has been proposed to control the radial migration of cortical neurons. Reelin signaling involves the lipoprotein receptors apolipoprotein E receptor 2 (ApoER2) and very low density lipoprotein receptor (VLDLR), the adapter protein Disabled1 (Dab1), and phosphatidylinositol-3-kinase (PI3K). Since in Reelin-deficient mutant mice reeler most cortical neurons are unable to migrate to their destinations in the cortical plate, we hypothesized that Reelin signaling plays a role in the dynamic cytoskeletal reorganization which is required for neurons to migrate.

In the present study we demonstrate that Reelin signaling leads to serine3 phosphorylation of n-cofilin, an actin-depolymerizing protein that promotes the disassembly of F-actin. Phosphorylation at serine3 renders n-cofilin unable to depolymerize F-actin, thus stabilizing the cytoskeleton. We provide evidence for ApoER2, VLDLR, Dab1, src family kinases (SFKs), and PI3K to be involved in n-cofilin serine3 phosphorylation and show that phosphorylation of n-cofilin takes place in the leading processes of migrating neurons. Immunostaining for phospho-cofilin in dissociated reeler neurons is significantly increased following incubation in Reelin-containing medium compared to control medium. By using a stripe choice assay, we show that neuronal processes are stable on Reelin-coated stripes but elongate on control stripes. Our findings suggest that Reelin-induced stabilization of neuronal processes attaches them to the surface of the cortex thereby providing the proper orientation for the migration process.

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Postnatal brain overgrowth in Bassoon-mutant mice involves increased hippocampal cell numbers and aberrant proliferation

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Mutant mice lacking the presynaptic active zone protein Bassoon develop spontaneous epileptic seizures and an altered pattern of basal neuronal activity is accompanied by enlargement of several brain regions with the strongest size increase in hippocampus and cortex. To unravel the development of this phenotype, manganese-enhanced magnetic resonance imaging (ME-MRI) and histological techniques were applied. Altered manganese distribution indicating aberrant basal activity patterns was found to precede abnormal brain growth which is first detected in the hippocampus after one month and subsequently accumulates to a size increase of 38% after 3 months in this brain region. Completing volumetric MRI data by stereological analysis of histological sections reveals both, increased hippocampal subfield volumes and numbers of NeuN and GFAP-positive cells, indicating that hippocampal overgrowth results from increased cell numbers. In addition to neuron counting mainly confined to principal cells of the hippocampus, also total numbers of parvalbumin-positive interneurons were increased. Their cell density, similar as for NeuN-positive hilar mossy cells, however, was reduced. Therefore, despite increased total cell numbers, reduced densities of these two vulnerable cell populations rather point to a partial seizure-induced cell loss. As hippocampal overgrowth is not detected until the first postnatal month, apart from disbalanced apoptosis, proliferation of glial cells or newly generated cells in the subgranular zone could account for its size. Although the morphology of GFAP-positive cells indicates a reactive gliosis, their unaltered cell densities do not support any seizure-induced astrocyte proliferation. In contrast, bromodesoxyuridine (BrdU) administration after one month reveals enhanced proliferation of cells in the subgranular zone that may contribute to the hippocampal enlargement. The results suggest that overgrowth of the hippocampus and other brain regions in Bassoon-mutant mice develops postnatally as a consequence of increasing numbers of neuronal and glial cells which may partially arise from aberrant proliferation of cells in the subgranular zone of the denate gyrus.

Phospho-cofilin, induced by Reelin signaling, is involved in the proper positioning of sympathetic preganglionic neurons in the spinal cord

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Reelin, an extracellular matrix protein secreted by Cajal-Retzius (CR) cells in the marginal zone of the cerebral cortex, plays a critical role in neuronal mitgration. Binding of Reelin to its receptors vldlR and ApoER2, results in the phosphorylation of Dab1, an intracellular adaptor protein. In reeler mutant mice lacking reelin and in Dab1 mutants neuronal migration is disrupted.

It has been shown that Reelin signaling is also involved in the positioning of sympathetic preganglionic neurons (SPN) in the spinal cord. Reelin is located in an area between the intermediolateral region (IML) and the central canal (CC). In wildtype mice, SPN migrate from the ventrolateral region dorsally towards the IML and stop their migration there. In Reeler mice, however, SPN continue to migrate to the central canal. Therefore it is assumed that Reelin functions as a stop signal for SPN in the spinal cord.

Here we were able to show that Reelin signaling leads to the phosphorylation of n-cofilin, an actin-depolymerizing protein that promotes the disassembly of F-actin. Its Reelin-induced phosphorylation stabilizes the cytoskeleton, thereby contributing to the correct positioning of SPN in the IML region.

Defining in vitro conditions for enrichment of stem-like cell population in primary human brain tumor cultures

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Current understanding of neuro-oncology links brain regions with life-long neurogenesis to high grade glial brain tumors, known for poor prognosis due to their strong chemo- and/or radio-resistance. Recently demonstrated stem-like cell population in adult and paediatric brain tumors suggest the involvement of neuronal stem cells in tumor initiation due to longitudinal accumulation of mutations and subsequent malignant transformation.

Gene-overexpressions in brain tumours for receptors of the growth factor family (EGF & FGF) or signal pathways like NOTCH, SHH and the canonical WNT/ ß-catenin cascade supporting this hypothesis. Inhibition of the mutated mechanisms (NOTCH and SHH) in glioblastoma derived cell lines or changes in the activity of aberrant signal transduction (i.e. via Bone Morphogenetic Proteins) resulted in significant reduction of brain tumor stem cell population as well as reduced *in vitro* proliferation and inability to form tumors *in vivo*. Amongst other relations this could lead to the possibly of developing novel approaches in brain tumor therapy.

Our project is focused on defining the in vitro cell culture conditions leading to enrichment in stem-like cell population. Freshly resected high grade glial tumors were carefully dissociated to single cell suspension and subsequently propagated as spherical cell aggregates under the influence of mitogens. Brain tumor cultures from different donors were characterised by growth kinetics, gene profiling and differentiation potential.

Two media-compositions (DMEM/F12 and Neurocult® based) and oxygen levels (21% and 3%) were analysed. Applying a longitudinal analysis with MTS assay, a suppressed cell growth in hypoxic conditions was demonstrated as compared to significantly faster proliferation in DMEM/F12 based media. FACS analysis for CD 133- a marker of stem-like cells showed significant increase of this cell population following cultivation in low oxygen combined with Neurocult® based media. DMEM/F12 based culture media seem to boost the proliferation of fast dividing progenitor cell population as revealed by a lower CD 133^+ level. Variations in differentiation potential (10 days in DMEM/F12 based media without mitogens) and gene expression profiles for oncogenic genes, SHH, WNT and stemness were assessed histochemically and by semi-quantitative PCR methods.

Distinct effects of post-weaning environmental enrichment and adult wheel running on neurogenesis and synaptic turnover in the hippocampus, subiculum and entorhinal cortex of mice.

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Neurogenesis in the hippocampal dentate gyrus (DG) is accompanied by synaptic turnover. Different environmental conditions and experimental manipulations influence neurogenesis; for instance, neurogenesis is significantly enhanced by wheel running (vanPraag et al. 1999). However, little is known about the response of other parameters of neural plasticity such as synaptic turnover at the level of the whole hippocampal network. The rate of neurogenesis in gerbils was found to be inversely related to the extent of synaptic turnover in the inner molecular layer of the DG (Butz et al. 2008).

We examined the effects of social and physical deprivation or enrichment during rearing (pd 21-60, two groups) and subsequent responses to voluntary wheel running in CD1 mice (two groups) by assessing cell-proliferation (CP) and cell-survival (CS) rates with the BrdU-method and synaptic-turnover rates with the Gallyas silver impregnation of lysosomal accumulations (Gallyas et al. 1981). We included the hippocampus (DG, CA1, CA3) as well as adjacent and associated areas (subiculum, entorhinal cortex) in our study.

The rate of neurogenesis in the DG was not affected by the conditions of social or physical deprivation/enrichment during rearing. However, rearing history significantly influenced the neurogenic response to wheel running in the young adult animals. Socially and physically deprived animals showed significantly higher rates of neurogenesis in response to the wheel-running challenge. Furthermore, a suppressive effect of wheel running on synaptic-turnover rates was found in enriched and deprived reared mice. The effects on synaptic turnover were restricted to the DG, CA1 and CA3. No effects were found in the subiculum and entorhinal cortex.

The results are discussed in relation to the inverse relationship between neurogenesis and synaptic-turnover rates found in gerbils (Butz et al. 2008) as well as under the aspect of a maturation-induced imbalance in the limboprefrontal system of socially and physically deprived animals.

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Development of the sensory innervation in the antenna of the grasshopper, Schistocerca gregaria

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The antenna is a segmented appendage of the deuterocerebral segment of the head. Its multiple sensory innervation allows it to play a decisive role in olfaction, audition, flight, optomotor and reproductive behaviour. The grasshopper antenna in particular, has also proven to be a model system for studying the development of a peripheral nervous system. Previous studies show that the antennal sensory nervous system is pioneered in a stepwise manner by cells from three zones (A1-A3) and in this respect reflects the developmental program found in the maxilla and the legs consistent with their serially homologous nature.
Here we investigate the establishment and differentiation of the sensory innervation of the grasshopper antenna from 30% of the embryonic development until the adult stage. We used immunocytochemical staining against the neuronal marker HRP to describe the morphology and topology of differentiating neurons both from receptive fields along the antenna and from scolopale organs such as Johnston`s organ. The first receptor cell differentiates from the epithelium at 35% at the tip of the antenna (A1). Further receptor cells arise in clusters within the three meristal annuli A1-A3. Such receptor units are not fully developed during embryogenesis, they first their adult morphology only in the late larva. The architecture of the late embryonic Johnston`s organ is very similar to the adult structure, only the number of its receptor cells is lower than in the adult.
We subsequently investigated the localisation of neurogenic zones during embryonic development using lachesin, a molecular marker for neuronal precursors in the locust nervous system. Neurogenic zones were described in three neighbouring segments simultaneously in epithelium in addition to the A1-A3 meristal zones. The spatial oganisation of these zones is developmentally dependent.
Using mitotic markers we found the highest mitotic activity within the epithelium of the three meristal annuli (A1-A3) and in the area of the developing Johnston`s organ. Our hypothesis is that not only neuronal elements of the sensory system such as pioneer neurons differentiate in the epithelial layer and migrate into the lumen of the antenna, but also subsequent previously undescribed neurons which are characterised here.

Integration of ES cell derived neurons into pre-existing neuronal circuits

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Mouse embryonic stem cells are an attractive source for generating all kinds of cell types – amongst others special populations of neurons. Following an established differentiation procedure involving retinoic acid they can be used to produce a defined lineage of neuronal precursors. Growing on cover slips in dissociated neuronal culture these precursors mature into a homogenous population of glutamatergic pyramidal cells (Bibel *et al.* 2004 nat. neurosci.).
We used an ES cell line carrying an EGFP transgene controlled by the tau-promoter present in various copies within the genome. Cells were differentiated according to the protocol and consecutively precursors were injected into mouse hippocampal slice culture.
Live imaging of slice cultures at different time points after injection showed a region specific integration into the pre-existing circuit of the conserved hippocampal structure. Depending on injected region and depth within the slice structurally matured pyramidal as well as granule cells could be found. These cells highly resemble local hippocampal CA1 and CA3 pyramidal neurons and dentate gyrus granule cells, respectively, as shown via Sholl analysis. Local clues therefore seam to determine their fate. They show postsynaptic spines that most likely carry active synapses.
Our approach serves as a model system to test for functional integration of ES cell derived neurons into existing networks. While comparing wild type ES cells to knock out or knock in cells carrying mutations relevant in the context of synaptic plasticity, several known molecular players can be examined in view of their role in maturation and cellular communication during development.

Neuropeptide and serotonin expression in the adult and developing central complex of the grasshopper, *Schistocerca gregaria*

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The central complex (CX) is a major integrative region within the insect brain. Conventionally, it consists of the neuropil divisions: the protocerebral bridge, the central body, the ellipsoid body, the noduli and the accessory lobes. Columnar and tangential projection neurons contribute to this neuroarchitecture. The CX plays a crucial role in spatial orientation and navigation and in the regulation of locomotor behaviour.

The grossmorphology of the CX of the grasshopper is well known and described at the single cell levels in the adult. This neuroarchitecture develop embryonically, several neural elements of the columnar system have been identified at this stage. In this current study we investigated the neurochemical architecture of the embryonic and adult CX using mass spectrometry (MS) imaging techniques and immunocytochemistry. In particular we established an MS protocol to map the neuropeptide inventory of the central body. With immunocytochemistry we described the temporal expression of several neuropeptides and serotonin in the developing CX. We found that members of the tachykinin and allatostatin family are expressed relative early (55% and 65% of the development) in highly specific regions in the embryonic CX. The biogenic amide serotonin is also expressed in the embryonic CX, albeit later (80%), while periviscerokinin-like peptides and FMRF amide-like peptides are initially expressed in larval stages. In the case of all the peptides investigated to date as well as serotonin the expression of neuroactive substances in tangential projection neurons precedes that in columnar neurons.

3D reconstruction of allatostatin-, tachykinin- and serotonin expression patterns showed a dorso-ventral layering of the immunostained projections in the CB. No co-localisation of the three substances could be discovered.

Further we analysed the topology of these three substances in neuroblast (NB) lineages, whose progenies build a columnar system of the CX. Our question is whether the temporal expression of these neuroactive substances reflects a position within a NB lineage.

Tamoxifen and Raloxifen change the amount and the subcellular localisation of the Gap Junction Connexin 43 in NTera-2/D1 Embryonal Carcinoma Cells

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Tamoxifen and Raloxifen are selective estrogen receptor modulators (SERMs) and widely used medical drugs. Tamoxifen is still the first line drug in the treatment of estrogen responsive breast cancer and is also used as a prophylactic agent in patients with an increased risk for developing this disease. Raloxifen is used as preventive drug for osteoporosis. Although estrogen signalling is known to affect neuronal development, brain effects of Tamoxifen and Raloxifen are only partially understood. Certain studies demonstrate that Tamoxifen impairs memory formation, a process which is closely linked to the formation of new neurons. Raloxifen in contrast does not have this negative side effect. With the following experiments we started to find out whether Tamoxifen and Raloxifen influence neuronal differentiation of NTera-2/D1 cells in order to identify mechanisms underlying the different modes of action. NTera-2/D1 cells can be differentiated by retinoic acid into neurons and astrocytes, a process being accompanied by distinct changes in connexin 43 (Cx43). Cx43 is a gap junction protein which is known to regulate early neuronal development in NTera-2/D1 cells. Here we show that in undifferentiated NTera2/D1 cells Tamoxifen and Raloxifen lead to a significant time and concentration dependent change in amount and subcellular localisation of Cx43. Cells incubated with these substances in concentrations between 10 nmol/l and 10 µmol/l were tested for Cx43 expression by western blot analysis. By this 10 µmol/l Raloxifen for 1 and 2 days lead to a transient 2-fold increase in the total amount of Cx43, which was no longer detectable after 4 days. However, a shift of the phosphorylated P1/P2-form to the unphosphorylated P0-form of Cx43 could be detected at this late time point. Also Tamoxifen at a concentration of 10 µmol/l lead to a significant increase in the total amount of Cx43 after 1 day, but in this case the shift to the P0-form could already be seen after 2-days. In general these results could be confirmed by immunofluorescent staining revealing an increase in Cx43 after a 1-day treatment with 10 µmol/l both in the cytoplasm and the plasma membrane. After 2 and 4 days nearly all Cx43 is located in the cytoplasm probably representing the P0-form of Cx43 detected in the western blot. This means at least for undifferentiated NTera2/D1 cells that there is no big difference between the effects of Tamoxifen and Raloxifen in the expression of Cx43, a result which is contradictory to our original hypothesis. Despite this, we suggest the drug dependent shift in Cx43 phosphorylation to result from apoptotic cell death in undiffentiated NTera2 cells.

Pax6 Promotes Neurogenesis in Human Neural Stem Cells

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During brain embryogenesis, transcription factors are key regulators of cell fate and differentiation. The *Pax* gene family members are important transcriptional regulators, influencing cell fate and proliferation in several organs (Chi and Epstein, 2002). Loss-of-function studies have shown a reduction in the number of neurons and increased precursor proliferation in the cortex of Pax6 mutant mice. These animals also exhibit improper cortical lamination, underdevelopment of the basal ganglia, failure to establish a boundary between the pallium/subpallium, and ectopic expression of other regulators (Caric, 1997; Chapouton, 1999; Götz, 1998; Heinz, 2002; Stoykova, 1996 and 2000; Toresson, 2000; Yun, 2001). Conversely, neurogenesis is increased by overexpression of Pax6 in mouse embryonic cortical and striatal cells (Hack, 2004; Heins, 2002), as well as in adult mouse subventricular zone (SVZ) cells both *in vitro* and *in vivo* (Hack, 2004 and 2005). However, the effects of Pax6 overexpression on human neural stem cells (NSCs) *in vitro* and after intracerebral transplantation are unknown.

In this study, we have explored the consequences of mouse Pax6 overexpression on proliferation, differentiation and survival of human fetal cortical and striatal NSCs *in vitro* and following transplantation into the striatum of neonatal rats. We show that Pax6 exerts a pro-neurogenic effect *in vitro* on striatal NSCs but not cortical NSCs derived from 6-9 week old human fetuses (Kallur, 2006). The increased neurogenesis was at the expense of the GFAP+ cell population but without affecting survival and proliferation. Overexpression of Pax6 produced increased numbers of GABA+ and DARPP-32+ (characteristic of striatum), but not glutamate+ (characteristic of cortex) neurons. Pax6-overexpressing cells survived and migrated to the same extent as control cells at 1 month after intrastriatal transplantation into newborn rats. Moreover, Pax6-overexpression led to decreased proliferation of the grafted cells, and a substantial increase of the numbers of DCX+ grafted cells in the grafted area.

The present study describes, for the first time, the fate and cellular properties of human fetal NSCs, derived from different forebrain structures, when genetically modified to express high levels of the pro-neuronal transcription factor Pax6. It is conceivable, that for the generation of large numbers of transplantable cells with ideal properties, Pax6 overexpression has to be combined with other factors. Furthermore, before considering transplantation of such modified cells in patients, it has to be shown that the genetic manipulation does not lead to any functional abnormalities or adverse effects. Nevertheless, the demonstration that NSCs of human origin, which will be required in order to move into a clinical setting, can be genetically modified to produce more neurons *in vitro* and after transplantation *in vivo*, supports the idea that stem cell-based strategies could be developed for the treatment of central nervous system (CNS) disorders.

Betulinic acid changes the amount and subcellular localization of Cx43 in human NTera-2/D1 cells

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Betulinic acid is a naturally occurring triterpene found as a secondary metabolite in several plant families. Whereas Betulinic acid is known to promote apoptotic cell death in several tumor cell lines of neuroectodermal origin, the question for effects of Betulinic acid on normal neuronal differentiation has not yet been addressed. We therefore used human NTera2/D1 cells, a cell line of human origin which can be differentiated under the influence of Retinoic acid into neurons and astrocytes. A cellular system known to be involved in the regulation of such differentiation processes is intercellular communication by gap junctions consisting of the gap junction protein connexin43 (Cx43). Here we present the first results about the influence of Betulinic acid on the expression of the gap junction protein Cx43 in undifferentiated NTera2/D1 cells, demonstrating a transient time- and concentration- dependent up regulation of Cx43 by Betulinic acid. Subconfluent monolayers of NTera2/D1 cells were treated with Betulinic acid in concentrations ranging from 10 nmol/l to 10 µmol/l for 2, 4 and 6 days and the total amount of Cx43 protein was quantified via Western blot analysis. After 2 days especially for the highest concentration of Betulinic acid a subsignificant increase of Cx43 was detected, followed by a significant increase after 4 days and a final decline after 6 days. Furthermore we investigated also effects of Betulinic acid on the intracellular localisation of Cx43. Again subconfluent monolayers of NTera2/D1 cells were treated with 10 µmol/l Betulinic acid for either 2, 4 or 6 days, followed by an analysis of Cx43 immunoreactivity by indirect immunofluorescent detection. At day 2 the Cx43 protein was located both in large gap junction plaques at the cell membranes and in a perinuclear compartment probably representing the Golgi apparatus. After 4 days Cx43 immunoreactivity revealed an overall increase, in combination with a shift from the cell membrane towards the Golgi apparatus. After 6 days an overall decrease in Cx43 immunoreactivity was observed, paralleled to an almost complete translocation to the Golgi apparatus. In conclusion, at least to our knowledge, this study is the first one to demonstrate transient effects of Betulinic acid on expression of Cx43 in undifferentiated NTera2/D1 cells. Impact of these changes on Betulinic acid dependent apoptotic cell death on one hand and on neuronal differentiation on the other will have to be clarified by future experiments.

Dopaminergic differentiation of immortalized neural progenitors of the cell line CSM14.1 in vitro

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The use of neural progenitor cells (NPCs) as grafts for the treatment of Parkinson´s disease shows prospect. Further studies have to examine the potential of NPCs to differentiate into dopaminergic neurons. This study examined the in vitro differentiation of the temperature-sensitive immortalized mesencephalic progenitor cell line CSM14.1 under defined culture conditions. Cells were cultured and expanded at 33° C in DMEM/F12 containing 10% fetal calf serum (FCS). Differentiation was induced by elevating the temperature at 39° C and a reduction of FCS (1%). For immunocytochemistry (ICC) cells were fixed with 3.7% paraformaldehyde after cultivation for 2 and 4 weeks at 39° C and compared with 33° C cells. Cell lysates from the same time points were prepared for Western Blotting and 2-dimensional gel electrophoresis (2-DE) for proteome analysis. By evaluating the ICC-labeling a significant decrease of the NPC-marker Nestin over time was observed. GFAP-expression in a proportion of differentiating cells was only detectable after 4 weeks. The neuronal (dopaminergic) differentiation was demonstrated by ICC against neuronal nuclei antigen (NeuN), neurofilaments (NF), microtubule associated protein 5 (MAP5), Synaptophysin and the pacemaker enzyme of dopamine synthesis tyrosine hydroxylase (TH). In undifferentiated cells none of these markers were detectable, whereas after 2 weeks of differentiation their expression increased and was more pronounced after 4 weeks. Moreover, an overlap of several neuronal markers was found. 2-DE has been performed and statistical evaluation of up- or down-regulated proteins is currently in progress and will be verified by Western Blotting. Compared to own previously published studies where the expression of NeuN and TH was not detectable by ICC during 2 weeks of differentiation, now the differentiation of CSM14.1-cells over a longer time period has led to a significant increase of NeuN and the dopaminergic marker TH. We acknowledge Antje Schümann for skillful technical assistance.

Proliferative response of distinct precursor subpopulations and neurogenesis after cortical infarcts in the young and aged dentate gyrus.

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During hippocampal neurogenesis slowly dividing precursor cells with astrocytic, radial glia-like properties could be distinguished from highly dividing neuronal precursors in the subgranular zone (SGZ). Several studies indicate that these distinct subtypes of precursors are differentially stimulated under pathophysiological conditions. Even the relatively quiescent radial glia-like precursors (type 1 cells) increase their proliferative activity within hours after cortical infarcts. It is the aim of the present study, to analyze whether the proliferative response is also present in the aged brain and if so, whether these precursor cell proliferation leads to an increased neurogenesis after cortical infarcts. To this purpose, we used the photothrombosis model to induce focal infarcts in the forelimb cortex of 3 and 16 months old transgenic mice expressing green-fluorescent protein (GFP) under control of the stem cell marker nestin. Sham-operated mice served as controls. To label proliferating cells the mice received three single injections of bromodeoxyuridine (BrdU, 50 mg/kg i.p. every 2 h) at day 4 after the infarct. Two hours after the last injection the animals were transcardially perfused and processed for immunocytochemistry using antibodies against BrdU, Nestin-GFP, glial fibrillary acidic protein and doublecortin. Stereological analysis of BrdU-positive cells in the SGZ revealed only 14 % of proliferating cells in aged compared with young controls. After cortical infarcts the total number of BrdU-positive cells remained stable in young animals whereas old mice showed a significant increase in proliferating cells (+40 %). Phenotype analysis of the proliferating cells using confocal laser scanning microscopy further showed that cortical infarcts stimulate radial glia-like as well as neuronal subpopulations in the young but only neuronal precursors in the aged brain. To analyze neurogenesis, an additional group of animals received two daily injections of BrdU (50 mg/kg i.p.) from day 3 to day 14 after surgery and survived for 7 weeks. Colocalization of BrdU with the postmitotic neuronal marker NeuN in aged animals revealed a shift of newborn neurons into the granule cell layer whereas in the young brain most newborn neurons were found in the SGZ. However, an increase in total number of BrdU-positive neurons was only found in the young dentate gyrus (+50 %). Our data demonstrate thathippocampalprecursor cells in the aged brain maintain their ability to respond to cortical infarcts even though their number is strongly reduced.

Self-renewal and differentiation in neural stem cell cultures are regulated by gp130-dependent signaling

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Cytokines of the IL-6 family have emerged as important regulators of neurogenesis and self-renewal of embryonic and neural stem cells. They bind to the common receptor subunit gp130 and signaling via gp130 activates both the janus-kinase/signal transducer and activator of transcription (JAK/STAT) and the mitogen-activated protein kinase (MAPK) pathways. In order to study the contribution of these two signaling pathways in mediating the effects of ciliary neurotrophic factor (CNTF) on adult neural stem cells, neurosphere cultures from two different signalling module mutation mouse strains were investigated. These mutations selectively inactivate either the cytokine activated JAK/STAT- or MAPK-pathway, which leads to over activation of the remaining intact signaling pathway. We find that, while basal proliferation in neurosphere cultures was not altered in the mutants, inhibition of the proliferation of progenitor cells by gp130-ligands depends on an intact JAK/STAT3-pathway,. On the other hand, the gp130-dependent stimulation of stem cell renewal, reflected by an increase in the number of secondary sphere forming cells, requires activation of both signal transduction pathways. Further analysis using immunocytochemistry and QPCR revealed signaling via STAT3 leads to a parallel increase of expression of neuronal (e.g. TUBB3/TUJ) and stem cells markers (GFAP, CD15, *Klf4*, *Notch1*, BLBP). In summary our results indicate, that neuropoietic cytokines stimulate both differentiation and self-renewal in neurosphere cultures via STAT3, most likely by acting differentially on different cell populations present within these cultures.

Gene Regulation of Tenascin C and its Isoforms in the Developing Mouse Central Nervous System and Neural Stem Cells

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During neurogenesis different crucial events determine the temporal and spatial generation and organisation of neural cells emerging from neural stem cells. Radial glia cells represent a distinct type of neural stem/progenitor cells in the brain that serve as source of neurons and glia cells. New-born neurons migrate along these cells to their final position, contacting the latter via cell adhesion and extracellular matrix molecules. Radial glia cells in the developing brain express the matrix glycoprotein tenascin C (Tnc) during neurogenesis. In these progenitor cells the expression of Tnc is modulated by several developmentally relevant extrinsic and intrinsic factors.

In this study, we show that the growth factors EGF and FGF-2, that stimulate proliferation of neural stem cells, strongly stimulate the expression of Tnc in neurosphere cultures which serve as *in vitro* model for neural stem/progenitor cells.

Structurally, Tnc consists of several protein domains including 8 constitutive fibronectin type-III (FNIII) domains. By independent alternative splicing of six additional FNIII domains, theoretically, up to 64 different Tnc isoforms could be generated, and in the cerebellum 27 different Tnc isoforms have been detected. The analysis of the complexity of Tnc isoform expression in neural precursor cells, grown as free-floating neurospheres, revealed 20 different Tnc isoforms to be present. During brain development we detect a differential expression of the alternatively spliced FNIII domains in regions of active cell proliferation and neuronal migration.

The expression of Tnc is intrinsically regulated by transcription factors that control the relative abundance of different isoforms of the molecule. We show here that in the *Pax6*-deficient *small eye (sey)* mutant the expression of Tnc is impaired, which mainly affects the large isoforms. The transcription factors *Pax6* and *Otx2* selectively regulate differently sized isoforms of Tnc. Upon transfection of neural progenitor cells with expression plasmids for various transcription factors, we found that *Pax6* and *Otx2* preferentially support the large Tnc isoforms that are important for cell migration. We could prove a direct binding of *Pax6* to different positions in the Tnc upstream regulatory sequence by ChIP assays.

Neurosphere cultures from *sey*-mutants show a higher proliferative capacity and impaired neurogenesis which is due to the loss of the neurogenic signal provided to neural precursor cells by *Pax6*. Under certain conditions neural precursor cells in culture build out radial cell processes which show characteristics of radial glia cells and serve as migration substrate for neurons. Cells in *sey* cultures also generated these functional radial glia cells although the morphology of this cell type *in vivo* is strongly disturbed. Time-lapse imaging of these cultures revealed that the migration of neurons along radial glia cells in *Pax6*-defective cultures is slowed down, which may result from the missing stimulatory influence of large Tnc isoforms.

Our results suggest that Tnc partakes in the regulatory and functionally relevant mechanisms of radial glia cells during embryonic development of the brain and that distinct isoforms of this molecule fulfil different functions.

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Intracellular signalling and cellular remodelling induced by Epidermal Growth Factor in the adult neurogenic subventricular zone *in vivo*

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There are two major regions in the CNS that retain the capacity to generate new neurons throughout adulthood: the subgranular layer of the hippocampus and the subependymal zone (SEZ) of the lateral ventricles. In the SEZ that lines the border between the ependyma and the striatum, astrocyte-like stem cells (Type B) reside and divide to generate transient amplifying Type C cells. The Type C cells belong to a fast proliferating cell population that in turn gives rise to neuroblasts (Type A cells). Neuroblasts migrate along the rostral migratory stream (RMS) into the olfactory bulb where they differentiate into interneurons. Adult neurogenesis involves a series of events, each of which is controlled by several factors. Understanding the interplay between these factors – how they govern proliferation, migration and differentiation - is of great interest, regarding potential treatments of neurogenic illnesses in the CNS.

Epidermal growth factor (EGF) is a common mitogen that stimulates the proliferation of various cell types. Recently it has been shown that EGF boosts the proliferation of the fast proliferating Type C cells in the adult SEZ in vivo and represses neurogenic differentiation (Doetsch *et al*. 2002). Furthermore, the ependyma appears to be partially dedifferentiated after long term activation of the EGF receptor (Gregg and Weiss 2003).

Here we show that in differentiated neural stem cells *in vitro* short time application of epidermal growth factor (EGF) evokes rapid phosphorylation of the MAPkinase ERK1/2 and the transcription factor CREB predominantly in GFAP+ astrocytes. In contrast none of the Tuj-positive neurons shows increased activation levels for either protein.

Using the phosphorylated form of these proteins as an indicator for EGF receptor activation, we demonstrate that intraventricular injection of EGF *in vivo* induces ERK and CREB phosphorylation not only of the Dlx2+ type C cells but also of the astrocytic stem cells (GLAST+ type B cells). In addition short time application of EGF causes an increase of pCREB and pERK in a subpopulation of ependymal cells. The DCX-positive neuroblasts did not reveal augmented ERK phosphorylation.

As previously described (Doetsch *et al*. 2003) long term infusion of EGF induces an increase in cell proliferation and a decrease in neurogenesis in the SEZ. An analysis of the long term EGF-infused brains revealed that a subpopulation of ependymal cells extended a basal process through the proliferative subependymal zone and aquired a morphology similar to the radial glia during brain development (Gregg and Weiss 2003). Interestingly, the S100ß+ **activated ependymal cells reexpressed the neural stem cell marker NTPDase 2 (an ecto-ATPase) and the glial fibrillary acidic protein (GFAP) but not the stem cell marker GLAST. In addition in the majority of these partially dedifferentiated ependymal cells CREB phosphorylation was detected.**

Taken together our results reveal new insights into EGF-induced SEZ plasticity*in vivo* and the potential of ependymal cells for morphological and biochemical plasticity.

Optical modulation of neuronal differentiation of mouse embryonic stem cells expressing Channelrhodopsin-2

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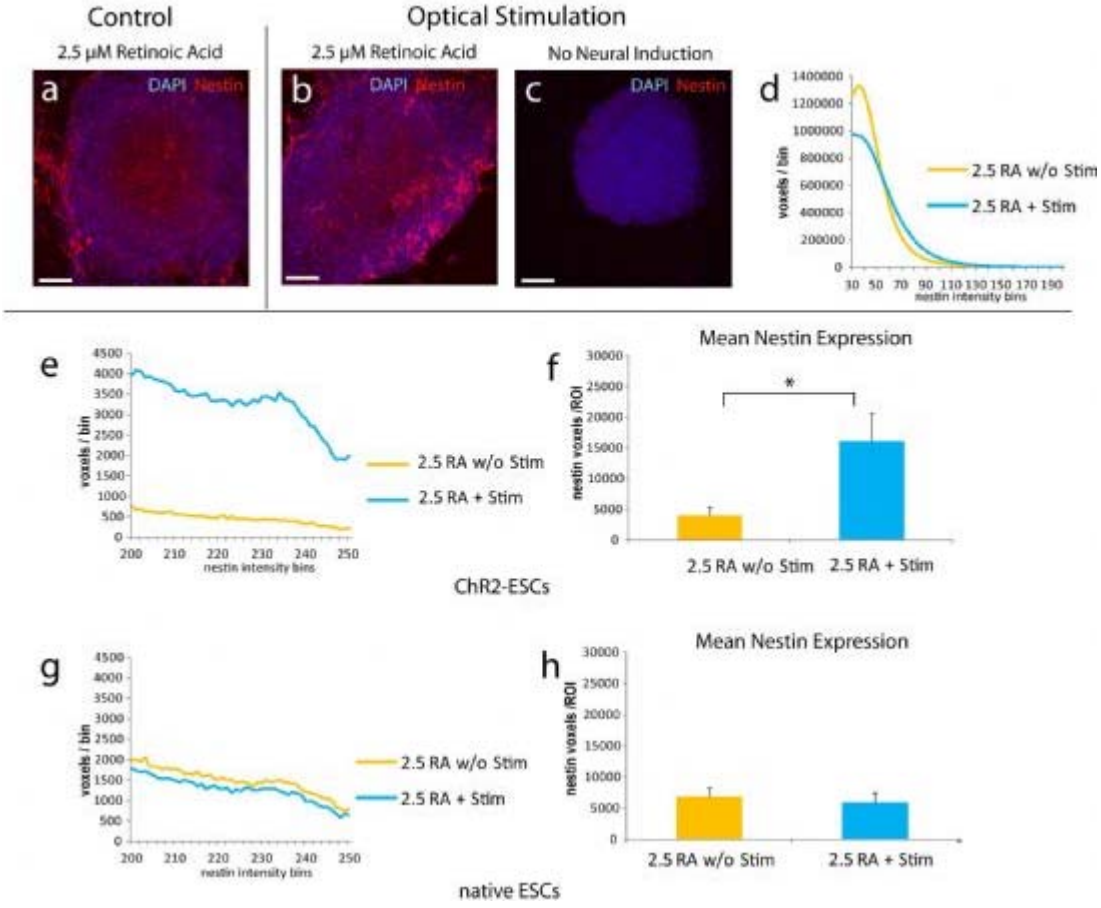
Stem cell techniques ultimately could lead to novel therapies for certain brain diseases including Parkinson's Disease and stroke. Our group recently developed methods for the transduction of neural stem cells with Channelrhodopsin-2 (ChR2), a rapidly-gated light-sensitive cation channel that allows optical control of membrane voltage. As membrane depolarization has been shown to play an important role in the neuronal differentiation of stem cells as well as in the survival and function of mature neurons, we sought to test whether we could non-invasively modulate differentiation processes in ESCs and proliferating progenitors. Mouse ESCs were transduced with a lentiviral ChR2-YFP construct under the control of the EF1-a promoter. Confocal microscopy demonstrated membrane localization of ChR2-YFP with high, uniform expression levels in the ESC population. ChR2-ESCs continued to express the embryonic stem cell marker SSEA1. Electrophysiologically, the ChR2-ESCs displayed typical outwardly rectifying currents and passive currents; illumination with blue light (470 nm) evoked strong inward currents (~300pA) in the voltage clamp recording configuration. Neural lineage differentiation was induced with 5µM retinoic acid for 8 days, followed by a neuronal differentiation medium containing sonic hedgehog (SHH) and FGF8b. By day 28 whole cell patch clamp recordings of the resulting ChR2-ESC-derived neurons displayed mature neuronal sodium currents including spontaneous activity, which could be blocked by CNQX and DAP5, proving excitatory transmission. To assess ChR-2 mediated cellular excitation, we performed Ca-imaging on ChR-ESCs after incubation with the Ca-channel activator BK 8644 and the Ca-imaging dye Fura-2. We observed an increase of intracellular Ca concentrations after light illumination (15 Hz, 10s). Immunofluorescence indicates expression of both L- and T-type voltage gated Ca-channels in embryonic stem cells in embryoid body stage. To test whether we could optically modulate neural differentiation, we stimulated ChR2-ESC during the first 5 days of neuronal differentiation with blue light (15 Hz) every hour in addition to the application of 2.5 mM retinoic acid using an automized photostimulation setup. Cells were immunostained for cellular nuclei (DAPI) and the neural marker nestin. Statistical comparison of fluorescence intensity histograms revealed a significant increase of nestin expression of about 40% after light stimulation. These data point to potential uses of ChR2 technology for noninvasive optical control both of proliferating stem/progenitor cells and the resulting differentiated neural progeny.


Control
2.5 µM Retinoic Acid
Optical Stimulation
2.5 µM Retinoic Acid
No Neural Induction
a
DAPI Nestin
b
DAPI Nestin
c
DAPI Nestin
d
voxels / bin
nestin intensity bins
2.5 RA w/o Stim
2.5 RA + Stim
e
voxels / bin
nestin intensity bins
2.5 RA w/o Stim
2.5 RA + Stim
ChR2-ESCs
f
Mean Nestin Expression
nestin voxels /ROI
*
2.5 RA w/o Stim
2.5 RA + Stim
g
voxels / bin
nestin intensity bins
2.5 RA w/o Stim
2.5 RA + Stim
native ESCs
h
Mean Nestin Expression
nestin voxels /ROI
2.5 RA w/o Stim
2.5 RA + Stim

Mapping of the embryonic isoform of the microtubule associated protein tau in the adult rat brain

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Tau is a microtubule-associated protein with a developmentally regulated expression of multiple isoforms. Is was thought that the shortest 0N/3R isoform present at birth is no longer expressed in the adult rat brain. Because the adult 4R tau isoforms bind to microtubules with higher affinity than embryonic isoform, this isoform switch may reflect a need for more dynamic microtubules during development. Interestingly, the embryonic 0N/3R isoform is not only expressed in neuronal precursor cells, but also in mature neurons in the olfactory bulb, magnocellular neurosecretory system, posterolateral hypothalamus, locus coeruleus, raphe nucleus, solitary nucleus, median septum and diagonal band, olfactory tuberculus and piriform/olfactory cortex. Further studies might address whether this unique expression pattern is associated with an functional significant neuronal plasticity.

Transforming Growth Factor ß Induces Neurogenesis through Activation of Nedd9

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Transforming Growth Factor ßs (Tgfßs) and their signalling effectors are expressed in the forebrain, but little is known upon the role of this multifunctional cytokine during forebrain development. Using hippocampal and cortical primary cell cultures of developing mouse brains, this study unravels that Tgfß induced cell cycle exit of neural progenitors, mainly through symmetric neurogenic division. Decrease in the cycling fraction of progenitors was accompanied by increased expression of neuronal markers, indicating neuronal differentiation. During this process, Tgfß not only induced exit from cell cycle through upregulation of specific inhibitors, but also regulates several other genes known to be involved in developmental processes of neuronal progenitors. Using siRNA-mediated knock-down of these Tgfß-induced target genes, we identified essential signalling components for Tgfß-dependent increase in neuronal differentiation. This study shows that Tgfß mainly induce the last mitotic division of neural progenitors by coevally transforming the progenitor pool into a status that mediates neuronal differentiation.

S-phase marker - 5-bromo-2-deoxyuridine bioavailability after intraperitoneal injection.

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Determination of mitotic activity and labeling the daughter cells are crucial tasks in research on embryonic and postnatal neurogenesis, or in design for new regenerative strategies on injured neural tissue. In despite of the variety of endogenous or exogenous S-phase markers, the 5-bromo-2-deoxyuridine (BrdU) is used in neurobiology probably the most frequently. Nevertheless, there are still lacking information about the bioavailability of BrdU in the host organism.

In our study we determined the time-dependent changes in dynamics of 5-bromo-2-deoxyuridine in the rat serum. The changes in dynamics of BrdU level in the rat serum were estimated using one of the most frequent doses (100 mg per kg of body weight) after intraperitoneal (i.p.) injection. After i.p. injection of BrdU, the rat serum was extracted and co-cultivated with the human adenocarcinoma cell line HCT-116. Consequently, the cells were processed according flow cytometry protocol.

Pursuant to presence of BrdU-labelled DNA in HCT cells we found, that BrdU appears in the rat serum during first 15 minutes after i.p. injection. Between 15^{th} and 60^{th} minute the BrdU level culminates. Outstanding decrease of BrdU level in rat serum occurs between 60^{th} and 120^{th} minute. Three hours after i.p. injection, the presence of BrdU is no more detectable, so the cells in *in vivo* conditions are certainly not able to incorporate BrdU into DNA.

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Role of the *Tshz1* gene in olfactory bulb development

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Tshz1 belongs to the Teashirt family of zinc finger transcription factors, which in the mouse consists of three genes. The three Teashirts were identified amongst more than one hundred genes with enriched expression in the dorsal spinal cord of the adult mouse, using Affymetrix microarray hybridization, and were selected for further functional analysis in the central nervous system. In Drosophila, Teashirt was shown to have a homeotic function in defining trunk identity and to play an important role in transmission of Wingless/Wnt signals. The murine homologues, Tshz1, Tshz2 and Tshz3, encode proteins with three Teashirt-like zinc-fingers (consensus: CX2CX6LX2L/MX2HMX4H) and with a homeodomain sequence, not found in the Drosophila Teashirt. We have generated a mouse strain carrying the mutated Tshz1 locus through the insertion of a membrane-anchored GFP in exon 2. Tshz1 mutant mice die few hours after birth for unknown reasons, they have swollen abdomen and breathing difficulties. Analysis of GFP expression in the Tshz1 GFP/+ mice revealed that Tshz1 is expressed in the developing dorso-lateral ganglionic eminence, dorsal migratory stream as well as in the granule cells of the olfactory bulb from development to adulthood. We found that in the developing olfactory bulb Tshz1 is necessary for the correct embryonic development of the outer ring of the granule cell layer. In the absence of Tshz1, the granule cells arrest their maturation process to an intermediate maturation stage and fail to distribute correctly from the center to the periphery of the bulb. Moreover, genome-wide expression analysis on control and mutant olfactory bulbs at P0 revealed that Tshz1 promotes the expression of the guidance molecule Sema3C, which provides a possible mechanism for the Tshz1 mediated regulation of the radial distribution of the developing granule cells.

Directed neural differentiation using protein nanoarrays on tissue-like soft substrates

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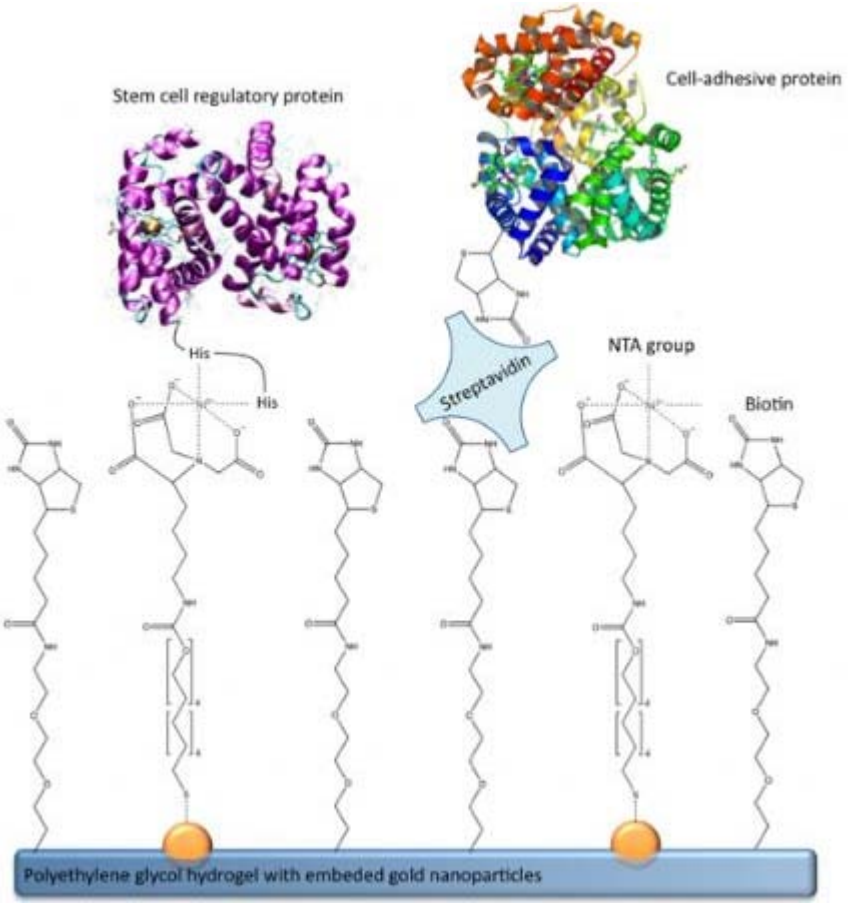
In the developing brain, neural stem cells give rise to almost all of the cells that constitute the central nervous system. Although the capacity of neural stem cells to generate the three main cell-types of the central nervous system, namely neurons, oligodendrocytes and astrocytes, has long been demonstrated, the underlying mechanisms and related factors in this process are still largely unknown. Recently, stem cells have been shown to react to mechanical cues in the extracellular environment, affecting their fate determination. However, the effect of substrate stiffness upon neural differentiation is still poorly understood.

Our goal is to develop an in vitro cell culture environment featuring biological and physical properties of the developing ventricular zone of the cerebral cortex, in order to recreate early stages of cortical development. For this, we plan to investigate the development and differentiation of neural stem cells derived from embryonic mouse brain on physically- and biologically-defined substrates. By using polyethylene glycol (PEG) hydrogels, we are able to mimic the stiffness of different tissues including brain. Furthermore, these hydrogels can be further functionalized by incorporating additional biological cues such as cell adhesive and cell regulatory proteins. Since the protein-repellent properties of PEG prevent nonspecific protein adsorption to the substrate, cell interaction is restricted to proteins specifically immobilized to the substrate using linker molecules. For this, we plan to use two different techniques for incorporating proteins into the hydrogel: 1) Biotinylated proteins are specifically immobilized to a biotin-conjugated hydrogel using streptavidin as a linker protein. 2) His-tagged proteins are immobilized via a nitrilo triacetic acid (NTA) group that is chemically coupled to the hydrogel. By combining these two techniques, two different protein species are simultaneously immobilized to the substrate in a chemically-defined manner that also allows a controlled protein orientation on the substrate. Moreover, the nanopatterning technique developed in our group enables us to incorporate nanometer-scale gold particle clusters of defined geometry that serve as anchor points to specifically immobilize proteins to the hydrogel using the aforementioned techniques (see figure). By varying the cluster density, the protein density at the substrate can therefore be controlled. This allows us to investigate the effect of cell-signalling molecules at different surface densities.

Together, these techniques enable us to produce biologically and physically defined materials in the nanometer scale. The protein immobilization techniques further allow us to adapt this platform to feature a large variety of cell-regulatory and cell adhesive proteins that influence nerual development. In this way, we plan to investigate the effect of different extracellular matrix components such as fibronectin and laminin, as well as regulatory proteins involved in neural differentiation. To this end, we have already successfully used nanoarrays of the Notch ligands Jagged and Delta to inhibit C2C12 myoblast differentiation in a density-dependent manner (unpublished data), and this approach is being applied to neural stem cell differentiation.


Stem cell regulatory protein
Cell-adhesive protein
His
His
Streptavidin
NTA group
Biotin
Polyethylene glycol hydrogel with embeded gold nanoparticles

Effects of human amyloid precursor protein on hippocampal neurogenesis in transgenic mice housed in enriched environment.

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Brain atrophy caused by substantial neuronal loss is a prominent pathological feature of Alzheimer's disease and has been linked to ectopic deposition of neurofibrillary aggregates and amyloid plaques. One of the principal components of senile plaques is the amyloid ß-peptide (Aß). The precursor protein of Aß (amyloid precursor protein, APP) is widely expressed throughout the nervous system, its physiological function in neurons, however, is largely unknown. The aim of our study was to characterize the role of APP in adult hippocampal neurogenesis. For this purpose, we used PDGF-APPwt mice (line 15) that overexpress human wild-type APP (hAPP). Promotion of neurogenesis was induced by housing mice in enriched environment. To assess the different stages of neurogenetic development comprising proliferation, survival and differentiation, mice were given i.p. injections of 5-Bromo-2-deoxyuridine (BrdU).The proliferation rate was evaluated one day after the last BrdU injection. To determine the survival rate of newly born cells, we analyzed the number of BrdU labelled cells four weeks after the last injection. The phenotype was mapped by triple fluorescent staining for BrdU, the neuronal marker NeuN, and the glial marker S100ß. Quantification was performed with unbiased stereological counting techniques. In both, wild type and hAPP transgenic mice, the number of proliferating and surviving cells is significantly increased under conditions of enriched environment. Comparing the relative increase in the number of newly generated cells revealed an enhanced proliferation in hAPP mice when compared to wild-type controls. In contrast, the survival of newly born cells was significantly reduced in hAPP mice under conditions of enriched environment. Analysis of cell fate revealed different effects of housing conditions on the phenotype in wild-type and hAPP transgenic mice. In wild-type mice, neuronal determination was promoted by housing in enriched environment. In hAPP transgenic mice, however, the fraction of presumably neuronal cells remained unchanged. Moreover, the frequency of phenotypically undefined cells was increased. Altogether, our results demonstrate opposing effects of elevated APP expression on neurogenetic proliferation versus survival of newly generated hippocampal cells.

Lipid phosphate phosphatases control cortical layering during embryonic development

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The neocortex contributes to the functional complexity of the mammalian brain, partly because of its striking organization into distinct neuronal layers. Disruption of neocortical development results in several disorders. It was shown that biologically active phospholipids like lysophophatidic acid (LPA) have an influence on the formation of neocortex. Thereby it has been suggested that LPA could play a role in controlling the balance between proliferation and apoptosis in cortical development. In this context a still open question is whether lipid phosphate phosphatases (LPPs) could play a role in cortical layer formation. LPPs are ectoenzymes, able to dephosphorylate and thereby control the levels of extracellular phospholipids such as LPA. It is known that the overexpression of LPP-1 shapes the LPA-induced migration of fibroblasts in wound-healing assays *in vitro*. However, their expression, distribution and function in the brain remain unclear.

In this study we could show by quantitative real-time PCR that LPP-1 and LPP-1a are constantly expressed during embryonic and postnatal development. Immunohistochemistry analyses also specified an expression of LPP-1/-1a in neurons.
We were able to show that overexpression of LPP-1 and LPP-1a causes enhanced neurite outgrowth in neuronal cells, whereas knock-down of endogenous LPP-1 and LPP-1a results in a reduction of neurite length. Furthermore we were able to show that LPP-formed neurites are resistant to collapse-inducing substances, such as LPA. *In vivo* analysis of LPP-1 and LPP-1a knock-down during development by *in utero* electroporation showed that cortical neurons that lack LPP-1/-1a expression are no longer able to migrate to their proper layer. To elucidate further the functional consequences of this cortical disorganization we now start with electrophysiological measurements.
So far our data provide evidence that the expression level of lipid modulators such as LPP-1 and LPP-1a and their control of the bioactive LPA-levels are important for neuronal migration and therefore cortical layer formation in early embryonic neocortex development.

From the Matrix to the Nucleus – The Sam68 gene family in neural stem cells

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Tenascin C (Tnc) is an important cue for embryonic neural stem cells (NSCs) in the mouse as the NSCs of Tnc-null mice show developmental defects. NSCs deficient for Tnc display a proliferation defect and a delayed maturation as the expression of the late-NSC marker EGFR is decelerated due to an altered growth factor response. However, target genes that are influenced by the Tnc signalling in NSCs were largely unknown. Previously we identified target genes of Tnc signalling in a genetic screen based on the induction gene trap technology. Sam68 was found among the Tnc responsive genes, which is an RNA binding protein of the STAR family. The regulation of Sam68 by Tnc was unambiguously confirmed *in vitro*. In addition, we could show in the developing brain that Sam68 and Tnc are expressed in a fashion compatible with the observed regulation in vitro. Furthermore, we could show that Sam68, which is a signal dependent regulator of alternative splicing, alters the splicing pattern of Tnc towards larger isoforms when overexpressed in NSCs. The results further indicate that Sam68's influence on Tnc splicing is dependent on phosphorylation of Sam68 by ERK1/2. Furthermore, Sam68 was shown to affect the proliferation of cultured neural stem cells. Overexpression of Sam68 in neural stem cells significantly reduced the proliferation of the cells as revealed by BrdU incorporation studies.

In the mouse two genes highly related to Sam68 (Khdrbs1) are present. Slm-1 (Khdrbs2) and Slm-2 (Khdrbs3) constitute together with Sam68 the Sam68 gene family. Due to their high conservation, it is probable that all three proteins make important contributions to brain development and it is conceivable that they may have redundant or complementary functions in this process. Therefore, we currently perform a thorough analysis of the entire Sam68 gene family in neural stem cells and the developing brain. Therefore, in addition to the results of the gene trap screen, first preliminary data of this project will be presented on the poster. This ongoing study should give new and important insights in the function of this class of RNA binding proteins for the biology of neural stem cells during embryonic forebrain development.

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Glial differentiation in the developing dentate gyrus of reeler mice

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The dentate gyrus is the primary entrance for incoming signals from the entorhinal cortex to the hippocampus and contributes to the formation of memories. Among the molecular cues that regulate the morphogenesis of the dentate gyrus during development is Reelin, a secreted extracellular matrix molecule that induces a signal transduction cascade in responsive neurons involving the lipoprotein receptors ApoER2 and VLDLR, the cytoplasmic adapter molecule Dab1, Src family nonreceptor tyrosine kinases and phosphatidylinositol 3-kinase. Absence of Reelin protein in the spontaneous mouse mutant reeler leads to a failure of dentate granule neurons to form a distinct cellular layer and to a pronounced malformation of the postnatal GFAP-positive radial glial scaffold (Foerster et al. 2002). In particular, GFAP-positive cells in the developing dentate gyrus of reeler mice mostly lack long radial processes and morphologically resemble mature glial cells (Weiss et al. 2003). To examine whether dentate radial glia prematurely transform into astrocytes in mice with defective Reelin signalling we compared the differentiation of dentate precursor cells. We observed no difference in the glial maturation of astroglial cells in wildtype and mutant mice both in vivo and vitro, which we monitored by immunocytochemical detection of different astroglial marker proteins. In line with this observation, addition of recombinant Reelin to acutely dissociated dentate gyrus cells did not alter the expression pattern of astroglial differentiation markers. In summary, our data indicate that the time course of glial transformation of precursor cells is not altered in the dentate gyrus of reeler mice. However, we observed a slight increase in the total number of astrocytes generated at different developmental time points in the dentate gyrus of mutant mice.

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J Comp Neurol. 19;460(1):56-65

A crucial role for primary cilia in cortical morphogenesis

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Primary cilia are important sites of signal transduction involved in a wide range of developmental and postnatal functions. Proteolytic processing of the transcription factor Gli3, for example, occurs in primary cilia, and defects in intraflagellar transport (IFT), which is crucial for the maintenance of primary cilia, can lead to severe developmental defects and diseases. Here we report an essential role of primary cilia in forebrain development.

The recessive mutation *cobblestone* (*cbs*) was identified in an ENU- based mouse mutagenesis screen designed to identify defects in early neurogenesis. The *cbs* mutation is distinguished by cortical "heterotopias", appearing at 10.5 d.p.c as protrusions from the pial surface of the dorsal forebrain. *cbs* mutants show defects in the formation of the dorsomedial telencephalon, including the formation of ectopic evaginating folds within the cortex that lead thereby to heterotopia formation. Later embryonic stages show a failure to form superficial cortical layers such as the subplate, the cortical plate and the marginal zone. *In situ* hybridization analysis indicated the formation of dorsal telencephalic structures such as the choroid plexus and the cortical hem, but not of the hippocampal anlage, and the morphology of dorsal structures was severely disturbed. Although the development of the ventral forebrain appeared largely normal, the formation/maintenance of proper boundaries in both the dorsal-ventral and rostral-caudal axes was compromised. We also observed misrouting and defasciculation of nerves arising from the trochlear, oculumotor and trigeminal mesencephalic nuclei. Standard genetic mapping and complementation analysis identified *cbs* as a hypomorphic mutation in the *Ift88* gene. Northern blot, real time RT-PCR, and Western blot analysis showed a 70-80% decrease in levels of both *Ift88* mRNA and protein, but the mRNA sequence was normal, indicating a mutation in a transcriptional regulatory site. Ift88 is a protein that is localized to cilia and plays a key role in intraflagellar transport (IFT), indicating that the developmental abnormalities in the *cbs* mutant reflects a defect in IFT.

Many of the phentoypes seen in the *cbs* mutant phenocopy the abnormalities seen in the *Gli3* mutant forebrain, and we show that Gli3 proteolytic processing is reduced, leading to an accumulation of the full length isoform. *In situ* hybridization for *Ptch1*, a downstream target of Shh signalling, indicated an increase of expression of the gene in the ganglionic eminences of the *cbs* mutant. The upregulation of *Ptch1* and also of *Gli1*, another downstream target of Shh signalling, was confirmed by real time RT-PCR. However, luciferase assays on *cbs* fibroblast cultures showed an inability to respond to acute Shh signalling. In addition, by *in situ* hybridization we observed an upregulation of *Wnt7b*, *Wnt8b*, and *Axin2* expression, the latter a readout of canonical Wnt signalling. Interestingly, the ultrastructure and morphology of ventricular cilia in the *cbs* mutant remains intact. Together, these results indicate a critical role for ciliary function in the developing forebrain. In addition, our data suggest that cortical defects like those seen in the *cbs* mutant could be involved in the mental retardation seen in ciliopathies such as Bardet-Biedl, Meckel-Gruber, Joubert, and oral-facial-digital type I syndromes.

Expression pattern of the 473HD epitope on dividing cortical progenitors: a novel evidence for regulation of neuron generation during corticogenesis

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The establishment of the cell heterogeneity in cortex begin at the early stages of embryogenesis and is orchestrated by intrinsic and extracellular signals. The processes involving in the generation of particular cell types are not completely understood. Since the 473HD epitope, a sulfation-dependent motif of chondroitinsulfate-glycosaminoglycans (CS-GAGs) is expressed on cycling neural stem/progenitor cells (NSPCs) at the early neurogenesis, it seemed promising to investigate whether the 473HD-expressing cells are involved in development of cellular heterogeneity. The first clue came from observation of the 473HD expression patterns to the ventricular dividing NSPCs in vivo showing that the perpendicular division of NSPC is closely associated with an equal 473HD epitope partitioning, while most of horizontally divided NSPC have an unequal 473HD distribution. Moreover, the expression of the 473HD epitope on NSPCs throughout mitosis and cytokinesis is closely associated with the generation of progenitors by symmetric and asymmetric divisions, while only a small fraction of newborn neurons was positive for 473HD. Because the NSPC heterogeneity in the developing cortex is given by the simultaneously existence of multipotent and lineage-restricted progenitors, we reasoned to enrich the 473HD+ cells by immunopanning and examined their progeny in details. According to the peak of neurogenesis, almost all 473HD+ cells signified the expression of Nestin, BLBP and FGFR. Interestingly, 15% of selected cells were identified as Tbr2+ intermediate progenitors (IPs) that revealed an exclusive co-expression of FGFR and EGFR. The predominant expression of FGFR on cycling 473HD+ cells can explain their dependency on FGF-2 rather than on EGF for cytokine-dependent proliferation. Furthermore, FGF-2 and EGF could differently modulate the choice of the 473HD+ precursor fate and lead to the generation of daughter cells with different phenotype. If daughter cells were generated in FGF-2-containing media, these had a higher probability of radial glia identity and occurs less frequently the expression of Tbr2 than EGF-driven ones. Hence, both growth factors could contribute to the temporal choices in the development of 473HD+ NSPC into early proliferative or later restricted progenitors. This is not entirely surprising, when considered with the fact that during cortical development, the proportion of 473HD+ cells declined at to half between E13 and E18, which fits well the predicted behavior for NSPCs because, as development proceeds, an increasing fraction of NSPCs is expected to leave the cell cycle and to become restricted. In order to test this assumption, we examined whether selective removal of CS-GAGs by a bacterial enzyme Chondroitinase ABC (ChABC) may be initiated the fate switch of the 473HD+ progeny. Thus, the digestion of the CS-GAGs caused a reduction of the cycling cell pool and their division number. In effect, the ChABC-treatment affected the FGF-2-responsive, but not the EGF-responsive 473HD+cells in term their proliferative behavior. More important, the addition of ChABC unaffected the generation of IPs, while the population of Tbr2-positive cells seems to expanded by terminal division of 473HD+ NSPCs. Altogether, our data suggested that CS-GAGs are crucial for expand and the spatiotemporal maintain of FGF-2-sensitive NSPCs by the simultaneous suppression of their functional maturation into restricted progenitors at the peak of neurogenesis in cortex.

Histone deacetyltransferase-mediated control of forebrain neurogenesis: Analyzing the role of specific HDACs through RNA interference

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The development of the nervous system in mammals is a complex and tightly regulated procedure that needs to take place in a highly coordinated manner. The expression of developmentally-relevant genes has to occur in a temporally and spatially regulated pattern in order to ensure correct development, positioning and differentiation of all the cell types of the nervous system. Histone deacetylases (HDACs) are a family of histone-modifying enzymes that lead to the compaction of chromatin and subsequent modification of gene transcription. They were recently associated with the control of both neuro- and astrogliogenesis in the developing brain. Here, the role of specific HDACs in the development of neural cells in the mouse brain is adressed.

Many HDACs are expressed in differentiating neural progenitor cells, therefore trichostatin A (TSA) was used to inhibit all class I and II HDACs. Inhibition of class I and II HDACs with TSA in *in vitro* differentiating neural precursors derived from embryonic striatum led to a dramatic reduction in neurogenesis (Shaked *et al*., PLoS ONE 2008). To further investigate this finding, several different approaches have been taken. Firstly, the expression of HDAC-regulated candidate genes responsible for controlling neurogenesis has been examined through real-time RT-PCR. Since the BMP2/4 signaling pathway had previously been implicated in the HDAC-mediated control of neurogenesis, the expression of genes encoding the corresponding growth factors, receptors, and downstream signaling proteins was analyzed. Upon HDAC inhibition with TSA, the expression levels of *Bmp2*, *Stat1*, *Stat3*, *Ngn1* and *Ngn2* were upregulated in cultures of *in vitro*-differentiating neural precursors from both the cortex and striatum. In contrast, the expression of *Smad7*, an inhibitor of bone morphogenetic protein 2 /4 (BMP2/4) signaling, decreased as a result of HDAC inhibition. These findings suggested that HDACs control neurogenesis by inhibiting the BMP2/4 signaling pathway, with the additional involvement of JAK/STAT signaling and the Ngn transcription factors. Secondly, in order to ascertain which HDAC family members influenced neurogenesis, the expression of individual HDAC genes was inhibited via RNA interference. Expression of HDAC 1, 2, 4 and 5 could be effectively downregulated, as seen on the mRNA level. The effect of the knockdown both on the protein level and on neurogenesis and astrogliogenesis will be elucidated and presented. Finally, the expression patterns of individual HDACs in developing brain tissue will be presented.

Functional analysis of Svet1 (Unc5h4) gene in the developing mouse neocortex

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Cerebral cortex is a highly organised structure that consists of many different neuronal cell types. In the mature cortex these cells form six different layers. All cortical projection neurons are derived from two proliferative zones: the cortical ventricular zone (VZ) and subventricular zone (SVZ). Svet1 cDNA was found to be specifically expressed in subpopulation of cells of SVZ but not VZ (Tarabykin et al., 2001) and therefore could be a marker of cells born in SVZ. This subpopulation of Svet1-expressing cells was designated UL2 (Upper Layer) cells. Svet1 is a non-coding RNA which is transcribed from intron 1 of the Unc5h4 gene. The Unc5h4 is a member of the netrin receptor family, which are known to be involved in axon navigation, cell migration and apoptosis. To analyse functional importance of the Unc5h4 gene in the development of neocortex we generated Svet1 knockout mouse line. Additionally we performed “gain-of-function” experimentsby electroporating Unc5h4 cDNA in utero into cortical cells. Phenotype analysis of both loss-of-function and gain-of-function experiments will be presented.

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shRNA-mediated knockdown of TNAP affects proliferation and differentiation in adult neural stem cell cultures

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During adult neurogenesis, multipotent GFAP-expressing neural stem cells (NSC) generate mitotically active progenitors which finally differentiate into mature neurons and glial cells. These transitions are controlled by signalling cues from the stem cell niche. Previous publications implicate signals acting on nucleotide receptors in this process (Mishra et al. 2006) and demostrate the presence of the ectonucleotidase triphosphate diphosphohydrolase 2 (ENTPDase2) and the tissue nonspecific alkaline phosphatase (TNAP) in neurogenic regions of the adult mouse brain (Braun et al. 2003, Shukla et al. 2005, Langer et al. 2007). To further investigate the role of nucleotidases, we used the transgenic mouse line *Tg(GFAP-TVA)5Hev/J* which harbours a copy of the quail *tva-receptor*-gene controlled by a 5 kbp-fragment of the human *GFAP*-promoter. Cells expressing the tva-receptor are highly susceptible to infection with the avian retrovirus RCAN(BP)A. Generating NSC-cultures from transgenic mice we are able to infect more than 50% of the cells with the RCAN-virus. For a specific and stable knockdown of ENTPDase2 and TNAP, we introduced shRNA-expression cassettes into the viral vector, thereby abrogating the associated protein function. We show that the knockdown of TNAP reduces cell proliferation as well as the differentiation of neurons and oligodendrocytes. In general, our experiments demonstrate the advantage of retroviral infection over prevalent transfection protocols and open the prospect of reliably establishing stable gain-of-function and loss-of-function conditions in highly proliferative NSC-cultures. Furthermore, they demonstrate a functional role of TNAP in the control of adult neurogenesis.

Sonic hedgehog is a polarized signal for motor neuron regeneration in adult zebrafish.

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In contrast to mammals, the spinal cord of adult zebrafish has the capacity to re-initiate generation of motor neurons form ependymo-radial progenitor cells after a lesion. During development the ventrally expressed morphogen sonic hedgehog (shh) polarizes the ventral neural tube, leading to discrete expression domains of specific transcription factors. This, in turn, is necessary for motor neuron development. Here we show that ventral neural tube markers (shh, nkk6.1, olig2, pax6) are expressed at low levels in ependymo-radial glial cells also in the unlesioned adult spinal cord in dorso-ventral expression domains that closely match those in the developing neural tube. After a lesion of the adult spinal cord, the central canal expands and expression of these markers is increased in proliferating ependymo-radial glial cells. However, the spatial relationship of expression domains is retained. Intraperitoneal injection of cyclopamine, an inhibitor of shh signaling, during regeneration significantly reduced the number of proliferating ependymo-radial glial cells and the number of regenerated motor neurons. Thus ependymo-radial glial cells of the adult zebrafish spinal cord retain the ventral polarity of the developing neural tube and shh signaling is essential for motor neuron regeneration in adult zebrafish.

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Expression and functional relevance of RPTPß/ζ Isoforms in progenitors of the developing visual retina

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Differentiation and morphogenesis of neural tissues involve a diversity of interactions between neural cells and their environment (Garwood et al., 2002). The receptor protein tyrosine phosphatase ß/ζ (RPTPß/ζ) is expressed in four splice variants two of which are secreted into the extracellular matrix whereas the other two forms represent transmembrane receptors with tyrosine phosphatase activity. Phosphacan, one secreted variant and the long receptor contain chondroitin sulfate glycosaminoglycan (GAG) chains. A third splice variant, the short RPTPß/ζ receptor form lacks the sequence that carries the GAG attachment sites. The fourth isoform, called Phosphacan Short Isoform (PSI) comprises approximately a third of the Phosphacan N-terminus. In the CNS, Phosphacan and the RPTPß/ζ receptors display restricted spatiotemporal expression patterns, which suggests potential roles in various developmental processes, including cell migration, differentiation, synaptogenesis, synaptic function and myelination. Recent studies revealed that the RPTPß/ζ isoforms are differentially expressed in the developing retina (Klausmeyer et al., 2007) but the particular cell type and putative functions during development remain to be elucidated. A special interest is focussed on the DSD-1-epitope that represents a unique chondroitin sulfate structure of RPTPb/z long and Phosphacan. Recently, it could be shown that this epitope is surface-expressed on cycling radial glia in germinal zones during forebrain development as well as on multipotent neural stem/progenitor cells derived from the adult subventricular zone (Von Holst et al., 2006). In the present study immunhistochemical analysis revealed that the Phosphacan/ RPTPß/ζ is expressed by actively cycling cells in the outer neuroblastic layer (ONBL) at different stages of retina development. It could be displayed that at early embryonic stages Pax6 and Nestin-expressing progenitor cells are immunopositive for KAF13, an antibody which recognizes all four RPTPß/ζ isoforms, and for mAb 473HD that reacts with the 473HD-epitope. To further quantify and characterize the RPTPß/ζ-expressing cell population, we established a culture system for retinal stem/progenitor cells in mice, called “retinospheres”. Immuncytochemical analysis of acutely dissociated cells from E13.5 to E18.5 revealed that 30% of 473HD-epitope-expressing cells actively proliferate. At E13.5 half of cells are Nestin-positive progenitors. This amount decreases at E15.5 and subsequently became increased at E18.5.

In summary, a high proportion of 473HD-immunopositive cells represent an actively cycling stem/progenitor cell population. This corresponds to previous studies in the developing and adult forebrain. Nevertheless, we could also detect a small population of more differentiated cells expressing RPTPß/ζ, dependent on the developmental state of the retina. These results indicate potential functional roles during proliferation, differentiation or maturation. Knockdown experiments using siRNAs *in vitro* and *in vivo* in a Whole Embryo Culture (WEC) system will give us further insight into the influence of RPTPß/ζ on retinal development.

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Protein-Tyrosine Phosphatase MEG2 is Spatially and Temporally Regulated in the Developing Retina and is Expressed in the Retinal Stem Cell Niche

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Protein-tyrosine phosphatases (PTPases) appear to coordinate many aspects of neural development, including cell proliferation, migration and differentiation.
To date, we focus on the potential role of the intracellular protein-tyrosine phosphatase MEG2 in the early embryonic mouse retina. Using quantitative real-time PCR and Western Blot analysis our previous studies reveal, that MEG2 is strongly expressed in the early developing retina (E13). In contrast, in the late embryonic (E15/E18) and postnatal (P0-P20) retinal development MEG2 appeared downregulated on mRNA- and protein-level. In double immunolabeling of PTP-MEG2 with BrdU, Ki67, Nestin and ß-III-Tubulin we demonstrate for the first time that MEG2 is strongly expressed by proliferative retinal stem/-progenitor cells and by postmitotic newborn retinal neurons. Furthermore, using an *in vitro* retinosphere differentiation assay we could demonstrate the *in vitro* expression of MEG2 in retinospheres. In this assay, colocalisation studies revealed that the expression of MEG2 is mainly restricted to Pax6-positive and ß-III-Tubulin immunoreactive cells. Taken together, these observations suggest that the intracellular protein-tyrosine phosphatase MEG2 plays an essential role in a variety of developmental processes such as in retinal progenitor/stem cell proliferation and differentiation.
To investigate the potential functional role of PTP-MEG2 in more detail, especially in retinal stem cell/progenitor cells, studies in PTP-MEG2 knock-out mice (Wang et al., 2005) are in progress. Furthermore, the use of short-hairpin plasmids, which should knock down MEG2 expression as well as the use of MEG2-overexpression plasmids are planned.
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After traumatic brain injury cells of the oligodendroglial lineage differentiate into protoplasmatic astrocytes

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Acute brain injuries like ischemic stroke or traumatic lesions of the cortex induce fast neuronal and glial cell death. Secondary injury processes involve activation of different cell types like astroglia, oligodendrocytes and microglia cells. A complex and yet not understood sequence of cellular responses initiate functional recovery after the neurodegeneration process.

The use of transgenic mouse models with green fluorescent astrocytes and red fluorescent oligodendrocytes allows the direct visualisation of cellular responses in acute injuries. After cortical stab wounds or middle cerebral occlusion (MCAO, the animal model of ischemic stroke). we were able to identify reactive astrocytes and oligodendrocytes in close proximity to the lesion site. Three days after a lesion glial cells appeared that displayed, both, astro- and oligodendroglial properties recognized by a simultaneous expression of EGFP and DsRed1. We named these cells AO cells for their astroglial and oligodendroglial characteristics due to the simultaneous activity of the astroglial GFAP and the oligodendroglial PLP promoter. Since AO cells could be labelled by oligodendroglial markers like Olig2 and Sox10, but not for neuronal markers or those demarcating mature astrocytes, we classified them as oligodendroglial progenitor cells. Since AO cells disappeared about 15 days after the injury, we could not follow their fate in the double-transgenic fluorescent mice. Therefore, we developed a mouse model in which the transient signal, i.e. co-activity of GFAP and PLP promoter upon injury, could be switched into a permanent one. We took advantage of the functional complementation of split Cre DNA fragments as coincidence detectors and generated transgenic mice in which N- and C-terminal Cre fragments (NCre and CCre) were targeted by the GFAP and PLP promoter. In cells of these transgenic mice (crossed to a ROSA26 reporter mouse) the fragments complemented each other when both promoters were simultaneously active. The functional Cre recombinase with reconstituted enzyme activity modified DNA sequences with flanking loxP sites. Using these new transgenic animals we could show that after acute injuries AO cells differentiate into protoplasmatic GFAP-positive astrocytes *in vivo*.

The novel transgenic mice with split-Cre expression allowed us to show a coincident activity of two glial promoters *in vivo* for the first time. Furthermore, we could demonstrate that after acute brain trauma oligodendrocyte lineage cells can differentiate into protoplasmic astrocytes.

The identification and cell biological characterization of the neural stem/progenitor cells throughout hippocampus at early stages of embryonic development

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Intense interest of this study has focused on the unique features of the hippocampus (HC) development because it continues to generate new neurons into adulthood. HC is constructed using a developmental plan unique within the CNS. Unlike other cortical regions, only one fraction of mitotic progenitors seems to migrate from hippocampal subventricular zone (HSVZ) into the dentate notch, where they establish a proliferative zone of dentate gyrus and retain the ability to generate neurons. Hence, progenitor diversity and their fate allocation are determined in developing HC at very early stages. Despite many years of study, the cell biological properties of the progenitor subsets throughout hippocampal neuroepithelium at begin of their formation are not well understood. In this study we identified the cell population within HSVZ and the dentate notch at E13 *in vivo*, which is identified by the expression of known progenitor markers, such nestin, LeX and BLBP. Important to note is the fact that these cells are exclusively designated with the 473HD-epitope (also known as DSD-1-epitope) that is expressed in CNS germinal zones, including subpopulation of neural stem cells with radial glia-like properties. A developmental analysis revealed that the prominent expression of the 473HD$^+$ progenitor cells in the hippocampal formation decreased throughout embryogenesis and could well determined in the SGL of adult HC. These observations prompted us to attempt the enrichment of this cell population from E13 hippocampus via immunopanning with mab 473HD. The immunological analysis of selected cell fraction reveled a highly significant increase in the number of BrdU-incorporated cells, supporting the interpretation that the selective isolated cell population consists of actively cycling, mostly nestin and BLBP- positive radial glia cells. Somewhat unexpectedly, around 80% of selectively isolated cells revealed a strong expression of calretinin at 2hrs after plating. This may reflect the onset of differentiation of radial glia cells into Cajal-Ratzius cells that among radial glia initially populate dentate anlage. Given the fate of Cajal-Retzius cells during neurogenesis in hippocampus, it is not surprisingly that the selected cells after 24hrs in serum-containing medium differentiated into bIIItubulin neurons. Thereby the number of neurons differentiating from the positive-selected cells was significantly increased compared to the yield obtained in negative-selected cell culture. These observations support the view that the 473HD-positive population of hippocampal progenitors can generate neurons throughout developing dentate gyrus. Most important, the immunoselected cell population contained also cells that exhibit the cardinal stem cell features of self-renewal and multipotency. In light of the heterogeneity of neural stem cells regarding their responsiveness to growth factors, the positive-selected cells give rise to neurospheres in presence of FGF-2 in culture medium, and not in response to EGF. Vice versa, the negative-selected cells generated significantly more neurospheres in EGF than in FGF-2-containing medium. Hence, in this study we identified and characterized the subpopulation of the FGF-sensitive stem/progenitor cells that populate the germinal regions of embryonic hippocampus. Since neurodegeneration within the hippocampus occurs in a variety of neurological disorders, understanding the development of the hippocampal stem/progenitor cells is critical for the advancement of novel therapeutic strategies to prevent or reverse neurodegenerative disorders.

Levels and regional expression patterns of major histocompatibility complex (MHC) class I genes in the brain of the common marmoset monkey (*Callithrix jacchus*)

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A crucial component of the vertebrate immune system is the major histocompatibility complex (MHC) gene family. It encodes cell-surface transmembrane proteins involved in the presentation of peptides derived from proteolytic processing of pathogens to T lymphocytes. Recent research in rodents and cats has implicated these molecules in synaptic plasticity processes in the developing mammalian visual system as well as in adult synaptic plasticity (Corriveau et al., 1998; Huh et. al, 2000). To extend these findings, we investigated the expression of MHC class I genes in the brain of a non-human primate, the common marmoset monkey (*Callithrix jacchus*).

In situ hybridization with probes detecting Caja-G (a classical MHC class I gene of marmosets) and beta-2-microglobulin (smaller subunit of the MHC class I dimer) revealed a strong expression in the hippocampal formation as well as in substantia nigra of adult animals. Furthermore, examination of silver grain distribution indicated that Caja-G expression is predominantly neuronal. Strong expression of Caja-G and beta-2-microglobulin was also observed in the neurons of the visual cortex, especially in layers IV and VI. Moreover, immunohistochemistry with different specific antibodies confirmed that MHC class I protein expression is neuronal. We have previously shown that OX-18, an antibody raised against the conserved residues of rat MHC class I molecules, stained strongly neurons in the marmoset substantia nigra and the hippocampus (Rölleke et al., 2006). A similar staining pattern revealed Q1/28, an antibody which targets the most conserved domain of the human MHC class I molecules and cross reacts with the marmoset MHC class I proteins. In summary, these results demonstrate that both the levels and the regional expression of MHC class I genes in the brain is well conserved from rodents to non-human primates and may indicate their potential role in brain development and neuronal plasticity.

Formation of GABAergic synapses occurs without the involvement of dendritic protrusions

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Synaptogenesis and the role of dendritic protrusions in this process is a well–studied phenomenon for glutamatergic synapses. Much less is known about the formation of GABAergic synapses which are located predominantly on the dendritic shaft. Here we used two–photon laser–scanning microscopy to examine contact formation between GABAergic axons and dendrites of CA1 pyramidal cells. We used hippocampal slice cultures of transgenic mice that express Green Fluorescent Protein (GFP) in 30-50 % of hippocampal GABAergic interneurons (López-Bendito et al. *Cereb Cortex* (2004) 14: 1122-1133). The postsynaptic neurons were filled with the red fluorescent dye Alexa 594 through a patch pipette.

Dendritic protrusions can distinguish and select between glutamatergic and GABAergic boutons. In contrast to contacts with glutamatergic boutons, which can be long–lasting, the contacts of dendritic protrusions with GABAergic boutons were always short–lived. Similarly, contacts made by GABAergic axonal protrusions were always transient. New putative GABAergic synapses were formed exclusively by new boutons appearing at pre–existing axon–dendrite crossings without the involvement of any dendritic or axonal protrusions. New GABAergic boutons formed synapses within several hours, as judged from post-hoc immunostaining for pre- and postsynaptic proteins. These findings imply that fundamentally different mechanisms underlie the generation of GABAergic and glutamatergic synapses.

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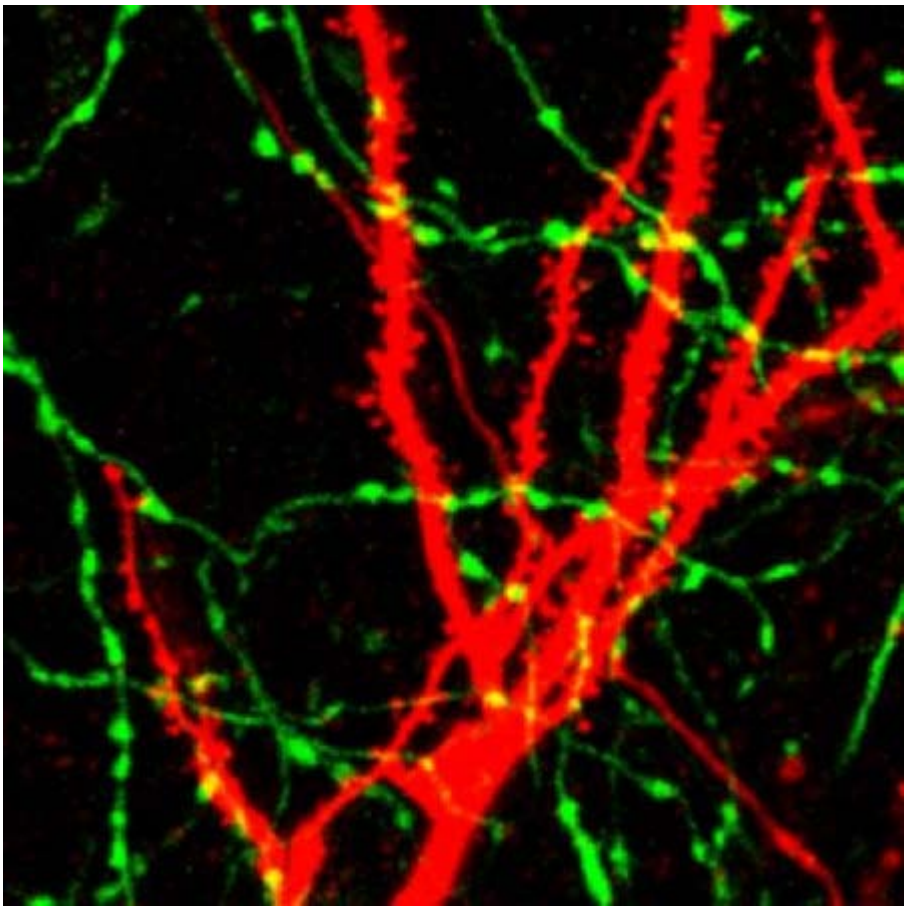
Figure: GABAergic axons (green) cross and make synapses onto dendrites of CA1 pyramidal neurons (red).



The actin binding protein profilin1 is critical for mouse CNS development

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Profilins are small, monomeric actin binding proteins essential for actin polymerization that were implicated in dendritic spine plasticity in the mature CNS. Moreover, analyses of *Drosophila* mutants revealed a critical role of profilins in the control of axonal outgrowth and nervous system development. Two profilin isoforms are expressed in the mammalian CNS: profilin1 with an almost ubiquitous expression pattern and the predominantly CNS-specific profilin2. However, phenotype analysis of $Pfn2^{-/-}$ mice demonstrated a critical role of profilin2 in synaptic function, while it is rather dispensable for CNS development.

As $Pfn1^{-/-}$ mice die during early embryonic development, a conditional approach is required for studying the neuronal function of profilin1. We generated such a mouse model ($Pfn1^{flx/flx}$) and crossed it into the Nestin-cre background resulting in effective CNS specific deletion of profilin1 starting around embryonic day 10.5. $Pfn1^{flx/flx,Nestin\text{-}cre}$ mice are viable and indistinguishable from their $Pfn1^{flx/flx}$ littermates by mere observation but display massive histological alterations. In $Pfn1^{flx/flx,Nestin\text{-}cre}$ mice fiber tracts like the corpus callosum or the anterior commissure are less pronounced. Furthermore, both cortex and cerebellum are smaller in size and the hippocampus shows an anterior displacement. While the cytoarchitecture of the neocortex is not affected, Purkinje cells and Bergmann glia of the cerebellum are less organized in mutant mice and show morphological defects.

Taken together, our data revealed a critical role of profilin1 in CNS development. Ongoing biochemical investigations as well as analysis of primary cell culture will clarify the molecular base of the $Pfn1^{flx/flx,Nestin\text{-}cre}$ phenotype.

Development of the nitric oxide/cyclic GMP signalling pathway in the nervous system of the locust embryo

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Nitric oxide (NO) is a transcellular messenger molecule with various functions in the adult nervous system and elsewhere. In addition, there is growing evidence for the involvement of NO signalling in nervous system development in insects. In the locust embryo, NO plays a role during neuronal migration in the developing enteric nervous system (Haase and Bicker, 2003), and for peripheral pioneer neuron outgrowth (Seidel and Bicker, 2000). In the central nervous system (CNS), NO promotes regeneration of injured axons (Stern and Bicker, 2008). Thus, it appears not unlikely that there is a developmental role for this pathway also during development of the ventral nerve cord.

In the present study, we traced the development of the NO-cGMP system during embryogenesis in whole mounts using various cytochemical techniques. We visualized NO-sensitive neurons by cGMP-immunofluorescence after incubation with an NO-donor in the presence of the cGMP-activator, YC-1 and the phosphodiesterase-inhibitor, IBMX. Cells of the ventral nerve cord respond to NO exposure as early as 37% embryogenesis.

Using the NADPH-diaphorase technique, we identified somata and neurites of possible NO-synthesizing cells in the ventral nerve cord. Some, but not all of these cells, stained with a universal antiserum against a conserved region of NO synthase (NOS) protein. The first NADPH-diaphorase positive cell bodies could be detected around 48% of embryogenesis. The number of positive cells increased with time to reach the full complement of cells visible in the adult CNS at 80%.

The NADPH-diaphorase reaction only indicates the possible presence, but not the activity of NOS. Since L-citrulline is a by-product of NO synthesis, antisera against citrullin can confirm the presence of NOS, and in addition indicate the physiological activity of the enzyme. When applied to whole mount embryonic ventral nerve cords, citrulline-immunolabelling was present in varying subsets of NADPH-diaphorase positive cells, but before 80% embryogenesis, the staining was very variable and often weak. In a regeneration paradigm however, where one of the two connectives between ganglia had been crushed in two places, strong, reliable staining was observed as early as 60% embryogenesis. Citrulline immunolabelling became visible 30 minutes post crush and persisted at least until 24 hours post crush in severed distal segments in the injured connective, whereas staining was absent or very weak on the untreated side. Inhibition of NOS by L-NAME, but not its inactive enantiomere D-NAME, completely abolished citrulline-immunofluorescence. Thus, citrulline-immunolabelling appears to reflect specific activity of NOS. These results are supported by data showing that in adult brains citrulline-immunoreactivity is found in neuronal cells that stain for NADPH-diaphorase, NOS, and have been shown to release NO.

Our results suggest that in the younger embryo, NOS, although present may not always be constitutively active, or only at a low level which is hard to detect using citrulline-immunostaining.

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Stern M., Bicker G. (2008) Dev. Neurobiol. 68:295-308

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Carbon monoxide organises NO-dependent neuronal chain migration.

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The enteric nervous system (ENS) of insects is a useful model to study cell motility. Using small molecule compounds to activate or inactivate biosynthetic enzymes, we demonstrate that the gaseous messenger molecules carbon monoxide (CO) and nitric oxide (NO) regulate neuron migration in the locust ENS. In the embryonic grasshopper, the nerve plexus of fore- and midgut including the enteric ganglia are formed by neurons that arise in three neurogenic zones on the stomodeum (Ganfornina et al. (1996), J. Comp. Neurol. 372:581-596). The midgut plexus in particular is established from neurons of the ingluvial ganglia that travel in a mode of chain migration on four defined pathways. During their migration on the midgut, the enteric neurons express immunoreactivity for NO-dependent cGMP as well as for carbon monoxide releasing heme oxygenase (HO) enzymes. While inhibition of nitric oxide synthase or application of the extracellular gaseous molecule scavenger hemoglobin reduce cell migration, we have shown that inhibition of HO by metalloporphyrins promotes enteric neuron migration in intact locust embryos (Knipp and Bicker, in press). Thus the blocking of enzyme activity results in a gain of function. The suppression of migratory behavior by activation of HO or application of a CO-donor strongly implicates the release of CO as an inhibitory signal for neuron migration in vivo. Moreover, we were able to visualise these findings by time lapse video microscopy. Apart from enhancing neuronal motility, pharmacological blocking of CO release leads also to a significant divergence of the cell bodies along the migratory pathway. Thus CO may provide a slow down signal to regulate the intercellular distance in the chain of migratory neurons.

The cellular distribution of NO and CO biosynthetic enzymes, together with the results of the chemical manipulations in whole embryo culture suggest CO as an intracellular modulator of transcellular NO signalling. We propose that CO and NO interact to organise the timing of the posterior directed cell migration along the midgut.

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Pre-synaptic Vesicle Exocytosis in Human Model Neurons Generated by Spherical Aggregate Culture Method

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The NT2 teratocarcinoma cell line is derived from human testicular cancer that has been induced to differentiate into fully functional postmitotic neurons; however, the differentiation process is rather lengthy (up to 2 months). We, therefore, established a new cell culture protocol using a spherical aggregate method that shortens the length of differentiation processes and produced postmitotic neurons (hNT2) that expresses various neuronal markers such as MAP2, tau and functional NMDA receptors (1, 2). Here, we report that the hNT2 neurons generated by spherical aggregate method express typical pre-synaptic proteins (synapsin and synaptotagmin) along the neurite. The expression of these proteins increases with the age of neurons in culture, indicative of synaptic maturation processes particularly between 1 and 2 weeks.We followed the kinetics of vesicle exocytosis in real time using a fluorescent styryl dye, FM1-43. Upon stimulation the neurons are labeled with FM1-43 and about 60-70 % of the dye is released within 5 minute, which implicates that hNT2 neurons undergo active vesicle recycling. The specificity of FM1-43 labeled punctuate staining was confirmed by co-localizing with synapsin immunoflourescence staining. Taken together our data indicate that the hNT2 neurons undergo pre-synaptic neurotransmitter release, and can be utilized as a model of human neurons to study synaptogenesis and vesicle recycling.

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SynCAM1 Overexpression Increases the Number of Excitatory Synapses, *in vivo*

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Synapses are asymmetric cell junctions with precisely juxtaposed presynaptic and postsynaptic sides. The assembly of synapses requires the precise initiation and control of cell signaling: Initially, the axon of the presynaptic cell and the dendrite of the postsynaptic cell have to come in physical contact. Many of these initial contacts are transient and will disappear again within minutes. Nevertheless, some contacts will be stabilized, will recruit pre- and postsynaptic machinery and will finally proceed into a synapse maturation phase.

Proteins that span the synaptic cleft and thereby enable communication between the pre- and the postsynaptic cell are known to control synaptogenesis. Our understanding of synaptogenesis, particularly of the late “maintenance phase”, has advanced significantly over the last few years. Still, the mechanisms of initial contact formation of axon and dendrite remain elusive.

The family of synaptic cell adhesion molecules (SynCAMs) comprises four transmembrane proteins that span the synaptic cleft. SynCAMs have been shown to play an important role in synaptogenesis, *in vitro*. Here, we present a transgenic mouse model with selective overexpression of SynCAM1 in neurons. In this model, SynCAM1 overexpression results in a significant increase in the number of excitatory synapses on a structural as well as on a functional level. The mice also show impaired spatial learning and an increase of anxiety-related behavior. On a single-synapse level, it is noteworthy that we neither saw a difference in paired-pulse facilitation (a measure for presynaptic properties) nor in the composition of the receptors on the postsynaptic side. Also, synaptic plasticity was unaltered in long-term potentiation experiments. Finally, basal network properties also remained unaltered in SynCAM1 overexpressing mice.

Taken together, our results show that SynCAM1 induces synaptogenesis but does not modulate the synaptic function itself. Conclusively, other mechanisms are known to control the maturation of new-born synapse, and determine the properties of synaptic transmission in synaptognesis while our data indicate that SynCAM1 might serve as a molecular switch that is instructive for the initial phase of synaptogenesis.

The role of SATB2 and CTIP2 in cortical connectivity and the elucidation of their downstream pathways

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Satb2 belongs to a novel class of transcriptional regulators that control neuronal differentiation by binding to multiple matrix attachment DNA regions (MARS), thereby controlling the transcription of multiple genes (Britanova et al. 2005). Satb2 controls the postmitotic identity of upper-layer (UL) neurons in the neocortex, partially by directly interacting with the upstream promoter region of Ctip2 and repressing its expression (Britanova et al. 2008). Ctip2 is a transcription factor expressed in the deep layer (DL) neurons (layer V) of the neocortex where it is required for the formation of the corticospinal tract. In Satb2 KO mice upper layer neurons fail to acquire their proper identity and as a consequence the corpus callosum fails to form, while some of the Cre expressing UL neurons are misrouted into forming connections normally comprised of DL neurons (e.g. internal capsule, celebral peduncle).
On the other hand, Ctip2 KO mice which appear to have normal callosal projections (e.g. corpus callosum), fail to form cortical connections to the spinal cord and no CSMN axons extent past the pons (Arlotta et al. 2005). Additionally, the zinc finger protein Fezf2 which is also expressed by CSMN and corticotectal projection neurons, appears as early as E8.5 in a pattern consistent with the location of neural progenitors (ventricular zone), suggesting that it might play a role in the initial fate specification of these neurons (Molyneaux et al., 2005). Similarly to Ctip2 mutants, the UL neurons in Fezf2 KO mice develop normally but there is a complete absence of CSMN and other subcerebral projection neurons of layer V. In order to investigate genetic interactions between Satb2, Ctip2 and Fezf2 as well as their role in the cortical connectivity we generated two types of double mutants Satb2;Ctip2 and Satb2;Fezf2. Resulting phenotypes will be presented and discussed.

Calneurons provide a Calcium-threshold for trans-Golgi network to plasma membrane trafficking

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Phosphatidylinositol 4-OH kinase IIIß (PI-4Kß) is involved in the regulated local synthesis of phospholipids that are crucial for trans-Golgi network to plasma membrane trafficking. In this study we show that the calcium sensor proteins Calneuron-1 and -2 physically associate with PI-4Kß, inhibit the enzyme profoundly at resting and low calcium levels and negatively interfere with Golgi-to-plasma membrane trafficking. At high calcium levels this inhibition is released and PI-4Kß is activated via a preferential association with the neuronal calcium sensor-1 (NCS-1). In accord to its supposed function as a filter for subthreshold Golgi calcium transients neuronal over-expression of Calneuron-1 enlarges the size of the trans-Golgi network due to a built-up of vesicle proteins and reduces the number of axonal Piccolo-Bassoon transport vesicles, large dense core vesicles that carry a set of essential proteins for the formation of the presynaptic active zone during development. A corresponding protein knockdown has the opposite effect. The opposing roles of Calneurons and NCS-1 provide a molecular switch to decode Golgi calcium transients and impose a calcium threshold for PI-4Kß activity and vesicle trafficking.

The histone deacetylase SIRT2 in axonal outgrowth and pathfinding

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SIRT2 is an unusual member of the histone deacetylase (HDAC) family acting amongst other substrates on acetylated a-tubulin. It is generally believed that acetylation of microtubules is associated with an increase and deacetylation with a decrease in their stability. As axonal outgrowth and pathfinding are dependent on dynamic rearrangements of the cytoskeleton, including microtubules, we wanted to analyse a potential SIRT2 function in these processes. We show that in neurons, SIRT2 is localised mainly to the soma and significantly less to neurites. Expression of wild type or a constitutively active SIRT2 in hippocampal neurons led to reduced neurite outgrowth and inhibition of ephrinA5-mediated growth cone collapse. The reverse was true for knockdown of SIRT2 using siRNA, whereby growth cones showed a collapsed phenotype even in the absence of ephrinA5. These results underline that SIRT2 plays a role in neurite outgrowth and axonal pathfinding. Furthermore we show that SIRT2 interacts with and is phosphorylated by cylin-dependent kinase 5 (CDK5) a key factor of neuronal migration and neuronal cytoskeleton remodelling. As shown by others, local microtubule stabilization in neurites is the signal specifying the axon during neuronal polarization. As SIRT2 deacetylates tubulin and could therefore change the acetylation status of microtubules we investigate SIRT2 localisation in different stages of neuronal polarity to analyse if SIRT2 is involved in specifying the future axon.

As mentioned above, a-tubulin might not be the sole SIRT2 substrate accounting for SIRT2's effects on neurons, so currently we try to find new SIRT2 brain substrates using deacetylation assays and proteomics for candidate identification.

BDNF/ephrin-modulated neuronal motility relies on SRF-dependent gene expression

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For the correct formation of neuronal projections axon guidance cues play a pivotal role. The refined orchestration of repulsive and attractive molecules enables the axonal growth cone to reach its proper target, where then synapses can be formed. The guidance cue brain derived neurotrophic factor (BDNF) has been demonstrated to elicit branch promoting activity which in vivo might result in e.g. initial overshooting of primary axons beyond their later termination zone. Contrastingly, ephrin-As - stimulating contact-mediated repulsion - have been implicated in axon retraction, a process also known as axonal pruning.

We are interested in the molecular mechanisms of this axon guidance process refining neuronal projections by investigating the interactions taking place between BDNF and ephrin-A signalling. Particular focus is given on potential downstream gene expression programs activated by this crosstalk governed by the Serum Response Factor (SRF). SRF – a transcription factor of the MADS-box family – was previously shown to function in (actin) cytoskeleton dynamics, neuronal migration, axonal pathfinding and synaptic plasticity. We are firstly analysing whether these guidance cues modulate gene expression and secondly whether this occurs in an SRF-dependent manner using forebrain-specific *Srf*-deficient mice. Furthermore we will provide data on neuronal morphology in vitro and in vivo (neurite branching, growth-cone dynamics) these guidance cues have independently from each other and in concert by co-application of BDNF and ephrin-As.

Fast synaptic signalling as a guiding cue for migrating precursors of cerebellar cortical inhibitory interneurons?

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The cells that form the adult nervous system typically originate far from their final positions and migrate there through well defined routes. Interneurons have recently been recognized to follow a long, and often tangential route from the neuroepithelium to their final destinations. Secreted signaling molecules, including classical neurotransmitters, constituents of the extracellular matrix, and direct cell-cell interactions have all been identified as being critical to neural migration. Restricted secretion of morphogens by specifically localized cells and direct cellular interactions between migrating neurons and radial glia are two well characterized mechanisms known to direct migration of radially translocating projection neurons. In contrast, structural or cellular elements which support, and possibly orchestrate interneuronal migration remain yet largely elusive.

To address this issue, we simultaneously analyzed electrophysiological properties and migratory behavior of individual GFP-tagged Pax2 positive precursors of inhibitory cerebellar interneurons in juvenile transgenic mice (p5-8). All cells have been studied in acute brain slices to minimize putative artificial side effects, which may occur under cell culture conditions.

In a first step, we were able to document, by whole cell patch clamp recordings, the functional expression of ionotropic AMPA and $GABA_A$ receptors in Pax2 positive cells. Next, we employed conventional confocal microscopy to achieve high resolution time lapse movies over a period of up to 4 hours in acute slice preparations. Morphological changes of individual precursor cells were observed and corresponding trajectories of their center of mass as well as velocity over time curves were analyzed quantitatively. The most surprising result was derived from a third set of experiments, in which we patched individual, migrating Pax2 positive cells. The mobility of these cells was verified by observing them immediately before patch clamping. In these migrating cells, we found robust, fast rising, spontaneously occurring postsynaptic currents (sPSCs). We demonstrated two types of postsynaptic events. The first was sensitive to the $GABA_A$ receptor antagonist bicuculline and showed a relatively slow current decay with time constants of about 20 ms. Reversal potential analysis revealed that these sPSCs were carried by chloride ions. The second type of sPSCs was sensitive to the AMPA receptor antagonist NBQX. These sPSCs decayed much faster with time constants of about 2-5 ms and reversed at 0 mV, as typical of non-specific cation conductivities. The frequency of miniature PSCs could be increased by application of the calcium ionophore ionomycin.

Taken together, we conclude that Pax2 positive precursor cells of inhibitory cerebellar interneurons receive synaptic input during migration. There are at least two types of afferents, glutamatergic and GABAergic. These data suggest that synaptic contacts may act as cues for proper path finding and/or translocation of immature interneurons in the cerebellum.

Srf mutant mice display Reeler-like phenotypes including hippocampal cell/fibre delamination and aberrant dendritic branching

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The murine hippocampus is a highly organised structure with clearly segregated cell and fibre layers. We are interested how guidance cues regulate gene expression programs to ensure proper assembly of neuronal networks during development.

By using forebrain-specific conditional *Srf* mutant mice we are investigating the role of SRF (serum response factor) - a MADS box transcription factor and major regulator of the actin cytosceleton - in hippocampal development and dendritic branching. In order to visualise hippocampal projections and individual neuron morphology, we crossed *Srf* mutant mice with a mouse strain expressing GFP driven by the *Thy1* promoter (GFP-M line). We observed abnormal dendritic branching of CA1 pyramidale neurons, a dispersed granule cell layer with the segregation between granule cells and commissural/associational fibres being lost and misrouting of mossy fibre axons. These phenotypes are very reminiscent of neuronal abnormalities described for the Reeler mouse mutant, a crucial secreted factor regulating cortical and hippocampal lamination. This encourages us to analyse a potential interaction of SRF and Reelin, with SRF-mediated gene expression being a target of the Reelin signalling cascade. Towards this aim, we employ recombinant Reelin in SRF-dependent reportergene-assays and neurite branching assays in vitro.

Analysis of neuronal membrane dynamics using farnesylated PA-GFP

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Biological membranes play an important role in almost all physiological functions of cells. Their basic structure is a lipid bilayer, a fluid structure, in which proteins are embedded in a highly organized manner. In nerve cells, an axonal and somatodendritic compartment can be distinguished, which each possess unique features with respect to their plasma membrane composition and function. Analyses of differences in the dynamics of axonal and somatodendritic membranes may contribute to a better understanding of their respective functional organization.

To analyze dynamics and fluidity of neuronal membranes, we created a farnesylated photoactivatable GFP (fPA-GFP). It is known from previous studies, that farnesylated GFP integrates into the lipid bilayer of the neuronal plasma membrane. After transfection in PC12 cells, we locally activated fPA-GFP by a UV pulse and followed its distribution over time using time lapse microscopy. The cell body, the middle part of the processes and the process tips were analyzed separately. We report that fPA-GFP dissipated much slower in all compartments compared to a cytosolic control construct. The mobility was further reduced in the cell body as well as in the tip of the process compared to the middle part of processes. A decrease of the fluidity of the membrane, e.g. through lowering of the temperature to room temperature lead to a marked reduction of the mobility, which was most evident in the processes.

To distinguish between the membrane dynamics of the axonal and somatodendritic compartment, lentiviral expression vectors were prepared, where the expression of fPA-GFP was under the control of a neuron-specific promoter. These construct were used to infect E16 primary cortical cultures from mice. We observed that the dissipation of fPA-GFP in the membrane of the cell body was similar for primary neurons and PC12 cells, suggesting that the fluidity of the lipid bilayer is the same in these cells. In contrast, the dissipation of fPA-GFP was much slower in dendrites than in axons of primary neurons, and was in both compartments much slower than in processes of PC12 cells.

The results show that fluorescence photoactivation provides a useful and sensitive method to determine protein mobility within different compartments of the neuronal plasma membrane. It will be interesting to use this method to systemically analyze compartment specific differences in the mobility of different classes of membrane proteins, such as integral and peripherally associated membrane proteins.

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Role of the microtubule associated tau protein in the shaping of neuronal dendrites

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Tau is a microtubule-associated protein (MAP) promoting tubulin polymerisation and stabilising microtubules. Thus tau is thought to be essential for neuronal cell morphogenesis, especially for axonal elongation and maintenance. However, its role in establishing correct axonal connection of brain areas has not been explored. We could show that tau knockout mice establish the ordered whisker representation (barrel array). To explore the role in shaping dendrite trees the three-dimensional branching pattern of Golgi-stained CA1 pyramidal cells was reconstructed. Sholl analysis revealed that dendritic complexity in apical trees is reduced by tau deficiency while basal dendrites remained unaffected. We conclude that the tau protein is dispensable for the establishment of axon connections in the embryonic brain, but it is involved in the regulation of dendritic branching. Currently we are investigating adult neurogenesis, microtubule stability in apical versus basal dendrites, distribution of other MAPs, and the clustering of synapses.

Survival Promoting Peptide/ Y-P30 enhances axon growth by activating Syndecan-3 signaling.

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Survival Promoting Peptide/ Y-P30 is a maternally derived peptide in the fetal brain. During pregnancy it is produced by peripheral mononuclear cells of the vascular system of rats and humans. From maternal blood Y-P30 peptide is imported to the fetal brain where it enriches in the cortical neurons. Previously, we have shown that Y-P30 enhances the survival and neurite outgrowth in thalamic neurons.

The current report focuses to find whether Y-P30 enhances dendrites or axon growth. The studies on neurons from retinal explants revealed that Y-P30 significantly increases the growth of axons. Next in order to understand the molecular mechanisms behind the action of Y-P30, we analysed alternation in gene expression in response to Y-P30 using microarray on cortical organotypic cultures. Interestingly, syndecan-3 was reported to be upregulated in presence of Y-P30. Syndecan-3 is a neurite outgrowth-promoting heparan sulphate proteoglycan. The mRNA expression of syndecan-3 was confirmed to be upregulated in retinal explants. Furthermore, enzymatic digestion of heparan sulphate side chain abolishes the effects of Y-P30 on axon growth.

The analysis of activation of intracellular signaling revealed that similar to syndecan-3, Y-P30 also activates c-src phosphorylation. Furthermore, src and MEK dependent activation of MAPK was observed after Y-P30 stimulation in cortical cultures. Y-P30 also induces localization of beta-actin protein in the growth cone of retinal neuron axon within 15 minutes, similar was observed on activation of syndecan-3 with pleiotrophin.

These evidences suggest that Y-P30 enhance axon growth by upregulating the expression of syndecan-3 and activating the downstream signaling.

In Vivo Imaging of Spontaneous Activity Patterns in the Developing Mouse Visual Cortex

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The development of neuronal networks is regulated by activity dependent mechanisms. Already during early stages of development, most brain areas generate action potentials and synaptic activity even without sensory input. For instance, *in vitro* as well as *in vivo* experiments revealed synchronous network oscillations that occur spontaneously in the developing cortex. This form of activity is believed to be important for the generation of functional networks. However, little is known about the patterns of activation on the cellular level during such network events in the living animal. In addition, how network activity affects neurons on the subcellular and synaptic level in the intact developing brain has not been studied so far. We have started investigating network and synaptic activity patterns in dendrites *in vivo* bycalcium imaging.

To study neuronal activity in cortical networks, we labeled populations of neurons in the visual cortex of anesthetized newborn mice (postnatal days 5–15) with the fluorescent calcium-sensitive dye Oregon Green BAPTA-1 (OGB) by means of “bolus loading”. In a second set of experiments we filled individual cortical neurons with OGB by single cell electroporation. Subsequently, we performed two photon time lapse imaging of network activity (2 Hz, pixel size: 0.2 µm) and dendritic calcium transients (5 Hz, pixel size: 0.2 µm).

Calcium imaging of populations of cortical neurons revealed two distinct modes of synchronous network activity. Recurrent synchronous network activations across the entire field of view (> 150 µm) occurred in all studied ages at frequencies of about 2 events / minute. During synchronous network activity, the neuropil was always active throughout the entire imaged area. In contrast, neuronal cell bodies contributed only intermittently to this network activity. In fact, only a minority of cells was active during each event, even though most cells were repeatedly active during the recording time. We did not observe any immediately apparent spatiotemporal rule of cellular activation. As a second mode of synchronous network activity, we occasionally recorded domain-like patterns in young animals consisting of synchronous activation of only a few cells and the surrounding neuropil (domain size: < 100 µm).

Dendritic calcium imaging demonstrated that cortical neurons exhibited both global and local calcium transients. Local calcium transients were restricted to short stretches (< 10 µm) of dendrite and showed varying durations. They were reminiscent of those calcium transients that regulate neuronal development in the developing retina and hippocampus as shown recently in *in vitro* experiments.

We are currently investigating the various activity patterns both in populations and individual neurons in more detail. In particular, we are focusing on the question how synchronous network activity determines the dendritic activation patterns in developing pyramidal cells and whether these patterns play a decisive role in synaptogenesis.

Global deprivation of brain-derived neurotrophic factor in the CNS reveals area-specific requirement for dendritic growth

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Neurotrophins are essential for multiple aspects of neuronal development and function, such as the maintenance of neuronal survival, regulation of neuronal architecture, and synaptic plasticity. By exogenous application in the developing CNS it was shown that Neurotrophins, upon Trk receptor activation, influence neuronal morphology by modulating both dendritic (for a review see McAllister et al. 1999) and axonal elongation and arborization (Cohen-Cory and Fraser, 1995). However, direct evidence for an *in vivo* role of BDNF in modulating the structure of developing CNS neurons has been difficult to provide. Conventional BDNF mutants, indeed, die within the first 3 weeks. Interestingly, BDNF expression in the hippocampus and cerebral cortex reaches adult levels between the 2nd and 3rd postnatal week. Therefore, a number of mouse lines have been generated using Cre-mediated excision of BDNF within selected brain areas. However, as BDNF is transported anterogradely throughout the brain and imported in the CNS from peripheral sensory ganglia, it has not been possible so far to study the effects of a global deletion of BDNF throughout the CNS.

We generated a new mouse line in which BDNF excision is induced by Cre recombinase inserted in the *tau* locus, characterized by a neuron specific deletion of BDNF at embryonic stages. Measurements with a sensitive immunoassay indicate that at most 5% of control levels of BDNF remain in some areas of the CNS of such animals. These BDNF-depleted animals survive for several months after birth and while the size of their brain is reduced, the effects of BDNF deprivation are surprisingly area-specific. Specifically, the volume of the hippocampus is not significantly affected, while the size of the striatum is reduced by about 35%. Accordingly, a detailed analysis of the morphology of CA1 pyramidal neurons revealed only minimal effects on dendritic length and branching as well as on spine density. On the contrary, the length of dendrites and spine density in medium spiny neurons of the striatum is highly significantly reduced. These results reveal surprising, area-specific requirements for BDNF in modulating the post-natal extension of dendrites. Future experiments will be addressing whether such area-specific requirements for BDNF may be due to intrinsic differences in the responsiveness of diverse neuronal populations to BDNF, possibly consequence of differences in the activation or transport of the specific TrkB receptor.

Specific role of ProfilinIIa as a mediator of structural plasticity in mature hippocampal neurons

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Among actin-binding molecules profilin performs a key function by interacting with G-actin and providing it to the growing filament. Up to four different profilin genes are expressed in phylogenetically disparate organisms as yeast, plants or vertebrates with profilinI being essential and ubiquitously expressed. Interestingly, ProfilinII (PFNII) shows highest expression levels in the brain whereas profilinIII and IV are expressed solely in testis.

Besides binding actin itself profilins are characterized by their interaction with several actin related proteins, among which the ARP 2/3 complex is especially important mediating organization of the submembranous actin network. In addition, two other protein motives are bound by profilins: poly-L-proline (PLP) stretches in proteins of e.g. the WAVE/ WASP family and membrane bound phospho-lipids like phosphatidylinositol-4,5-bisphosphate (PIP2).

Although a variety of interaction partners are known, the role of profilin and especially of the brain specific form PFNIIa in the central nervous system remains poorly understood. Previous evidence suggests both pre- and postsynaptic functions. Specifically, profilins have been shown to effect dendritic spine stability and synaptic plasticity in vitro and in vivo. In addition PFNIIa has been shown to interact with the small GTPase RhoA through the RhoA specific kinase ROCK to affect spine morphology in an activity-dependent manner involving activation of glutamate receptors (Schubert *et al*. 2006).

In this study we used RNAi mediated knockdown of PFNIIa in organotypic hippocampal slice cultures (14 DIV) to investigate the role of the brain specific isoform in dendrite morphology and spine stability of mature pyramidal neurons. We biolistically transfected CA1 cells with a shPFNIIa expression vector and we observed a significantly reduced dendrite complexity as well as lower dendritic spine density suggesting an important role for PFNIIa mediated actin stability in these structures. Moreover, we could confirm that such alterations are specific for the loss of PFNIIa as a coexpression of shPFNIIa and RNAi resistant PFNIIa showed a complete rescue of the phenotype. Furthermore comparable experiments using profilinI coexpressed with shPFNIIa did not show any rescue effects. Experiments using PFNIIa mutants revealed that actin binding but not interaction with PLP are responsible for the PFNIIa dependent maintenance of dendrites and spines.

Our results indicate a PFNIIa specific function independent of PFNI in stabilizing neuronal morphology in mature hippocampal pyramidal cells. Ongoing experiments are aimed at describing whether the stabilizing function of PFNIIa might be regulated in an activity-dependent manner.

MOLECULAR AND CELLULAR CHARACTERIZATION OF METHYLMERCURY AND SELENIUM SYNAPTOTOXICITY IN THE DEVELOPING HIPPOCAMPUS OF RATS AND MICE

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Methylmercury (MeHg) exposure from occupational, environmental, and contaminated foods is a significant threat to public health. MeHg readily passes the brain barrier causing severe psychological and neurological problems. The developing nervous system is particularly vulnerable to the toxin, and although MeHg poisonings have been studied for decades, the molecular components and pathways involved in the cellular pathology are largely unknown. Several studies have demonstrated that selenium can be also neurotoxic, yet protect against the MeHg-induced pathology. The molecular mechanisms involved in the selenium induced neurotoxicity and protection are ill-defined. An aim of this studie is to evaluate the neurotoxic effects of MeHg and selenium on developing neurons, and to identify cytoskeletal and synaptic proteins that may contribute to the neuropathology. Hippocampal primary cell cultures from rats and mice at 14 days were exposed to either MeHg or sodium selenide (Na2Se) (0-50 mM), or in combination, for 24 hours and the number of dead cells determined using propidiumiodide as a marker for cell death. Immunocytochemical quantification of dendritic and synaptic proteins, including MAP2 and synaptophysin, was evaluated. A concentration-dependent increase in mortality was observed with single toxin exposures in either species, and results in dendritic fragmentation, loss of cellular integrity, and dramatic changes in a number of cytoskeletal and synaptic proteins. In contrast, co-exposure with the toxins resulted in maintenance of staining. Toxin-induced changes in the dendritic spines (size, volume, contact to presynaptic structures), as well as synaptic protein expression and localization are also described.
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The IRM proteins and their involvement in optic lobe development

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Visual pathway formation in the fly is independent of experience and takes place during pupal development. We are interested in the mechanisms by which visual neurons of a functional pathway make contact to each other. In this context we focus on a small, evolutionarily conserved group of proteins of the immunoglobulin superfamily. In Drosophila this protein family comprises Irregular Chiasm C/Roughest (IrreC/Rst), Kin-of-irre (Kirre), and their interacting protein partners Sticks-and-Stones (SNS) and Hibris (Hbs). We have named this ensemble of proteins the Irre Cell Recognition Module (IRM). IRM proteins function together in various cellular interactions including myoblast fusion, cell sorting in imaginal discs, axonal pathfinding and target recognition in the optic neuropils of Drosophila. Understanding IRM protein function will help to unravel the epigenetic rules by which visual pathways are formed.

Counter-balancing of Eph forward and ephrin reverse signaling explains topographic guidance of retinal ganglion cell axons

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Topographic projections are a preeminent feature of embryonic brain wiring. The retinotectal projection is the best-studied model for this type of neural connectivity. A crucial leap in understanding its formation was made when graded repulsive ephrin ligands on tectal cells and corresponding graded axon guidance receptors of the Eph family on retinal ganglion cell (RGC) growth cones were discovered. Those findings essentially corroborated the gradient chemoaffinity model of topography formation. Although having considerable predictive power, this model is not fully consistent with the outcome of the stripe assays used to investigate topography formation *in vitro*. In particular, it fails to explain the absence of true topographic differentiality of axonal growth in stripe assays. Moreover, the chemoaffinity model does not include recent experimental evidence: the existence of counter-gradients of Eph receptor and ephrin ligand in retina and tectum and fiber-fiber interactions have been suggested as well. We developed a novel computer model with counter-balancing Eph forward and ephrin reverse signaling to facilitate the understanding of the *in vitro* data and to address the underlying complex interplay of the signals involved. Our model provides topographic mapping which is at the same time robust against perturbations of gradients and tectal size. More importantly, the model predicts a topographic differential behavior, when RGC axons are challenged by a substrate with alternating stripes of ephrin and Eph. Replication of this experiment *in vitro* confirmed this prediction: nasal axons grew on ephrinA2 stripes whereas temporal axons grew on EphA3 stripes. Therefore, we provide evidence for the first time under defined *in vitro* conditions of topographic differential guidance of nasal and temporal axons.

Mapping the 'synaptome' of spontaneous synaptic calcium transients reveals distance dependent patterns of synaptic activation in dendrites of developing hippocampal neurons

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During development, spontaneous neuronal activity occurs in many brain areas and shapes the formation of neuronal networks. Recently, it has been proposed that the development of precise synaptic connections does not only require correlated firing of action potentials of entire neurons, but also specific spatio-temporal patterns of synaptic activation in dendritic segments. However, the dendritic patterns of synaptic activation during spontaneous neuronal activity are currently unknown, mostly because electrophysiological methods do not provide information about the spatial aspects of synaptic function.

In this study we used calcium imaging to investigate the spatio-temporal relationships of spontaneous postsynaptic activity in dendrites of pyramidal neurons in organotypic slices of the hippocampus from developing rats. To visualize the synaptome – the population of synaptic calcium transients from (almost) the entire dendritic tree - we combined high-speed calcium imaging using a CCD camera with piezo-controlled z-stepping. Simultaneously, we performed whole-cell voltage-clamp recordings to measure synaptic currents. We then used custom-made software to detect dendritic calcium transients and to determine their spatio-temporal characteristics.

The majority, but not all dendritic calcium transients were correlated with synaptic currents. Recordings in the presence of APV and NBQX demonstrated that the calcium transients that were correlated with synaptic currents were also glutamate receptor dependent and thus most likely synaptic. Further support for this conclusion came from an independent set of experiments where we elicited local calcium transients by presynaptic electrical stimulation. Stimulated calcium transients reliably occurred at putative synapses.

Many spontaneous synaptic calcium transients occurred simultaneously with barrages of synaptic inputs, which are characteristic for the developing hippocampus (giant depolarizations). We additionally observed that different subsets of synapses were activated during successive barrages. Quantitative analysis of the spatio-temporal patterns of synaptic calcium transients revealed that the correlation of activation between two synapses was dependent on their distance. Specifically, synapses that were located close to each other (< 20 µm) were more likely to be active simultaneously than those that were farther apart from each other.

This finding suggests a local rule for connecting neurons at specific dendritic segments: synapses that are located within the same dendritic segment may be more likely to be stabilized or strengthened if they fire in a correlated fashion. In addition or alternatively, neighboring synapses may be weakened or eliminated, if they are "out of sync". Such a mechanism may serve to establish connections with subcellular precision. Interestingly, this high level of wiring specificity would be expected for independent information processing in individual dendritic segments.

Paternal care is essential for dendritic and synaptic development of pyramidal neurons in the somatosensory cortex

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Sensory stimulation during early development is essential for the adequate functional maturation of sensory systems. During the neonatal phase the main source of sensory stimuli are the parents and siblings. Clinical studies have revealed the educational importance of paternal care, and evidence is accumulating that growing up without father increases the risk of abuse, mental dysfunctions, low educational performance and criminal outcome. More recently, the importance of paternal care is also emerging from studies in a variety of animal models. Using the degu (*Octodon degus*), a biparental rodent as an animal model, we investigate the maternal and paternal contribution to the maturation of brain and behavior. The aim of the present study was i) to quantify the amount of paternal care in relation to total parental investment and ii) to test whether paternal somatosensory stimulation affects dendritic and synaptic development of pyramidal neurons in the somatosensory cortex.

Behavioral analysis included nursing, huddling of mother or father with any pups and licking/grooming any pup by mother or father as well as playing of any pup with mother or father. Because of the specific contribution of paternal care, two groups were compared at the age of 21 days: a) pups reared by both parents and b) pups reared without father. 6 biparental degu families were videotaped during the first 21 postnatal days. Video recordings of 20 minutes were taken at 8am, 11am, 14pm and 16pm daily.

In biparental families paternal care comprises 37 % of total parent-offspring interactions, and somatosensory stimulation provided by the fathers primarily consists of huddling, grooming and playing. In fatherless families we have previously shown that the single mother does not compensate the lack of paternal care, thus, these pups are raised under partly deprived social family conditions. Since paternal care is a major a source of somatosensory stimulation via various body contacts, its contribution on neuronal and synaptic development was analyzed in the somatosensory cortex. We found that pups raised with father displayed significantly higher spine numbers on basal dendrites of both hemispheres, and higher spine numbers and longer and more complex basal dendrites in the left hemisphere than in single-mother reared pups. In addition, single-mother reared degus showed significant effects of hemispheric lateralization of neuronal morphology, revealing longer and more complex basal dendrites in the right hemisphere compared to the left hemisphere. Thus, paternal care seems to be essential for dendritic and synaptic development of pyramidal neurons in the somatosensory cortex.

Investigation of developing nerve fibres in mouse embryos by ultramicroscopy

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Ultramicroscopy is a valuable approach for the 3D-analysis of complex morphological structures. It allows three-dimensional reconstructions of cm-sized specimens with micrometre resolution (Dodt et al., 2007). Therefore, ultramicroscopy fills a gap between confocal microscopy and macroscopic imaging techniques. Ultramicroscopy is based on the principle of light sheet illumination (Fig. 1). Since mechanical slicing is avoided, many drawbacks
of histology, like mechanical distortions and misalignments are eliminated.
In an ongoing study, we applied ultramicroscopy to the investigation of nerve fibre growth in the hindlimb of mouse embryos. E12.5 embryos were labelled with neurofilament-160 antibody and a secondary fluorescent antibody. As ultramicroscopy requires translucent specimen, we rendered mouse embryos transparent by a chemical clearing procedure. By detecting the fluorescence signal, we are able to threedimensionally visualize the complete topograhic organization of nerve projections to specific targets in the hindlimb. This approach allows detailed developmental studies on axon guidance and branching defects in the peripheral nervous system of genetically and pharmacologically altered mice. We present examples for the detection of axon guidance defects in different mouse mutants.

The study was supported by the SFB 391 and the Hertie Foundation.

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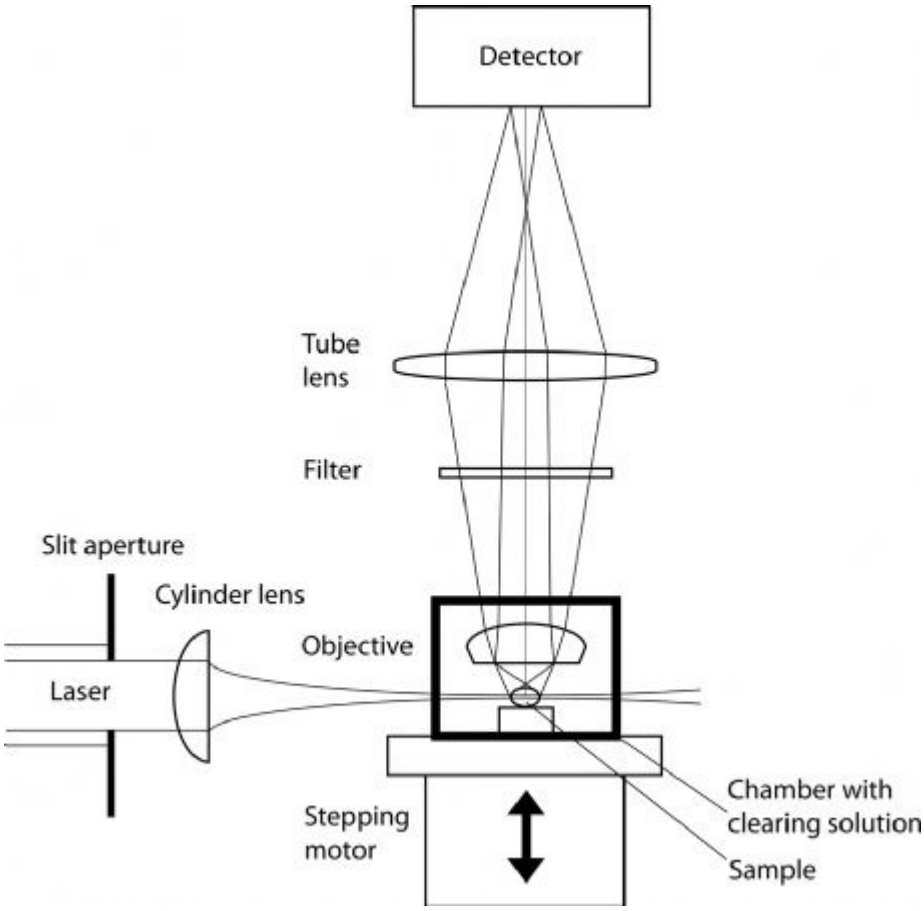
Jährling, N. et al. (2008) Med. Laser Appl. *in press*

Figure 1: The transparent specimen is illuminated by a thin sheet of laser light perpendicular to the imaging light path. Fluorescent light is thus emitted only from a thin optical section and collected by the objective lens. Figure from Jährling, N. et al., (2008).


Detector
Tube lens
Filter
Slit aperture
Cylinder lens
Objective
Laser
Stepping motor
Chamber with clearing solution
Sample

Neuroligin1 regulates presynaptic maturation

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Presynaptic nerve terminals pass through distinct stages of maturation after their initial assembly. Here we show that the postsynaptic cell adhesion molecule Neuroligin1 regulates key steps of presynaptic maturation. Presynaptic terminals from Neuroligin1 knockout mice remain structurally and functionally immature with respect to active zone stability and synaptic vesicle dynamics, as analyzed in cultured hippocampal neurons. Conversely, overexpression of postsynaptic Neuroligins in young neurons enhances the same parameters to the levels of mature cultures. The extracellular domain of Neuroligin1 is sufficient to induce assembly of functional presynaptic terminals, while the intracellular domain is required for terminal maturation. These data show that induction of presynaptic terminal assembly and maturation involve mechanistically distinct actions of Neuroligins, and that Neuroligin1 is essential for presynaptic terminal maturation.

Nogo-A/RTN4-A regulates dendritic growth in developing primary hippocampal neurons.

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Multiple reticulon (RTN) proteins are expressed in the mammalian brain. RTN-4A/Nogo-A is probably the most prominent family member and is well known as a potent myelin-derived inhibitor of neurite growth. Like RTN-1A, Nogo-A is also widely expressed by principal neurons in the developing and adult mammalian central nervous system, but the intracellular functions of both proteins in neurons are unclear. We have assessed the potential intracellular role of Nogo-A and RTN-1A in developing primary mouse hippocampal neurons by manipulating their expression levels. Increasing the intracellular level of Nogo-A specifically promoted dendrite elongation and branching, while sparing axon development. In contrast, increasing intracellular RTN-1A levels reduced axon outgrowth, but did not affect dendritic development. Consistent with a role of Nogo-A in dendritic development, gene silencing of Nogo-A by RNA interference (RNAi) caused impaired dendritic elongation and branching. Immunofluorescence studies revealed a partial colocalization of Nogo-A with membrane vesicles and cytoskeletal components. Our findings demonstrate that reticulons can differentially regulate axonal and dendritic morphogenesis of cultured mouse hippocampal neurons. Moreover, they highlight a novel function of Nogo-A as a regulator of dendritic development and suggest that its intracellular levels are important during critical stages of dendritic morphogenesis.

Calsyntenin-1 and APP specify distinct endosomal compartments in the axonal growth cone

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The type-1 neuronal transmembrane protein calsyntenin-1 was recently identified as a novel cargo-docking protein for Kinesin-1-mediated axonal transport of tubulovesicular organelles. In addition, a role of calsyntenin-1 in the stabilization of amyloid precursor protein (APP) was suggested based on the observation of the formation of a tripartite complex between calsyntenin-1 and APP via the common cytosolic ligand X11L/Mint-2. To further define the function of calsyntenin-1, we immunoisolated calsyntenin-1 organelles and determined their proteome. We found that calsyntenin-1 organelles are enriched with components characteristic for vesicles of the endosomal recycling pathway. In addition, we found that immunoisolated calsyntenin-1 vesicles were enriched for APP. Selective immunoisolation of early and recycling endosomes and their biochemical analysis demonstrated that full-length calsyntenin-1, but not APP, is present in recycling endosomes, while both APP and calsyntenin-1 are colocalized in an early endosomal compartment. The differential distribution of calsyntenin-1 and APP to distinct organelles of the endosomal recycling pathway was confirmed by microscopic analyses of axonal growth cones of cultured hippocampal neurons using endosomal marker proteins for distinct subcompartments of the endosomal recycling pathway. Our results indicated that neuronal axons and growth cones contain two distinct calsyntenin-1-containing transport packages, one characterized as early-endosomal, APP-positive, and the other one as recycling-endosomal, APP-negative. We suggest that calsyntenin-1 serves as a cargo-docking protein for distinct early-endosomal and recycling-endosomal vesicles for their exocytic delivery along the axon towards the growth cone surface. In this function, calsyntenin-1 may contribute to developmental processes, such as neuronal polarization, neurite extension, and axonal pathfinding.

The RNA-Binding Protein MARTA2 Regulates Dendritic Targeting of MAP2 mRNAs

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Dendritic targeting of mRNAs encoding the microtubule-associated protein 2 (MAP2) in neurons involves a cis-acting dendritic targeting element (DTE). Two rat brain proteins, MARTA1 and MARTA2, bind to the DTE with both high affinity and specificity. In this study, affinity-purified MARTA2 was identified as orthologue of human far-upstream element binding protein 3. MARTA2 is strongly enriched in brain polysome fractions. In neurons, it associates with the cytoskeleton and MAP2 mRNAs, and resides in somatodendritic granules and dendritic spines. Expression of a dominant-negative variant of MARTA2 disrupts dendritic targeting of endogenous MAP2 mRNAs, while not noticeably altering the level and subcellular distribution of polyadenylated mRNAs as a whole. Finally, MAP2 transcripts associate with the microtubule based motor KIF5c and inhibition of KIF5c but not cytoplasmic dynein function disrupts extrasomatic trafficking of MAP2 mRNA granules. Thus, in neurons MARTA2 appears to represent a key trans-acting factor involved in KIF5c-mediated dendritic targeting of MAP2 mRNAs.

Nitric Oxide Regulates Development of Zebrafish Motoneuron Axons

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Nitric Oxide (NO) is a highly reactive free radical gas that has recently been shown to regulate the development of nervous tissue. Despite this, we still know relatively little of how NO influences *in vivo* nervous system development. In order to address this issue, we have used the zebrafish, a popular model organism for developmental studies, to determine how NO signalling affects the ontogeny and maturation of spinal cord neurons.

Here, using NADPH diaphorase histochemistry, we report that putative NO generating cells are detectable in the neuropil of the zebrafish spinal cord as early as the second day of development. In addition, using immunohistochemical methods, we demonstrate that manipulation of NO levels during development markedly affects the outgrowth of spinal motoneuron axons. We show that larvae which have been raised in the NO-donor DETA-NO (500µM) throughout early development exhibit a marked decrease in the arborisation of motoneuron axons when compared to control fish. In contrast, when neuronal nitric oxide synthase expression is perturbed from the onset of development using anti-sense morpholino oligonucleotides (AMO), the converse effect is observed. These AMO-injected larvae exhibit a dramatic increase in the number of axonal arborisations when compared to control fish. Our results implicate NO as an important mediator of motoneuron development during ontogeny of the zebrafish nervous system.

Neuroligin 2 Drives Postsynaptic Differentiation at Inhibitory Contacts Through Gephyrin and Collybistin

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Each neuron typically receives thousands of presynaptic contacts of diverse transmitter types. Proper neuronal function requires local postsynaptic differentiation of the plasma membrane with the accumulation of transmitter-specific receptor, scaffold and adhesion proteins in precise apposition to these contact sites. We identified an assembly mechanism where the synaptic adhesion protein Neuroligin 2 functions as a core organizer of inhibitory postsynaptic differentiation at sites of inhibitory axon contact. Neuroligin 2 interacts with the postsynaptic scaffolding protein Gephyrin, through a novel cytoplasmic motif, and activates the signaling protein Collybistin to tether the inhibitory postsynaptic scaffold to the plasma membrane. This protein assembly is sufficient to recruit and retain inhibitory transmitter receptors as shown by reconstitution of GABAergic postsynaptic elements in non-neuronal cells. Deletion of Neuroligin 2 in mice leads to deficits in inhibitory postsynaptic scaffold recruitment and to diminished GABAergic and glycinergic transmission in vivo, exemplify the central role of Neuroligin 2 in driving inhibitory postsynaptic differentiation.

3D Quantification of Morphological Alterations of Coincidence Detector Neurons in the Medial Superior Olive of Gerbils During Late Postnatal Development

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Neuronal coincidence detection is used to process interaural time differences (ITD) allowing the azimuthal location of sound sources. In the medial superior olive (MSO) neurons compute this sound localization by a highly precise coincidence detection mechanism. Sound localization and its neuronal processing has been subject to many model predictions based on *in vivo* and *in vitro* data obtained in gerbils, cats, bats and for the avian analog in chicken and owl. It is generally assumed that dendritic signal processing of MSO neurons affects this computation. However, little quantitative data about the morphology of these coincidence detector neurons is available, limiting computational models and theoretical approaches that incorporate cellular dimensions. . We used single cell electroporation, optical 3D-reconstruction, and compartmentalization to gain anatomical parameters of MSO neurons in order to quantitatively describe their morphological alterations during late postnatal development. We find that between postnatal day 9 and 37 the length of the main dendritic tree is shortened, the total cell surface area becomes reduced by a third, and the dendritic arborization decreases. Surprisingly the cell volume increases more than 1.5-fold during this developmental time window, a change which can be attributed to a steady increase in dendritic diameter. Together the developmental changes indicate that MSO neurons mature to a morphologically compact, cylinder-like cell. From the obtained results we suggest that full morphological maturation of these neurons is reached around P27, about two weeks after hearing onset. However, activity dependent processes predicated on adequate auditory cues might help to shape the mature MSO cell morphology. Additionly we confirm that the location of axonal origin of gerbil MSO neurons is mainly somatic.

Neuronal M6-proteolipids are required for neurite extension

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The function of mature neurons relies critically on the developmental outgrowth of their cellular processes. The increase abundance of the neuronal glycoprotein M6A at the onset of neurite outgrowth, its particular enrichment at the leading edge of growth cones, and *in vitro* overexpression studies have suggested that M6A may enhance neurite extension. Here we analyzed neurite extension in the persistent absence of M6A or a second neuronally expressed member of the proteolipid protein (PLP) family of tetraspan membrane proteins, termed M6B. M6A and M6B are 75% similar at the amino acid level and colocalize on neuronal processes. We generated both M6A^{null} and M6B^{null} mice that presented no apparent phenotypical abnormalities, possibly due to functional compensation. To assess the effect of the chronic lack of both neuronal M6-proteins we have analyzed M6A^{null}*M6B^{null} double mutant mice. These mice had a significantly increased mortality rate after weaning, and survivors were moderately impaired in their motor abilities. No major differences were observed by histological analysis. Chronic lack of M6-proteins significantly impaired neurite extension in cerebellar granule cells and cortical neurons *ex vivo*. The oligodendroglial homologue PLP associates with cholesterol, and predicted cholesterol-binding motifs are also present in M6A and M6B. Indeed, addition of cholesterol enhanced neurite outgrowth, but the effect was diminished in double-mutant neurons compared to controls. We propose that the neuronal glycoproteins M6A and M6B contribute to neurite extension in a cholesterol-dependent manner.

Neuronal bHLH Transcription Factors NEX and NDRF are Essential Regulators of Cortical Axon Tract Formation

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Targeted axogenesis of cortical projection neurons and the subsequent formation of major white matter tracts are fundamental to the establishment of functional brain circuitry. However, the genetic program that specifies the cortical projection neuron phenotype is still poorly understood. Neuronal basic helix-loop-helix (bHLH) transcription factors NeuroD, NDRF (NeuroD2) and NEX (MATH2, NeuroD6) are expressed in cortical projection neurons throughout development and in the adult. Their expression profile and reported neurite outgrowth promoting activity in vitro make these factors attractive candidates to control aspects of the projection neuron phenotype in vivo. In the absence of distinct neocortical defects in NeuroD, NDRF and NEX single mutants (suggesting functional redundancy), we generated compound double and triple mutants to study the role of neuronal bHLH proteins in cortical development. In NDRF*NEX double mutants interhemispheric axon tracts of neocortical origin (corpus callosum and anterior commissure) are completely absent, whereas the archipalliar hippocampal fissure (originating from hippocampal pyramidal cells) forms properly. Probst bundles or other aberrant axon aggregates were not observed, suggesting a fundamental role of NDRF and NEX already in axon outgrowth. In contrast, survival, migration and dendritogenesis of projection neurons and neocortical layer formation appeared largely unaffected. While prominent neocortical NeuroD expression in wildtype perinatal mice is restricted to the subventricular zone, NeuroD expression in NDRF*NEX double mutants persists in pyramidal neurons of the cortical plate and might (partially) compensate for the loss of NDRF and NEX. Accordingly, neocortical layer formation is severly impaired and hippocampal pyramidal neurons are absent in NeuroD*NDRF*NEX triple mutants. Moreover, intercortical and corticofugal projections fail to develop and thalamacortical projections are severely impaired in NeuroD*NDRF*NEX triple mutants. Taken together, we conclude that NEX and NDRF act cooperatively as regulators of cortical pyramidal neuron axogenesis and that neuronal bHLH proteins are essential for pyramidal cell development and maintenance.

Imaging the outgrowth of spinal nerves in Semaphorin3A mutant mouse embryos

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Innervation of the embryonic forelimb begins with the formation of long processes by motor neurons in the ventral spinal cord and sensory neurons in the dorsal root ganglia. These axons undertake a complicated journey to find their ultimate target within the periphery. The neuronal chemorepellent Semaphorin3A and many other proteins regulating axonal guidance have been identified by the analysis of dissociated neurons *in vitro*. However one would like to work with a system that allows for the observation and manipulation of axonal outgrowth and pathfinding as it occurs *in situ*. Preparing transverse and sagittal slices of mouse embryos expressing GFP specifically in newborn neurons (*tauGFP*: Tucker *et al.*, Nature Neurosci 2001), we were able to reproduce normal developmental innervation patterns by the spinal nerves of the forelimb and to follow this outgrowth using fluorescence microscopy (Brachmann *et al.*, Dev Dynamics 2007). The advantage of our system is that slices can be manipulated not only pharmacologically, but also genetically, by crossing the *tauGFP* line with mice containing targeted mutations in genes of interest. This was done for mice deficient in the gene encoding Semaphorin 3A (*Sema3A*). In transverse slices obtained from E10.5 *Sema3A* -/- embryos we could clearly observe premature outgrowth and strong defasciculation of spinal nerves, compared with wild type and *Sema3A* +/- *tauGFP* littermates. Furthermore, we were able to detect ectopic, Brn3a- and GFP-positive neurons that could reflect a sensory neuronal identity. The results of high magnification time-lapse imaging of wildtype and *Sema3A* -/- slices prepared at younger embryonic stages will be presented.

6-hydroxydopamine lesions of nigrostriatal neurons in the mouse induces tyrosine hydroxylase expression in striatal neurons

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The goal of this study was to establish a mouse model of Parkinson's disease by inducing a stable unilateral dopaminergic cell loss in the substantia nigra pars compacta (SN). A mouse model is of special interest, because by using transgenic or knock-out animals the effects of various factors on such a dopaminergic deafferentiation could be investigated. Male C57BL/6-mice (n = 17, about 22 g) were lesioned by a stereotactic 6-OHDA-injection into the right medial forebrain bundle (2 µl, containing 5 micrograms 6-OHDA; coordinates according to bregma: AP –1.2; L -1.1; V -5). One, 2 and 3 months after lesion contralateral apomorphine- and ipsilateral amphetamine-induced rotations were evaluated. Stride length measurements and forepaw preference (cylinder test) of lesioned mice were compared with age matched intact controls (n = 9). Three months after lesion, mice were perfused with 3.7% PFA and Nissl stainings or immunohistochemistry to visualize tyrosine hydroxylase (TH) immunoreactive neurons in brain sections were performed. In the apomorphine test (0.5 mg/kg, 40 min) we observed significantly different rotational behaviours during one to three months postlesion: one group demonstrated rapid increasing numbers of high rotations over time (from 13.8 to 24.1 rot/min, n = 9), whereas the other group showed low and stable amounts of rotations (from 6.8 to 7.6 rot/min, n = 8). Intact controls performed only -0.6 rot/min. Lesioned mice also had significantly shorter stride lengths. The high rotating animals preferentially used their right forepaws as compared to the low rotating or intact mice. However, to our surprise lesioned animals showed no significant changes in the amphetamine test. Histology and TH-immunostainings revealed a nearly complete unilateral dopaminergic cell loss in the SN and a dopaminergic deafferentiation, resulting in loss of TH-ir nerve terminals in the ipsilateral striatum. However, in all lesioned animals we observed respective numbers of TH-containing neurons in the dopaminergic deafferentiated striatum. Stereologic countings of these cells is performed currently and will be compared to the outcome of the several behavioural tests.

Control of neuronal apoptosis by electrical activity

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Electrical activity and sufficient supply with survival factors play a major role in the decision of developing neurons to undergo apoptosis or persist. In particular high frequency neuronal activity that has been shown to efficiently release neurotrophic factors is essential for the survival of immature neurons. We aimed at correlating neuronal activity and apoptosis in the developing neocortex. Therefore dissociated cortical cultures of the newborn mouse were transfected with a plasmid coding for a caspase-3 sensitive fluorescent protein (Golbs et al., J. Neurosci. Methods, 2007). This approach allowed real-time analysis of caspase-3 dependent apoptosis in individual neurons. In addition cortical cultures were grown on MEA chips to determine the patterns of network activity that promote cortical survival. We found that in conditions of trophic deprivation elevated extracellular potassium concentrations lead to an increase in the abundance of high frequency events, termed bursts. Moreover increased extracellular potassium concentrations could attenuate caspase-3 activation in cortical neurons and promoted an overall increase in neuronal survival under trophic deprivation. These data indicate that increased network activity can protect immature cortical neurons against trophic deprivation induced apoptosis.

ADAM17 overexpression induces angiogenesis by increasing the proliferation of pericytes during chicken brain microvessel development<

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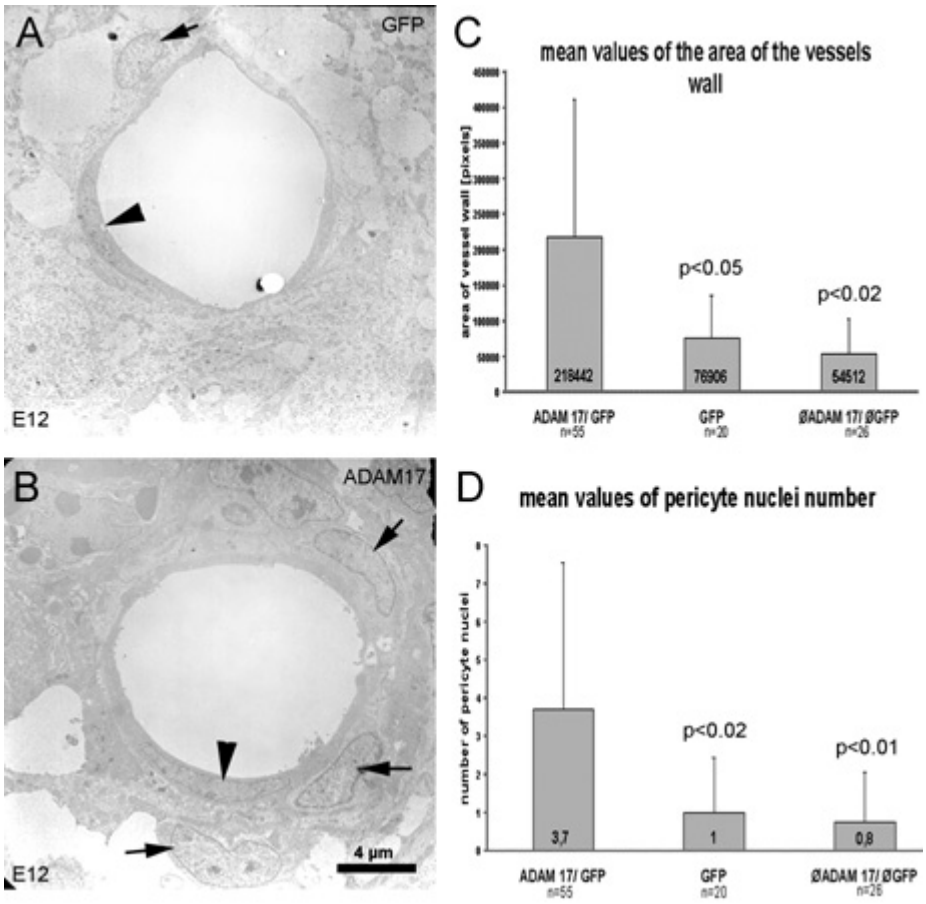
ADAMs (a disintegrin and metalloproteases) are a family of transmembrane metalloproteases with multiple functions; they are involved in cell-cell and cell-matrix interactions and potentially mediate proteolysis, adhesion, and cell signal transduction. ADAM17, also named TACE (tumor necrosis factor-alpha converting enzyme), is the best-characterized member of the ADAM family. It is a major sheddase that releases the extracellular domain of many proteins, including receptors and precursors of cytokines and growth factors. In this study, the cDNA of chicken ADAM17 was cloned and its expression was analyzed for the first time during chicken brain development, focusing on the optic tectum. Semi-quantitative RT-PCR revealed no significant difference at different embryonic stages, although expression seemed to decline slightly at later stages (E16-E20). Results from in situ hybridization indicated that, during tectal development, ADAM17 is expressed in a distinct spatiotemporal profile in the different layers of the tectum. By ex ovo electroporation, we overexpressed ADAM17 in chicken developing optic tectum and analyzed its effect on the development of brain microvessels by immunohistochemistry and transmission electron microscopy. Compared to both wild type and GFP (green fluorescent protein) controls, ADAM17 overexpression increases the width of the walls of radial microvessels. The number of pericytes, but not of endothelial cells, is increased significantly (Figure). Our results suggested that ADAM17 can induce the proliferation of the pericytes during brain microvessels development. We here speculate that the observed increase in the number of pericytes is caused by a remodeling of the extracellular matrix environment of the vascular cells due to ADAM17-induced changes in cell-matrix interactions, matrix protein shedding, or cell signal transduction.

Figure. Results from transmission electron microscopy at E12 (A,B) and statistical analysis (C,D). A, GFP electroporation alone (control); B, ADAM17 overexpression sample. The arrowheads indicate the nuclei of endothelial cells, and the arrows indicate the nuclei of the pericytes. Scale bar, 4µm. C shows the mean values for the area occupied by the blood vessel walls in the electroporated area or controls. D shows the mean values for the number of the pericyte nuclei in the blood vessels walls (n=55 for ADAM17/GFP co-electroporation; n=20 for electroporation of GFP alone; and n=26 for the wild type control).


A
GFP
E12
B
ADAM17
E12
4 µm
C
mean values of the area of the vessels wall
area of vessel wall [pixels]
p<0.05
p<0.02
218442
76906
54512
ADAM 17/ GFP
n=55
GFP
n=20
ØADAM 17/ ØGFP
n=26
D
mean values of pericyte nuclei number
number of pericyte nuclei
p<0.02
p<0.01
3,7
1
0,8
ADAM 17/ GFP
n=55
GFP
n=20
ØADAM 17/ ØGFP
n=26

Differential expression of apoptosis inhibitor survivin in rat olfactory epithelium

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Neuronal cell death often occurs via apoptotic mechanisms. However, because of the very limited proliferation in neuronal tissues, apoptosis needs to be controlled very tightly, which is realized by apoptosis inhibitors. Some neuronal tissues such as the olfactory epithelium however show a life-long proliferation and neurogenetic activity with continuous neuron turnover. The question arises: does the olfactory epithelium express apoptosis inhibitors as well and if so, whether the expression increases with the decreasing proliferation rate during postnatal development.

We investigated the expression of a very potent apoptosis inhibitor, survivin, in olfactory mucosa of rats at different postnatal ages (postnatal day P10-P900) using RT-PCR and duplex-RT-PCR followed by electrophoretic separation of the products in ethidium bromide containing agarose gel. Semiquantitative analysis was performed with the Phoretix 1D Quantifier.

Our experiments show, that survivin is expressed in olfactory mucosa at all postnatal ages. Additionally, contrary to our expectations, the expression level is much higher in young animals with a high proliferation rate compared to older ones when a low neurogenetic activity is observed. The postnatal decrease quantitatively parallels the postnatal proliferation density decrease (Weiler & Farbman, J Neurosci 1997, 17, 3610-22).

These results indicate, that the apoptosis inhibitor is <u>not</u> required for the long survival time of a neuron but for the early vulnerable phase in the young neurons life. In young animals, when proliferation is high, many olfactory sensory neurons compete a) for the space within the olfactory sheet and b) for the target cells in the olfactory bulb. Without getting sufficient support for survival from the target cells by the appropriate kind and number of synapses, neurons are doomed to die. During extension of the axon to the target cells, olfactory neurons therefore are very vulnerable to apoptotic processes so that apoptotic inhibitors can effectively promote survival until the neuron establishs the proper synapses. In older animals, when proliferation is low, the turnover pressure is low and apoptosis inhibitors are not necessary as much. Thus we conclude, that the apoptosis inhibitor survivin is expressed to help neurons to survive during their competition for target contacts until they establish enough stable synapses, which then take over the survival function.

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Hippocampal Slices as Model for Evaluation of Neuronal Stem/Progenitor Cells to Identify Regenerative Therapies in Bacterial Meningitis

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Background: Bacterial meningitis causes life-long disabilities in up to 50% of the survivors. Brain injury caused by bacterial meningitis prominently affects the hippocampus a brain region involved in learning and memory function. In experimental bacterial meningitis hippocampal injury is characterized by apoptosis of cells in the subgranular zone of the dentate gyrus (DG). In the DG neurogenesis occurs lifelong and this brain structure is therefore potentially well equipped for brain repair.
Multipotency and the capacity for continuous self-renewal make embryonic stem cells attractive candidates for cell-replacement studies.
Aim: To investigate tissue repair mechanisms by stem/progenitors cells grafted into organotypic hippocampal slice cultures.
Method: An *in vitro* co-culture model combining long-term hippocampal slice cultures from postnatal rats (P4/5) with embryonic stem/progenitor cells from the subventricular zone (E14-17) was established to assess whether the stem/progenitors cells survive and integrate into the host tissue. To this end, we expanded stem/progenitor cells from the subventricular zone of embryonic day 14 to 17 rats as neurospheres *in vitro*. To track the fate of transplanted cells in the host tissue, cells were either labelled chemically or cells were isolated from transgenic rats expressing green fluorescent protein (GFP). Chemically labelled as well as GFP-expressing cells were then grafted into organotypic hippocampal slices in the hilus region of the DG. Cells were allowed to grow in co-culture conditions with the addition of epidermal (EGF) and basic fibroblast growth factor (bFGF). The survival and integration of grafted cells was examined on cryosections of organotypic slice cultures and the differentiation stage was assessed by immunohistochemistry.
Results: Histomorphologic analysis revealed migration and neurite outgrowth of both types of cells into the DG at day 7 after engraftment. In the presence of bFGF and EGF both chemically labelled and GFP-expressing neurosphere cells were able to differentiate and to mature into neurons.
Conclusion: Embryonic derived stem/progenitor cells grafted into organotypic slice cultures survive, migrate, proliferate, differentiate and integrate into the host tissue. The transplantation of neurosphere derived stem/progenitor cells may hold promise for regenerative therapies aimed at repair of apoptotic brain damage in the hippocampus of patients suffering from neurofunctional sequelae after bacterial meningitis.

The role of serum response factor in axonal regeneration

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In contrast to the peripheral nervous system (PNS) the central nervous system (CNS) shows almost no regeneration. Reasons for the poor regeneration can be divided into intrinsic and extrinsic. Intrinsic factors cover for instance the lack of growth-promoting factors in CNS neurons themselves, whereas molecules in the surrounding environment present an extrinsic obstacle. The latter, namely Nogo, myelin-associated glycoprotein (MAG), oligodendrocyte-myelin glycoprotein (OMgp) and e.g. chondroitin sulfate proteoglycans (CSPGs) are inhibitors presented by the myelin sheath or reactive astrocytes and microglia.

We are interested as to what extent gene expression programs, particularly those initiated by Serum Response Factor (SRF) are contributing towards axonal regeneration in vitro and in lesion models. SRF, a MADS box family transcription factor, regulates multiple target genes including Immediate Early Genes (IEGs; e.g. c-fos, Egr1) and cytosceletal genes (e.g. various actin isoforms), thereby allowing SRF access towards modulation of cytosceletal dynamics.
Here, we demonstrate a role of SRF-mediated gene transcription in improving neuronal growth blocked by various myelin-associated inhibitors, thereby increase regeneration of CNS neurons. These experiments involve virally-mediated expression of a constitutively-active SRF variant, SRF-VP16, both in vitro and in vivo. Complementarily, we provide data investigating whether SRF-mediated target gene modulation is subject to a signalling cascade initiated by myelin-associated inhibitors. These data raise the possibility that interference with coordinated SRF gene expression is a contributing factor to the intrinsic inability of CNS neurons to regenerate.

A potent anti-NgR antibody with ligand-blocking properties does not robustly ameliorate functional deficits in two rat models for spinal cord injury

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An insult to the mammalian spinal cord often results in persistent functional deficits. This is partially due to inhibitory molecules in CNS myelin blocking neurite growth, including Nogo-A, oligodendrocyte myelin glycoprotein (OMgp), and myelin-associated glycoprotein (MAG), which all bind to the neuronal Nogo-66 receptor (NgR1) and thus block neurite outgrowth. Neutralizing the interaction between the inhibitory ligands and NgR1 may alleviate the inhibition and may, therefore, result in increased neuronal regeneration after injury. Consequentially, antibodies neutralizing this interaction might have therapeutic value.

From our anti-NgR1 antibody program, two antibodies (mAb50 and mAb51) were selected, binding with high affinity (approximately 100 pM) to human and rat NgR1. Both antibodies, reduce the inhibitory effects of a ligand peptide derived from Nogo-A (Nogo66) on neurite outgrowth in differentiated human NTera2 cells and rat dorsal root ganglion neurons. We chose mAb50 to test its efficacy in two rat models of spinal cord injury. In a spinal cord over-hemisection model mAb50 increased behavioral recovery over the time period of 6 weeks. However, in histological studies no sprouting of corticospinal tract fibers was observed, and local markers for glial, neuronal, and synaptic functioning were unaltered. Moreover, in a spinal cord contusion model, behavioral outcome was identical among groups.

Our results suggest that neutralizing NgR1 with a ligand-blocking antibody is not sufficient to induce robust long-range regeneration and functional recovery in spinal cord injury and indicate that besides NgR1, independent inhibitory systems are relevant for neurite outgrowth inhibition.

A novel synthetic silica hydrogel for culturing rat hippocampal neuronal cells in 3D

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Cells are surrounded by a highly intricate and dynamic three-dimensional (3D) matrix *in vivo* which regulates cell growth and migration. These interactions may be lost in conventional two-dimensional monolayer cultures, which prompt the development of innovative 3D cell cultures using hydrogels. Here, we investigate the potential of a novel silica hydrogel, Laponite (Rockwood Clay Additives LTD), as a 3D cell culture matrix for rat hippocampal neurones. Laponite, or hydrous sodium magnesium silicate, is capable of forming sturdy gels are very low concentrations (2% wt/vol) as well as showing thixotropic properties. It is a unique physical hydrogel which changes its rheological properties in response to shear stress, exhibiting high viscosity at low shear rates, low viscosity at high shear rates and progressive restructuring after shear.

In this study we have analysed the Laponite hydrogel as a potential 3D culture medium. Initial attempts at assessing cell viability by growing hippocampal monolayers under gel found that gel cover resulted in cell behaviour reminiscent of preservation/fixation with monolayers showing no spatial or morphological changes over time. One possible explanation for this behaviour may be the high gel osmolarity (400-450 mOsm). In contrast, cell seeding showed positive results, with an increased tendency for cells to adhere to the Laponite gel forming distinct adherence patterns.

Ciliary neurotrophic factor stimulates axon outgrowth of mature retinal ganglion cells directly via the JAK/STAT3- and PI3K-signaling pathways and indirectly via MAPK/ERK-signaling pathway dependent glial activation

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Mature retinal ganglion cells (RGCs) normally do not regenerate injured axons and instead undergo apoptosis after optic nerve injury. However, if lens proteins are either intravitreally injected or slowly released from the injured lens, RGCs are switched to an active regenerative state, enabling them to regenerate axons into the damaged optic nerve. Astrocyte-derived CNTF has been proposed to be one of the major contributing factors. Consistent with this hypothesis, intravitreal injections of exogenous CNTF exert similar effects on RGCs in vivo. Nevertheless, controversy exists over the ability of endogenous or exogenous CNTF to directly stimulate axon regeneration of mature RGCs. Here we demonstrate, using dissociated retinal cell cultures, that CNTF directly and potently stimulated axon outgrowth of mature RGCs and that cAMP elevation significantly increased these effects. JAK/STAT3- and PI3K/AKT-signaling pathway inhibitors compromised its beneficial effects, whereas the inhibition of MAPK activity alone or together with CNTF enhanced axon outgrowth in vitro. In vivo, intravitreally applied CNTF directly activated the JAK/STAT3-signaling pathway in RGCs and the MAPK/ERK-signaling pathway in retinal glia. As a consequence, astrocytes increased endogenous CNTF expression in a manner that depended on the MAPK/ERK-signaling pathway. In addition to the JAK/STAT3- and PI3K/AKT-, MAPK/ERK-pathway inhibitors also compromised the CNTF-induced transformation of RGCs into a regenerative state in vivo. These data suggest that exogenously applied CNTF directly stimulates mature RGCs, whereas indirect effects, which may be mediated by astrocyte-derived CNTF or other glial-derived factors, contribute to its full effects in vivo.

BMP signaling induces transdifferentiation of the NR into RPE

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Bone morphogenetic proteins (BMPs) play multiple roles during chick eye development, being for example involved in both dorsal and ventral patterning of the NR and in RPE specification. In this study, we analysed the potential of BMP5 in inducing transdifferentiation of the NR at later stages of chick eye development. Similar to Bmp7, Bmp5 transcripts are localised to the developing RPE. Implantation of BMP5-soaked beads at E4 and E5 induced RPE development in the ciliary margin of the chick eye, as determined by the expression pattern of RPE- and NR-specific genes, such as Mitf, MMP115 and Chx10. Retina stem/progenitor cells exist in the ciliary margin of the chick and in other species, including humans. Thus, our findings provide insights on how retinal stem cells can be activated for possible regenerative therapies.

Suppression of fibrous scarring following spinal cord injury results in different axon growth behaviour of various spinal cord fiber tracts

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Traumatic injury of the central nervous system results in a collagenous basement membrane-rich fibrous scar which is a major barrier for axonal regeneration. This dense collagen IV meshwork serves as a binding matrix for numerous other extracellular matrix (ECM) components and axon growth inhibitory molecules. Recently, we established a treatment to suppress collagenous lesion scarring by local application of a potent iron chelator (BPY-DCA), which deprives prolyl-4-hydroxylase, a key enzyme of the collagen biosynthesis, from its cofactor Fe^{2+}. We further added cyclic AMP to inhibit proliferation and ECM production of meningeal fibroblasts (Klapka et al., Eur.J.Neurosci. 22, 2005, 3047-58). This anti-scarring treatment (AST) resulted in a delay of fibrous scarring, enhanced regeneration of corticospinal tract (CST) axons, retrograde neuronal protection and functional recovery. The current study focuses on the comparison of the axon growth capacities of different spinal cord fiber populations following the AST treatment in the adult rat. We have investigated calcitonin gene related peptide (CGRP), dopaminergic, serotonergic, corticospinal (CST), and rubrospinal (RST) fibers at 5 and 12 weeks after dorsal hemisection at thoracic level T8 with/without AST treatment. Axon fragments present within the lesion area and beyond were counted in 50 µm parasagittal cryo-sections. At both time points all fiber tracts studied showed significantly increased axon growth in AST- treated animals as compared to controls (buffer injection). Anti-CGRP immunopositive fibers show the highest regenerative capacity at 5 weeks, followed by the 5-HT-positive serotonergic and TH-positive dopaminergic fibers. Both anterogradely BDA-traced long distance motor axon tracts (RST and CST) show a lower capacity to regenerate, of which the CST has got the lowest capacity of all examined fiber populations. Interestingly at 12 weeks after injury and treatment the overall number of growing axon has further increased and the serotonergicfibers showed the most prominent growth response upon AST. We conclude that the various fiber populations investigated showed different capacities of AST-promoted axon growth.

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Use of different gel matrices as implants after scar resection in chronic spinal cord injury to promote axonal regeneration

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The fibrous scar which forms after a spinal cord lesion – with a collagen IV meshwork being the substrate binding several inhibitory molecules - is a major impediment for axonal regeneration. We have previously developed a pharmacological treatment to transiently suppress the formation of the collagenous fibrous scar. Treatment consists of local injection of an iron chelator and cyclic AMP application to inhibit a key enzyme of collagen IV-biosynthesis in acute spinal cord injury (SCI) of adult rat (Hermanns et al., J Neurosci Methods, 2001). Treated rats showed axonal regeneration, neuroprotection of projecting pyramidal neurons and functional recovery in different motor tasks after acute SCI (Klapka et al., Eur J Neurosci. 2005). The majority of human SCI patients suffer not from acute SCI but from a chronic injury in which the lesion scar is fully developed. We therefore established an injury model that comprises the resection of a lesion scar via aspiration five weeks post-injury. In the same surgery implantation of a gel matrix into the resulting gap was carried out. Tested matrix materials were a lipid formulation, polyethylene glycol, and alginate hydrogel. These matrices were either implanted alone or in combination with an iron chelator (either deferroxamine or 2,2'-bipyridine-5,5'-dicarboxylic acid) to find out whether the positive effects seen in the acutely injured rats after chelator treatment were also apparent in the chronically injured animals after matrix implantation. Axons were traced via BDA injections into the spinal cord rostral to the resected area seven days prior to sacrifice. Control-injured animals either received no resection of the lesion scar or no implantation of any matrix material into the resection cavity. Five weeks after implantation, we compared the different matrices with respect to their tissue compatibility and histologically examined scar formation in and around the lesion site. Furthermore, we tested their axonal regeneration promoting features and possible side effects on function (tested with the BBB score). Five weeks after resection, regenerated axons were found in all implants. These axons were either traced via BDA-injections or visualized by PAM-staining. Quantification of these ingrowing axons revealed differences in axonal regeneration promoting capacities of the tested matrices. These results show for the first time a direct comparison of matrices in chronic SCI and their influence on spontaneous axonal regeneration.

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Glucose-dependent insulinotropic polypeptide (GIP) and its receptor (GIPR): cellular localization, lesion-affected expression, and impaired regenerative axonal growth.

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Glucose-dependent insulinotropic polypeptide (GIP) was initially described to be rapidly regulated by endocrine cells in response to nutrient ingestion with stimulatory effects on insulin synthesis and release. Previously, we demonstrated a significant up-regulation of GIP mRNA in the rat subiculum after fornix injury.

To gain more insight into the lesion-induced expression of GIP and its receptor (GIPR) expression profiles of the respective mRNAs were studied after rat sciatic nerve crush-injury in (i) affected lumbar dorsal root ganglia (DRG), (ii) in spinal cord segments, as well as in (iii) proximal and distal nerve fragments by means of quantitative RT-PCR. Our results clearly identified lesion-induced as well as tissue type-specific mRNA regulation of GIP and its receptor.
Furthermore, comprehensive immunohistochemical stainings not only confirmed and exceeded the previous observation of neuronal GIP expression, but also revealed corresponding GIPR expression implying putative modulatory functions of GIP/GIPR signaling in adult neurons. Complementary, we also observed expression of GIP and its receptor in myelinating Schwann cells and oligodendrocytes. Polarized localization of GIPR in the abaxonal Schwann cell membranes, the plasma-membrane-associated GIPR expression of satellite cells as well as ependymal GIPR expression strongly suggests complex cell type-specific functions of GIP and GIPR in the adult nervous system that are presumably mediated by autocrine and paracrine interactions, respectively.
Notably, *in vivo*-analyses using GIPR-deficient mice suggest a critical role of GIP/GIPR signal transduction in promoting spontaneous recovery after nerve crush, as traumatic injury of GIPR-deficient mice sciatic nerve revealed impaired axonal regeneration when compared to wildtype mice.

THERAPEUTIC EVERY-OTHER-DAY FASTING IMPROVES RECOVERY FROM CERVICAL AND THORACIC SPINAL CORD INJURIES

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Caloric restriction and intermittent fasting are well known for their live-span prolonging and neuroprotective benefits. We recently discovered that every other day fasting (EODF) resulted in significantly improved functional outcomes after partial cervical spinal cord injuries in rats (dorsolateral funiculus crush). Interestingly, these benefits were not only seen with every other day fasting starting weeks prior to trauma but also when we begun this fasting regimen not until after the spinal cord injury. We coined the term therapeutic every other day fasting as opposed to prophylactic fasting. The mechanisms of the behavioural benefits triggered by therapeutic EODF are largely unknown, and we attribute them in part to reduced spinal cord lesion sizes, possibly due to elevated levels in neuroprotective beta-hydroxybutyrate on the fasting days. Moreover, rats on therapeutic EODF showed significantly more corticospinal axon arborisations in the gray matter of the injured spinal cord indicative of axonal sprouting. Whether this is causally related to a massively increased ratio of full-length versus truncated trk B receptors after therapeutic EODF remains to be shown. More recently, we tested if therapeutic EODF would improve recovery after a moderately severe (1.5 mm displacement) low thoracic contusion injury in adult male Sprague Dawley rats. Both, the prophylactic EODF as well as therapeutic EODF rat groups performed significantly better than ad-lib-fed or a pair-fed controls. Significant benefits were also seen in footprint/gait assessments using the Catwalk (Noldus). Histological analysis and studies of inflammatory parameters are currently ongoing. In summary, therapeutic EODF appears to be a safe and simple treatment with robust effects in two different animal spinal cord injury paradigms, making it an interesting candidate for clinical translation. Supported by grants from Canadian Institutes of Health Research, Christopher and Dane Reeve Foundation and the Craig H. Neilsen Foundation.

Studies on a collembolan brain: Neuroanatomy and Immunocytochemistry

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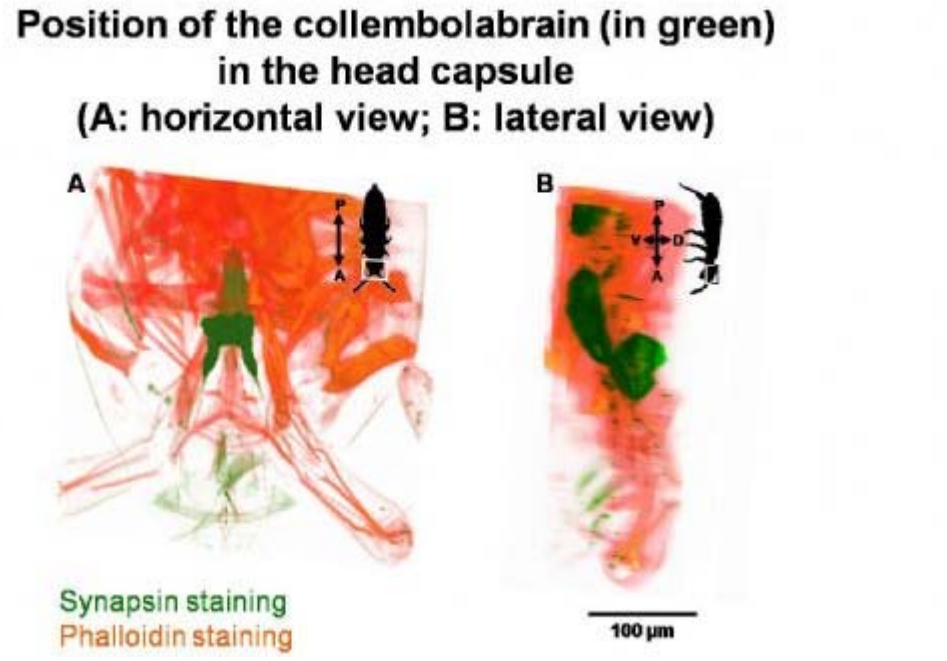
The large and diverse group of the arthropods consists of hexapoda, crustacea, myriapoda, and chelicerata. Despite this classification has been known for a long time, there is still some debate about their phylogenetic relation. Most recent studies suggest that collembola (springtails) represent a basal group of the hexapoda. However, comparative neuroanatomy and immunocytochemistry of those basal animals is largely missing.

To obtain data of brain neuroanatomy and immunocytochemistry of these minute animals, we examined brains of three collembolan species (*Folsomia candida*, *Protaphorura armata*, and *Tetrodontophora bielanensis*). Immunolabelings were carried out with an antiserum against synapsin in combination with several antisera against neuromediators and peptides known for specifically labeling neuropils in insect brains. To analyze these stainings, we used a confocal laser scanning microscope, and reconstructed several brain neuropils.

With these methods we visualized the gross brain anatomy and identified several brain regions that putatively correspond to established neuropils of the insect brain. These include the central complex with the associated lateral accessory lobes, the antennal lobes with olfactory glomeruli, and a structure which seems to resemble the mushroom bodies.

Eventually, comparison of these data with data obtained from insects and decapod crustaceans showed that the collembolan brains share more features with insect brains than with decapod brains.



Does Nitric Oxide affect neuronal plasma membranes and morphological differentiation?

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Nitric oxide (NO) is synthesized from arginine by three different types of NO-synthases (NOS). NO has been shown to have numerous effects on intracellular cell signalling by binding to enzymes containing hem groups in their active center such as guanylate cyclase or by direct protein nitrosylation at the amino acid residue cysteine such as Ras-GTPase. Recently, we could show by fluorescence recovery after photobleaching (FRAP) that NO decreases the plasma membrane microviscosity of endothelial cells [Grote-Westrick et al. (in preparation)]. Using NBD C6-sphingomyeline as a fluorophore, we show that a similar effect occurs on neuronal secondary cell lines: in SHSY-5Y neuroblastoma- and in PC12 pheochromocytoma cells a NO-mediated decrease in membrane microviscosity was found.
By the use of NOC18 as NO donor and L-NAME as NOS inhibitor we observe changes of retinoic acid or nerve growth factor induced morphological differentiation in SHSY-5Y and PC12 cells, respectively. In order to be able to discriminate between protein and membrane mediated effects we use a NO-nonresponsive Ras mutant (C118S) and an inhibitor of NO-sensitive guanylate cyclase as well as specific knock-out cells. As a non-NO compound affecting the microviscosity of plasma membranes more selectively we use benzyl alcohol in the context of morphological differentiation. Our results are compatible with the assumption that NO modifies morphological differentiation as a membrane active substance.

Neuronal integration of neurotransmitter inputs in *Drosophila* Kenyon cells

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Understanding how different environmental stimuli are integrated within the brain of an organism is an exciting challenge for modern neurobiology. Especially the studies addressing the coincidence detection in neuronal plasticity demonstrate that integration of different inputs in time can occur at different levels; the network, the single neuron, and single molecules. In *Drosophila*, the powerful molecular genetics combined with the behavioral analysis has been instrumental to identify the neuronal network implicated in olfactory associative learning. Although this analysis reveals a critical contribution of defined sub-sets of mushroom body intrinsic neurons, the Kenyon cells, the neuronal integration of the inputs into the Kenyon cells remains unclear.

To address this problem we used cameleon, a genetically- encoded fluorescent indicator for Ca^{2+}, to characterizetransmitter-induced modulations in Ca^{2+} in cultured sub-sets of Kenyon cells. We focused our study on acetylcholine (ACh), g-amino butyric acid (GABA), glutamate, octopamine, and dopamine, neurotransmitters that all play a role in neuronal plasticity. After verification of functional receptors we used pharmacological tools to confirm that ACh acts via nicotinic ACh-receptors that trigger a rapid increase in intracellular calcium by depolarization. Although GABA, glutamate and octopamine do not directly influence intracellular calcium concentrations their combination with ACh leads to neuromodulatory effects in the Kenyon cells. The results presented in this study provide the basis to characterize the neuronal integration of different neurotransmitter inputs in distinct sub-sets of *Drosophila* Kenyon cells with the aim to identify the neuronal integration during learning.

Taurine specifically activates GABAergic networks in the developing cerebral cortex

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Taurine is one of the most abundant free amino acids in the brain and has been identified as an endogenous agonist of both $GABA_A$ and glycine receptors. While we recently showed that taurine mediates an excitatory action on identified neurons in the developing neocortex, its action on the overall excitability and information processing in neuronal circuits during postnatal development remains elusive. Therefore we recorded identified neurons in neocortical slices (400 µm) of mice between the first and fourth postnatal day using whole-cell patch clamp recordings with Cl^--based pipette solutions and used the properties of postsynaptic currents (PSCs) to analyse the action of taurine, applied either by bath or be focal pressure application, on network activity.

Bath application of taurine significantly increased in a dose dependent manner the frequency of excitatory PSCs recorded in pyramidal cells. Since 300 µM taurine was sufficient to induce maximal responses, further experiments were performed with 300 µM taurine. In the presence of 0.2 µM TTX the taurine-induced PSCs were completely abolished, while a taurine-induced inward current remained in most of the recorded neurons. The taurine-induced PSCs were not affected by CNQX (10 µM) or APV (60 µM), antagonists of AMPA/Kainate and NMDA receptors, respectively, demonstrating an obvious lack of a glutamatergic component in taurine-induced postsynaptic activity. In addition, experiments with low-Cl^- pipette solutions revealed that the taurine-induced PSCs reversed their direction at E_{GABA}, suggesting that they are exclusively mediated via ligand-gated Cl^- channels. The $GABA_A$ antagonist gabazine (3 µM) completely abolished the taurine-induced PSCs, which can be explained by effects of taurine on the postsynaptic receptors or on presynaptic cells responding to taurine. A blockade of glycine receptors by 1 µM strychnine induced a reduction in the frequency of taurine-induced PSCs and inward currents, indicating that activation of glycine receptors was also involved in mediating these taurine-induced effects in the presynaptic elements of the activated circuits. In addition, this result also indicates that the taurine-induced PSCs observed in the recorded neurons are mediated mainly by postsynaptic $GABA_A$ receptors. To identify the presynaptic neurons responding to taurine, we pressure-applied taurine focally to individual neurons in different regions of the cortical plate far from the recorded neuron. We could identify only few neurons at which focal application of taurine results in synchronised activity at the postsynaptic recorded cell, indicating that only a small fraction of neurons is involved in taurine-induced network activity.

These results demonstrate that taurine can activate neuronal networks in the immature neocortex and suggests that this activity is mediated via GABAergic synapses by only a limited subpopulation of neurons in the developing neocortex. Thus taurine may function as an endogenous regulator of excitability in the developing neocortex.

Action of NOS inhibitors and an NO scavenger on the expression of aggression in the cricket *Gryllus bimaculatus* (De Geer)

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Agonistic encounters between male crickets *Gryllus bimaculatus* follow a stereotype pattern that results in an aggressive winner and a submissive loser that avoids aggressive conflict for several hours (Stevenson et al. 2005, J. Neurosci. 25: 1431–1441). Since the unconventional gaseous neuromodulator nitric oxide (NO) may depress aggressiveness in mammals, we test whether NO underlies loser depression in crickets. Mature adult male crickets were isolated for 24 hours and received head injections (10 µl) of: **1)** insect ringer, **2)** D-Name (D-N^G-Nitroarginine methyl ester), an inactive isomer of **3)** L-Name (L-N^G-Nitroarginine methyl ester), a competitive inhibitor of nitric oxide synthase (NOS),**4**) L-NNA (L-N^G-Nitroarginine), also a competitive NOS inhibitor, **5)** PTIO (2-Phenyl-4,4,5,5,-tetramethylimidazoline-1-oxyl-3-oxide), an NO scavenger. Contests were then staged between pairs of weight-matched crickets in a small arena (initial fight) and again 15 minutes later between the winner and loser (re-engagement); in each case we evaluated the "level of aggression" on a scale of 0-6 (cf. Stevenson et al. 2005). Whereas D-Name had no influence, L-Name, L-NNA and PTIO all significantly increased the level of aggression at the initial fight. Furthermore, while in the control groups the losers usually avoided the winners (level 1), in the test groups (L-Name, L-NNA, PTIO) they frequently exhibited aggressive behaviour (level 3 or higher) (see Figure). These data support the notion that the natural release of NO suppresses the expression of aggression and/or enhances escape/retreat behaviour in the cricket. We are currently testing other modulators of the nitric oxide system for influences on cricket aggression.

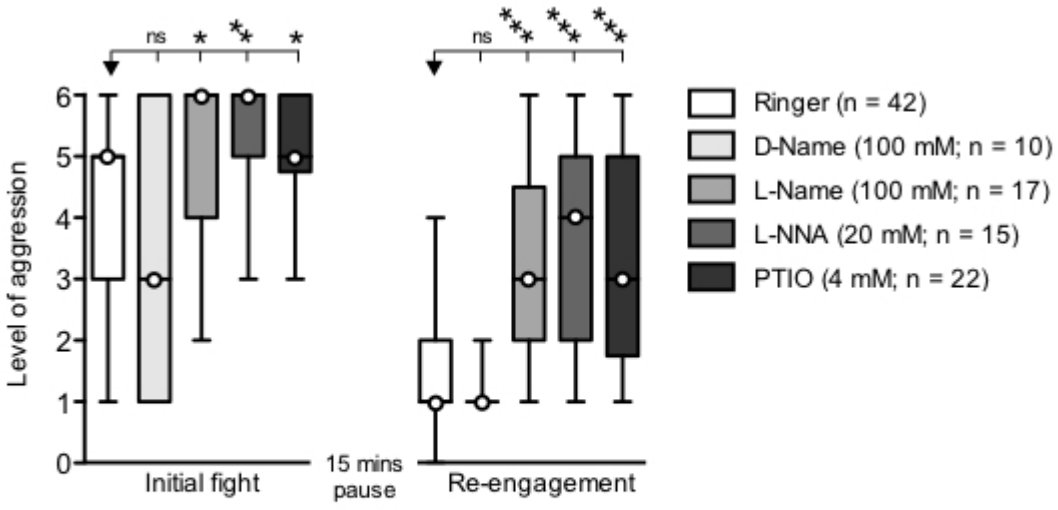

Figure: Box whisker plots showing the level of aggression (circles: median; bars: iqr; whiskers: min - max) for the treated groups of adult male crickets (see key) at an initial fight (left side) and of the winners versus the losers in a subsequent fight (right side, 15 minutes later). Significant differences to the ringer group are indicated (U-test: *, **, ***: $p < 0.05, 0.01, 0.001$).

SIFamide in the brain of the honeybee

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A variety of neurotransmitters, including acetylcholine, glutamate, GABA, histamine and biogenic amines contribute as primary signaling substances to information processing in the nervous system. Neuropeptides form a very large family of additional signaling molecules, but their distribution and function in the brain is less well known. In this study, we focus on the adult honeybee brain, and a specific peptide: SIFamide. SIFamides share a conserved c-terminal sequence across a number of arthropod species. They were originally identified in four neurons of the median neurosecretory cell cluster of flies. Here, we performed immunocytochemistry and immunogold-labeling of SIFamide, followed by confocal scans and electron microscopy.

Our stainings revealed that SIFamide is present in almost all neuropils of the honeybee brain, including those commonly associated with the olfactory system but excluding the first optic neuropil, the lamina. The stained neurites originated from four somata with a diameter of about 25µm in the pars intercerebralis located above the anterior rim of the central body, and from two somata in the subesophageal ganglion. The primary neurites of the four somata in the protocerebrum had an initial diameter of about 4 µm. They projected posteriorly and bended ventrally between the central body and the protocerebral bridge. They followed the posterior midline ventrally and turned anteriorly beneath the bottom of the b-lobes. They subsequently separated in pairs, which further projected posterior-laterally towards the posterior outer rim of the dorsal lobes. Several side branches left the primary neurites in that area and supplied all neuropils by a huge arborizing network of SIFamide-positive neurites, with generally decreasing diameters after each branch point. These branches carried strings of varicosities, 1-2 µm in diameter and up to 2 µm long, which were linked by narrow (0.2-0.7 µm, in diameter) inter-varicosity segments of variable length. Terminal branches commonly carried single- or multi-headed boutons, indicative of release sites. In the antennal lobes, each glomerulus was wrapped by SIFamide-positive neurites, only few boutons were found in the central core of glomeruli. No SIFamide-positive neurites were found in the antennal nerves.
Neuropeptides are commonly thought to be contained in dense vesicles, which are prominently present in insect brains. We investigated the distribution of SIFamide in dense vesicles in the antennal lobe of honeybees and found that SIFamide-positive vesicles were considerably different from non-stained dense vesicles. Gold particles were found in clearly delineated neuritic profiles, often on dense vesicles with a diameter of about 85 nm without an apparent core. These profiles also contained small clear vesicles indicating that they likely use an additional transmitter. Dense vesicles in SIFamide-negative profiles were smaller with diameters of about 60 nm and often displayed a dense core.
The distribution of SIFamide-positive neurites of common cellular origin over the whole brain of the bee suggests that the peptide may contribute to the shaping or modulation of brain activity in a global manner.

We like to thank Dr. Akikazu Yasuda for the gift of the antibody.

Modulation of a locust flight steering muscle by octopamine and tyramine

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In the invertebrate nervous system the neuromodulator and –hormone octopamine is known to account for a range of effects which altogether seem to modify the organism's state of motivation in a distinct and consistent manner ("orchestration hypothesis"). In addition, there is now good evidence that the immediate precursor of octopamine in biosynthesis, tyramine, serves as a neuroactive substance itself. Respective data on tyramine so far comprise the characterization of purely tyramine-immunoreactive neurons, the identification of tyramine-receptors and the assignment of a specific function in different invertebrate species like *Caenorhabditis*, *Drosophila*, *Manduca* and *Locusta*.

Our approach to investigate the significance of tyramine and octopamine in the insect nervous system (*Schistocerca gregaria*) focuses on the peripheral distribution and changes of these biogenic amines, especially if animals undergo different behavioral treatment. For that purpose we make use of a novel, reliable antibody to tyramine to stain the axonal terminals of tyraminergic/octopaminergic fibers. We also ask whether tyramine could act as a possible transmitter/modulator at neuromuscular junctions. Observations on the contraction mode of stimulated flight steering muscles under the influence of tyramine and octopamine suggest an individual effect of tyramine. The organism’s ability to physiologically meet behavioral demands may thus be more precisely regulated than previously expected.

New, cryptic peptide derived from the rat neuropeptide FF precursor

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Recently, we reported discovery of a novel sequence derived from NPFF precursor: NAWGPWSKEQLSPQA, which blocked the expression of conditioned place preference induced by morphine and reversed the antinociceptive activity of morphine (5 mg/kg, s.c.) in the tail-immersion test in rats. The sequence was named as NPNA (Neuropeptide NA from its flanking amino acid residues). Mechanism of its binding and action remains unknown. The C-terminal end of the NPNA sequence cannot be amidated, in contrast to other peptides derived from this precursor (NPFF, NPAF, NPSF), where a glycine residue from the C-terminal side of the peptide's sequence is a donor of $-NH_2$ group for amidation. Amidation of these peptides is crucial for interaction with opioid receptors. Synthetic NPNA injected intracerebroventricuraly inibits the expression of mRNA coding for Ga(i1), (i2), and (i3) protein subunits in isolated structures: hippocampus, striatum and frontal cortex. The results bring the evidence that a yet another bioactive sequence might be present within the NPFF precursor.

How does Tan maturation and reaction kinetic affect histamine recycling from carcinine?

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In the visual system of *Drosophila*, histamine is the neurotransmitter used for stimulus transmission from retina photoreceptor cells to postsynaptic neurons. There is ample experimental evidence supporting the idea of a histamine recycling pathway in a shuttle between photoreceptor axonal endings and surrounding epithelial glia. The enzymes Ebony and Tan are involved in the recycling process. Tan has been localized to photoreceptor cells, where it hydrolyses b-alanyl-histamine (carcinine) to b-alanine and histamine. Active Tan, as a member of the family of cysteine peptidases, is generated in a slow maturation process of internal proteolytic cleavage. We investigate the contribution of this maturation process and of the reaction kinetics of Tan on histamine recycling and visual performance. In aiming at these goals we are pursuing two strategies. First, Tan expression and purification schemes are outlined and kinetic parameters of Tan activity are determined. In addition, we are generating maturation mutants *in vitro*. After transformation into the genome they will be used to investigate the effect of Tan processing on enzyme activity as determined by visual performance *in vivo*. Here, we report on expression, purification reaction kinetics of Tan.

Altered expression of peripheral blood cytokines in migraineurs.

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Background:
Recent findings suggest that patients with migraine and especially patients with high attack frequency show changes in pro- and antiinflammatory cytokine profiles not only during but also outside attacks.
Objective:
The aim of this study was to investigate whether chronic inflammation could be a contributing factor to migraine chronification. Interictal levels of cytokines like TNF-alpha, IL-1 beta, IL-4, IL-6, and IL-10 were compared between patients with migraine and headache free healthy controls.
Methods:
Cytokine mRNA levels were measured in the peripheral venous blood of 28 migraineurs using Real Time Quantitative Polymerase Chain Reaction.
Results:
Levels of inflammatory cytokines like IL-6 and IL-1ß were significantly increased in migraine patients. Levels of antiinflammatory cytokines like IL-4 and IL-10 were decreased. There were no differences between patients with chronic or episodic migraine or between patients with migraine with or without aura. TNF-alpha levels were significantly decreased in patients compared with controls.
Discussion:
Our results point to a contribution of cytokines to migraine pathogenesis. The lack of a difference in cytokine mRNA levels of episodic migraineurs and chronic migraineurs suggests that cytokines are not involved in the chronification of migraine.

Effects of serotonergic compounds in Retinal Spreading Depression

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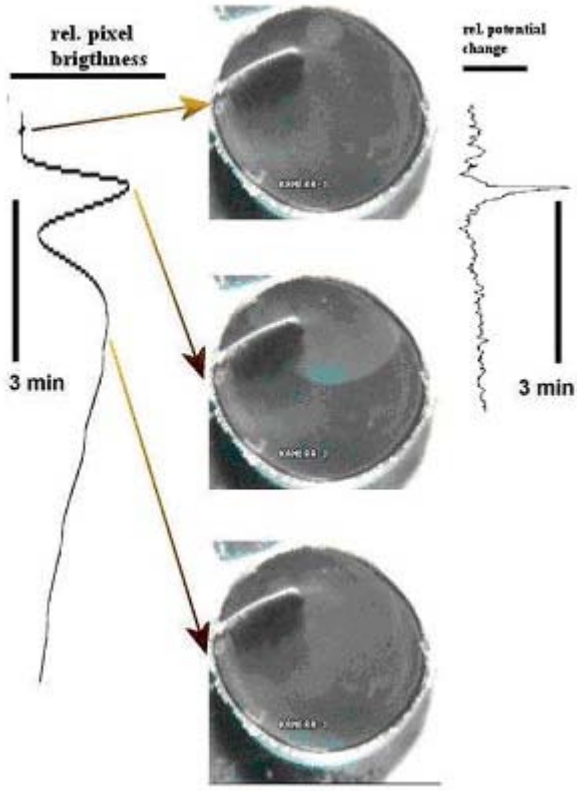
The SD is related to a variety of functional syndromes of the CNS such as **migraine**, transient global amnesia and certain forms of epilepsy. Furthermore it has been shown to be dependent on the action of a wide variety of neurotransmitters and drugs, and it can be controled by small external forces.
The retinal spreading depression (rSD), an excitation depression wave in neuronal tissue, has been verified to be a useful tool to study the systemic action of neuropharmacologically active drugs. According to the high intrinsic optical signal (IOS) compared to brain-slices, concomitant with the retinal spreading depression (rSD), it can be monitored with standard video imaging techniques in space and time. Thus the retina has been utilised in our studies as a model system. In this retinal IOS there is also a pronounced second peak of long duration (ca. 10 min), which is mainly related to metabolic processes, and is not given in elelctrophysiological recordings, and which in brain slices is a 100 to a 1000 times smaller. Thus an investigation is difficult or impossible except in the retinal SD, although metabolic processes by sure are highly important in the physiological concomittants of SD (migraine -pain).
We have combined optical recordings with electrophysiologcal measurements to obtain detailed information about the action of drugs wich are relevant for migraine therapy (serotonergic drugs as well as some other substances relevant in the field).
SD is defined among others by its propagation velocity, but also by the latency of its set-up after a proper stimulus. Especially this latency has been shown to be directly correlated with the excitability of the tissue. Thus influencing this latency by drugs must be a major aim of migraine pharmacological treatment.
In the presented experiments we investigated the effects of a variety of migraine relevant drugs (5HT, 5CT, LSD, melatonin and a variety of non-serotonergicc drugs used in migraine therapy) with the rSD. In our experiments both, the propagating properties and especially the initiation-phase (latency) of the rSD have been found to depend on the serotonergic pharmacology, as well as the accompanying potential shift. We have furthermore found neuroprotective effects of some of the drugs under investigation.
Propagation of a SD-wave in a retina monitored by a video set-up. The wave was elicited mechanically and then propagates concentrically after a short latency. The IOS is given left to the 3 video frames. The recording of the bulk potential change is given right to the video frames.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A comparison of the secretion of BDNF from hippocampal cultures induced by high-potassium depolarisation and backpropagating action potentials

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The mammalian neurotrophin BDNF is an important regulator of activity-dependent plasticity processes. BDNF is stored in secretory granules and is released in response to high-frequency electrical stimulation of cultured neurons. At the cellular level, the characteristics of BDNF-release in hippocampal neurons have been analysed in more detail by using GFP-tagged BDNF and monitoring the change in fluorescence intensity of BDNF-GFP containing secretory granules during high-potassium induced depolarisation of hippocampal neurons. Moreover, the activity-dependent release of BDNF-GFP could be shown recently to be induced by physiological patterns of electrical stimulation (Kuczewski et al., 2008).

We have now investigated the release of BDNF-EGFP induced by backpropagating action potentials and compared it to the kinetics and the signalling cascades driving release of BDNF-EGFP after high-potassium (50 mM KCl) depolarisation. Dissociated rat hippocampal neurons were transfected with EGFP-tagged BDNF and the release of BDNF-EGFP was analysed by monitoring the GFP-fluorescence intensity of BDNF-EGFP containing secretory granules using time lapse video microscopy. In one series of experiments release measurements were performed in the presence of the fluorescence quencher bromphenol blue to investigate differences in fusion pore dynamics. The efficiency of BDNF-release in response to different patterns and numbers of repetitions of backpropagating action potentials (theta burst stimulation, high frequency tetanus, spontaneous synaptic network activity) were analyzed. While the release kinetics turned out to be very similar between electrical stimulation and high-potassium depolarization, the subcellular sites of secretion were different between both paradigms: BDNF-release occurred relatively unselective from the soma, dendrites and at postsynaptic sites, in response to high-potassium depolarisation. In contrast, a less generalized BDNF-EGFP release could be observed after electrical stimulation, and was often confined to selected vesicle clusters in dendrites. Differences in the kinetics of fusion pore openings and of vesicle movements prior to release were further analysed for both stimulation paradigms. Our results suggest that BDNF-EGFP release after high-potassium induced depolarization and following physiological electrical activity share similar kinetics and mechanisms, but that due to the overall depolarisation of the cell by superfusion with high-potassium the release sites are more distributed over the cell, whereas release in response to groups of action potentials can occur in a more confined fashion that might underly synaptic input specific plasticity.

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The impact of chronic and acute administration of IGF-I on activity-dependent release of BDNF

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The neurotrophic peptides BDNF and IGF-I control similar cellular functions in the mammalian brain. BDNF as a member of the mammalian neurotrophins is a secretory protein and is ubiquitously expressed in the CNS throughout life. IGF-I which belongs to the insulin-like growth factor family is highly expressed in the young and developing rodent brain. With increasing age the main source of IGF-I for the brain is the circulating IGF-I which is synthesized in peripheral tissues and reaches the brain through the blood-brain barrier. In recent studies, it could be shown that IGF-I induces a change in the BDNF level in the brain. Furthermore, in the context of physical exercise, elevated concentrations of IGF-I and BDNF were observed in the brain and blood stream. The cellular mechanisms connecting increased IGF-I and elevated BDNF levels, however, are largely unknown. An effect of IGF-I on the expression level of BDNF could be demonstrated. Therefore the question arised if IGF-I can directly modulate the release properties of BDNF.
We have now investigated the chronic and acute effects of IGF-I on the activity-dependent release of BDNF and on the fusion pore opening of BDNF-containing secretory granules. Dissociated cultures of rat hippocampal neurons were transfected with EGFP-tagged BDNF. The depolarisation-induced release of BDNF-EGFP was analysed by monitoring the intracellular GFP-fluorescence intensity using time lapse video microscopy. The characteristics of activity-dependent fusion pore opening of BDNF containing secretory granules were studied just in time by quenching intravesicular fluorescence of BDNF-GFP through the extracellular application of bromphenol blue, which readily crosses the secretory granule fusion pore.
Long-term application of IGF-I (24 h; 50-400 ng/ml) to dissociated hippocampal neurons had no influence on activity-dependent release of BDNF: the delay between start of the depolarisation and the onset of BDNF release (20-30s under control conditions and after IGF-I treatment) were unaffected. The kinetics of fusion pore opening of secretory granules were also not modified by chronic IGF-I stimulation. Likewise, the amount of released BDNF 5 min after stimulation with 50 mM KCl did not reveal any significant effects of IGF-I (depolarisation induced BDNF release without IGF-I incubation: 15,1 % ± 2,1 %; with IGF-I incubation: 15,3% ± 1,7 %). Furthermore, the characteristics of secretory granules were unchanged. Neither the vesicle size, nor the packaging density of BDNF containing secretory granules were modified by chronic IGF-I stimulation. Similarly, the acute application of IGF-I to BDNF-EGFP expressing neurons did neither induce release of BDNF nor facilitate depolarisation induced (50 mM KCl) secretion of the neurotrophin.
Taken together these results suggest that neither chronic nor acute incubation with IGF-I has an influence on the release properties of BDNF.

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siRNA mediated knockdown of BDNF in CA1 pyramidal neurons of mouse hippocampal slice cultures

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The protein BDNF is a member of the mammalian neurotrophin family. Besides the important role of BDNF in neuronal survival and differentiation, BDNF is a critical regulator of acute and long-term changes in synaptic plasticity. At the synaptic level, BDNF has both, pre- and postsynaptic effects. The application of BDNF acutely stimulates neurotransmitter release and phosphorylation of ionotropic receptors, which can drive fast changes of glutamatergic and GABAergic synaptic transmission. Long-term application of BDNF to neuronal cultures regulates morphological synaptic changes, enhances glutamatergic as well as GABAergic transmission by pre- and postsynaptic mechanisms and is also a mediator of synaptic homeostasis. Moreover, studies with BDNF knockout mice confirm the significance of BDNF in acute and long-term regulation of synaptic transmission. While many of these studies analysing the effect of BDNF on synaptic transmission have been performed by unrestricted administration or withdrawal of BDNF in complex neural networks, understanding more subtle mechanisms of synaptic changes by BDNF requires single cell overexpression or knockdown of the neurotrophin.

In order to analyse the consequences of a BDNF-deficit at a cellular level, we have developed an efficient method to knockdown BDNF expression in single CA1 pyramidal cells. Hippocampal slices from newborn mice were prepared according to the Stoppini method. Using single cell electroporation, CA1 pyramidal cells were cotransfected at 10 DIV with a validated siRNA against BDNF and a fluorescent dextran for later cell retrieval. Two days after transfection whole cell patch-clamp recordings of transfected cells were performed to analyse excitatory synaptic transmission. Electrophysiological properties (miniature synaptic currents, evoked IPSCs and EPSCs, paired-pulse facilitation, synaptic fatigue) of BDNF-deficient CA1 pyramidal cells were compared to those of CA1 pyramidal cells transfected with control siRNA, and to untransfected neighbouring cells in the same slices. Our results suggest similar basal electrophysiological and synaptic properties of BDNF-deficient cells grown in a BDNF-containing cellular context, suggesting that BDNF-withdrawal in a single cell can be compensated by the neighbouring BDNF-expressing cells.

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Stimulation of $P2Y_1$ receptors in the rat prefrontal cortex impairs sensory information processing

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The medial prefrontal cortex (mPFC) of mammals is essential for the regulation of sensory modulated attention and sensorimotor gating functions. Previous pharmacological studies in vivo have shown that G_q-protein coupled P2Y-receptors at the rat nucleus accumbens, sensitive to extracellular ADP/ATP, modulate neuronal transmission and reward-related behavior. In the present study cognitive responses to $P2Y_1$ receptor stimulation in the rat mPFC were investigated. In the social novelty discrimination of two juveniles (familiar and unfamiliar) by adult rats the microinfusion of the selective $P2Y_1$ receptor agonist MRS2365 into the mPFC caused a deficit in investigatory performance by a diminished selective attention to the unfamiliar juvenile. The microinfusion of MRS2365 also reduced the prepulse inhibition of the acoustic startle response as a sign of disturbed information processing in the mPFC. More complex cognitive functions were investigated using a spatial delayed win shift task at the radial arm maze. The treatment with MRS2365 and the introduction of a delay after acquisition of the non-delayed task led to an increase of across phase errors, but not of within phase errors, an indication for impaired monitoring and processing rather than short-term mnemonic functions in the mPFC. These results are supported by immunocytochemical localization of $P2Y_1$ receptors at neurons and glial cells in the mPFC. The presented data indicate that $P2Y_1$ receptors are involved prefrontal information processing and that they may play a role in cognitive-executive dysfunctions in psychiatric illnesses.

Involvement of P2Y receptors in signal transduction pathways in development and growth

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There is increasing evidence that neurotransmitters in the immature nervous system can act as trophic factors influencing development and growth. ATP exerts trophic as well as toxic effects through the stimulation of P2X/Y receptors. Thereby P2Y receptor-mediated effects may be regulated by various crucial signalling cascades, e.g. the mitogen-activated protein kinase/extracellular signal regulated protein kinase (MAPK/ERK) or the phosphoinositide 3-kinase/serine-threonine kinase Akt (PI3K/Akt) pathways.

In our previous studies, using organotypic tissue slice co-cultures, the expression of purinergic receptors as well as the growth promoting effect of P2 receptor agonists has been shown. The aim of the present study was to identify and to characterize signal transduction pathways responsible for the observed fibre outgrowth after P2 receptor stimulation using organotypic slice co-cultures of the dopaminergic system (ventral tegmental area/substantia nigra-(VTA/SN)-complex; prefrontal cortex (PFC)). With the help of multiple immunofluorescence labelling and Western blot analysis we confirmed changes in the expression of phosphorylated Akt (pAkt) and pERK after treatment with different P2 receptor agonist and antagonists, respectively. Hence, the expression of the $P2Y_1$ receptor, pAkt and pERK at the fibres interconnecting the VTA/SN-complex and the PFC was exposed immunocytochemically. Moreover the selective P2Y agonist adenosine-5'-O-(2-thiodiphosphat) significantly increased both the outgrowth of biocytin labelled fibres to the target region PFC and the quantitative expression of pAkt. The pre-treatment with the PI3K-inhibitor Wortmannin inhibited the studied effect.

In conclusion, the data revealed a P2Y receptor-mediated PI3K/Akt-stimulation suggesting to a role of this cascade in developing, differentiating dopaminergic organotypic co-cultures.

Effects of High- and Low-Frequency rTMS on the Inhibitory Systems in Adult Rat Cortex

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Repetitive transcranial magnetic stimulation (rTMS) is widely used to modulate human cortical excitability with the intention of studying cortical function or as a possible therapeutic intervention in neuronal disorders related to abnormalities of cortical excitability. Findings to date suggest that the modulatory effects of rTMS are depending on the stimulation parameters used, with stimulation frequency being the main factor. High-frequency rTMS leads primarily to facilitation, whereas low-frequency rTMS leads to suppression of cortical excitability. Knowledge concerning the cellular mechanisms is limited but synaptic plasticity has been suggested as a key process. However, cortical excitability is primarily a matter of complex inhibitory control and cellular mechanisms of inhibition have been found to be also plastic in an activity-dependent manner. The two isoforms of the glutamic acid decarboxylase (GAD), GAD65 and GAD67, the rate-limiting enzymes of gamma-aminobutyric acid (GABA) synthesis, are known to be regulated by phosphorylation in an activity-dependent manner. Therefore, we investigated whether the expression of GAD is affected by rTMS stimulation protocols inducing either low- or high-frequency activity within the cortical network.

Rats received either a 1 Hz rTMS (3 blocks of 20 min, 3600 stimuli in 70 min), or 5 blocks of an intermittent theta-burst like rTMS protocol (iTBS, 10 trains of 3 pulses/60ms [50 Hz, intertrain interval 200 ms, 5 Hz resp.] repeated 20 times every 10 seconds, 3000 stimuli in 63 min) at an intensity previously found to be peri-threshold for eliciting action potential activity (about 30% of max. output). Stimuli were applied via a figure-of-8 coil (MagStim) centred 10 mm (verum-stimulation) or 80 mm (sham-stimulation) above the rat's brain with mediolateral current direction to induce primarily callosal activity. Following rTMS, rats were perfused and visual, somatosensory, motor and frontal cortices of each hemisphere were processed for immunohistochemical studies.

Our results show that both high- and low-frequency rTMS induced a reduction of GAD67 in all cortical areas, reaching statistical significance only in the frontal cortex, but only iTBS significantly enhanced the GAD65 expression. Our results are in accordance with the finding that enhanced neuronal activity activates GAD65 but deactivates GAD67 via phosporylation [1]. They further fit to our previous finding of increased zif268 expression following iTBS but enhanced c-fos due to 1 Hz rTMS [2], since both GAD65 and zif268 are controlled by protein kinase C, while GAD67 and c-fos are regulated via protein kinase A. Our results indicate that high-frequency stimulation of the cortical network shifts the GABA synthesis ratio from the cytosolic to the synaptic location. This may be a fast response to prevent the genesis of epileptic activity. This study has been supported by the Deutsche Forschungsgemeinschaft, DFG (SFB 509, TP C12).

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Modulation of neuronal excitability through $GABA_B$ receptor-mediated tonic inhibition in the rat medial prefrontal cortex *in vitro*

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$GABA_B$ receptors are coupled to G proteins mediating their effects via second message pathways. They are located extrasynaptically and can be activated by "spill-over" of GABA from adjacent synapses. Activation of postsynaptic $GABA_B$ receptors results in the opening of potassium conductances of the GIRK (G-protein-coupled inwardly rectifying K^+) type to hyperpolarize the membrane potential and inhibit activities of respective cells, leading to tonic inhibition. Here we show that $GABA_B$ receptor-mediated tonic inhibition is powerful enough to modulate the excitability in layer 2/3 pyramidal neurons of the rat prefrontal cortex in acute slices.

Potassium currents of 106 ± 7 pA (mean ± s.e.; n=18, holding potential Vh = -50 mV) are induced by the specific $GABA_B$ receptor agonist baclofen (25 microM) in pyramidal neurons. These currents showed no or little accommodation and could be abolished by the application of 1 microM CGP 52432, a selective $GABA_B$ receptor antagonist. The selective GIRK1/4 channel blocker rTertiapin-Q (500 nM) blocked approximately 40% of the baclofen induced current. Furthermore, in the presence of barium (300 microM), baclofen was still able to induce a substantial outward current. Therefore, up to 60% of the total baclofen induced current is not mediated by GIRK1/4 channels.

Application of CGP 52432 (1 microM) revealed a tonic $GABA_B$ receptor-mediated current of -19.4 ± 3.7 pA (n=5), in the presence of GABA (3.3 microM), and NO-711 (2.5 microM), a specific GABA uptake inhibitor (GAT1), and blockers of excitatory synaptic transmission (APV 50 microM, CNQX 10 microM). Co-application of gabazine (10 microM) indicated an additional current of similar amplitude, which could be attributed to tonic $GABA_A$ receptor activation. Testing furthermore for its impact on the input-output response function of the pyramidal neurons, we found that $GABA_B$ receptor activation shifts the input-output function to the right. Moderate extracellular stimulation (5 pulses, 70Hz) strongly activated a $GABA_B$ receptor-mediated outward current. This suggests that synaptic release of GABA in the prefrontal cortex is pooled to a sufficiently high concentration for activating $GBAB_B$ receptor-mediated inhibition.

In a modified artificial cerebral spinal fluid (mACSF) with increased K^+ and decreased Mg^{2+} and Ca^{2+} concentrations, the mean EPSC frequencies increased in both pyramidal cells and interneurons with application of CGP 52432 and decreased after application of baclofen. These experiments could give a measure of the overall activity in all the connected presynaptic neurons. In young rats, blockade of $GABA_B$ receptor-mediated tonic inhibition increased the duration of spontaneous depolarized states of the membrane potential observed with mACSF. Large-scale epifluorescence Ca^{2+}-imaging experiments further indicated that $GABA_B$ receptor-mediated tonic inhibition is capable of regulating the duration and size of individual depolarized states.

These results suggest that ambient GABA can induce a persistent tonic inhibition in the prefrontal cortex, which is mediated by extrasynaptic $GABA_B$ receptors and is large enough to modulate neuronal excitability.

Pituitary adenylate cyclase-activating polypeptide (PACAP) selective receptor (PAC1R) expression during the earthworm embryogenesis

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Recently we have shown the up-regulated expression of PACAP-like compounds in the special copulatory structure (clitellum) of mating earthworms, in deposited cocoons, and developing embryos. Recent work focusing on the expression of PACAP sensitive receptor (PAC1R) in earthworm *Eisenia fetida* embryonic tissues gives experimental evidences that it is present from the early developmental stages of earthworms. Expression of PAC1 receptors has been identified with western blot and far western blot analysis. Results of western blot analysis showed that PAC1R is a 45 kDa molecular weight protein in embryos. According to the conserved regions of PAC1R mRNAs of several species we designed primer sets. Polymerase chain reaction (RT-PCR) was performed using freshly prepared *Eisenia fetida* cDNA from adult neural tissues. The results of the PCR indicated a 300 base pairs long product which might be a partial sequence of the PAC1R mRNA. Exact anatomical location of PAC1-receptors has been determined by light and electron microscopic immunocytochemistry. PAC1R-like immunoreactivity was restricted to distinct neuron set of the developing central nervous system and some cells of the body wall epithelium. Electron microscopic immunocytochemistry revealed that PAC1 receptors were located on plasma membranes and intracellular membranes of both differentiated tissues (neurons, epithelial cells) and distinct cell sets of germinal layers. Our present results suggest that PACAP-like compounds play a role in the earthworm development and tissue differentiation participating the mediation of the differentiation of germinal layer cells to various tissues.

Identification of a neuropeptide S responsive circuitry connecting endopiriform cortex and amygdala

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The 20 residue peptide neuropeptide S (NPS), termed after its N-terminal serine residue, has recently been identified as the endogenous agonist for the orphan G-protein coupled GPR154 receptor now referred to as NPSR. Analysis in heterologous expression systems revealed a likely G_q protein-mediated mobilization of intracellular Ca^{2+}, suggesting that NPS may enhance neuronal excitability. Based on expression pattern and NPS application *in vivo*, roles in olfaction, anxiety, arousal, hippocampus-dependent learning and memory, as well as energy balance and food intake have been suggested [Xu et al. 2007, J Comp Neurol 500: 84-102.]. Anxiety, arousal and hippocampus-dependent memory formation depend critically on the function of the basolateral amygdala (BLA). In the current study we investigated the role of NPS in the control of neuronal activity in the endopiriform nucleus (EPN) and its potential to modulate activity in the BLA as well as BLA-dependent expression of conditioned fear behavior.
We applied electrophysiological and pharmacological techniques in the slice preparation of the mouse *in vitro* to characterize direct and indirect cellular effects of NPS in native neuronal cells. We demonstrate that NPS directly activates an inward current in 20 % of EPN neurons and evokes an increase of glutamatergic excitation in this nucleus. Excitation of the EPN is responsible for a modulation of BLA activity through NPS, as was shown by cut experiments and local application of NPS. As a result, GABAergic inhibition via local interneurons is increased and spike activity in a subset of BLA projection neurons is enhanced. Finally, local administration of NPS to the EPN / piriform cortex *in vivo* resulted in a selective disturbance of contextual, but not auditory cued fear memory responses. By testing plus maze behavior immediately prior to the fear memory, we could rule out a general effect of our treatment on anxiety-like behaviors as seen upon intracerebroventricular injection at the dosage used [Xu et al. 2004, Neuron 43: 487–497]. Together, these data suggest the existence of a specific NPS-responsive circuitry between EPN and BLA, likely involved in contextual aspects of fear memory.

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Analysis of the gustatory receptor mediated signaling cascade in *Drosophila melanogaster*

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Drosophila melanogaster has approximately 60 gustatory receptor (GR) genes, which are expressed in the proboscis, first legs and wings, as well as in the female genitalia. Each gustatory receptor neuron expresses a subset of these GR genes. GRs are seven transmembrane proteins, which usually couple to G-protein linked signaling cascades. The aim of our study is to unravel the intracellular signaling cascade in the gustatory neurons, with special emphasis to the role of heterotrimeric G-proteins. Two of these receptors, GR5a and GR66a, have well understood response profiles to the tastants trehalose and caffeine. We investigated the involvement of different Gα-subunits in sugar taste transduction in behavioural assays with transgenic flies expressing mutated G-proteins and RNAi directed against different G-proteins. We also analysed the expression of Gα-subunits in the proboscis by quantitative RT-PCR and immunohistochemistry. The physiological properties of gustatory sensory neurons in response to sugar stimuli were then measured applying the tip recording technique.

Analysis of RNA editing of the mouse 5-HT$_{2C}$ receptor by Real-time PCR

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Among the 14 kinds of serotonin (5-HT) receptor subtypes, the 5-HT$_{2C}$ receptor has been extensively studied because of its pathophysiological significance in the brain. It represents an important target for antidepressants and, based on the fact that the receptor modulates dopamine release, is discussed as a potential target for schizophrenia as well. However, its contribution to brain functions is very complex due to the fact that the receptor RNA is post-transcriptionally modified by RNA editing. Altered 5-HT$_{2C}$ RNA-editing patterns have been reported in depression as well as in schizophrenia and suicide. Furthermore, it has been shown that antidepressants like fluoxetine as well as antipsychotics such as haloperidol or risperidone influence the editing of the 5-HT$_{2C}$ mRNA.

So far, editing of the 5-HT$_{2C}$ receptor mRNA has been analyzed by either direct sequencing or pyrosequencing, two methods that are time consuming and do not allow specific investigation of transcripts with a certain editing pattern.

We report a new technique based on real time-PCR to investigate 5-HT$_{2C}$ mRNA editing in the mouse brain. This method facilitates the analysis of mouse 5-HT$_{2C}$ receptor RNA editing patterns directly from cDNA. We analyzed cDNA from 11 different brain regions using TaqMan assays which selectively recognize the non-edited, the fully edited (ABECD) as well as five other highly abundant isoforms (A, AD, ABD, ACD, ABCD) of the mouse 5-HT$_{2C}$ receptor mRNA. We found that, with the exception of the cerebellum, all brain regions show similar frequencies of the investigated transcripts. The transcripts edited at positions ABD showed the highest abundance while the non-edited transcript constitued only a low percentage of the total mRNA. The other partially edited transcripts showed medium abundances, which differed only slightly between brain regions. Our results are in good agreement with reported data obtained by sequencing methods. In contrast, the non-edited transcript made up the vast majority of transcripts found in the cerebellum. We did not detect the fully edited transcript in any of the brain regions analyzed. Furthermore, the abundance of editing at the five positions is similar to rats but differs between mice and humans. In addition, we found that treatment with the selective 5-HT$_{2C}$ agonist lorcaserin (10 mg/kg i.p.) significantly increased the overall amount of transcript in a number of brain regions leaving the editing pattern unaffected.

Editing of the 5-HT$_{2C}$ receptor mRNA has a strong effect on the pharmacological properties of the receptor. The pharmacological properties of the isoforms of the mouse 5-HT$_{2C}$ receptors analyzed in this study are comparable to the respective human receptor isoforms. However, when analyzing pharmacological data obtained in mice, one has to keep in mind that the editing patterns in the two species are different.

Carbachol promotes cortical differentiation in rat

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Immature neuronal networks display special synchronous activity pattern during the late embryonal and early postnatal period. They occur for instance in retina, spinal cord, hippocampus and neocortex and are termed calcium waves, retinal waves, giant depolarising potentials or early network oscillations. Early synchronous activity patterns are believed to contribute to the selforganisation of neuronal ensembles which then become modifiable by experience-dependent plasticity during the postnatal critical period.
In the cortex, calcium waves and synchronous activity can be electrically evoked and induced by certain transmitter receptor activation for instance by Carbachol (CCH) which activates primarily the muscarinic acetylcholine receptors.
However, less is known about the consequences of these synchronous activity on structural and neurochemical differentiation. Early activity patterns may modify gene expression, presumably in a specific manner and depending on the pattern of electrical activity and the frequency of events.
To enlarge on these questions we used the model system of newborn rat organotypic visual cortex cultures. First, we analysed the mRNA expression status of the muscarinic receptors M1-M4. We found out that all receptor mRNA´s are expressed in organotypic cultures. In response to CCH stimulation Ras andMAPK 42/44 (ERK1/2) are transiently activated. Already 15 minutes after CCH treatment Ras is activated and MAPK phosphorylation increases, without affecting the MAPK 42/44 protein expression. CCH transiently increases the mRNA expression of c-fos, BDNF and NT-3, whereas NPY mRNA expression is unaltered. Furthermore, the mRNA and protein expression of several oligodendrocyte-specific genes are upregulated with CCH.
On the structural level CCH is able to promote pyramidal cell differentiation by increasing length and branching of apical dendrites in layer II/III and VI, as well as basal dendrites in layer VI. Furthermore, it promotes interneuronal maturation by increasing the soma size and the length and segments of primary dendrites.
In conclusion, the cholinergic agonist Carbachol promotes differentiation of rat visual cortex on neurochemical and morphological level.
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Dual modulatory role of GABA(B) receptors in hipoocampal parvalbumin-expressing cells

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Metabotropic ?-aminobutyric acid receptors (GABABRs) mediate modulatory influence of the GABAergic system on both excitatory and inhibitory neurotransmission in the hippocampus. We used a combination of ultrastructural and pharmacological approaches to elucidate the pre- and postsynaptic localization and function of GABABRs in inhibitory fast-spiking parvalbumin-expressing (PV+) basket cells. At the light microscopic level, weak immunostaining for the GABAB1 protein was found in somato-dendritic compartments of PV+ interneurons. At the electron microscopic level, the labeling for GABAB1 was abundant postsynaptically where immunoparticles were mainly found to be localized to the extrasynaptic membrane of dendritic shafts. Quantitative analysis further revealed an association of GABAB1 to asymmetrical putative glutamatergic synapses on dendritic shafts. Using transgenic animals, in which either the GABAB1a or GABAB1b isoform of the receptor subunit was knocked-out, we found that the GABAB1b protein was predominant in this compartment. Presynaptically, the immunoreactivity for GABAB1 was sparse but immunogold particles for the receptor subunit were consistently present in axon terminals of PV+ cells. Consistent with results of the immunocytochemistry, puff-application of 100 ?M baclofen, a GABABR agonist, to the apical dendrites of PV+ cells resulted in a slow inhibitory current in PV+ cells. Furthermore, putative PV+ basket cell-mediated monosynaptic IPSCs in pyramidal cells, elicited by electrical stimulation in the somatic layer in the presence of ionotropic glutamate receptor blockers, were dramatically reduced by 5 ?M baclofen applied to the bath. These results show that functional GABABRs are present in the dendrites and terminals of hippocampal PV-containing basket cells. The localization of postsynaptic receptors suggests that they are involved in the modulation of glutamatergic inputs to the neurons. In contrast, presynaptic GABABRs modulate the inhibitory output by regulating GABA release from these interneurons. (Supported by DFG: SFB 780, project A2)

Differential Expression of inhibitory neurotransmitter receptor subunits in the thalamocortical system

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The thalamocortical network is characterized by the generation of low frequency oscillatory burst activity during slow wave sleep as well as spike and wave discharges observed during episodes of absence epilepsy. The cyclic interaction between specific pacemaker and Ca^{2+} currents which is responsible for generating rhythmic burst firing in a number of thalamic cell types critically depends upon membrane hyperpolarization. Inhibitory currents generated by $GABA_A$ receptors ($GABA_AR$) are an important source of hyperpolarizing membrane currents. In the thalamus, the conventional form of transient synaptic responses that underlie phasic inhibition and a tonic inhibition depend on differential activation of specific $GABA_AR$ subunits. In a rat model of absence epilepsy, the WAG/Rij rats, it has been shown that expression of $GABA_AR$ a4 and d subunits was increased in sensory relay nuclei. Furthermore it was recently recognized that glycine (the classical inhibitory transmitter in the spinal cord) mediates neurotransmission in the ventrobasal thalamus by activating glycine receptors (GlyR). The possible contribution of GlyR to absence epilepsy has yet not been investigated. To further appreciate the role of inhibitory neurotransmitter receptors in the generation of rhythmic activity in the thalamocortical system we commenced an extensive expression analysis of $GABA_AR$ and GlyR in several thalamic nuclei (dorsal part of the lateral geniculate nucleus, dLGN; ventrobasal thalamic complex, VB; nucleus reticularis thalami, NRT; centromedial nucleus, CM; paracentral nucleus, PC; centrolateral nucleus,CL) and primary visual (V1) and somatosensory (S1) cortical areas. Standard PCR analyses of different $GABA_AR$ (a1-a5, ß1, ß3, γ2-γ3) and GlyR (a1- a3, ß) revealed a differential expression in the thalamocortical system of non-epileptic ACI and epileptic WAG/Rij rats. The $GABA_A$ γ2 subunit for example was highly expressed in dLGN but was barely detectable in NRT. Furthermore, the GlyR a3 subunit expression levels were high in VB whereas in dLGN the signal was much weaker. Currently experiments are performed to unravel possible differences in receptor expression levels in WAG/Rij and ACI rats. Furthermore immunohistochemical studies will be performed to identify protein expression and cellular localization of inhibitory receptors. In summary, preliminary data indicate a nucleus-specific expression of $GABA_AR$ and GlyR which may underlie the generation of distinct synaptic and extrasynaptic potentials and the occurrence of epileptic activity in the thalamocortical system.

Molecular and pharmacological characterization of cockroach biogenic amine receptors

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Biogenic amines are important messenger substances in the central nervous system as well as in the periphery of both vertebrates and invertebrates. These small organic compounds modulate and regulate various physiological and behavioral processes as diverse as motor patterns, secretion, aggression, circadian rhythm, and learning. Biogenic amines mediate these multifaceted effects predominantly by binding to members of the superfamily of G protein–coupled receptors. The molecular identification as well as the functional and pharmacological characterization of these receptors is crucial for the comprehension of the intracellular signaling pathways activated by biogenic amines. Here, we focus on our results concerning cDNA sequences of a serotonin and a dopamine receptor of the cockroach *P. americana.* Cockroaches are not only important pest species but also established model organisms for neurobiological and physiological research.

Using a strategy based on degenerate primers and RACE PCR we obtained cDNA sequences encoding for a serotonin (Pea*5-ht1*) and a dopamine (Pea*dop2*) receptor of *P. americana.* Both proteins display the major characteristics common to all G protein-coupled receptors. The tissue-specific expression pattern of the receptor mRNAs has been investigated by RT-PCR. High expression levels for both receptors were found in brain tissue and salivary glands. The distribution of the receptor proteins was analyzed by immunohistochemistry with specific affinity-purified antibodies. In order to clarify the functional and pharmacological properties of the cloned receptors, we studied HEK293 cell lines, stably expressing these receptors.

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Functional characterization of a novel splice variant of the human orexin type-2 receptor

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Orexin A and orexin B are hypothalamic peptides that regulate sleep and wakefulness, feeding behavior, neuroendocrine and autonomic functions. The orexin receptors OX1R and OX2R are members of the G-protein coupled receptor family and act via Ca2+ mobilizing signals. We isolated a novel splice variant of the human OX2R using RACE-cloning. This new isoform lacks the seventh transmembrane domain and contains an alternative non-homologous sixth transmembrane domain.

To identify potential functional effects of the OX2R splice variant on wild type OX2R function both isoforms were expressed in Xenopus laevis oocytes via mRNA microinjection. Under voltage clamp conditions, application of orexin A induced a Ca2+ dependent chloride current in oocytes expressing the wild type OX2R while no effects of orexins were found in oocytes solely expressing the OX2R splice variant. Coexpression of the splice variant with the wild type OX2R resulted in significantly lower amounts of the evolved currents. A similar effect of the OX2R splice variant was observed in a human adrenocortical carcinoma cell line (NCI-H295R) and in Chinese hamster ovary (CHO) cells. We transfected different amounts of OX2R splice variant DNA into NCI-H295R cells that endogenously express the wild-type OX2R. CHO cells which do not express orexin receptors were treated in the same manner, but were additionally transfected with wild-type OX2R DNA. Intracellular calcium concentrations before and after orexin A application were monitored using the fluorescent Ca2+ indicator Fluo-4 AM and a fluorescence microplate reader. Both cell lines showed up to 50% reduction of the Ca2+ response following orexin application depending on the expression of the splice variant OX2R.

In CHO cells transfected with wild type- and splice variant OX2R-EYFP fusion protein, we found wild-type OX2R mainly located within the plasma membrane while the OX2R splice variant was expressed in the endoplasmatic reticulum. This phenomenon was already described for other G-protein coupled receptors and their dominant-negative splice variants e.g. the human histamine H4 receptor. A possible mechanism of interaction could be the dimerization of wild type OX2R and OX2R splice variants. We used the method of BRET (Bioluminescence resonance energy transfer) to analyze OX2R dimerization. CHO cells were cotransfected with OX2R-Rluc (Renilla luciferase) as BRET donor and OX2R-EYFP as BRET acceptor and coelenterazine-h was used as substrate for Rluc. The intensity of the obtained BRET ratio was compared to different control samples and suggests dimerization of the OX2Rs.

Our results suggest that the novel OX2R splice variant is predominantly expressed in the endoplasmatic reticulum and modulates the wild-type OX2R function in a dominant-negative manner probably via heterodimerization that may lead to retention of the wild-type OX2R within the endoplasmatic reticulum. Our findings reveal new possibilities concerning regulation and signalling of the orexin system.

Serotonin receptors 5-HT1A and 5-HT7, that activate different signalling pathways, form heterooligomers

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The neurotransmitter serotonin (5-HT) is involved in the regulation of major physiological functions. Cellular responses to serotonin are mediated through the activation of different 5-HT receptors. The large majority of 5-HT receptors (excluding 5-HT3) belongs to the family of G-protein coupled receptors (GPCR). Many GPCRs have been shown to form oligomers rather than function only as monomers activating one heterotrimeric G-protein in order to transduce the signal. Oligomerisation, especially if occuring between different receptor subtypes, can influence pharmacological and signalling properties of receptors and therefore provide an additional mechanism to modulate signal transduction. We found that 5-HT1A and 5-HT7 receptors, that couple to different G-proteins, form heterooligomers. While 5-HT1A couples to inhibitory Gi-protein, which inhibits adenylate cyclase activity, stimulation of 5-HT7 leads to activation of stimulatory Gs-protein followed by the increase of adenylate cyclase activity. In addition, 5-HT7 couples to G12-protein leading to activation of small GTPases of the Rho family. By using biochemical, electrophysiological and biophysical approaches we analysed the role of heterooligomerisation in regulation of receptor functions, including coupling to G-proteins, intracellular transport and cellular excitability. To study, whether the heterooligomerisation takes place early in the synthetic pathway or occurs at the cell membrane, a trafficking trap assay was established. Furthermore, we analysed oligomerisation dynamics in response to receptor stimulation.

A possible role for TRPC channels in synaptic signals of olfactory bulb granule cells

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In the mammalian olfactory bulb, granule cells mediate self- and lateral inhibitory interactions between mitral/tufted cells via a dendrodendritic reciprocal synapse. Although axonless, granule cells (GCs) spike during odorant stimulation. We have found previously in juvenile rat GCs that synaptically evoked spikes cause a prolonged depolarization and extra calcium influx throughout the dendrite in comparison to current-evoked spikes. Here we describe a similar effect in adult mouse GCs, using conventional current-clamp recordings and two-photon imaging in acute brain slices and sensory-like stimulation, i.e. extracellular activation of a glomerulus.
We observed a prolonged depolarization following the synaptically evoked spike with a half duration of 125 ± 210 ms (n = 33 cells). This afterdepolarization was significantly different from what is observed when the GC spike is evoked by somatic current injection with respect to both amplitude and duration ($P < 0.001$). Application of the NMDAR blocker APV (20 µM) significantly reduced the extra depolarization in all tested GCs (n = 10).
The putative mechanism underlying this plateau current is a non-specific cationic conductance. Therefore we tested various TRPC receptor knockout animals (courtesy M. Freichel, Homburg). In the knockout of both TRPC1 and TRPC4 we observed a considerable change in the plateau (n = 21 cells): the amplitude and duration of the synaptic afterdepolarization were significantly smaller than in the wild type ($P < 0.001$ and 0.05 respectively, Mann-Whitney test), and there was no significant difference between somatically evoked and synaptic afterdepolarizations in these double-KO animals. The single KOs TRPC1-/- (n = 7) and TRPC4-/- (n = 9) showed less pronounced reductions of the synaptic afterdepolarization.
We are currently testing whether there is also a reduction in extra calcium entry following synaptic spikes in TRPC14-/- animals.

Our results imply that TRPC1 and TRPC4 channels contribute to GC signaling upon strong sensory stimulation.

In search of delta receptor-interacting proteins

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Ionotropic glutamate receptors (iGluRs) are ligand-gated ion channels that mediate the vast majority of excitatory neurotransmission in the mammalian brain. The subfamily of delta receptors, delta1 and delta2, within the family of iGluRs is based solely on sequence similarity. No agonist is known to activate the delta receptors, and it is not known how they work. Nevertheless, they are important, given that delta2 plays a crucial role in cerebellar function as mice that lack the gene encoding delta2 display ataxia and impaired synaptic plasticity. However, the mechanisms by which delta2 participates in cerebellar functions are largely unknown.

Recently, a study by Naur et al. (2007) showed that the ligand binding core of delta2 binds D-serine and glycine. However, no detectable currents were observed after application of D-serine or glycine to Xenopus oocytes injected with cRNA encoding wild type delta2. But, amazingly, both molecules reduced the spontaneous currents through delta2 receptors containing the lurcher mutation. The lurcher mutation is a point mutation exchanging alanine 618 for threonine and results in a constitutively active delta2 receptor.

The study of Naur et al. demonstrates that delta2 can bind ligands and that cleft closure of the ligand-binding core can induce conformational changes that alter ion permeation. It is possible that additional subunits or accessory proteins that are not found in heterologous expression systems are essential for the function of delta receptors as ion channels.

We try to identify such potentially interacting proteins of delta receptor subunits by a combination of affinity purification and analysis by Multidimensional Protein Identification Technology (MudPIT).

Inhibition by U73122 of GIRK and BK Channels in a Phospholipase C-Independent Fashion

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Phospholipase C (PLC) regulates phosphatidylinositol 4,5-bisphosphate (PIP_2) levels in the plasma membrane and generates the second messengers inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). The PLC inhibitor U73122 is widely used to explore the various facets of PIP_2-associated signaling, including Ca^{2+} release from IP_3-sensitive calcium stores, DAG-mediated activation of protein kinase C, and PIP_2-dependent regulation of ion channel activity. However, the selectivity of U73122 has been disputed. In the ion channel field, a particular concern is that U73122 appears to exert a direct inhibition of Kir3 (GIRK) channels that is not associated with its action on PLC (Meyer et al., 2001; Lei et al., 2001; Cho et al., 2001; Filippov et al., 2004; Sickmann et al., 2008). In a previous study in cardiac cells, Cho et al. (2001) proposed that U73122 blocks Kir3 channels by interfering with their PIP_2 binding site. To elaborate on this major side effect, we examined the action of U73122 and U73343, a structurally related, but not PLC-inhibiting analogue, on Kir1.1, Kir2.1 or Kir3.1/3.2 channels expressed in HEK293 cells. Both compounds (10 µM) displayed an unusual degree of selectivity for Kir3, even superior to that of tertiapin, which discriminates between Kir3 and Kir2, but also inhibits Kir1.1. Recordings from mutant Kir2 and Kir3 channels showed that U73122 is unlikely to block Kir3 by interfering with binding of phosphatidylinositol 4,5-bisphosphate, nor did U73122 seem to act like a pore blocker. Unexpectedly, U73122 and U73343 also suppressed Ca^{2+}-activated K^+ channels of the large-conductance type (MaxiK, BK) in a PLC-independent fashion. In single-channel recordings, both compounds significantly decreased open probability of BK channels and slowed their ultra-fast gating ("flickering") at very depolarized potentials. Alignment of the amino-acid sequences of Kir3 and BK channels suggested that the highly selective effect of U73122/U73343 is mediated by a homologous domain within the long C-terminal ends. In fact, mutations in the C-terminal region of Kir2 and Kir3 channels significantly altered their sensitivity to the two compounds. Our data strongly caution against the use of U73122 when exploring signaling pathways involving Kir3 and BK channels. However, the apparent binding of U73122/U73343 to a common structural motif might be exploited to develop drugs selectively targeting Kir3 and BK channels.

Low Voltage Activated Calcium Channels Are Coupled to Ryanodine Receptors in Neurons of the Thalamic Reticular Nucleus

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Neurons of the thalamic reticular nucleus (NRT) exhibit a transient depolarisation termed low threshold spike (LTS) following release from sustained hyperpolarisation. This is caused by the activation of low voltage activated Ca^{2+} channels (LVACC). Although the role these channels play in thalamocortical oscillations was studied in great detail, little is known about the downstream intracellular Ca^{2+} signalling pathways. This study combines electrophysiological techniques and two-photon Ca^{2+} imaging to show that Ca^{2+} release from intracellular stores indeed occurs in NRT neurons. Firstly, release of Ca^{2+} from intracellular stores was demonstrated in NRT neurons. Secondly, the neurons revealed a cell-type specific coupling of LVACC and ryanodine receptors (RyR) to facilitate Ca^{2+} induced Ca^{2+} release (CICR). CICR could be evoked by repetitive LTS generation and contributed ~30% to the resulting build-up of the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). It was abolished by a specific blocker (cyclopiazonic acid, CPA) for sarco(endo)plasmic reticulum calcium ATPases (SERCAs) or by high concentrations of ryanodine. High voltage activated Ca^{2+} channels (HVACC) did not contribute to CICR, and action potentials contributed little to the build-up of $[Ca^{2+}]_i$ upon repetitive LTS generation. Finally, Ca^{2+} released from intracellular stores significantly reduced the number of action potentials during the LTS. In conclusion, activation of LVACC but not HVACC causes CICR via RyR in NRT neurons, thereby adding a Ca^{2+} dependent intracellular route to the mechanisms determining rhythmic oscillatory bursting in the NRT.

Antidepressant-induced internalization of serotonin transporters in serotonergic neurons

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A deficiency of serotonergic signaling is thought to be involved in the etiology of depression. Thus, drugs blocking the reuptake of serotonin back into the neurons are widely used in treatment of this disease; however, their delayed effect in remission of patients suggests that the clinical response does not rely on simple serotonin uptake inhibition but may include further regulatory mechanisms. We have analyzed cellular serotonin transporter (SERT) expression on exposure to the selective serotonin reuptake inhibitor citalopram in serotonergic neurons expressing the native SERT allele in its natural surroundings. Biotinylation of SERT-expressing HEK293 cells, as well as confocal microscopy analysis in these cells and in serotonergic neurons, revealed that exposure to citalopram time dependently reduces the amount of cell surface-expressed SERT. Furthermore, in serotonergic neurons, longer exposure to citalopram not only caused an internalization of SERT proteins from the cell surface but also induced a redistribution of SERT from neurite extensions into the soma. This process was reversible on drug removal. Microarray analysis performed on citalopram-treated serotonergic neurons revealed that antidepressant treatment does not alter SERT mRNA expression, suggesting that SERT trafficking from and to the cell membrane is regulated on the posttranscriptional level. Taken together, our results provide evidence that antidepressant-treatment induced transporter internalisation is an important means to modulate synaptic plasticity without interference on the gene expression level.

Kv7/M-type potassium channels are critical determinants of neuronal network activity in neonatal mouse brain

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The mechanisms of synchronization and epileptogenesis in hyperexcitable neonatal neuronal networks are only partially understood. To investigate pathophysiological mechanisms underlying brain developmental abnormalities and epileptogenesis linked to Kv7/KCNQ/M-type potassium channel deficiency that is associated with a neonatal epilepsy syndrome in humans, (BFNC), we generated conditional transgenic mice with attenuated M channel activity. M channels were suppressed by the expression of a dominant-negative KCNQ subunit resulting in a functional knockout. Tet-Off system-mediated restriction of transgene expression to defined developmental periods revealed a critical role for M channels in neonatal brain development. Suppression of M channels during this period resulted in a severe phenotype that included neurodegeneration and neuroinflammation in the hippocampus, increased seizure susceptibility, spontaneous epilepsy, and marked behavioral changes in adult mice. Absence of transgene expression during the first two neonatal weeks prevented phenotype development. We hypothesized that attenuated M-channel activity causes changes in neuronal network activity in neonatal brain. Therefore, we performed simultaneous acute depth profile recordings of local field potentials and unit activity in visual cortex (V1) and hippocampus in awake head-fixed P5-7 pups. V1 network activity in control mice consisted of short spindle-like bursts in superficial layers with frequencies in the beta2 range (10-30 Hz). Activity in the hippocampal CA1 region mainly consisted of sharp waves with maximum amplitudes in stratum radiatum and phase reversal in the pyramidal cell layer reflecting Schaffer collateral activation. In V1 of mutant mice spindle burst were more frequent and increased in frequency, amplitude and duration. Occasionally, long-lasting spindle oscillations propagated to deeper cortical layers where they initiated phase-locked unit activity. The topographical activity pattern in mutant hippocampus was not altered, however, sharp wave frequency in CA1 stratum radiatum was also increased.

Acute treatment with the NKCC1 blocker bumetanide that lowers $[Cl^-]_i$ in immature neurons reduced cortical and hippocampal network activities and specifically suppressed spindle bursts in V1. Chronic bumetanide treatment during the first two neonatal weeks prevented morphological hippocampal changes and improved the behavioral phenotype to comparable levels as did doxycycline-suppressed transgene expression.

The data suggest that cortical M-channels are critical for the control of network oscillations in the neonatal mouse brain.

Role of thyroid hormone receptors alpha and beta for the postnatal regulation of Cav1.3 currents in mouse inner hair cells

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Thyroid hormone (TH) controls many processes during the critical period of final differentiation of the cochlea, and TH deficiency causes hearing loss due to structural and functional deficits of the organ of Corti. TH acts through the nuclear receptors TRalpha1 and TRbeta1 which regulate transcription. TRbeta1-deficient mice are deaf, whereas TRalpha1-deficient mice have normal hearing.

In rats and mice, the critical period extends from E17 to P12 including a phase of spontaneous action potential activity in inner hair cells (IHC) evoked by regenerative activation of voltage-gated Cav1.3 Ca2+ channels and delayed rectifier K+ channels. In this phase of spiking, the amplitude of IHC Cav1.3 currents is being up-regulated until P7/P11 and then down-regulated to a lower mature level. In IHCs of hypothyroid rats and athyroid Pax8-/- mice, peak IHC Ca2+ currents were doubled and their down-regulation was delayed (Brandt et al. J. Nsci. 2007, Sendin et al. J. Nsci. 2007) which led to the question which of the TH receptors controls Cav1.3 expression. We therefore recorded Ca2+ currents using Ba2+ (IBa) in IHCs of TRbeta1-/- and TRalpha1-/- mice. IHCs of TRbeta1-/- mice showed an increased peak of IBa (120 % of control) at P9-P11, which was much smaller than in hypothyroid or athyroid animals, suggesting a contribution of TRalpha1 in the developmental regulation of Cav1.3. Indeed, TRalpha1-deficient mice showed an accelerated up- and downregulation of peak IBa amplitude. We also analyzed mice with a targeted mutation of TRalpha1, TRaAMI/S, that blocks TH-mediated relieve of gene repression (Quignodon et al., Mol. Endocrinol. 2007). Here, the developmental up-and downregulation of IBa was altered in comparison with the WT, further indicating a role for TRalpha1 in controlling Cav1.3 expression. To conclude, a concerted action of both TRalpha1 and TRbeta1 seems to be necessary for the developmental up- and downregulation of IHC Cav1.3 current that is decisive for the control of Ca2+ action potentials, exocytosis and gene expression.

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Mutation of extracellular cysteines differentially effect the K^+-Cl^--cotransporters KCC2 and KCC4

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Cation chloride cotransporters (CCCs) play a pivotal role in chloride homeostasis, transepithelial salt transport and cell volume regulation. The gene family comprise the Cl and NKCC2 and the Cl^--extruders KCC1 to KCC4. Malfunction of these proteins is associated with severe pathological conditions, such as epileptic seizures, neuropathic pain, renal tubular disorders, and deafness. Furthermore, some of the cotransporters serve as molecular targets of diuretics, which represent some of the most commonly prescribed drugs.

Yet despite their physiological and medical importance, only few studies on structure-function relationships have been conducted. Here, we report on a comparative mutational analysis of KCC2 and KCC4, which display 72% identity on the amino acid level. Both proteins comprise 12 transmembrane domains with a large extracellular loop between transmembrane domains 5 and 6. This loop contains 4 cysteines, which are conserved from *drosophila* to man, and between all KCCs.

To evaluate the impact of these cysteines on KCC2 and KCC4 function, we mutated all 4 cysteines in both proteins and determined their transport activity after heterologous expression in HEK293 cells. The transport activity of the KCC4 quadruple mutant was unchanged compared to the wild type. This indicates that the investigated cysteines are not required for intramolecular disulfide bridges. In contrast, the transport activity of the KCC2 quadruple mutant was completely abolished. Immunocytochemistry and biotin surface labelling experiments demonstrated that the KCC2 quadruple mutant was expressed and trafficked to the plasma membrane. Identical substitutions of single cysteines in KCC2 and KCC4 also resulted in marked differences in transport activity between these two transporters. This selective outcome of cysteine mutations indicates cotransporter-specific conformation of the extracellular loop. Furthermore, our data demonstrate that data on structure-function relationships obtained even for highly conserved amino acid residues cannot simply be transferred between members of the CCC protein family.

Live-cell single molecule analysis of subunit stoichiometries of ion channels and receptors

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Many ion channels and receptors in nerve cells exhibit a heteromeric composition with the different subunit types assembled in a defined or random fashion. Biochemical methods have certain limitations that can make it difficult to determine the exact number and the stoichiometry of the subunits.

We developed an approach based on the observation of single fluorescent molecules in the membrane of a living cell that allows us to clearly determine the number of subunits of a protein and to determine the stoichiometry of heteromeric proteins. We measure the fluorescence emitted by GFP fused to the protein of interest and count photobleaching steps of single GFP tags. The number of photobleaching steps from a single channel equals the number of GFP tags and thereby unveils the number of subunits the protein is composed of. In an extension of this approach, we label different subunit types with green and red fluorescent proteins. The frequency of co-localization of the different tags reflects the chance of co-assembly of the different subunit types into the same receptor. From the degree of co-localization, we can determine the rule that underlies subunit assembly, and analyze heterogeneous populations of membrane protein complexes.

We used this approach to test the proposal in the literature that NMDA receptor subunits assemble into tri-heteromeric receptors when co-expressed. We demonstrate an ability to discriminate both theoretically and experimentally between different assembly mechanisms, including random assembly, stoichiometric assembly and mutual exclusion of certain subunits from the same receptor. We find that certain NMDARs employ a mutual exclusion rule to prevent formation of tri-heteromeric receptors and others assemble in a tri-heteromeric stoichiometric fashion.

STIM1 EXHIBITS DIFFERENT NEURONAL EXPRESSION THAN STIM2 AND SHOWS PUNCTA-LIKE COLOCALIZATION WITH ORAI1 UPON DEPLETION OF ER CALCIUM STORE

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It is now well established that cooperation of ER calcium sensors STIM1 or STIM2 with the plasma membrane calcium channel protein ORAI1 is crucial for a proper functioning of Store Operated Calcium Entry (SOCE) in non-excitable cells. However, little is known about these proteins in excitable cells. Although SOCE is ubiquitous in non-excitable cells, it is also crucial for the neuronal cells. Alterations of SOCE may lead to pathology like Alzheimer's and Huntington's disease.
We identify STIM1 and STIM2 proteins in mouse brain and in cultured cortical and hippocampal neurons using various techniques. We show that the protein and mRNA levels of STIM1 and STIM2 vary in different brain regions. For STIM1 the highest level is present in the cerebellum and for STIM2 in the hippocampus. Immunohistochemistry of brain sections shows a distinct distribution of both proteins mostly in the hippocampus, cerebellum and amygdala. We also demonstrate that STIM1 and STIM2 are present in cultured neurons and their expression is accumulated mainly in the cell bodies. However, strong dendritic immunostaining is observed for STIM1, but not for STIM2. In addition, our data revealed that depletion of the ER calcium store in cultured cortical neurons triggers translocation of YFP-STIM1, YFP-STIM2 and ORAI1 from disperse, in untreated, into punctuate structures in thapsigargin (TG) treated cells.
In an attempt to understand the different localization of STIM1 and STIM2 and mechanism of their translocation, we are further studying the effects of simultaneous expression of ORAI1 and STIM1 or ORAI1 and STIM2 on SOCE in cortical neurons. We measure intracellular calcium level during SOCE using a Ca^{2+} imaging method and FURA-2. This analysis is performed after depletion of intracellular Ca^{2+} stores by treating cells with TG in Ca^{2+} free medium, and during subsequent incubation of neurons in 2 mM Ca^{2+} media. The inhibitor ML-9 of SOCE is also being used. Our data allow us to propose that, in neurons, just as in non-excitable cells, the ORAI1 and STIM proteins are involved in Store Operated Calcium Entry.

Modal gating, not window current, is responsible for persistent Na^+ current in neocortical pyramidal neurons

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In neocortical pyramidal neurons, the persistent Na^+ current (I_{NaP}), which is only a small fraction of the total Na^+ current, plays a crucial role in synaptic integration, by amplifying excitatory and inhibitory synaptic potentials and determining spike threshold. Two mechanisms, which are not mutually exclusive, have been offered to explain I_{NaP}: 1) the "window current", which is an integral feature of the Hodgkin Huxley formalism, and 2) "modal gating", which entails periodic failure of individual channels to inactivate. Although the window current is expected to be active over a very wide range of voltages, like the actual I_{NaP}, comparison of whole-cell data with model simulations reveals that it does not account for the experimental observations. Thus, during slow voltage ramps, the window conductance would be expected to decline after reaching a peak, whereas the actual non-inactivating Na^+ conductance persists. Furthermore, were I_{NaP} to be a window current, it would be expected to entail the activation of a large number of channels, each with a low probability of opening. Number of channels (N) and open probability (P_o) can be estimated from fluctuations in membrane current. Because the variance of I_{NaP} recorded during slow voltage ramps from -70 to 0 mV is related to the current fluctuation, we used non-stationary noise analysis to evaluate N and P_o. As the current increases, the variance rises dramatically, reaching a maximum at around -40 mV, and then declines while the current continues to rise. Thus, P_o of the channels underlying I_{NaP} reaches 0.5 when I_{NaP} is still rising, indicating that the open probability of these channels in not low and that N must therefore be relatively small. This is consistent with our previous findings that I_{NaP} is almost entirely generated by the relatively low number of Na^+ channels located in the axon initial segment (Astman et al., J Neurosci 26:3465-73, 2006), and leads us to the conclusion that the persistent Na^+ current does, indeed, reflect "modal gating" of a select population of channels.

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Cell-autonomous homeostatic regulation in muscle cells of the fruit fly drosophila melanogaster.

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In the last few years homeostatic plasticity has been recognised as an important mechanism for excitable cells to keep their activity within functional limits (Turrigiano and Nelson, 2004; Davis and Bezprozvanny, 2001). Such a mechanism is particularly important during development, when the size and morphology of these cells change considerably or during activity dependent forms of plasticity such as LTP or LTD, which could drive a neurons activity out of functional limits. The mechanisms, however, are diverse and not well understood but include changes in synaptic size, presynaptic release, postsynaptic transmitter sensitivity and balance of voltage gated channels. To investigate and understand the mechanisms of homeostatic regulation at the molecular and cellular level a simple model system is needed. We took advantage of a Drosophila mutant (Electrical knock out, EKO, White et al., 2001) which carries a modified potassium channel gene. Muscle specific expression of the EKO gene with the Gal4-UAS binary system results in larvae expressing shaker potassium channels with lowered activation threshold and virtually absent inactivation. These larvae should have severely reduced excitability and thus contractions. Surprisingly, larvae were viable and muscle contractions were unchanged. In addition they did not exhibit changes in neuromuscular transmission (White et al., 2001) or pattern and frequency of motoneuronal input compared to control groups. On the level of muscle fibre properties we detected an increased calcium-current. The peak currents is elevated to about 140 ± 15% (n=8, N=9) compared to wildtype (Canton S) 100 ± 10% (n=7, N=8) and phenotypical wildtypes (UAS-EKO without Gal4 expression) 85 ± 13% (n=7, N=8) and their activation threshold is lowered by about 10 mV. To prove these findings we analysed the expression of voltage-gated calcium channels by RT-PCR and also found an increased expression of the calcium channel ß subunit.
We conclude that an increase of the calcium current is at least one of the mechanisms that compensate the effect of expressing EKO in muscle-cells.

Nanomolar ambient ATP decelerates $P2X_3$ receptor kinetics

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P2X receptors belong to the superfamily of ligand gated ionotropic receptors. There are seven P2X receptor cDNAs currently known, which have been termed $P2X_{1-7}$. Functional P2X receptors assemble as trimers, formed by subunits with two transmembrane domains and intracellular N- and C-termini. Activated at very low ATP concentrations they open nonspecific cationic channels, causing cell depolarization and increase in intracellular calcium concentration. In general this ability led to the assumption that P2X receptors may modulate synaptic transmission. Homomeric P2X receptors differ in their electrophysiological and pharmacological profiles. In the central nervous system, $P2X_3$ receptors are involved in pain signalling pathways, with ATP acting as the endogenous agonist. However, analysis of $P2X_{1,3}$ receptor functions is complicated, particularly because of the complexity of interactions between agonist binding and receptor gating. This is characterized by a rapid rise in receptor current accompanied by an extremely fast desensitization, which is followed by a very slow recovery from the desensitized state. In addition, modulatory effects on $P2X_3$ receptors have been reported for low concentrations of ATP [ATP], leading to both, enhancement and reduction of receptor currents. The former has been reported to be mediated by activation of ectoprotein kinases and the latter by high affinity desensitization (HAD). Both processes influence amplitudes rather than kinetics of evoked receptor currents.

Here we describe a new phenomenon, the modulatory influence of ambient low [ATP] on $P2X_3$ receptor kinetics. First, we studied in HEK cells whether persistent ATP affects current decay. To this end, $P2X_3$ receptor mediated currents, elicited by pressure application of saturating [ATP], were analyzed after pre-application of low [ATP]. Second, UV-flash photolysis of ATP was employed to investigate whether submicromolar [ATP] affects receptor activation. Finally we confirmed the effetc of nanomolar [ATP] on native $P2X_3$ receptors of neurons freshly isolated from rat dorsal root ganglia.

We found that persistent low [ATP] caused pronounced deceleration of receptor current activation and decay. This priming effect indicates a mechanism different from HAD. It could be explained by a pre-opening receptor isomerization, induced by the occupation of a high affinity binding site already at the resting state. The observed modulation of the receptor kinetics could be considered as physiological fine tuning mechanism of the nociceptive system, driven by the actual ambient agonist concentration.

The role of sodium channel availability in determining action potential conduction velocity in unmyelinated axons

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Repetitive impulse activity in somatic axons results in a progressive reduction in axonal conduction velocity. Since impulse activity produces a concomitant Na-K-ATPase dependent hyperpolarisation this has hitherto been deemed causal for the reduction in conduction velocity. To test this putative causality changes in axonal conduction velocity produced by repetitive activity were examined in single unmyelinated axons innervating the rat cranial meninges. The Na-K-ATPase was blocked with Ouabain (10µ-1mM), by cooling (24-35 °C), by reducing available ATP with cyanide (100-500µM) and by reducing enzymatic substrate either by lowering the extracellular K^+ concentration or by replacing extracellular Na^+ with Li^+. In the 42 axons examined Na-K-ATPase blockade increased the magnitude of activity-induced conduction velocity slowing thereby demonstrating that electrogenic Na-K-ATPase activity is not causally responsible for conduction velocity slowing. We propose instead that the most prominent influence of membrane potential on conduction velocity is secondary to its influence on the time constant of sodium channel slow inactivation. In accord with this idea, the sodium channel blockers lidocaine, carbamazepine and phenytoin (10-500µM) affected activity-induced conduction velocity slowing in a dose-dependent manner. At low doses (10-50µM) activity-induced slowing was slightly increased. At higher doses (>100µM) the degree of slowing decreased with increasing dose and at sufficiently high doses, activity-induced slowing was completely blocked. Activity-induced conduction velocity slowing is therefore proposed to be due to the progressive accumulation of sodium channels in their slow inactivated state.

Functional interaction between T-type Ca^{2+} and A-type K^+ currents in intralaminar thalamocortical relay neurons

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The thalamocortical network is the neuronal substrate for the generation and maintenance of low frequency oscillatory activity during slow wave sleep as well as spike and wave discharges observed during episodes of absence epilepsy. Thalamic relay nuclei are a critical component of this network. Beside the 'specific' nuclei, and the 'higher order' nuclei, 'unspecific' nuclei represent a mixed group of cells receiving ascending afferents from the cortex, and sending their axons to widespread cortical and striatal areas. They also receive massive monosynaptic input from the brainstem, and thereby are considered to mediate widespread neuronal excitation and arousal. These nuclei include the intralaminar nuclei (for instance, the centrolateral nucleus, CL). Within the thalamus, the slow oscillations are accompanied by burst firing in thalamocortical relay (TC) neurons. Burst firing in TC neurons depends on the activation of T-type Ca^{2+} currents (I_T). These currents generate a low-threshold Ca^{2+} spike (LTS), which triggers a high frequency burst of action potentials. Although the intralaminar nuclei in general are not well studied, it is known that intralaminar TC neurons exhibit pronounced bursting with unusual high frequency firing of action potentials and express a prominent I_T with an activation threshold around -70 mV.

A-type K^+ channels represent an important functional antagonist of T-type Ca^{2+} channels by generating a 4-aminopyridine (4-AP)-sensitive transient outward current (I_A) which activates below the threshold of action potentials. To determine the influence of I_A on the unusual bursting behavior of intralaminar TC neurons, its voltage-dependent and pharmacological properties were analyzed in acutely isolated TC neurons of the CL by using whole-cell patch-clamp techniques. The steady-state voltage dependency of activation was investigated by stepping neurons to increasingly positive test potentials (from -70 to +30 mV, 200 ms duration) from a conditioning potential of -120 mV (1000 ms duration). Construction of a steady-state activation curve revealed a threshold of around -50 mV and a half maximal voltage (V_h) of activation at -18 ± 2 mV (n = 8). Steady-state inactivation was investigated by holding neurons at increasingly positive conditioning potentials (from -120 mV to -20 mV, 1000 ms duration), and stepping to a constant analyzing potential of -20 mV (200 ms duration). Construction of a steady-state inactivation curve revealed a V_h of -64 ± 2 mV (n = 7). Application of different concentrations of 4-AP (0.1 – 10 mM; n = 5 -12) revealed a doses-responses characteristic with am IC_{50} value of 1.0 mM.

During current clamp recordings amplitude and duration of the LTS was increased and prolonged in the presence of 4-AP, respectively.

These results point to a scenario in intralaminar TC neurons where I_T activates at hyperpolarized membrane potentials of around -70 mV followed by the activation of a conventional I_A at more positive potentials of around -50 mV. Therefore I_A has only little influence on the regenerative initiation of an LTS but shapes its amplitude and duration.

A TTX resistant sodium current in presubicular neurons

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The presubiculum is a region situated between the subiculum and the entorhinal cortex which appears to have a specific role in the coding of space. Some subicular cells are sensitive to head direction (Taube *et al.*, 1990), others signal a combination of place and direction of movement (Cacucci *et al.*, 2004). Neuronal coding and the transmission of information between neurones depends significantly on sodium channels. Most identified Na channels are antagonised by tetrodotoxin (TTX), but several isoforms have been identified that are resistant to TTX. In this study on pyramidal cells in the rat pre-subiculum we observed an inward current that seems to be carried by Na and that is not suppressed by TTX.

Using whole-cell patch clamp recordings we show that firing in presubicular cells is characterized by action potentials with a large overshoot and a low frequency adaptation during repetitive firing. We noted that an inward current persisted in the presence of TTX (1 to 10 µM) and the Ca channel antagonists nifedipine and mibefradil. The current was not affected by the omission of external Ca ions, nor by the Ca-channel antagonists agatoxin IVA, and conotoxin GVIA or by the TRP channel blocker Flufenamic acid. Equimolar substitution of sodium by the impermeable cations NMDG or TEA completely abolished the TTX resistant current. Nearly complete current block was obtained after 15 min of exposure to lidocaine. These findings suggest that the current may correspond to a TTX resistant Na current.

With a midpoint of activation at -21 mV and inactivation half-voltage at -36 mV, the properties of the TTX-R sodium current were not identical with those of currents generated by isoforms containing the Nav1.5, Nav1.8 and the Nav1.9 alpha subunits. They were most close to those of the Nav1.8 subunit but the current was not abolished in pyramidal cells of mice lacking the Nav1.8 subunit. This TTX-R current could contribute to the coding functions of pre-subicular cells, specifically the maintained firing that is associated with their signalling of a stable head position.

Modulation of the Ca^{2+}-conductance of Nicotinic Acetylcholine Receptors by the endogenous protein Lypd6

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The agonist binding sensitivity and desensitisation kinetics of nicotinic acetylcholine receptors (nAChRs) can be modulated by snake venom neurotoxins and related endogenous small proteins of the uPAR-Ly6 family. We have identified Lypd6, a distantly related member of the this family as a modulator of nAChRs in neurons. Transgenic mice overexpressing Lypd6 display behaviors that were indicative of an enhanced cholinergic tone, such as a higher locomotor arousal and hypoalgesia. These mice are also more sensitive to the analgesic effects of nicotine.

In trigeminal ganglia cells Lypd6 selectively enhanced the Ca^{2+}-component of nicotine-evoked currents through nAChRs, as evidenced by comparative whole-cell patch clamp recordings and Ca^{2+}-imaging. In contrast, a knockdown of Lypd6 expression using siRNAs selectively reduced nicotine-evoked Ca^{2+}-currents. Pharmacological experiments with blockers such as alpha-Bungarotoxin or methyllycaconitine revealed that the nAChRs involved in this process are heteromers.

Taken together, Lypd6 seems to constitute a novel modulator of nAChRs that affects receptor function by selectively increasing Ca^{2+}-influx through this ion channels.

A novel family of proteins that control trafficking and gating of AMPA receptors

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Glutamate receptors of the AMPA subtype (AMPARs) together with the transmembrane AMPAR regulatory proteins (TARPs) mediate fast excitatory synaptic transmission in the mammalian brain. Here, we show by proteomic analysis that the majority of AMPARs in rat brain are co-assembled with isoforms of a novel family of transmembrane proteins, referred to as auxiliary AMPAR subunits (auxAMPARs). Functionally, auxAMPARs promote surface expression and profoundly alter channel gating by slowing deactivation and desensitization kinetics. These results introduce a novel auxiliary subunit of native AMPAR complexes providing a novel molecular determinant for modulating excitatory neurotransmission in the CNS.

Contribution of A-type potassium current I_{SA} in b-AP and EPSP attenuation in hippocampal CA1 pyramidal neurons

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A subthreshold activating A-type current (I_{SA}) shows a positive density gradient with distance from the soma in CA1 pyramidal neurons of the hippocampus, whereas sodium and calcium current densities stay constant. I_{SA} controls dendritic excitation in different ways: Most importantly, I_{SA} suppresses the retrograde spread of excitation, and back-propagating action potentials (b-APs) show a strong decrease in amplitude as they travel along the apical dendrite. But I_{SA} also quenches excitatory postsynaptic potentials (EPSPs). Finally, inactivation of I_{SA} at the sites of excitatory synaptic input allows for local b-AP recovery and strong depolarization as a base for long term potentiation. Thus, I_{SA} acts as a “dendritic shock absorber” and at the same time plays an important role in dendritic integration and synaptic plasticity.

The molecular correlate of I_{SA} in CA1 pyramidal dendrites are voltage-dependent potassium (Kv) cannels of the Kv4.2 subtype. These Kv4.2 channels have Kv Channel Interacting Proteins (KChIPs) bound, accessory subunits known to cause high channel surface expression and to modulate channel gating properties. Notably, in an animal model of temporal lobe epilepsy (TLE) functional Kv4.2 expression and total Kv4.2 protein levels have been shown to be heavily down-regulated.

The depolarization provided by a b-AP leads to an influx of calcium through voltage-dependent calcium channels. As the peak of the b-AP amplitude decreases with distance from the soma the associated calcium influx shows a parallel decrement. Therefore, we use calcium imaging techniques to study the attenuation of b-APs. In acute hippocampal slices from 3 weeks old C57bl/6 mice CA1 pyramidal cells are filled with bis-Fura-2 via the patch-pipette during the first 20 – 30 min of somatic whole-cell recordings. APs are generated by brief current injections (4 ms, 1000 pA), and changes in the fluorescence signal of the apical dendrite (250 mm) are measured with a high resolution CCD camera at a frame rate of 90 Hz. As a function of distance from the soma the normalized calcium signal slightly increases within the proximal part of the dendrite (127%) but then shows a gradual attenuation in the more distal dendrite (down to 57%).

By stimulating the Schaffer-collaterals we elicit single EPSPs or short bursts of five EPSPs (100 Hz) in CA1 pyramidal neurons. Somatic whole-cell recordings show a linear relation between slope and amplitude of single EPSPs. High frequency trains of EPSPs exhibit temporal summation with differences in the successive slopes. Relative to the slope of EPSP 1 the values first increase (EPSP 2 and 3), while the slopes of EPSP 4 and 5 are decreased.

Both the gradual decline of the back-propagating calcium signal and the decrease in late EPSP slope reflect the action of I_{SA} and are therefore sensitive to the drugs 4-aminopyridine (4-AP) and heteropodatoxin-2 (HpTx-2).

With the above tools in hand we want to study the effects of TLE models in wild-type mice and in knock-in mice expressing a KChIP binding-deficient Kv4.2 mutant.

The chloride channel ClC-2 regulates neuronal excitability

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ClC-2 belongs to the CLC-superfamily of voltage gated chloride channels. ClC-2 mediates an inward rectifying chloride current. It is activated by hyperpolarization, osmotic cell swelling and acidic extracellular pH. This plasma membrane channel is almost ubiquitous expressed. In mice, loss of ClC-2 leads to vacuolation of the white matter of the CNS (Leukoencephalopathy), male infertility and blindness.
It was hypothesized, that ClC-2 regulates neuronal chloride homeostasis by preventing chloride accumulation. This would result in a decreased GABAergic inhibition and in turn to an increased excitability after ClC-2 disruption. Consistent with this idea, human mutations in the gene encoding for ClC-2 have been found in patients with distinct forms of epilepsy. In contrast, mice did not show an increased susceptibility for seizures.
We now study the function of ClC-2 and its effects in hippocampal neurons. As no selective antagonist for ClC-2 is available we compare WT and ClC-2 KO (*Clcn-2*$^{-/-}$) animals. First we showed, that ClC-2 is functionally expressed in CA1 pyramidal neurons and absent in *Clcn-2*$^{-/-}$ mice. To test network excitability we recorded extracellular filed potentials from the dendritic and somatic area of the CA1, and compared the slope of the dendritic field to the spike height of the somatic field. Hippocampal slices from *Clcn-2*$^{-/-}$ animals showed in this so called E-S coupling a strongly increased excitability. Interestingly, basal synaptic transmission is significantly reduced in *Clcn-2*$^{-/-}$ animals. Taken together, this data perfectly matches an epileptic phenotype. A reduction of synaptic transmission might reflect homeostatic effects of the network preventing an epileptic phenotype.
It will be interesting to see whether changes in the intracellular chloride regulation are the cause for the observed phenotype. To clarify the underlying mechanisms of the impaired synaptic transmission we will study cellular parameters, synaptic transmission in whole cell recordings.

Mechanisms of mGluR1-mediated synaptic signaling in central neurons

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The metabotropic glutamate receptor subtype 1 (mGluR1) is widely expressed in the CNS. Its activation at parallel fiber-Purkinje cell synapses is crucial for cerebellar function. Synaptic stimulation of mGluR1 at parallel fiber synapses is followed by two G-protein-dependent processes: production of IP3 leading to release of Ca^{2+} ions from intracellular stores (Takechi et al., 1998) and initiation of a slowly activating EPSC (sEPSC). We studied mGluR1-dependent signaling in mice deficient for different subunits of transient receptor potential channels (TRPCs) by using whole-cell recordings and Ca^{2+} imaging in acute cerebellar slices. In contrast to earlier suggestions, the mGluR-mediated sEPSC persists in the absence of TRPC1 and in the combined absence of TRPC1, TRPC4 and TRPC6. We found that mGluR1-mediated synaptic transmission at the parallel fiber-Purkinje cell synapse requires TRPC3. Both the synaptically evoked slow EPSC and inward currents evoked by local application of the mGluR-specific agonist DHPG are completely absent in TRPC3-deficient mice. Ca^{2+} release from internal stores was not affected by the absence of TRPC3. In line with the electrophysiological data single cell quantitative RT-PCR analysis showed that TRPC3 is the by far dominating TRPC subunit of Purkinje cells. Importantly, TRPC3-deficient mice show a distinct defect in their walking behavior while the other TRPC-mutants examined (TRPC1-/-, TRPC1/TRPC4 (-/-)[2] and TRPC1/TRPC4/TRPC6 (-/-)[3]) are behaviorally inconspicuous. Thus, our results establish TRPC3 as a new type of postsynaptic channel that mediates mGluR-mediated transmission in cerebellar Purkinje cells and is important for motor coordination (Hartmann et al., 2008). Next, using high-speed two-photon Ca2+ imaging we detected the synaptically evoked TRPC3-dependent Ca^{2+} influx signal in Purkinje cell spiny dendrites. Our results show that the mGluR-dependent Ca^{2+} signal has two distinct components: a large Ca^{2+} release signal from stores and an about 10-fold smaller Ca^{2+} influx signal. The two Ca^{2+} signaling components are detected both in spines and dendrites, but have distinct kinetic features. Local application of the mGluR-agonist DHPG to Purkinje cell dendrites mimics the main properties of the synaptically evoked responses. Taken together, our results identify the important new features of mGluR1 synaptic signaling, including the TRPC3-dependence and the identification of two distinct Ca signaling components.

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TASK-3-like currents in the medial amygdala of mice and rats

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Two-pore-domain potassium channels (K_{2P}) are widely expressed in non-neuronal and neuronal tissue. In mammals fifteen different K_{2P} channel subunits (KCNK) have been identified and, due to structural and functional characteristics, they are divided into several subfamilies, e.g. acid-sensitive TASK and lipid-sensitive mechano-gated TREK/TRAAK channels. One member of the TASK family, TASK-3, is prominently expressed in the medial part of the amygdala of rats that has been shown to play an important role in processing unconditioned fear and aggressive behaviour. The excitability of these neurones is supposed to be regulated by a background potassium conductance of so far unknown molecular composition. In our study we tried to unravel the role of TASK-3 on neuronal excitability in the medial amygdala of rats and mice.

Previously, we demonstrated TASK-3 channel expression in medial amygdala from adult rats by in-situ hybridisation. Correspondingly, we also detected a TASK-3-like current by electrophysiological whole-cell measurements in acute brain slices. To identify the contribution of TASK-3 to the standing outward current (IKso) upon depolarising pulses we used the selective TASK-3 antagonist ruthenium red (RR) or acidification to pH 6.4. RR- or pH-sensitive neurones (-64,5 % of all medial amygdaloid neurones) showed a more hyperpolarised resting membrane potential (-59.7 mV ± 1.52; n = 16) compared to neurones lacking TASK-3-like currents (-43.34 mV ± 2.09; n = 9; $p < 0.01$). In addition, RR enhanced action potential frequency and action potential width during current injections in the more hyperpolarised cells.

Next, we asked the question whether these results obtained from rats are also true for mice. Therefore, we compared cellular excitability in TASK-3 wildtype and TASK-3 knockout mice. Surprisingly, we could not detect significant differences in parameters defining the shape of an action potential, i.e. amplitude (wildtype 65.02 mV ± 3.95; n = 5 vs. knockout 62.86 mV ± 2.74; n = 11; p = 0.66), action potential overshoot (wildtype 29.56 mV ± 4.32; n = 5 vs. knockout 29.93 mV ± 2.04; n = 11; p = 0.94), action potential width (wildtype 2.29 ms ± 0.16; n = 5 vs. knockout 2.04 ms ± 0.18; n = 11; p = 0.41) and number of action potentials during a current injection pulse of 20 pA over 200 ms (wildtype 0.86 ± 0.46; n = 7 vs. knockout 0.96 ± 0.33; n = 12; p = 0.86). Only the rheobase current to elicit an action potential seemed to be different between genotypes although it did not reach significance (wildtype 23 pA ± 15.94; n = 5 vs. knockout 8 pA ± 10.75; n = 10; p = 0.4). In addition, neurones from TASK-3 wildtype and knockout mice did not show any sensitivity to RR.

Taken together, our data suggest that TASK-3 channels are very important in controlling cellular excitability of medial amygdaloid neurones in rats. In contrast, TASK-3 channels in mice seem not to play this outstanding physiological role in processing fear and anxiety by controlling the activity of the medial amygdala.

Species Differences in Reactivation of Diaphragm Muscle Force Generation after Organophosphate Poisoning

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The main mechanism of action of organophosphorus compounds (OP), i.e. nerve agents and pesticides, is the inhibition of acetylcholinesterase (AChE). The failure of inhibited AChE to hydrolyze the neurotransmitter acetylcholine results in an endogenous acetylcholine intoxication followed by an over-stimulation of cholinergic receptors, a massive disturbance of numerous body functions and finally in death by peripheral and central nervous induced respiratory arrest. While anti-muscarinics, e.g. atropine, are highly effective in antagonizing acetylcholine at muscarinic receptors, these drugs are ineffective at nicotinic receptors, such as the neuromuscular synapses. Here, oximes, which are reactivating inhibited AChE, could restore the neuromuscular function. One of the major problems in the assessment of oxime efficacy in humans is the difficulty to extrapolate animal data to humans. In the present work it could be shown that the assignable cause seems to be a substantial and to date unconsidered species difference of OP and oxime effects on respiratory muscle function. Isolated hemidiaphragms of different animal species were used as an experimental model for testing oxime effects. For that, a 12-chamber organ bath system with vertically arranged force transducers and stop-flow superfusion was used. Indirect stimulation of muscle force was induced by an electrical field. The muscle was stimulated during 2 s with 20, 50 and 100 Hz. Diaphragm hemispheres were incubated with the OP tabun until contraction by indirect stimulation was blocked. Control measurements of the muscle force generation by direct stimulation demonstrated that only the neuro-muscular transmission was disturbed. Thereafter, the effect of the oximes HI 6 and obidoxime on impaired neuromuscular transmission was tested. Guinea pig diaphragm muscle required a considerably higher dose of tabun to inhibit muscular function in comparison to rats and mice. After incubation with oxime the recovery of muscle force generation in guinea pig diaphragm was nearly twice as large compared to rat and mice diaphragm. These species differences may be due to different kinetic properties of AChE regarding inhibition by OP and reactivation by oximes.

Glycine receptor subunits in the murine cochlea: expression, localization and developmental regulation

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Objective:
The cochlear efferent feedback system modulates auditory nerve activity and hair cell function, thus protecting the inner ear against acoustic injury. In our previous work, we gave a first description of glycine receptors (GlyR) in the rat cochlea, suggesting a role of glycinergic neurotransmission in the efferent innervation of the inner ear. The inhibitory GlyR is a pentameric postsynaptic chloride channel. Four temporo-spatially regulated ligand-binding GlyR{alpha} subunits (GlyR{alpha}1-{alpha}4) and one GlyR{beta} subunit are known in vertebrates. The GlyR{beta} polypeptide contributes to binding of the GlyR to the cytoplasmatic anchoring protein gephyrin. In mammalian spinal cord, the GlyR{alpha}2 subunit is replaced by GlyR{alpha}1 within 2–3 weeks after birth. Whereas GlyR{alpha}1 mediates motor inhibition in spinal cord and brainstem, the GlyR{alpha}3 subunit is predominantly found in brain regions associated with sensory integration in adult mammals.
Here, we give a first insight into expression and developmental regulation of GlyR subunits in the murine cochlea with respect to the possible role of GlyR in inner ear efferent innervation.
Methods:
Quantitative reverse transcription (qRT)-PCR, hair-cell specific *nested* RT-PCR, *in situ* hybridization and immunofluorescence staining were employed.
Results:
Quantitative RT-PCR identified GlyR{alpha}3 as the predominant GlyR{alpha} subunit in P20 murine cochlea, whereas both GlyR{alpha}1 and {alpha}2 transcripts prevailed before the onset of hearing (P0 - P9). High mRNA levels of GlyR{beta} and the anchoring protein gephyrin were detected from P0 – P20. Hair-cell specific *nested* RT-PCR analysis demonstrated expression of GlyR and gephyrin transcripts in inner hair cells (IHC) before the onset of hearing and in outer hair cells (OHC) afterwards, resembling the switch of efferent innervation from IHC to OHCs around the onset of hearing (~P10 in rodents). In accordance with RT-PCR results, *in situ* hybridization and immunofluorescence staining detected GlyR in OHCs of adult mice.
Conclusions:
GlyR{alpha} subunits display a distinct developmentally regulated expression pattern in the murine cochlea, which is different from spinal cord. The prevalence of the GlyR{alpha}3 subunit in adult murine cochlea is in line with growing evidence for the importance of GlyR{alpha}3 in sensory signal transduction. The switch of GlyR subunits from IHC to OHC parallels developmental changes in the efferent feedback system around the onset of hearing and supports a role of glycinergic transmission in the efferent innervation of the cochlea. Further studies will be necessary to elucidate functional implications of the distinct GlyR subunits in the mammalian inner ear.

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Functional expression of purinergic P2X receptors in the rat suprachiasmatic nuclei.

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Purinergic P2X-type receptors (P2XR) represent a novel structural type of ligand-gated ion channels activated by extracellular ATP. In mammals, seven P2XR subunits have been found in excitable as well as non-excitable tissues. Little is known about their structure and localization, and the role played by the ATP and specific P2XRs in the central nervous system. It is well established that hypothalamus is a prominent region of P2XR expression. Previous studies have shown that the subregions of the hypothalamus with the highest expression of P2XRs in neurons are the paraventrical and supraoptic nuclei (SON) where the $P2X_2$ immunoreactivity has been found at both pre- and postsynaptic sites. The aim of this work was to investigate functional expression of P2XR in the suprachiasmatic nuclei (SCN) that control circadian changes in a variety of physiological and behavioral functions, including metabolism, body temperature, hormone secretion, feeding and sleep and activity cycles. We examined the expression of seven P2XR mRNAs and proteins in the SCN, and compared it with SON, of the rat brain slices using in-situ hybridization and immunohistochemistry combined with confocal microscopy. Acutely isolated slices loaded with the calcium ions sensitive dye, Fura 2-AM, were used to examine ATP-induced increase in intracellular concentration of calcium ions ($[Ca^{2+}]_i$). The effect of glutamate that induces an increases in $[Ca^{2+}]_i$ practically in all living and well stained cells at both SCN and SON was used for comparison. Experiments were performed on adult or 21-day-old rats. In-situ hybridization showed the presence of $P2X_2$, $P2X_3$ and $P2X_4$ subunit transcripts both in the SON and SCN. Immunohistochemistry revealed the presence of $P2X_2$, $P2X_3$, $P2X_4$ and $P2X_5$ receptor proteins in the SON but not in the SCN. ATP application induced calcium responses in cell of both areas. In contrast to the SON, there was a lower percentage of ATP-sensitive cells and lower amplitude of ATP-induced calcium increases in the SCN. The ATP-induced increase in $[Ca^{2+}]_i$ of the SCN were reduced, but not abolished, after removal of extracellular Ca^{2+}, attenuated by PPADS (pyridoxal-phosphate-6-azophenyl-2',4'-disulphonic acid) and suramin. Ivermectin, a specific allosteric modulator of $P2X_4R$, prolonged the decay of the responses after ATP removal. Purinergic P2Y receptor (P2YR) agonist ADP induced larger increases in $[Ca^{2+}]_i$ than UTP or UDP, and MRS2179, a specific $P2Y_1R$ antagonist, significantly reduced ATP-induced $[Ca^{2+}]_i$. increases in the SCN. Thus both Ca^{2+} influx through the pore of P2XRs and P2YR-mediated release of Ca^{2+} from intracellular stores contributed to the ATP-induced increase in $[Ca^{2+}]_i$ in the SCN. These results demonstrate differential distribution of P2X receptors within hypothalamus and support functional interaction of extracellular ATP with these receptors in the SCN. ATP is considered to be a dominant extracellular messenger for neuron-glia and glia-glia communication. Further studies are required to elucidate whether low functional expression of P2X receptors in the SCN is related to its specific function as endogenous clock pacemaker and/or is due to low number of glia cells in this region.

Role of GlyR alpha2 subunit for KCC2 expression

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Glycine receptors (GlyR) are predominantly expressed in spinal cord, brain stem and lower brain regions. They are pentameric ligand-gated ion channels. Whereas the hetero-oligomeric adult form consists of two a1 and three ß subunits, juvenile GlyRs are thought to be homo-oligomeric channels composed of a2 subunits only. It is believed that the subunit switch occurs during the 2nd postnatal week. Due to a negative chloride potential in young neurons juvenile GlyRs elicit excitation. The chloride potential shifts around the time, when juvenile GlyR are replaced by adult type GlyR. Therefore adult-type GlyRs are inhibitory. Activation of early GlyR is crucial for the formation of postsynaptic gephyrin scaffolds, which subsequently trap adult-type glycine receptors via binding of the glycine receptor ß subunit cytoplasmic pole.

In this work, the expression of GlyR subunits during in vitro differentiation of spinal neurons was investigated. We also want to know if the depolarizing phase of the GlyR is essential for the subunit switch.
First the expression of the diverse GlyR subunits over a defined time period was determined by RT-PCR and Real Time PCR. It was found that mRNAs encoding the four GlyR subunits can be detected at all stages investigated. The mRNA level of the a1, a3 and ß subunits increases during development, whereas that of the a2 subunit decreases.
To find out whether the depolarizing state of the GlyR plays a role in the subunit switch, the neurons were treated with strychnine, which is a known antagonist of the GlyR. A significant down-regulation of the a1, a2, a3 and ß subunit mRNA levels is seen at div04 and div 28. The ß subunit mRNA level also shows a significant down-regulation at div 14 and 21.
As KCC2 (potassium chloride co-transporter) expression is known to be crucial for the depolarizing phase we decided to investigate its expression. This transporter is responsible for the changes of the intracellular chloride concentration during maturation of the neurons. KCC2 carries chloride ions together with potassium ions out of the cell. The intracellular chloride concentration decreases and the cell becomes hyperpolarizing.
We wanted to know, whether the activity of the GlyR affects the expression of KCC2.
The cells were treated with strychnine to block glycinergic transmission. The same was done with bicuculline and both strychnine and bicuculline to block the GABAAR mediated transmission as well.
Obviously blocking of the GlyR affects the KCC2 mRNA expression. The KCC2 mRNA level of cells treated with all three mentioned variants is in all investigated div stages significantly lower than in control cells.

IDENTIFICATION OF INTERACTIONS OF THE NOVEL TROUT *SHAKER* a-SUBUNIT TSHA3 WITH OTHER *SHAKER* SUBUNITS

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Voltage gated potassium channels (K_v) are essential for neuronal signal processing as they perform the repolarization during the action potential. They are built of four a-subunits, which contain six membrane spanning helices and a cystosolic amino- and carboxy-terminus. Additionally four ß-subunits can be added at the cytosolic site of the channel to form an octameric complex. It is still under discussion, if the association of ß-subunits is facultative or obligatory for functional K_v *in vivo*. The bony fish *Oncorhynchus mykiss* (trout) expresses a K_v, termed Tsha3, which was classified as a novel member of the subfamily of *Shaker* channels by computer-assisted structural analyses. This subunit co-localizes with the K_v1.2 homologue Tsha1 in the brain (Piwowarski et. al (2004)). Interestingly, the sequence of Tsha3 shares most similarity with the human epithelial potassium channel KCNA10 which is only weakly characterised until now. Upon *in vitro* expression of Tsha3 no voltage gated potassium currents (I_{Kv}) were identified, but in co-expression of Tsha1 and Tsha3 an inactivating component was added to the non-inactivating Tsha1 currents. The region of Tsha3 with the most prominent differences to *Shaker* a-subunits is the amino-terminus. This stretch harbours the tetramerisation domain, which mediates subtype specific tetramerisation of K_v a-subunits, interactions between K_v and other proteins (e.g. the ß-subunits), and can be involved in channel gating. The amino-terminal end of Tsha3 is elongated by nearly 90 amino acids similar to the inactivation particle of certain *Shaker* (K_v1.4) or *Shaw* (K_v3.4) channel subunits. Another interesting feature at the amino-terminus is the sequence of the site for interaction with the ß-subunit, which is localized within the T1 domain. While the consensus sequence for this site is MXYFDPLRNEY in the mammalian shaker channels (Long et. al (2005)), in Tsha3 the sequence is MXYFDP**MK**NEY. Circular dichroism- (CD) spectroscopy and electron paramagnetic resonance (EPR) studies were performed to gain information about the structure and dynamic of the amino-terminal part of Tsha3. As a prerequisite for these studies, the soluble amino terminal domain was expressed in *E. coli* and affinity purified. The data obtained from CD-spectroscopy indicated a high percentage of random coil structure. Concomitantly the EPR data point out that Tsha3 is very mobile at the free amino-terminus. Taken together these data suggest, that the free amino-terminus of Tsha3 is a highly flexible domain. It is remarkable that no coiled structure resembling an inactivation ball was identified. Furthermore the core domain of the ß2-subunit was expressed und purified. In pull down assays, we could observe stable interactions between the amino-termini of Tsha3 and Tsha1 as well as between Tsha3 and the ß2-subunit. In future experiments the interaction between Tsha3 and the ß2-subunit will be analysed in a cell culture based approach. This will allow the identification of the effects of the ß2-subunit on the gating properties of Tsha3. Supported by the DFG- Sonderforschungsbereich 431: “Membranproteine- funktionelle Dynamik und Kopplung an Reaktionsketten”.

Chloride imaging in trigeminal sensory neurons of mice

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The trigeminal nerve functions as a warning system that protects the body from potential noxious stimuli. In the periphery, free nerve endings innervate the face including the mucous membranes of eyes, nose and mouth. Receptors located on these nerve endings detect different environmental stimuli like temperature, touch and chemicals. Well-known examples for trigeminal chemodetection are TRPV1 and TRPM8 cationic channels that are activated by capsaicin (chili peppers) and menthol (peppermint), respectively. Moreover, it has been shown that other ligand-gated cation channels like P2X-receptors are involved in trigeminal chemoperception.

Until now, a potential role of ligand-gated chloride channels in trigeminal chemodetection has not been investigated. In neurons the intracellular chloride concentration is controlled by members of the $Na^+K^+2Cl^-$ (NKCC) and K^+Cl^- (KCC) cation-chloride cotransporters families. In contrast to central neurons, which change the expression of these transporters postnatally, trigeminal ganglion (TG) neurons keep high levels of NKCC1 and thereby probably accumulate chloride intracellularly. This has already been shown for other peripheral neurons like dorsal root ganglion and olfactory sensory neurons. Given a high intracellular chloride concentration, opening of a chloride conductance leads to a chloride efflux and thus depolarizes the neuron. Indeed, previous work in our lab showed Ca^{2+} transients upon stimulation with γ-aminobutyric acid (GABA) in TG neurons of mice in Ca^{2+} imaging experiments.

Here, we use the chloride imaging technique to investigate changes of intracellular chloride concentration upon GABA application on dissociated TG neurons. Cells isolated from embryonal (E19), early postnatal (P0-P5) and adult mice were loaded with the cell-permeable Cl^- sensitive fluorescent dye MQAE (N-[ethoxycarbonmethyl]-6-methoxyquinolinium bromide). We show that GABA stimulation of these cells leads to a chloride efflux in soma and neurites. Removing Cl^- from the extracellular solution and thereby creating a higher Cl^- gradient across the cell membrane enhances the GABA-induced Cl^- efflux.

In further experiments we will characterize this Cl^- efflux using pharmacological tools (e.g. the NKCC1 blocker bumetanide as well as chloride channel and GABA receptor antagonists). Since this technique leaves the intracellular Cl^- concentration undisturbed, we also aim to determine $[Cl^-]_i$ of TG neurons of mice.

Our data show that opening of a chloride conductance by GABA leads to an efflux of Cl^- and thus to a depolarization of trigeminal sensory neurons. Neuronal excitation by activation of GABA receptors makes them potential targets for the trigeminal perception of toxic chemicals.

Characterisation of GABA Induced Responses of Trigeminal Sensory Neurons

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The peripheral sensory system is able to detect a wide variety of stimuli. In the facial mucosae and skin trigeminal ganglion neurons (TGNs) mediate nociception, mechano-, thermo- and chemosensation, while in the body this information is detected by dorsal root ganglion (DRG) neurons.
It has recently been described that GABA acts excitatory on DRG neurons of different vertebrate species. This effect was observed in neurons taken from postnatal as well as adult animals. In contrast to neurons of the central nervous system, DRG neurons maintain high levels of the $Na^+K^+2Cl^-$-cotransporter (NKCC1) even at adulthood. Thus, NKCC1 accumulates chloride in these cells resulting in a depolarisation upon GABA stimulation.
Here, we examine the effect of GABA on sensory neurons isolated from murine trigeminal ganglia. Using whole-cell patch-clamp we could show that GABA elicits responses in approximately 100% of TGNs in a dose-dependent manner. Furthermore, GABA stimulation leads to a robust increase of intracellular calcium observable in calcium-imaging experiments. This GABA-induced excitation is independent of animal age and culturing conditions, as it can be seen in neurons isolated from embryonal (E19), early postnatal (P0-P5), and adult (> 7 weeks) mice as well as under different culturing conditions. The GABA-induced calcium rise is inhibited by the NKCC1 blocker bumetanide in early postnatal as well as adult neurons, suggesting that an increased intracellular Cl^- concentration is essential for the response. Pharmacological characterisations showed that responses to GABA are mediated by $GABA_A$ receptors as they are sensitive to the $GABA_A$ antagonists bicuculline and gabazine but cannot be inhibited by TPMPA (1,2,5,6-Tetrahydropyridin-4-yl methylphosphinic acid) or strychnine thus ruling out an involvement of $GABA_C$ and glycine receptors respectively.
Ion exchange and pharmacological experiments reveal that the calcium signal observed upon GABA stimulation is produced by an influx of extracellular calcium via voltage gated calcium channels (VGCCs).
In summary, we suggest an intracellular Cl^--accumulation in TGNs produced by NKCC1 which leads to a depolarising efflux of chloride upon $GABA_A$ receptor opening followed by an influx of extracellular Ca^{2+} through VGCCs.
In future studies we aim to examine the role of GABA receptors in the detection of environmental chemicals. A large variety of receptors such as members of the transient receptor potential (TRP) channel family expressed in TGNs is capable of detecting potentially harmful chemicals. Therefore, a possible function of GABA-induced excitation of TGNs could be the perception of naturally occuring $GABA_A$ agonists with toxic potential.

Fragrant dioxane derivatives, a new class of positive modulators of $GABA_A$ receptors with selectivity for receptors containing $ß_1$ subunits

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g-Aminobutyric acid (GABA) is the most important inhibitory neurotransmitter in the mammalian brain, which regulates excitability in neuronal circuitries mainly through the fast ionotropic $GABA_A$ receptors ($GABA_A$Rs), heteropentameric proteins constructed from subunits derived from several related gene families. Subunit combinations are restricted in their number with the $a_1b_2g_2$ receptor-type being dominant in the brain (ca 60% of total brain $GABA_A$Rs) followed by the $a_2b_{2/3}g_2$ receptor-type (ca 15%). Native receptors may contain two different b- and two different a- subunits co-assembled in one receptor, providing large heterogeneity of native $GABA_A$Rs. Positive allosteric modulators of $GABA_A$ receptors ($GABA_A$R) are of high clinical importance as anxiolytics, anesthetics and sedatives. In total, the $GABA_A$R incorporates more than ten distinct modulatory binding sites targeted by anticonvulsive, antiepileptic, sedative, hypnotic and anxiolytic compounds belonging to chemically different structural classes. Cis-jasmone (the active compound from jasmine oil) has sedative properties and interacts with $GABA_A$Rs. Further natural odorous compounds like terpenoids and fragrances potentiating $GABA_A$Rs have been described in recent years. Searching for further compounds, we screened several libraries of odorants and terpenoides. We characterized the action of several odorants on $GABA_A$Rs. We discovered a new structural class of $GABA_A$R modulators: fragrant [1,3]-dioxane derivatives (FDD) that, similar to propofol, enhance the action of GABA and directly activate $GABA_A$R at higher concentrations. At heterologously expressed $a_1b_xg_{2L}$ (x-for 1,2,3) $GABA_A$R these odorants showed higher potency at b_1-versus b_2 and b_3-containing receptors (EC_{50}s ca 30 µM vs ca 200 µM, respectively). Substitution of the a_1-subunit with a_2- in the $a_1b_xg_{2L}$ $GABA_A$R did not significantly change the EC_{50} for FDD. The mutation b_1-M286W nearly abolished potentiation by FDD. When compared to propofol, FDD produced a similar modulation of spontaneous neuronal firing in striatal, hippocampal and hypothalamic cultures. In hypothalamic histaminergic neurons expressing the $GABA_A$R $ß_1$-subunit FDDs were more potent in modulating GABA-responses than in $ß_1$-negative Purkinje neurons of the cerebellum (EC_{50}s 33 µM vs 120 µM, respectively). Spontaneous GABAergic inhibitory postsynaptic currents (sIPSCs) were significantly prolonged by FDD starting at 1 µM in hypothalamic neurons and 25 µM in Purkinje cells, indicating synaptic localization of b_1-containing $GABA_A$Rs. In conclusion, with the help of FDDs we define here the phenotype of b_1-containing $GABA_A$Rs and their contribution to synaptic currents in comparison to the unspecific action of propofol. Our findings shed light on the physiological role of b_1-$GABA_A$R and pave a new avenue for the design of ß-subunit- specific therapeutics.

Clinically used antidepressants augment cellular excitability by inhibiting tandem-pore domain potassium channels

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The molecular correlate of potassium background currents has been identified as two-pore-domain potassium channels (K_2P), which are widely expressed in the central nervous system and in peripheral organs. Their name is derived from their molecular structure of the subunits in which two pore domains flanked by transmembrane helices are located in tandem. They stabilise the resting membrane potential, thereby controlling cellular excitability. Some of these K_2P channels are coupled to seven-helix receptors like the 5-HT2c receptor. Serotonergic signaling pathways are intimately involved in the manifestation of mood disorders and are subject to therapeutic treatment by antidepressants. Their use in clinical praxis is limited because of unwanted cardiac side effects probably due to an interaction with K_2P channels which are also expressed in heart tissue. Recently, it has been shown that one member of the K_2P channel family, TREK-1, as downstream target of the serotonergic pathway can interact with antidepressants. However, it is not clear whether other K_2P channels are also modified by these substances. Furthermore, the mode of interaction of antidepressants with K_2P channels is completely unclear at the molecular level.

TREK-1 is sensitive to selective serotonin reuptake inhibitors as well as tri- and tetracyclic antidepressants. When expressed in HEK-293 cells, human TREK-1 current is reduced upon application of Fluoxetine (100µM) by 76.4%, Citalopram (1mM) inhibits current amplitude by 70.8% and Venlafaxine (1mM) by 29.4%. This corresponds to the finding that Venlafaxine exerts the least cardiac side effects in clinical praxis. Maprotiline (1mM) inhibits the current by 77.4 %, Doxepine (1mM) by 53.4 % and Mirtazapine (1mM) by 65.8%.

Next, we investigated the putative interaction site of the channel protein with antidepressants. Therefore, we engineered a C-terminal deletion mutant of TREK-1, that, unfortunately, did not express functionally in our expression system. Hence, we constructed a C-terminal deletion mutant of the closely related K_2P channel TASK-1 lacking the least 163 amino acids (TASK-1[DC163]). When expressed in *Xenopus* oocytes, current amplitude of TASK-1 wildtype (-76.4%) as well as TASK-1[DC163] (-60.2%) was reduced to the same amount upon application of Fluoxetine (1mM). Another TASK-1 mutant (TASK-1[D243-248) with a deletion in a region responsible for interaction with volatile anesthetics and receptor coupling was also still inhbited by Fluoxotine (reduction of about 60 %).

Deletion mutants of TASK-1 suggest that neither the C-terminus of K_2P channels nor an important regulatory region next to the fourth transmembrane segment [D243-248] are necessary to exert the inhibitory effect of antidepressant drugs.

Finally, the prominent expression of K_2P channels in heart an brain corroborates our hypothesis that their blockage may be responsible for both antidepressant effects as well as life-threatening cardiac side effects.

Taurine is a full agonist at native and recombinant GABAA receptors.

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The efficacy and potency of taurine were investigated on native GABAA receptors from tuberomamillary nucleus (TM) neurons lacking glycine receptors (GlyR) using whole cell recording and single cell RT-PCR. Taurine (100 mM) activated currents comprising 35-63% of the maximal GABA (0.5 mM) evoked current. The potency of taurine (EC50 ca 10 mM) was not different between neurons. In a subpopulation of TMN neurons from mice lacking gamma subunit-expression taurine acted as a full agonist. Taurine-mediated currents were comparable or even larger in amplitude than maximal GABA-currents in oocytes injected either with alpha1/beta2, alpha2/beta3 or beta3/gamma2L subunits. Co-injection of the beta subunit with alpha and gamma subunits resulted in a partial agonism of taurine: maximal taurine-evoked responses represented ca. 60-80% of maximal GABA-mediated responses. In concatenated GABAA receptors with a defined subunit composition of alpha1-gamma2L-beta2-alpha1-beta2 type responses to taurine represented 35% of maximal GABA-evoked currents. As the alpha/gamma interface contains the diazepam-binding site, we hypothesize that this site is essential for the self-inhibition of taurine binding. Consistent with this, sub-maximal taurine responses in TMN neurons expressing the gamma2 subunit of the GABAA receptor were potentiated by diazepam-site antagonists.We conclude that taurine is a full agonist at native and recombinant GABAA receptors devoid of a diazepam-binding site.

Expression and role of Ca2+ channel alpha2delta subunits in the peripheral auditory system of mice

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Voltage-activated Ca2+ channels consist of a pore-forming alpha1 subunit (SU), a beta, an alpha2delta (and sometimes also a gamma) SU. Expression of alpha1 SU is cell-specific as is the co-assembly with the auxiliary subunits, giving rise to Ca2+ channels with different, tissue-specific properties. Inner (IHC) and outer hair cells (OHC) express predominantly voltage-activated Ca2+ channels with the pore-forming subunit Cav1.3 (Platzer et al., Cell 2000; Michna et al., J. Physiol. 2003), whereas spiral ganglion neurons (SG) express Cav1.2, Cav2.1 and Cav2.2 channels (Roehm et al. Mol. Cell. Neurosci. 2008). Alpha2delta SU 1-4 play a critical role in trafficking alpha1 SU to the plasma membrane and moreover modulate Ca2+ channel properties. Only recently, alpha2delta1&2 were identified as pharmacological targets for treatment of chronic pain e.g. by gabapentin. So far, the expression of alpha2delta SU in inner and outer hair cells and SG neurons is unknown.

Using RT-PCR and in situ hybridization, we detected alpha2delta1,2,3 in the organ of Corti and the cochlea. A mouse model with a targeted deletion of alpha2delta-3 (Jackson Laboratories) reported to have a reduced startle response showed increased ABR thresholds despite normal DPOAEs. LacZ reporter staining due to excision of the of alpha2delta-3 gene in alpha2delta-3-/- mice indicated expression of alpha2delta-3 in spiral and vestibular ganglia whereas it was absent in IHCs. Ba2+ currents in IHCs of alpha2delta-3 ko mice were unaltered, confirming that the alpha2delta-3 SU is not crucial for IHC Ca2+ currents.

In conclusion, the hearing deficit of alpha2delta-3-/- mice is not due to malfunction of IHCs or OHCs but may rather be caused by altered presynaptic Ca2+ currents of spiral ganglion neurons.

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T-type Calcium Current is upregulated by zinc in the hippocampus

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A single episode of status epilepticus (SE) caused by pilocarpine, triggers multiple changes in the brain which ultimately lead to chronic epilepsy. This process is referred to as epileptogenesis. One striking change is the upregulation of CaV3.2 T-type calcium current, which causes many hippocampal pyramidal cells to display intrinsic bursting behavior. The processes which couple SE to T current upregulation are not known. We hypothesized before that SE-induced accumulation of zinc may play a role in these processes and we have found, that intracerebroventricular injection of zinc transforms about 60% of CA1 pyramidal cells into bursting neurons. Therefore, we examined here whether zinc also induces an increase in T current density in these neurons.

Method:

Saline was injected with or without ZnCl2 (45 mg) into the right ventricles of adult Sabra rats. On different days after injection we prepared acute hippocampal slices and used sharp electrodes and whole-cell patch-clamp recordings as well as immunostainings for CaV3.2 to analyze the effects of zinc.

Results:

Two days following zinc injection the density of T current was markedly increased compared to neurons in the control group and stayed elevated for a period up to 14 days. Congruently, immunostainings disclosed an increase in CaV3.2 abundance in CA1 pyramidal cells in hippocampi from zinc injected rats. Finally, we found that the bursting activity in 1/3 of the tested CA1 pyramidal cells was suppressed by specific blockage of the T-type calcium current with small concentrations of nickel.

Conclusion:

Our findings show that an increase in brain zinc causes upregulation of CaV3.2 abundance leading to an increase in T current and associated neuronal bursting. They support our hypothesis that an increase in free zinc during SE may contribute to epileptogenesis. These findings have therapeutic implications for the prevention of epilepsy following brain insults

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Large Dense Core Vesicle Exocytosis in Mouse Chromaffin Cells is regulated by Munc13s and Baiap3

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Members of mammalian uncoordinated 13 protein family (Munc13s) are essential for SV exocytosis in neurons and have also been implicated in LDCV exocytosis in chromaffin cells. However, the *C.elegans* ortholog Unc-13 appears to be dispensable for LDCV exocytosis, raising the question whether SNARE-mediated exocytosis of SVs and LDCVs is controlled by distinct regulatory proteins. We therefore analyzed LDCV exocytosis in cultured chromaffin cells taken from knockout mouse lines of four members of the Munc13 protein family, Munc13-1, Munc13-2, Munc13-3 and Baiap3. Baiap3/Bap3 (brain specific angiogenesis inhibitor 1 associated protein 3), was identified and named as an interaction partner of BAI 1 (brain specific angiogenesis inhibitor-1), and is a Munc13 homologue of unknown function. Its closet known relative, the non-neuronal Munc13-4, has been shown to be essential for the secretion of lymphocyte cytolytic granule content. Unlike Munc13-4, Baiap3 expression is largely restricted to brain and adrenal gland, with low levels also present in lung. To determine whether LDCV exocytosis is impaired in the absence of Munc13-1, Munc13-2, Munc13-3 or Baiap3, we combined flash photolysis of caged Ca^{2+} and membrane capacitance measurements in cultured chromaffin cells. Our analysis of exocytosis in chromaffin cells shows that Munc13-1 and Munc13-2 act as positive regulators of LDCV exocytosis and play a more critical role in the release of this vesicle type than their *C.elegans* ortholog Unc-13. Based on sequence similarity, Baiap3 is a member of the Munc13 protein family. However, unlike Munc13-1, -2, -3 and -4, which function as essential positive regulators of SNARE-mediated exocytosis, our data indicate that Baiap3 negatively regulators exocytosis, at least in chromaffin cells. In this study, we found that exocytosis in chromaffin cells from Munc13-1 deficient mice and mice lacking both Munc13-1 and Munc13-2, showed a dramatic reduction in LDCV exocytosis. In contrast to this, chromaffin cells deficient for Baiap3 showed increased exocytosis and Baiap3 overexpression in WT cells suppressed LDCV release. The exact molecular mechanism by which Munc13s regulate SNARE-mediated exocytosis remains to be elucidated. However, we found that Baiap3 interacted with both Munc13-1 and Syntaxin in co-sedimentation assays, which indicates that competition for binding to SNARE complex components is a possible explanation for the opposing functions of Munc13-1/2 and Baiap3 LDCV exocytosis.

Aberrant function and structure of photoreceptor ribbon synapses in the absence of Complexins 3 and 4

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Complexins (Cplxs) regulate the speed and Ca^{2+}-sensitivity of SNARE-mediated synaptic vesicle fusion at chemical synapses. Interestingly, two of the known vertebrate Cplxs, Cplx3 and -4, are specifically localized to retinal ribbon synapses. To test whether the function of Cplx3 and -4 contributes to the highly efficient transmitter release of ribbon synapses, we studied retinal function and structure in Cplx3 and -4 single and double knock-out mice. By comparing the electroretinographic recordings (ERG) from single and double mutants we found a cooperative effect of Cplx3 and -4 deletion in the attenuation of transmitter release from photoreceptor ribbon synapses resulting in a decreased b-wave amplitude. Most of the other detected effects in the ERG in both plexiform layers were additive. Light and electron microscopic analyses revealed a disorganized outer plexiform layer in the retina of mice lacking Cplx3 and -4, where a significant proportion of photoreceptor terminals contained spherical-shaped free-floating ribbons. Our results show that Cplx3 and -4 are essential regulators of transmitter release at photoreceptor ribbon synapses. Their loss leads to aberrant adjustment and fine-tuning of transmitter release at the photoreceptor ribbon synapse, alterations in transmission at bipolar cell terminals, and changes in the temporal structure of synaptic processing in the inner plexiform layer of the retina.

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The proteome of the presynaptic active zone: from docked synaptic vesicles to adhesion molecules and maxi-channels

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The presynaptic proteome controls neurotransmitter release and the short and long term structural and functional dynamics of the nerve terminal. Using a monoclonal antibody against synaptic vesicle protein 2 we immunopurified a presynaptic compartment containing the active zone with synaptic vesicles docked to the presynaptic plasma membrane as well as elements of the presynaptic cytomatrix. Individual protein bands separated by SDS-PAGE were subjected to nanoscale-liquid chromatography electrospray ionization-tandem mass spectrometry (nanoLC ESI MS/MS). Combining this method with 2-dimensional BAC/SDS-PAGE and MALDI-TOF and immunodetection we identified 240 proteins comprising synaptic vesicle proteins, components of the presynaptic fusion and retrieval machinery, proteins involved in intracellular signal transduction, a large variety of adhesion molecules and proteins potentially involved in regulating the functional and structural dynamics of the presynapse. Four maxi-channels, three isoforms of voltage-dependent anion channels and the tweety homolog 1 were co-isolated with the docked synaptic vesicles. As revealed by *in situ* hybridization, tweety homolog 1 reveals a distinct expression pattern in the rodent brain. Our results add novel information to the proteome of the presynaptic active zone and suggest that in particular proteins potentially involved in the short and long term structural modulation of the presynaptic compartment deserve further detailed analysis.

Characterization of SV31, a novel synaptic vesicle membrane protein and putative transporter

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Synaptic transmission relies upon synaptic vesicles, abundant organelles of the presynaptic nerve terminal of neurons. Therefore, the analysis of the synaptic vesicle proteome is essential for identifying components involved in vesicle mobilization, migration to the presynaptic plasma membrane, docking and fusion as well as recycling of synaptic vesicles via endocytosis. To date, a considerable variety of synaptic vesicle proteins that govern these processes has been identified.
To detect novel synaptic vesicle proteins that has escaped previous biochemical analyses, we subjected synaptic vesicles to different gel-based approaches in combination with mass spectrometry. By analyzing the proteome of synaptic vesicles we identified a large number of proteins, 4% which were not previously assigned to the synaptic vesicle compartment. One of those we named SV31 (synaptic vesicle protein of 31 kDa), a membrane protein with predicted 6 transmembrane helices. Analysis of the subcellular distribution of recombinant SV31 in PC12 cells reveals an association with punctuate and reticular structures and suggests a vesicular location. Immunohistochemical analyses of rodent brain sections reveal a broad expression in many brain tissues. In cultured hippocampal neurons SV31 partially colocalizes with the synaptic vesicle proteins VAMP-2, synaptophysin and the vesicular glutamate transporter, however there was no colocalization with glutamic acid decarboxylase. SV31 binds the cations Zn^{2+} and Ni^{2+}, but not Co^{2+}, Mn^{2+}, and Ca^{2+}.

Taken together, these data suggest that the novel integral synaptic vesicle protein SV31 is present in subpopulations of synaptic vesicles and represents a novel cation binding protein and a putative transporter. Further analyses will concentrate on the functional characterization of the newly identified protein.

Syndapin I deficiency leads to profound changes in dynamin localisation, synaptic vesicle morphology and recycling at presynaptic terminals.

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The F-BAR domain containing protein family of syndapins/pacsins has been suggested to interconnect membrane trafficking events with the closely associated actin cytoskeleton. The brain specific protein syndapin I has been implicated in synaptic vesicle recycling at presynaptic terminals. F-BAR domains have been shown to provide membrane curvature sensing and/or curvature inducing entities. To get more insights into the functional relevance of these proteins, not only on the biochemical level but up to levels of learning and memory formation processes, we have set out to create a gene targeting strategy for the murine syndapin I gene.

Complete loss of syndapin I protein was confirmed in brain homogenate and in primary hippocampal neurons. Syndapin I-deficient mice are viable, but have reduced fertility and body weight. In behavioural tests mutants performed extraordinarily well on the rotarod. Strikingly, knockout mice developed generalized seizures without loss of consciousness on new challenges.

Biochemical fractionation of detergent soluble and insoluble pools showed a significant increase in solubility for dynamin, a major binding partner of syndapin I. Solubility of a comprehensive set of other proteins was not affected. In line with these results, a significant increase in dynamin solubility was also observed in primary hippocampal neurons. Compensatory effects as tested by quantitative immunoblot analysis of brain homogenates were not noticed for a plethora of proteins, including dynamin. Ultrastructural analysis of synaptic vesicle morphology revealed an increase in vesicle diameter in knockout mice. Electrophysiological investigation in the CA1-region of the hippocampus did not show any significant differences in input/output curves or changes in paired pulse facilitation, however. SynaptopHluorin measurements in primary hippocampal neurons from wildtype and knockout animals revealed delayed repriming kinetics. Taken together, the data suggest that impaired localisation of dynamin subcomplexes together with loss of syndapin I as a curvature sensing and/or inducing factor leads to changes in synaptic vesicle morphology and strongly impaired repriming kinetics at presynaptic terminals.

These analyses emphasize a critical requirement for a tightly regulated interplay between changes in membrane morphology, curvature sensing/inducing factors and the scission machinery during membrane remodelling processes.

Rapid time-course of back-propagating action potentials in dendritic spines of cortical neurons

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Fast, axonally initiated sodium action potentials that back-propagate into the dendritic tree (bAPs) play a key role in dendritic signal integration. However, the exact regional distribution of the size and waveform of these signals in the entire dendritic structure including basal and apical dendrites and dendritic spines remain elusive and controversial mainly due to technical limitations of electrode recordings from small dendritic processes as well as to relatively low sensitivity of voltage-sensitive dye imaging (Vm-imaging). We achieved a decisive improvement in Vm-imaging by a combination of measures, including: (a) the optimization of both the resting florescence light intensity and the relative fluorescence change in response to Vm change by utilizing monochromatic excitation light from a laser emitting at 532 nm in wide-field epi-fluorescence measurements; (b) the optimization of the slicing procedure that resulted in the preservation of healthy neurons and dendritic processes close (10-30 µm) to the surface of the slice (where light scattering is low). These improvements allowed us to reliably record bAP signals with high signal-to-noise ratio from all of the dendritic branches in the arbor as well as from individual dendritic spines. At relatively low optical magnification (field of view in the object plane 300 x 300 µm)we compared bAP-signal waveform from different dendritic regions. Because the response time of the charge-shift voltage-sensitive dyes is in the µs range and about 100 fold faster than the fastest component of the AP signal, the dye signal tracks the membrane potential without distortion and the time course of the signal is obtained directly from optical data. Surprisingly, we found that the time course of bAP signals was independent of the location in the apical, basal and oblique dendrites. At relatively high optical magnification (field of view in the object plane 30 x 30 µm) we monitored and compared bAP-signal size and waveform from individual dendritic spines and parent dendrites. The data argue clearly that the amplitude and the time course of the bAP in spines and dendrites did not significantly differ. The rapid and invariant time-course of the bAPs in the entire dendritic structure including spines has important implications for synaptic plasticity rules.

Effects of nicotine on memory-related hippocampal network oscillations in the adult rat in vitro

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During memory acquisition, neuronal networks in the hippocampus proper display synchronized theta oscillations, which are superimposed by small-amplitude gamma network activity. In contrast, memory consolidation has been suggested to depend on hippocampal sharp wave-ripple complexes (SPW-Rs) by which stored information is transferred from the hippocampus into the cortical mantel. Nicotine is thought to exert beneficial effects on various cognitive brain functions such as attention and memory. Here we used an *in vitro* paradigm to investigate the effects of nicotine on both the induction and the expression of stimulus-induced SPW-Rs in area CA3 of the adult rat hippocampus. In addition, we assessed the effects of nicotine on both pharmacologically-induced gamma oscillations in area CA3 and stimulus-induced gamma activity in the CA1 region. We show that nicotine facilitated the induction of SPW-Rs in a dose-dependent manner. Furthermore, when nicotine was applied on readily induced SPW-Rs, it significantly augmented ripple oscillations during SPW-Rs. This increase was depended on the activation of alpha 7 n-AChRs. In a concentration of 500 µM, nicotine transformed SPW-Rs into prolonged events reminiscent of paroxysmal depolarizing discharges. Intracellular recordings from CA3 pyramidal cells revealed that nicotine (100 µM) almost doubled the number of action potentials (APs) during SPW-Rs. This was accompanied by a significantly increased spike-timing precision of APs to the synchronized ripple oscillations. Unexpectedly, nicotine mildly reduced the power of kainate-induced gamma oscillations while, it strongly reduced stimulus-induced gamma oscillations. Together, our results suggest that activation of alpha 7 n-AChRs may exert facilitatory effects on processing memory consolidation.

Characerization of the synaptic role and localization of the family of Liprins-alpha

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In the presynaptic nerve terminal fusion of synaptic vesicles is restricted to a specialized subcellular membrane compartment, the active zone. The active zone is defined by the associated dense protein network, which ensures the high speed of synaptic transmission. The molecular mechanisms that control synapse formation, maturation, and stabilization as well as active zone assembly and organization are still not understood. Recent studies, mainly from the invertebrates C. elegans and Drosophila, point toward crucial roles for Liprin-a proteins in these processes. Whereas invertebrates contain a single Liprin-a gene, four genes are present in the vertebrate genome. To date very little is known about the mammalian Liprin-a genes and proteins.

Here, we analyzed the expression of Liprin-a1-4 in the developing and adult rodent brain on the mRNA and protein level. Liprin-a1-4 exhibited regional colocalization throughout the brain, albeit with diverging levels of expression. Immunocytochemistry showed that Liprin-a 1-4 colocalize with synaptic markers but are not exclusively localized at synapses. In a comparitive analysis of alternative splice patterns we observed that the four Liprin-a isoforms are differentially modified by alternative splicing and that these modifications are developmentally regulated.

With regard to disease-associated neuronal reorganization, we observed that the expression of the individual Liprin-a genes is controlled independently as Liprin-a4 is significantly down-regulated at all time points after Status Epilepticus (SE), whereas Liprin-a2 is strongly up-regulated only in the acute phase (6h) after SE.

Furthermore, diverging as well as overlapping functions of Liprins-a in neurons are being addressed.

Activity dependent shift in GABAergic action in identified neurons of the immature neocortex

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GABA evokes a membrane depolarization in immature neurons due to a high intracellular Cl^- concentration ($[Cl^-]_i$). Using gramicidin-perforated patch-clamp recordings, we could show that the Cl^- uptake in Cajal-Retzius cells is accomplished by a Na^+ dependent K^+-$2Cl^-$-cotransport, which operates at a very low capacity (Achilles et al., 2007, J. Neurosci. 27:8616). To investigate the functional consequences of such an inefficient homeostatic process, we performed gramicidin-perforated and whole-cell patch-clamp recordings of visually identified Cajal-Retzius cells in tangential slices of neonatal (P1-3) rats.

While GABA had an excitatory effect on Cajal-Retzius cells under resting conditions, focal application of GABAergic pulses with high frequency or a burst of Carbachol-induced GABAergic postsynaptic currents reduced the excitatory potential of GABAergic depolarizations. To investigate to which extent activity dependent alterations in $[Cl^-]_i$ contribute to the switch from excitatory to inhibitory action, we performed whole-cell recordings using defined pipette solutions containing 10, 15, 20 and 30 mM Cl^-. In these experiments we paired subthreshold and suprathreshold current pulses of different amplitudes with subthreshold depolarizations induced by focal GABA applications and found that the excitatory potential of GABA (as defined by the average reduction in the threshold current to evoke action potentials) significantly depends on the Cl^- concentration of the pipette solutions ($[Cl^-]_p$). In further experiments we altered the timing between GABA pulse and injected current, which revealed that the shift between excitatory and inhibitory GABA responses appeared at lower Cl^- concentrations if both stimuli were applied simultaneously as compared to a non-simultaneous application of the stimuli. If the current pulse was delayed by 200 ms inhibitory effects were observed at a considerably higher $[Cl^-]_p$, revealing that the timing between excitatory and GABAergic inputs also influences the $[Cl^-]_i$ dependency of GABAergic actions. Combined current clamp recordings and impedance measurements demonstrated that the complex interplay between GABAergic effects and the $[Cl^-]_i$ depends on the balance between membrane depolarization and a decrease in input resistance. In contrast, neither the slope of the depolarization nor the $[Cl^-]_i$ had a direct effect on action potential threshold.

In summary, these results suggest that a $[Cl^-]_i$ decrease within the physiological range will change GABAergic effects in a temporally complex manner. Thus activity-dependent alterations in $[Cl^-]_i$ will modify GABAergic influences on excitability and information processing in the immature neocortex.

Activity-independent recruitment of functional AMPAR at neurexin/neuroligin contacts<

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A combination of cell culture and animal studies has recently shown that adhesion between neurexins and neuroligins played important roles in synapse initiation, maturation, and function. Binding of neurexin-1ß to neuroligin-1 triggers the post-synaptic clustering of the scaffold protein PSD-95, but the composition and timing of accumulation of glutamate receptors at those nascent contacts remain unclear. Using glutamate uncaging and calcium imaging, iontophoresis and patch-clamp recordings, we identified functional AMPARs and NMDARs at PSD-95 clusters induced by neurexin-1ß coated microspheres on primary hippocampal neurons. The recruitment of AMPARs occurred as early as 2 hr after initial contact, and was not blocked by TTX/APV treatment. The differential recruitment of recombinant subunits GluR1 and GluR2, as well the absence of rectification in voltage/current curves, further showed that neurexin/neuroligin contacts primarily recruit GluR2-containing AMPARs. Finally, AMPARs participated in calcium entry at b-neurexin-induced post-synapses, most likely through the activation of voltage-gated calcium channels. These results demonstrate that the accumulation of AMPARs at nascent neurexin1ß-induced post-synapses occurs rapidly and does not require neuronal activity.

Neurobeachin: A novel regulator of functional receptors

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Neurobeachin (Nbea), one of BEACH (beige and Chediak-Higashi) protein family is cytosolic protein and found predominantly in neuronal tissue. It is closely related to LYST (lysosomal trafficking protein), and thought to act as an AKAP (A Kinase Anchor Proteins). Although neuromuscular junctions lacking the Nbea show no evoked release by action potential similar to those seen after Munc13s deletion, the functions of Nbea in central nervous system still remain illusive. The Nbea mutant die before birth and displays a cleft palate and omphalocele, which is a similar phenotype to vesicular inhibitory amino acid transporter deficient mice. To investigate the physiological role of Nbea in synaptic transmission, we took advantage of primary autaptic cultured hippocampal glutamatergic neurons and striatal GABAergic neurons from E18.5 Nbea KO embryo and wild type or heterozygous littermate controls. We detected no obvious morphological differences between neurons of the tested genotypes. The evoked release triggered by action potential and hypertonic sucrose in Nbea deletion neurons are significantly reduced although the number of presynaptic terminals are identical between KO and control neurons. Also, the responses to exogenous application of glutamate and GABA are significantly reduced in Nbea mutant without changes in total expression level of glutamate and GABA receptors. These data suggest that the Nbea is likely to be a novel regulator for trafficking/transporting of functional AMPA and GABA receptors to cell surface and thus result in determining the synaptic strength in neuronal network activity.

Spine neck plasticity controls depolarization in the spine head

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The majority of excitatory synapses in the brain is located on small dendritic protrusions called spines. The function of dendritic spines is not fully understood. Imaging experiments have shown that spines restrict the diffusion of calcium and other second messengers, forming tiny biochemical compartments that isolate neighboring synapses from each other. In addition to chemical compartmentalization, spines could play a role in shaping spine head depolarization. It has been suggested that the electrical resistance of the spine neck could enhance the local depolarization of the spine membrane (spine-EPSP), potentially activating a positive feedback loop through voltage-gated channels. Here, we use the voltage-dependence of NMDA receptors to estimate the depolarization in individual spines. Two-photon imaging allowed us to measure NMDA receptor-mediated calcium currents in CA1 pyramidal cells. In addition, we have measured the diffusional coupling between spine heads and parent dendrites of CA1 pyramidal cells using fluorescence recovery after photobleaching (FRAP). Our data indicate that spine necks in acute slices are highly plastic and can rapidly change their diffusional resistance by a factor of 10. We demonstrate a correlation between spine neck resistance and voltage-gated calcium influx during the spine-EPSP, suggesting that spine necks control spine calcium transients via electrical compartmentalization. We hope that our findings will help to resolve several long-standing controversies in the field of synaptic plasticity.

The mechanism of reliable synaptic transmission in layer 4 of the visual cortex

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Neurons in layer 4 of the primary visual cortex (V1) play an important role in transferring signals from thalamus to other layers of V1. Understanding the properties of synaptic transmission between layer 4 neurons helps us to gain a clearer picture about how layer 4 neuronal network cooperates to process visual information. Here, we have determined the quantal size (q), the size of readily releasable vesicle pool (N) and the release probability (p) of excitatory connections within layer 4 of V1 in mice. Compared to the excitatory synapses in the somatosensory cortex, those in V1 show relatively low release probability and have a larger pool size. This suggests that excitatory connections within layer 4 can transmit high frequency signals more reliably than those in the somatosensory cortex. It also implies that in different brain regions, synaptic transmission is tuned in a different manner to perform specific tasks.

Impact of presynaptic voltage transients on postsynaptic spike timing at a graded synapse in the fly motion vision system

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The frequency and timing of action potential firing of a neuron is largely controlled by the activity of its presynaptic input ensemble. We analyzed how presynaptic signals with different dynamics interact to control postsynaptic activity. In the blowfly's visual system we simultaneously recorded *in vivo* from an identified motion-sensitive neuron (V1) and from elements of the presynaptic ensemble (VS). The presynaptic cells themselves are electrically coupled among one another and convey both graded and spike signals to their common postsynaptic target. Spikes in the postsynaptic neuron were elicited by voltage-clamping one of the presynaptic neurons to various holding potentials. The time course of the holding current was analyzed to monitor the input received by the presynaptic cell. We found current transients in the clamped presynaptic cell to coincide with postsynaptic spikes in most cases. The current transients were highly variable in amplitude and occasionally absent during postsynaptic spiking. These characteristics imply that the current transients in the voltage-clamped neuron result from spikes in electrically coupled co-members of the presynaptic ensemble. We found a close temporal correlation between the current transients and postsynaptic spikes. This correlation was present regardless of the holding potential of the voltage-clamped cell and the resulting postsynaptic activity level. These results imply that the graded response components of the presynaptic cells effectively control the postsynaptic firing rate on a coarse time scale while the precise timing of the postsynaptic spikes is a consequence of spikes superimposed on the graded signals of the presynaptic neurons. This conclusion was corroborated by experiments, in which we elicited single presynaptic spikes by application of brief current pulses of varying amplitude at several different membrane potential levels. Regardless of a de- or hyperpolarized membrane potential presynaptic spikes led with a high probability to closely timed postsynaptic spikes.

Multiquantal Release Underlies the Distribution of Synaptic Efficacies in the Neocortex

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Neocortical inter-pyramidal connections are characterized by a wide range of EPSP amplitudes. This range could potentially be accounted for by differences in all three parameters of the quantal model of synaptic transmission, i.e. the number of release sites, release probability and quantal size. Here, we present a rigorous statistical analysis of the transmission properties of excitatory synaptic connections between layer-5 pyramidal neurons. Our central finding is that the EPSP amplitude is strongly correlated with the number of estimated release sites, and that the number of release sites can be an order of magnitude higher than the typical number of synaptic contacts for this type of connections. Our findings indicate that transmission at stronger synaptic connections is mediated by multiquantal release from their synaptic contacts. We propose that modulating the number of release sites could be an important mechanism in regulating neocortical synaptic transmission.

Identification and functional characterization of *Drosophila* SRPK3, a Ser/Thr kinase required for proper distribution of the active zone protein Bruchpilot in larvae

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In collaboration with the group of S. Sigrist we have identified and characterized the Bruchpilot protein (BRP) as an essential structural component of the presynaptic active zone of *Drosophila*. Suppression of this protein blocks the formation of presynaptic T-shaped ribbons (T-bars), leads to semi-lethality, and in escapers causes severe behavioural impairments including unstable flight (hence Bruchpilot = German for crash pilot; Wagh et al., 2006; Kittel et al., 2006). In a screen for genes which interact with the *bruchpilot* gene we identified a mutation that causes aggregates of BRP to form in axons of larval motor neurons. The corresponding gene codes for several isoforms of a serine/threonine protein kinase which shows high homology to mammalian SR protein kinases (SRPKs). Since proteins expressed from this gene *in vitro* phosphorylate typical SR protein substrates the gene has been named *Srpk3*. By RT-PCR four differently spliced mRNAs can be identified. GFP-labeled SRPK3 protein co-localizes with BRP at active zones. A direct interaction at the active zone may therefore take place. The BRP aggregates in larval motor nerves are not due to a general axonal transport defect. Hypomorphic or null mutations of the *Srpk3* gene lead to similar but less severe behavioural deficits in adult flies as were observed for BRP-RNAi-knock-down animals. The *Srpk3* mutant flies are impaired in flight and walking, and in addition display a dramatically reduced lifespan compared to wild-type flies. The behavioural phenotypes of the mutants can be rescued by transgenic neuronal *Srpk3* cDNA expression, life span is rescued partially. Ultrastructural comparison of null-mutant and wild-type larval motor nerves identified only in mutant axons conspicuous electron-dense structures of various shapes which are surrounded by vesicles. These vesicles, however, do not contain typical presynaptic vesicle proteins. Immuno-gold labelling of these structures and the identification of interaction partner candidates by a yeast-two-hybrid screen are presently attempted.

Synaptic activation of group II metabotropic glutamate receptors (mGluRs) in the dentate gyrus of the rat

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Pharmacological activation of mGluRs potently modulates excitatory synaptic transmission throughout the brain. However, little is known about the physiological conditions under which these receptors are activated by synaptically released glutamate. We employed the glutamate transporter inhibitor DL-threo-beta-benzyloxyaspartate (TBOA) to facilitate synaptic activation of group II mGluRs at the lateral perforant path to granule cell synapse in rat dentate gyrus. 100µM TBOA decreased fEPSPs which could partially be reversed by application of the group II mGluR antagonist (2S)-2-Amino-2-[(1S,2S)-2-carboxycycloprop-1-yl]-3-(xan th-9-yl) propanoic acid (LY341495). This inhibition by TBOA even occurred if stimulation was stopped during drug application, suggesting it is due to spontaneous, action potential independent, transmitter release. Next, 40µM TBOA (plus 50µM APV and 50µM MK-801) was used to facilitate the synaptic activation of mGluRs and to avoid inhibition of baseline transmission. To test for activation of mGluRs by action potential driven glutamate release we applied a conditioning burst (7 stim @ 100Hz) and assessed the resulting activation of mGluRs by the LY 341495-induced increase of a test response applied after different post-burst intervals (PBI) (0.03, 0.05, 0.2, 0.3, 0.8, 1, 3, 6, 20sec). The strongest mGluR effect on the test fEPSP was observed at PBIs 0.1 and 0.3 sec (15 ± 2.7%, n= 8) and the inhibition was detectable even after 0.5 – 0.8sec (8.9 ± 1.6%, n= 6). The latter suggests that a fraction of receptors remains activated for up to 1sec PBI. Therefore, we tested whether there is a cumulative mGluR effect following a series of conditioning bursts (4 bursts, 4/7 stim @ 100 Hz, 0.5sec inter-burst interval (IBI), 0.2sec PBI; 10 bursts, 2 stim @ 100 Hz, 0.05sec IBI, 0.2sec PBI; 15 bursts, 3 stim @ 50Hz, 0.3sec IBI). However, the group II mGluR mediated inhibition of fEPSPs did not further increase with these paradigms. In the next step we tested different numbers of stimuli in the conditioning burst for the effectiveness of mGluR activation (1, 3, 7, 14, 25, 28, 50, 56, 100, 200 stimuli @ 100Hz and 0.2sec PBI). mGluR mediated inhibition increased with the number of stimuli and the inhibition reached a plateau value of 31 ± 10% (n= 5, 100 stim @ 100Hz, 0.2sec PBI). Finally, we tested different frequencies (3, 5, 20, 100, 200Hz) in the conditioning burst with a nearly constant duration between 500 - 700ms and found that the degree of activation of mGluRs saturated at 200Hz with a value of 22 ± 2.5% (n= 5). We conclude that group II mGluRs seem to have a fast activation time constant (~ 50 ms) and a much slower deactivation time constant (500 ms). Further, the degree of synaptic activation of mGluRs under physiological and complex patterns of activity is not only limited by the glial glutamate uptake rate but also by the amount of releasable vesicles per time. The slow deactivation kinetics of group II mGluRs and the fact that the synaptic transmission is maximally inhibited by <31%, let us propose that the functional significance of mGluRs as presynaptic autoreceptors is not the degree but rather the long persistency of inhibition of glutamatergic transmission.

Ambient GABA concentration in layer I of the postnatal cortex

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GABA is the main inhibitory neurotransmitter in the adult brain. Synaptically released GABA activates postsynaptic $GABA_A$ receptors giving rise to GABAergic postsynaptic potentials. The latter terminate when GABA diffuses out of the synaptic cleft or is removed by GABA transporters. In addition to "phasic" transmission, ambient GABA which is normally present in the extracellular space can activate extrasynaptic GABAA receptors ($GABA_A$Rs) and/or presynaptic GABAB receptors ($GABA_B$Rs). Persistent activation of postsynaptic $GABA_A$Rs leads to a "tonic" inhibitory current that controls the level of excitability of neurons, while tonic activation of presynaptic $GABA_B$Rs can regulate the strength of glutamatergic and GABAergic inputs. Given $GABA_A$Rs or $GABA_B$Rs are not saturated by ambient GABA levels, physiological activity and/or manipulations that change the extracellular GABA levels can strongly influence cell excitability and plasticity of neuronal networks.

Cajal-Retzius (CR) cells represent a major class of early-generated neurons in the marginal zone (later layer I) of the neocortex. CR cells receive excitatory GABAergic inputs and the strength of these projections is controlled by presynaptically located $GABA_B$Rs. The latter are persistently activated by ambient GABA. Interestingly, the extracellular GABA level in this preparation is set by the activity of GABA transporters, namely GAT-3 releases GABA and GAT-1 operates in the uptake mode (Kirmse, Kirischuk J Neurosci., 2006, v.16, p.4216). Therefore, we suggested estimating the levels of ambient GABA by using presynaptic $GABA_B$Rs as GABA detectors.

Experiments were performed using two age groups (postnatal days 1-3 (P1-3) and P5-7) of mice. Evoked GABAergic postsynaptic currents (eGPSC) were recorded using the whole-cell configuration of the patch-clamp technique. eGPSCs were elicited by minimal electrical stimulation in layer I of the cortex. Paired-pulse protocol (50 ms inter-stimulus interval) was applied throughout. The mean amplitudes of eGPSCs, pair-pulse ratios (PPR) and failure rates were used to assess the degree of $GABA_B$ receptor activations under the following conditions:

1) When GABA-releasing GAT-3 has been blocked by SNAP-5114 or GAT-3 and GAT-1 were blocked by SNAP-5114 and NO-711, respectively, an addition of 0.5 µM GABA to the extracellular solution returned all selected parameters to the control values independently of the age of animals used.

2) When glutamate decarboxylase (GAD) was blocked by 3-mercaptopropionic acid, 0.5 µM GABA at P5-7 and 1.5 µM GABA at P1-3 were needed to reach the control values.

We conclude that extracellular GABA concentration is about 0.5 µM during the first postnatal week and this level is strongly dependent on the activity of GAD at P1-3.

Loss of Neuroligin2 disrupts GABA receptor integrity and leads to functional deficits in the mouse retina

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Neuronal connectivity relies on the accurate juxtaposition and composition of pre- and postsynapses. In the retina, processing of visual information occurs through a well-layered neuronal network, in which a main excitatory pathway is modulated by an inhibitory surround at two synaptic (inner / outer plexiform) layers. Neuroligins (NLs) are postsynaptic transmembrane adhesion proteins, essential for synaptic function, through interactions that are not entirely clarified. In this study, we chose the mouse retina, which allows for precise morphological and functional analysis, as a model to understand the importance of NL2 in the establishment and maintenance of a neuronal network.
In keeping with its distribution in the brain, NL2 was localized specifically at inhibitory postsynapses of both plexiform layers. However, in the retina, NL2 was found to be preferentially associated with GABA receptor subunits and did not associate with Glycine receptor clusters. Morphological analysis of the NL2 deletion-mutant mouse retina using cell-type and synapse specific markers revealed that the basic retinal architecture was intact even in the absence of NL2. However, a deficiency of NL2 lead to a dramatic reduction of specific $GABA_A$ receptor subunits coupled with a possible upregulation of the glycinergic amacrine circuitry. Moreover, NL2 deletion was accompanied by impaired processing of light stimuli: Responses of photoreceptors and bipolar cells to repetitive stimulation were reduced in NL2-deficient retina, and the baseline activity of retinal ganglion cells increased. As a consequence, stimulus-locking of retinal output spikes was reduced. Taken together, NL2 is required for the functional integrity of GABAergic postsynapses in the retina and its absence alters transmission in this sensory network.

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Active Zone scaffolding protein SYD-2 regulates Kinesin-3 activity

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Kinesins are molecular motors that carry out various intracellular transport processes along microtubule tracks. UNC-104 a *C. elegans* ortholog of Human kinesin is one such molecular motor of the kinesin-3 family that is involved in transporting synaptic vesicles to synapses in *C. elegans*. In contrast to most other kinesins being dimers, UNC-104 is a monomeric kinesin that does not show fast, processive movement in single molecule motility assays. However, *in vivo* UNC-104 can move fast and processively. We hypothesized that UNC-104 interacting proteins might play a role in regulating the non-processive to processive transition. One molecular player that could fulfill this role is SYD-2. SYD-2 is important for the organization and architecture of a synapse interacting with many scaffolding molecules at the active zone (AZ). Furthermore SYD-2 binds UNC-104 thus making it a prime candidate for regulatory protein. We confirm their interaction by pull-down assays and measure the velocity and processivity of the motor *in vitro* in the presence and absence of SYD-2. Our preliminary results show that SYD-2 increases the velocity of UNC-104 in a multi-motor assay (microtubule surface gliding assay) as well as on a single molecule level (analysis by TIRF microscopy).

SNARE-dependence of the exocytosis at the inner hair cell ribbon synapse

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Processing of acoustic information requires transduction of sound stimuli in the inner ear encoded by hair cells. The synapses of the inner hair cells (IHCs) with the auditory nerve fibers are characterized by an electron dense structure called synaptic ribbon, which is packed with synaptic vesicles and anchored to the presynaptic membrane. During exocytosis in most synapses, a four-helical coiled coil protein complex is formed, between the three SNARE proteins syntaxin 1, synaptobrevin 2 and SNAP-25, bridging vesicle and plasma membrane. This SNARE complex, which is essential for synaptic vesicles exocytosis, is expressed in the inner ear. However, the function of the SNARE proteins at this ribbon synapse had never been investigated.

To study the SNARE function on exocytosis of the IHCs, we took advantage of botulinum neurotoxins serotypes E (BoNT/E), C (BoNT/C) and D (BoNT/D) light chains, which cleave SNAP-25, syntaxin and synaptobrevin, respectively. Efficiency of these toxins was demonstrated *in vitro* by the cleavage of SNAP-25, syntaxin and synaptobrevin at nanomolar concentration and *in vivo* by block of large dense core vesicle exocytosis in chromaffin cells poisoned with either of the neurotoxins. Moreover, the use of these neurotoxins conjugated to Alexa-488 demonstrated a reliable loading of the toxins inside the IHCs. However, IHCs infused during ten minutes with BoNT/E, BoNT/C and BoNT/D light chains still displayed robust neurotransmitter release monitored as cell capacitance changes triggered by successive calcium influx. Next, we analyzed the IHC exocytosis in the absence of both synaptobrevin II and III< (double knock –out) and from the lethal-wasting mouse strain, which is characterized by a loss-of-function mutation of *VAMP1* (synaptobrevin I). However, IHC neurotransmitter release in the loss-of-function mutants was comparable to the wild type. We then finally investigated the expression pattern of the SNARE proteins in the inner ear using immunocytochemistry. Surprisingly, we could only detect SNARE expression (SNAP-25, syntaxin and synaptobrevin) in the efferent neuron that innervate the afferent auditory fibers but not in the IHCs. Together, these data suggest the provocative hypothesis that IHC exocytosis is SNARE independent.

N-cadherin controls vesicle clustering during early synapse maturation

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Synapse formation is thought to be initiated and regulated by the transsynaptic interaction of synaptic adhesion molecules. Classical cadherins, e.g. neural (N)-cadherin, represent a family of synaptic adhesion molecules that are well known to be localized perisynaptically close to the active zone and the postsynaptic density. However, relatively little is known about the functional role of N-cadherin in synapse formation, stabilization and plasticity. We have used a live imaging approach to study presynaptic vesicle accumulations in N-cadherin deficient, ES cell-derived neurons. The use of ES cell-derived neurons enabled us to study N-cadherin knockout synapses, because this circumvented the early embryonic lethality of the N-cadherin knockout mice. To fluorescently label presynaptic vesicles, cultured ES cell-derived neurons were acutely transfected with a DsRed2-SynaptobrevinII fusion proteins. At 6-7 DIV the density of presynaptic vesicle clusters was significantly reduced in N-cadherin deficient neurons. Live cell imaging of individual vesicle accumulations revealed an impaired addition of synaptic vesicles at nascent synapses in the absence of N-cadherin. This finding was confirmed by FRAP experiments, suggesting that N-cadherin plays an important role in controlling the accumulation of vesicles at immature synapses. At later maturational stages N-cadherin appeared to be dispensable because the vesicle accumulation process had almost ceased. In summary, our results indicate that N-cadherin plays an important role in regulating presynaptic vesicle accumulation thus promoting presynaptic differentiation.

The LEM-domain protein MAN1 is required for presynaptic function at the *Drosophila* neuromuscular junction

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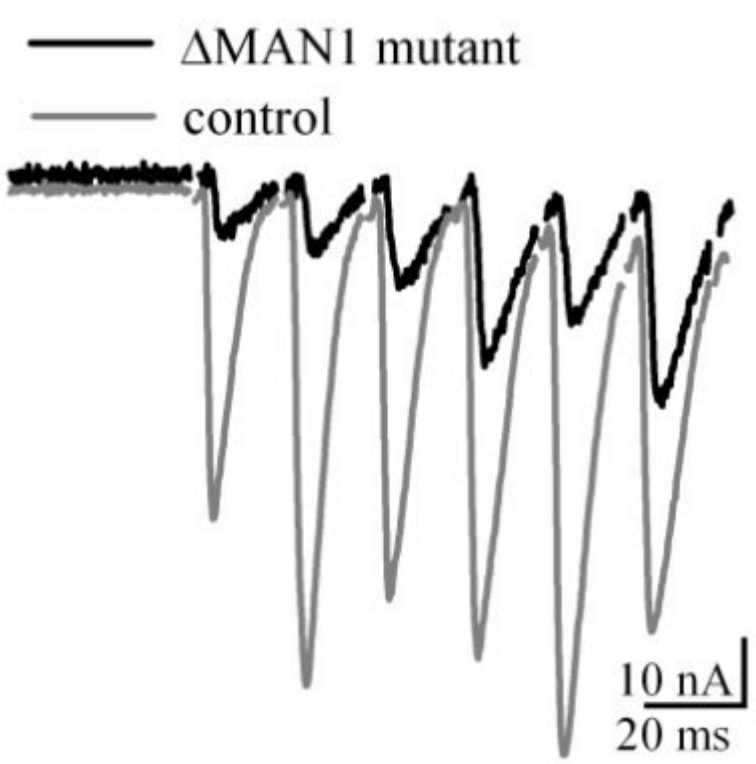
Bone morphogenic proteins (BMPs) are a family of growth factors belonging to the transforming growth factor beta (TGF-ß) superfamily.
At the *Drosophila* neuromuscular junction (NMJ), BMP signalling is involved in synapse development and possibly synaptic homeostasis through retrograde signalling.
The LEM-domain protein MAN1 is located in the inner nuclear membrane and acts as a negative regulator of BMP signalling during wing vein formation in *Drosophila*.
A deletion of the sequence coding for the C-terminus of MAN1 was generated, which we refer to as ΔMAN1.
In the present study, we investigate synaptic function in ΔMAN1 mutant animals.
We performed two electrode voltage clamp (TEVC) measurements on muscles of wild type *Drosophila* larvae and of animals homozygous for the ΔMAN1 mutation.
In the ΔMAN1 mutants, the amplitude of evoked excitatory postsynaptic currents (eEPSCs) was 16.6 ± 3.5 nA (n=6), corresponding to 26 % of the wild type amplitude (63.9 ± 6.1 nA; n=6). The amplitude of miniature EPSCs (mEPSCs) was similar in wild type (1.3 nA ± 0.1; n=4) and ΔMAN1 mutants (1.3 nA ± 0.1; n=5). During high-frequency-stimulation (6 pulses at 60 Hz), facilitation was more pronounced in ΔMAN1 mutants than in controls, indicating a reduction of release probability in ΔMAN1-mutants. The figure shows representative traces of EPSCs in a 60Hz train in wild type and ΔMAN1 mutant larvae.
Synaptic transmission could be partially rescued by neuronal expression of MAN1 in the mutant background, while expression in muscles was less effective. Our results suggest a crucial role for MAN1 in presynaptic function and probably retrograde BMP signalling at the *Drosophila* NMJ.


ΔMAN1 mutant
control
10 nA
20 ms

NR2B-containing receptors activated by ERK enhance presynaptic glutamate release in epileptic mice

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NR2B-containing NMDA receptors in the hippocampus are predominantly expressed in early developmental stages whereas NR2A-containing receptors occur later in development. This developmental switch has important consequences for synaptic transmission and plasticity. As we have shown recently, increased levels of NR2B were detected in the hippocampus of mice expressing a constitutively active form of MEK1 (caMEK1). These mice develop normally and were indistinguishable from control littermates until early adulthood but exhibit spontaneous epileptic seizures from 6-8 weeks of age. Here, we examine the effect of ifenprodil, a selective noncompetitive blocker of the NR2B-subunit, on whole cell currents of CA3 cells in hippocampal slices of juvenile (18-23 days of age) and adult (>42 days of age) caMEK-mice. The frequency of spontaneous EPSCs (sEPSCs) recorded in artificial cerebrospinal solution (ACSF) at a holding potential of -70 mV was generally reduced in the presence of ifenprodil. In slices of adult control mice the frequency reduction was less marked than in slices of adult caMEK mice. To investigate postsynaptic effects of ifenprodil on sEPSCs we recorded in Mg^{2+}-free ACSF at potentials of -60 and -40 mV in the presence of the AMPA receptors blocker DNQX. Although most of the activity vanished under these conditions we detected a decrease in amplitude and a faster decay of sEPSCs in control as well as in caMEK mice after application of ifenprodil.

Although we can not completely exclude a postsynaptic effect, our results indicate that the enhanced glutamate release in adult caMEK-mice might contribute to the epileptic phenotype.

Different input patterns to stellate versus pyramidal cells in the Entorhinal Cortex.

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Recent evidence suggest an independent role of the medial entorhinal cortex (mEC) in spatial navigation in rodents. *In-vivo*, many cells across the different layers of the mEC exhibit grid-like firing properties. Since not much of the intra- and inter-laminar connectivity of the different cell types in this region is clearly known, it is hard to come up with models on how the interplay between cellular and network-level mechanisms gives rise to the formation and functioning of grid-cells. In our study, we have developed a software to perform photostimulation experiments to elucidate the connectivity pattern of a cell in *in-vitro* brain slices. Using this technique, we look at the connectivity of layer II (LII) stellate cells and further compare this with the other excitatory cell in this layer, the LII pyramidal cells. Our results show that true synaptic events could be faithfully evoked and detected by flash-photolysis of caged-MNI-glutamate followed by a self-written detection algorithm. The local excitation profile (in LII) was broader for stellate cells as compared to the pyramidal cells. However, the pyramidal cells received inputs from the deeper layers (LV and LVI) preferentially in contrast to the stellate cells. As the cells in the deeper layers are reported to have feedback loops to the superficial layers and also convey information about the velocity of an animal in an open-space, this difference in connectivity between the two cell-types in LII could have important implications.

Optical recording of single synaptic vesicle fusion

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During synaptic transmission quantal packages of neurotransmitter (NT) are discharged from the pre-synaptic terminal by fusion of release competent synaptic vesicles (SVs) with the plasma membrane. Post fusion the vesicle constituents are recycled from the cell surface for further rounds of use by compensatory endocytosis. One of the key steps in the recycling process is the efficient capture of SV membrane proteins post fusion from the pre-synaptic surface in the right stochiometry and their subsequent assembly to generate a fusion-competent SV.

To understand exo-endocytic coupling and how the SV constituents are re-sorted, it is important to study the immediate fate of single vesicle proteins post fusion. We label individual SVs with a fusion construct of a pH-sensitive variant of GFP, pHluorin, attached to a lumenal domain of specific SV membrane proteins by transient overexpression in cultured hippocampal neurons. We then optically track single SV fusion and the spatio-temporal dynamics of their vesicle components at individual pre-synaptic boutons using high-resolution microscopy. Based on quantitative single molecule experiments we calibrate the number of pHluorin-tagged vesicle membrane proteins per SV. Our data suggest that upon overexpression, only 1-5 copies of these fluorescent reporters are able to replace their endogenous counterparts. This low number (sometimes 1 molecule per SV) highly limits the possibility to optically monitor the fate of single SV components after exocytosis. However, upon fusion of multiple SVs or a single SV with higher number of fluorophores, fast diffusional spreading can be resolved within the bouton membrane indicating, that most SVs fully collapse, and vesicle proteins disperse rapidly along the bouton membrane post fusion, instead of remaining clustered in raft like patches. Our results, however, underscore the necessity to increase the number of reporter molecules per SV by either over-expression on a null background or by complete replacement of the endogenous protein with the corresponding reporter construct using a knock-in approach.

Deletion of either one of the NO receptors alters excitatory synaptic neurotransmission in the hippocampal CA1 field

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Nitric oxide (NO) is a multifunctional messenger in the central nervous system (CNS) that can act bidirectionally across synapses during synaptic plasticity. Most signal transductions of this rapidly diffusing gas are mediated through the NO receptor guanylyl cyclase (NO-GC) and its substrate cyclic guanosine-3',5'-monophosphate (cGMP). The NO-GC exists in two functional relevant isoforms NO-GC1 and NO-GC2. Recently, it has been suggested that both NO-GC receptors are required for the induction of long-term potentiation in the visual cortex and in the CA1 region of the hippocampus. However, only little is known yet about their synaptic localizationsand functions. In order to obtain further insights, we are investigating their contributions to the excitatory synaptic transmission in the hippocampal CA1 field of mice, which are deficient in either one of the twoNO receptors.

Patch-clamp recordings in slices showed an approximately 50% reduction in the frequency of miniature excitatory postsynaptic currents (mEPSCs) in NO-GC1knockout (-/-) mice compared to wild-type (WT) and NO-GC2 (-/-) animals (n=10 for WT; n=8 for NO-GC1 (-/-), p=0.0001; n=13 for NO-GC2 (-/-), p=0.9). Similar results were also observed during minimal stimulation experiments, which highly indicates an impairment in the initial release propability for glutamate at the excitatory presynaptic sides (n=14 for WT; n=23 for NO-GC1 (-/-), p=0.0001; n=17 for NO-GC2 (-/-), p=0.7). However, upon prolonged repetitive stimulation at 20 Hz the steady-state release was much higher in NO-GC1 (-/-) mice than in WT animals (n=10 for WT; n=13 for NO-GC1 (-/-), p=0.0001; n=14 for NO-GC2 (-/-), p=0.7).

In addition, experiments concerning postsynaptic functions showed a significant difference in the amplitudes and kinetics of evoked NMDAR currents in the NO- GC2 (-/-) mice compared to WT and NO-GC1 (-/-) animals (n=7 for WT; n=8 for NO-GC1 (-/-); n=7 for NO-GC2 (-/-), p=0.001).

These data indicate dinstinct localisations of both NO-GC receptors in the hippocampal CA1 region. While the NO-GC1 isoform is suggested to be presynaptically located in glutamatergic terminals, the NO-GC2 is rather found at postsynaptic sides. Since the two NO-GC receptors are localized in different synaptic compartments and the knockout models show different effects, we propose the existence of two different NO/cGMP-mediated pathways, which play distinct roles in synaptic neurotransmission.

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All Munc13 isoforms are expressed and differentially distributed in the mouse retina.

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In mammals, the Munc13 protein family comprises three highly homologous members, Munc13-1, bMunc13-2/ubMunc13-2 (splice variants of the Munc13-2 gene) and Munc13-3, which are essential to synaptic vesicle priming, and thereby to neurotransmitter release. Munc13 isoforms exhibit distinct regional, and cellular patterns of expression in the brain, however, their precise localization and functional significance in the retina remains unknown. In the present study we have raised isoform-specific polyclonal antisera against the Nterminal fragments of the respective rodent Munc13 isoforms. Western blots performed with purified antisera showed that all Munc13 isoforms/splice variants are present in the adult mouse retina. Using double immunolabeling and confocal microscopy, we show that Munc13 isoforms are differentially distributed throughout retinal plexiform layers, where they are localized to presynaptic sites, but essentially do not colocalize with each other. Interestingly, ubMunc13-2 was the only Munc13 isoform expressed at photoreceptor ribbon synapses of the outer plexiform layer, where it was found to colocalize with the cytomatrix protein bassoon, but not the ribbon marker C-terminal-binding protein 2 (CtBP2). Experiments were performed on Munc13-2 deficient mice to investigate the functional and morphological properties of specific retinal synapses in the absence of Munc13-2. Preliminary results from electroretinogram recordings point towards a selective alteration in transmission at photoreceptor ribbon synapses lacking Munc13-2, but not Munc13-3. The morphological correlates of this synaptic dysfunction are currently being analysed by ultrastructural approaches.

Sequential Binding of Synaptobrevin to SNARE Partners Drives Priming and Fusion in Calcium-Mediated Neurotransmitter Release

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SNARE-complex assembly is an essential prerequisite for neurosecretory vesicles undergoing membrane fusion. During exocytosis a four-helical coiled-coil is formed by the SNARE proteins syntaxin, synaptobrevin and SNAP-25. Recent findings using mutagenesis studies of SNAP-25 promoted the notion that sequential N- to C-terminal assembly of the SNARE motif is responsible for vesicular priming and fusion (Sørensen et al. 2006. EMBO J. 25:955-966). In the present study we investigate the role of the vesicular SNARE protein synaptobrevin 2. In chromaffin cells, vesicular fusion can be studied in the knockout background by reconstitution of protein variants using viral overexpression into synaptobrevin/cellubrevin nulls (Borisovska et al. 2005. EMBO J. 24:2114-2126). Mutant proteins bearing destabilizing point mutations in the hydrophobic layers of the SNARE motif were used to test the hypothesis of sequential SNARE complex assembly. N-terminal double point-mutations of synaptobrevin 2 were without effect on release kinetics, but showed a reduction in vesicle pool size, indicating that N-terminal interaction is crucial for vesicle priming. Mutations in the C-terminal end of the SNARE domain affected kinetics of release, suggesting that C-terminal assembly of the SNAREs provides energy for the final steps of vesicular fusion in calcium-triggered release. The effect of local destabilization of SNARE interaction on single vesicle fusion was investigated by single spike amperometry. C-, but not N-terminal destabilization of the SNARE motif displayed a selective and significant decrease of the pre-spike foot duration, a parameter thought to reflect fusion pore stability. The observed region-selectivity of mutations strongly supports the idea of sequential N- to C-terminal SNARE complex assembly with a partly assembled complex corresponding to the primed vesicle state.

Dendrite arborization and synapse maturation DASM1 - involvement in synaptic transmission

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The immunglobulin superfamily member Igsf9 was shown to be involved in the development of the nervous system. Initially it was discovered in Drosophila, where it was named turtle, as mutant larvae show impaired movement. In mammals it was named DASM1, as RNAi knock-down led to impaired dendritic arborization (DA) and synaptic maturation (SM). DASM1 is a transmembrane protein with five extracellular immunglobulin domains, two extracellular type-III-fibronectin domains, and a putative PDZ signaling motif at the intracellular C-terminus.

Shi and coworkers showed via RNAi knock-down, and over-expression of a dominant-negative mutant, that DASM1 is involved in synaptic trafficking of AMPA receptors. In addition downregulation of DASM1 led to a reduced arborization of the dendrites. We showed in neurons from knock-out animals, that dendrite arborization is not altered compared to WT. Moreover, transfecting neurons with the before used RNAi sequences led to the same phenotypes in both KO and WT, strongly suggesting an off-target effect.

Next we studied excitatory synaptic transmission in more detail in the hippocampus of DASM1 KO animals. First, we used extracellular field potential recordings to compare the strength of synaptic transmission between control and KO mice. The size of the presynaptic fiber volley (input) was compared to the size of the field excitatory postsynaptic potential (fEPSP) response (output). Surprisingly, no differences were observed. Second, whole cell recordings revealed an elevated AMPA / NMDA ratio, while miniature excitatory postsynaptic currents were not altered. This suggests that the impaired ratio is probably caused by alterations of the NMDA receptors. Other parameters of basal synaptic transmission seem unaltered.

This study demonstrates, that unlike previously shown, DASM1 is not required for AMPA receptor trafficking. As our preliminary results support a role of DASM1 in NMDA receptor mediated synaptic transmission, we will continue to study in more detail the effects on NMDA receptors.

Cold-stable microtubule and associated proteins in the brain of hibernating hamsters

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In Alzheimer's disease the increase in tau phosphorylation is thought to be correlated with a decrease in the amount of tau associated with the cytoskeleton and decreased microtubule stability. The associated impairment of axonal transport finally leads to synapse regression and cognitive decline. During hibernation in syrian hamsters reversible tau phosphorylation associated with impaired memory was observed. Surprisingly, tubulin posttranslational modifications commonly used as a marker of microtubule stability did not change during hibernation, indicating a high degree of cold stability of tubulin. Microtubules isolated from brain and reassembled did hardly depolymerize during cold exposure. Several microtubule associated proteins were cosedimented which may account for the cold stability. They will be isolated by size exclusion chromatography, tested for promoting tubulin polymerisation and microtubule cold stabilisation, and lated identified.

Local vs. global temporal integration of excitatory input via activity-dependent dendritic spike attenuation.

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In both apical oblique and basal dendrites of pyramidal neurons, local membrane potential nonlinearities, termed dendritic spikes, can be elicited by synchronous activation of a sufficient number of individual synapses. Dendritic spikes can trigger action potential output with high temporal precison and are an important associative signal for induction of synaptic plasticity. Here we show using multiphoton glutamate uncaging in basal dendrites of CA1 pyramidal neurons that dendritic spikes are strongly modulated by prior neuronal activity. While synaptic input subthreshold for dendritic spike initiation does not affect dendritic spikes, synchronous input that elicits a dendritic spike inhibits nonlinear dendritic processing for several hundred milliseconds. This local mechanism remains spatially restricted to the stimulated branch. In contrast, synchronous stimulation that gives rise to a dendritic spike sufficiently large to generate a back-propagating axonal action potential causes a wide-spread, more global attenuation of dendritic spikes. These mechanisms subserve a novel form of branch-specific vs. global temporal integration. They are active within the time domain of common neuronal rhythmic forms of activity such as theta activity.

Role of the 65-kDa isoform of glutamic acid decarboxylase in GABAergic synaptic transmission in the lateral amygdala

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The amygdala plays a crucial role in the emotional evaluation of sensory input from the environment, and alterations of amygdaloid functions characterize of certain psychiatric illnesses such as anxiety disorders as well as the pathogenesis of temporal lobe epilepsy. Physiological functions are ensured by a fine-tuned influence of the inhibitory neurotransmitter γ-amino-butyric-acid (GABA). In fact, deficits in GABA-metabolism are closely related to pathological conditions both in animal models and humans. Two isoforms of the enzyme glutamic acid decarboxylase, GAD67 and GAD65, provide GABA in the mammalian brain. Previous studies of our lab have shown that a genetically determined deficiency of GAB65 results in generalization of conditioned fear, impairment of fear extinction and increase in seizure susceptibility.

Therefore the present study was undertaken to characterize the cellular and synaptic correlates of the GAD65-related alterations in the amygdala. Whole-cell patch-clamp recordings were performed in the lateral amygdala (LA) in brain slices of juvenile (postnatal days 15-30) and adult (6-8 week) mice with genetically determined GAD65 deficiency (GAD65-/-) and of their wild-type littermates (GAB65+/+). Intrinsic electrotonic and electrogenic membrane properties of projection- and local circuit interneurons in the LA were not significantly different between genotypes. Next, inhibitory postsynaptic currents (IPSCs) mediated by $GABA_A$ receptors were examined in LA projection neurons. Both, miniature and spontaneous IPSCs were significantly increased in frequency and decreasesd in amplitude in the LA of young GAD65-/- animals. At an adult age, differences between genotypes persisted with respect to spontaneous but not miniature IPSCs. Furthermore, monosynaptic GABAergic IPSCs evoked upon minimal electrical stimulation within the LA were significantly affected by a GAD65 deficiency, as indicated by a decrease in both success rate and input/output relationship.

In conclusion, the present finding indicate a critical role of GAD65 for GABAergic synaptic interactions in the amygdala, which may well contribute to generalized fear memory and impaired fear extinction during GAD65 deficiency.

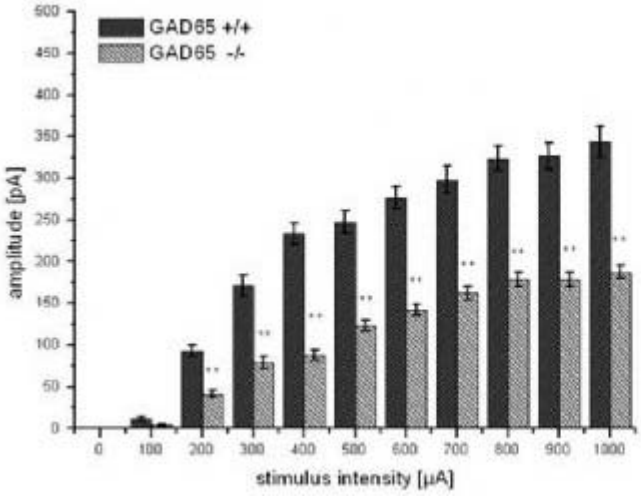

Fig 1: Mean peak amplitudes of evoked $GABA_A$-mediated IPSCs in LA projection neurons to increasing steps of stimulus intensities. GAD65-deficient mice reveal a highly significant 2-fold reduction of the amplitude [$n_{(+/+)} = 9$, $n_{(-/-)} = 17$]. (**) $p<0.01$ according to student's t-test.

Modulation of synaptic plasticity: functional characterization of the effect of extracellular matrixcomponents on hippocampus neurons

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The extracellular matrix (ECM), is a complex and highly interacting network of macromolecules including glycoproteins, polysaccharides and proteoglycans. Tenascins and chondroitin sulfate proteoglycans (CSPGs) are essential components of hippocampal ECM.

In the nervous system the ECM influences interactions of neuronal and glial cells, regulating cell migration, survival, differentiation, axonal pathfinding and synapse formation.

In order to investigate the involvement of ECM molecules on synaptic transmission we established astrocyte neuron cocultures and removed several ECM components by treatment with appropiate degrading enzymes. Electrophysiological characterization of cultures treated with the chondroitinase ABC (ChABC) and control cultures of astrocytes and embryonal hippocampal neurons (E18) was performed. We measured the sodium currents, action potentials and pharmacologically isolated mini excitatory and inhibitory postsynaptic currents (mEPSCs, mIPSCs). For pharmacological isolation we used differents neuroblockers: TTX, DNQX or PTX, for blocking the sodium currents, excitatory or inhibitory postynaptic currents, respectively, and analyzed amplitude, frecuency, charge, rise and decay time of postsynaptic events.

No effect was seen for mIPSCs but we identified a significant difference in the amplitude of mEPSCS. Median EPSCs amplitudes of neurons treated with ChABC were found to be smaller than in untreated controls. In general, an effect on the amplitude of synaptic events can be induced by pre- or postsynaptic mechanisms, namely by reducing the amount of transmitter per vesicle (presynaptic) or by reduced conductance of glutamate receptor channels and/or a reduced number of receptors per synapse (postsynaptic). Further experiments will be performed to clarify the underlying mechanisms.

In another set of experiments, we use the Transwell coculture system in which neurons and astrocytes are not in direct contact, but allow the interaction of cells via some unknown diffusible molecules which probably play an important role in synaptic transmission. We perform electrophysiological recordings of hippocampal neurons grown with ChABC and cultures grown without the enzyme. Both cellcultures shown synaptic activity. Data will be compared with results obtained in normal neuron astrocyte cocultures.

Furthermore, synaptic activity in embryonal cell cultures of transgenic mice lacking four ECM components, brevican, neurocan, Tenascin-C and Tenascin-R will be investigated. Working with four different combinations of KO and wildt type hippocampal neurons in coculture with astrocytes will help us to understand the synergistic function of several ECM compounds on synaptic transmission.

Bruchpilot controls the temporal precision of neurotransmitter release

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Synaptic communication is very rapid, and correspondingly chemical synapses display several important features that enable the presynaptic influx of Ca^{2+} to be followed by a postsynaptic response on the sub-millisecond time scale. To promote spatially and temporally precise stimulus-secretion coupling the specialised presynaptic region of exocytosis, the active zone, displays clusters of voltage-gated Ca^{2+} channels close to vesicle docking sites.

The *Drosophila* Bruchpilot (BRP) protein is required for the structural and functional integrity of the active zone including the clustering of presynaptic Ca^{2+} channels (Kittel *et al*., 2006). In BRP mutants, transmitter release is severely reduced and occurs less synchronously. The present study focuses on the mechanisms by which BRP influences the kinetics of transmitter release. We investigated whether the kinetic change in BRP mutants arises from an increased distance of vesicles to Ca^{2+} channels or from alterations of the vesicle fusion process itself. While synaptic delay is increased in BRP mutants, we neither find evidence of an altered action potential waveform, nor of changes in the kinetics of fusion pore dilation. Together with data from reducing Ca^{2+} channel expression independent of BRP, our results indicate that the delayed release of BRP mutants is not simply due to fewer Ca^{2+} channels at the active zone.

Novel interaction partners of AKAP79/150 in the synapse

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AKAP79/150 is a multi-adaptor protein in the postsynaptic density (PSD). Due to its binding to the kinases PKA and PKC, the phosphatase PP2B/CaN, scaffolding proteins such as SAP97 and PSD-95, as well as G-protein-coupled receptors like the beta1- and beta2-adrenergic receptor, and the calcium sensor calmodulin (CaM), AKAP79/150 is likely to play a key role as an important molecular interface in signal transduction. Calcium is critically involved in synaptic signalling, which is mediated not only by CaM but also by the calcium-binding protein caldendrin. Using GST pulldown and coimmunoprecipitation assays with endogenous proteins from rat brain extracts we found caldendrin as a second calcium-binding protein that interacts with AKAP79/150. Unlike the ubiquitously expressed CaM, caldendrin is almost exclusively expressed in the nervous system and highly enriched in the PSD. Caldendrin shares large similarity with CaM in its C-terminal part containing four EF-hand motifs, which are crucial for calcium binding and interactions with other proteins. It differs from CaM in having a larger hinge region between both EF hand pairs and a distinct molecular surface. Furthermore, the N-terminal part of caldendrin has no similarity to other known proteins. Therefore CaM and caldendrin not only share common interaction partners but each protein also has specific and unique binding partners. Interestingly, CaM and caldendrin do not seem to compete for binding to AKAP79/150, which makes caldendrin a unique neuronal calcium sensor protein and its interaction with AKAP79/150 another important jigsaw piece in the complexity of neuronal signalling.

Identification and characterization of proteins of the *Drosophila* nervous system.

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The project aims to identify proteins from the nervous system of *Drosophila melanogaster* and study their function. We began with screening of monoclonal antibodies from the large collection of monoclonals raised against fruit fly brain homogenate (Hofbauer 1991, Hofbauer *et al.* 2008) for a clear signal on western blots. Two of these antibodies, namely na21 & ab52, produced distinct signals on western blots of fly head extracts. na21 recognizes an antigen with M_r ~ 10 kD, while ab52 recognizes an antigen with M_r ~ 95 kD.

On cryosections of adult fly heads, na21 stains specific synaptic layers of the optic neuropil, like the most proximal layer of medulla and parts of lobula and lobula plate. It also stains sensory cells in the antennae, most neuropil regions in the central brain, the thoracic ganglia and a pair of organs near the tip of the abdomen. ab52 apparently stains the entire synaptic neuropil of the brain. Another monoclonal antibody called nb181 seems to be eye-specific as on cryosections it stains only eyes and photoreceptor terminals in the lamina and medulla.

Ultracentrifugation studies indicate that the antigen for na21 remains in the membrane pellet, from which it is not completely extracted by Na_2CO_3 treatment, hence it seems to be a membrane protein. The antigen for ab52 is present in the supernatant fraction of ultracentrifuged lysates, thus it seems to be a soluble (cytosolic) protein. Attempts to purify these antigens by immunoprecipitation using the corresponding monoclonal antibodies did not succeed so far. We are using alternate approaches like detergent treatment of membrane pellets to release the na21 antigen, Tricine-PAGE, etc to purify the antigens for MS based identification. The antigen for ab52 was found to be resolved as a single distinct spot in the *pI* range of 6-7 using 2DE (IEF-PAGE). Finally, upon identification, depending on what is known about the nature of the proteins, we will try to generate mutants for the genes and study their phenotypes in order to elucidate the function of the proteins.

Excitatory actions of GABA and tyramine in the cockroach salivary gland complex.

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The cockroach *Periplaneta americana* has acinar type salivary glands. The secretory acini consist of C-cells, responsible for electrolyte and water secretion and P-cells that secrete protein into the saliva. Salivation is controlled by the dopaminergic and GABAergic salivary neurons SN1 and SN2, and by several smaller serotonergic neurons. Tyramine may also play a role in salivation since tyramine receptors are expressed in the salivary gand complex. We studied the physiological role of the GABAergic innervation and the actions of tyramine in an isolated nerve-gland preparation. Using electrical salivary duct nerve (SDN) stimulation and simultaneous recording of the basolateral membrane potential (V_{bl}) from acinar cells we found: (1) GABA and tyramine enhance V_{bl} changes induced by electrical activity of the SDN and (2) GABA and tyramine act presynaptically. Secretion measurements revealed that GABA enhances the rates of fluid and protein secretion significantly. Pharmacological experiments indicate that $GABA_B$ receptors are involved in mediating the excitatory GABA effect. The presence of a tyraminergic innervation is currently under investigation.

Synaptic depression at individual synapses of pyramidal neurons is governed by spine microanatomy

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To maintain balance between learning and maintenance of stable memory traces, the brain has to regulate plasticity and stability on the level of single synapses. How this is achieved and whether all synapses have the same potential for plasticity is unknown.
Here we use two-photon imaging and glutamate uncaging to investigate how the subsynaptic presence of endoplasmic reticulum (ER) affects synaptic function and plasticity in individual spines of CA1 pyramidal cells. ER was frequently found in large spines that contained strong synapses, rarely in small spines. Low frequency stimulation of synapses on ER containing spines produced delayed calcium release events and mGluR-dependent synaptic depression. Both phenomena were dependent on mGluR activation and IP3 signaling and were never observed in spines without ER. We conclude that in pyramidal cells, spine ER controls the potential for synaptic depression on the level of single synapses. Spine ER represents the structural correlate for a mechanism by which strong synapses can be weakened in response to new experience.

Translaminar connectivity in superficial layers of the neocortex

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Layer 1 neurons of the neocortex are exclusively GABAergic. Networks of layer 1 interneurons are electrically coupled, and provide inhibitory synaptic input to fellow layer 1 interneurons and layer 2/3 pyramidal neurons. However, little is known about the translaminar excitatory innervation of layer 1 interneurons.

Here, we performed paired-recordings from layer 1 and layer 2/3 neurons in adult neocortical slices. We classified layer 1 interneurons according to their action potential firing pattern, their frequency versus current relationship and their anatomical features. We investigated how efficiently layer 2/3 pyramidal neurons excited layer 1 interneurons, and compared the properties of unitary EPSPs with those recorded between pairs of layer 2/3 pyramidal neurons. The properties of unitary EPSPs were found to be dependent on the identity of the postsynaptic neuron. The unitary EPSP amplitude was 1.1 ± 0.2 mV (average ranged from 0.3 to 2.4 mV) in layer 2/3 – layer 1 connections, and 0.5 ± 0.1 mV (average ranged from 0.1 to 1.0 mV) in layer 2/3 – layer 2/3 connections. Subdividing different interneurons, we found an unitary EPSP amplitude of 1.0 ± 0.3 mV in fast-spiking interneurons and 1.3 ± 0.4 mV in classical accommodating interneurons, respectively. Our results suggest that L2/3 pyramidal neurons will more efficiently recruit L1 interneurons than L2/3 pyramidal neurons providing a substrate for strong feed-forward inhibition, which might contribute to the sparse firing of L2/3 pyramidal neurons observed *in-vivo*.

Stimulus induced translocation of alpha Ca^{+2}/Calmodulin Dependent Kinase II (a-CaMKII) to the electrical synapse protein connexin 36 (Cx36)

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Gap junctions are the structural equivalents of electrical synapses in the central nervous system(CNS), which can be formed by one or more of connexin (Cx) isoforms.
Connexin 36 (Cx36) is one of the major components of electrical and mixed synapses in the CNS, and may provide a substrate for changes occurring during molecular processes underlying plastic changes at sites of electrical synapses. Recent studies imply that Cx36 is a putative partner for several binding proteins, including alpha Ca^{+2}/Calmodulin Dependent Kinase II (a-CaMKII). Studies from our laboratory indicate that cytoplasmic domains of Cx36, carry binding as well as phosphorylation motifs for a-CaMKII.

Here, we firstly aimed to clarify whether binding of a-CaMKII to Cx36 is subject of activity dependent mechanisms such as translocation during glutamate/glycine stimulation. For this purpose, cultures of primary hippocampal neurons were transfected with EYFP-Cx36 and ECFP-a-CaMKII and subjected to live cell imaging using confocal laser scanning microscopy. Our results demonstrate a reproducible and significant translocation of a-CaMKII to Cx36 after stimulus application. However, neurons transfected with Cx36 mutants which lack the a-CaMKII binding sites, fail to show stimulus induced translocation of a-CaMKII. In addition to these findings, the autophosphorylation-deficient mutant of a-CaMKII (T286A) does not show colocalization with Cx36 as well as constitutively active a-CaMKII mutant (T286D).
Secondly, we performed experiments using a fluorescence resonance energy transfer (FRET) protocol in the same culture model. Experiments to measure FRET were performed after photoinactivation of EYFP-Cx36 with a 514nm laser after stimulation with glutamate/glycine. Our data show that the increase in the donor fluorescence (ECFP-a-CaMKII) was inversely proportional to the decrease of the acceptor fluorescence (EYFP-Cx36) which suggests close proximity and physical interaction between Cx36 and a-CaMKII.
Taken together, these studies support our concept, that Cx36 is subject of acitivity dependent interaction with a-CaMKII. Our data open new avenues to further dissect the molecular and cellular mechanisms contributing to neuronal plasticity at sites of electrical synapses.

Central glutamatergic transmission is controlled by lysophosphatidic acid

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At neuronal synapses the transmission is based on the regulated exocytotic fusion of synaptic vesicles filled with neurotransmitter. The vesicle recycling is orchestrated through the combined actions of proteins and lipids. Recent research shows that lipids, especially phospholipids, play a major role of endo- and exocytosis processes, including synaptic vesicle cycling. In our study we analyzed lysophosphatidic acid (LPA) effect on primary hippocampal neurons in synaptic transmission. LPA is a small phospholipid with a variety of biological activities. This mediator produces diverse cellular and biochemical responses in a range of different cells and acts trough G-protein coupled receptors (LPA1-5).

Our previous experiments showed a high level of LPA-2 receptor mRNA in hippocampal neurons, LPA-1 and LPA-4 mRNA was very low whereas LPA-3 and LPA-5 stayed undetected. In our recent study we could show that the extra cellular LPA level regulates receptor-dependent excitatory transmission. This going specifically through the LPA-2 receptor, demonstrated by using mice that lack LPA-2 receptor. The signaling cascade analysis were done by pharmacological tests and showed that LPA-induces this effect on differentiated hippocampal neurons by coupling of G_i-proteins. Following activation of inositol (1,4,5) trisphosphate pathway leads to Ca^{2+} release from intracellular stores. This in turn triggers calcium influx through the P/Q-type channels, which are expressed at the presynaptic membrane. Electrophysiological measurements shows that this moderate Ca^{2+} increase alters the basal release probability and the rate of vesicle cycling specifically on glutamatergic synapsis.

We could show in our experimental results, that LPA, apart from the already known effect on the clathrin-mediated vesicle cycle, also plays a novel role in the regulation of glutamatergic transmission in the clathrin-independent vesicle cycle.

Quantitative and qualitative modulation of synapsins by SAP47 in *Drosophila*

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SAP47 (synapse associated protein of 47 kDa) and synapsins are highly conserved synaptic vesicle associated proteins in *Drosophila melanogaster*. Analysis in vertebrates indicates that synapsins are phosphoproteins that bind synaptic vesicles to the cytoskeletal mesh in a phosphorylation-dependent manner, whereas no information on the molecular function of SAP47 or its homologues in invertebrates or vertebrates has been reported so far. In *Drosophila* it is observed that Syn^{97} and $Sap47^{156}$ null mutants have defects in learning and memory (Chen et al., this volume; Saumweber et al., this volume) but have no clear defects in viability or fertility. We now report that western blots of brain homogenates from $Sap47^{156}$ null mutants show an additional synapsin signal in comparison to wild type (CS). However, if the homogenates are treated with phosphatase the additional signal is lost. Quantification of synapsin and SAP47 protein content by ELISA in Syn^{97} and $Sap47^{156}$ null mutants and in CS flies reveals that the $Sap47^{156}$ null mutant flies show a 2.7 fold increase in synapsin content compared to CS flies. Thus the results suggest a qualitative and quantitative modulation of synapsin by SAP47. The possibility of a direct molecular interaction between SAP47 and synapsin is currently being investigated by co-immunoprecipitation (Co-IP) experiments. To investigate differences in expression and/or localization of synapsins in SAP47 null mutants or SAP47 in synapsin null mutants, cryosections of adult heads of Syn^{97} and $Sap47^{156}$ null mutants were stained with monoclonal antibody nc46 (anti-SAP47) and 3C11 (anti-synapsin), respectively, but no obvious differences in staining patterns were observed. The possibility of SAP47 as a modifier of synapsin at the genetic level is being investigated using homozygous double mutants of SAP47 and synapsin. The Syn^{97};$Sap47^{156}$ double mutants are viable but have a reduced life span, decreased negative geotaxis and walking activity when compared to CS. Behavioral read-outs like optomotor responses, learning and memory, and ethanol tolerance are currently being used to characterize the various defects in Syn^{97} and $Sap47^{156}$ null mutants and the double mutants. Also, phosphorylation of synapsins in *Drosophila* is presently being analyzed by expression of *in-vitro* mutagenized transgenes and immunoprecipitation followed by 2-D gel electrophoresis and mass spectrometry.

NCAM ubiquitination is mediated by the SCF ubiquitin ligase HOS (betaTrCP2)

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The neural cell adhesion molecule NCAM plays an important role during neural development and in the adult brain. Recently we showed that NCAM is endocytosed and recycled in different cell systems. To study the regulation of NCAM trafficking we investigated the ubiquitination of NCAM. We found that NCAM is mono-ubiquitinated in response to endocytosis induction. As a responsible ubiquitin ligase that mediates the attachment of ubiquitin to target proteins we identified HOS (beta TrCP2). HOS is a member of the SCF (Skp1-Cullin1-F-box-Roc1) family of ubiquitin ligases which in general recognizes phosphorylated substrates. Furthermore, overexpression of HOS resulted in significantly increased endocytosis of NCAM in different cell systems whereas a dominant-negative form of HOS had no effect on NCAM endocytosis. Therefore, we identified NCAM as a new substrate for the ubiquitin ligase HOS.

Analysis of the secretory apparatus of Trichoplax adhaerens

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The nervous system has constantly increased its complexity and differentiation throughout metazoan evolution. A major transformation of the nervous system occurred during the rise of vertebrates, as the gene repertoire increased due to genome duplications. Still, the basic gene repertoire of lower metazoans such as the cnidarian *Nematostella vectensis* is quite similar to that of vertebrates. This also applies for proteins known to be involved in the fast Ca^{2+} regulated release of transmitter molecules from neurons.
To study the evolution of the molecular machinery of the secretory apparatus we chose the animal *Trichoplax adhaerens*, a species of the phylum placozoa. It is clearly one of the most basal metazoans as it lacks symmetry, basal lamina, organs, muscle cells and a nervous system. Despite the fact that no distinct nerve cells have been described, we have identified several proteins thought to be involved in regulated secretion, e.g. SNARE proteins, in the *Trichoplax* genome.
To identify the cells containing these secretory proteins, we first investigated the so far poorly understood morphology of *Trichoplax* through electron microscopy. We used the new techniques of high-pressure freezing, freeze-substitution and low temperature embedding to prepare the animals. We found differences to the schematic rendering published in 1972 by Grell (Zoomorphology, 73(4):297-314). Our analysis suggests that the cavity between the upper and lower epithelia does not exist. Also the degenerating cells between the so-called cylinder cells and polynuclear fiber cells could not be found.
To further identify specific cells or compartments with secretory functions, we are currently performing immunostainings for secretory SNARE proteins. We believe that such cells could be the ancestors of nerve cells found in all other metazoans.

Towards a Mechanistic Model of Signaling Events Underlying Inhibitory Synapse Assembly

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GABAergic and glycinergic synapses, mediating fast inhibition in the CNS, balance the activity of neural circuits. At the inhibitory postsynapse, GABA and glycine receptors accumulate around a scaffold composed of the protein Gephyrin. Gephyrin is recruited to sites apposed to GABA or glycine releasing terminals through interaction with Neuroligin 2, a member of the Neuroligin family of synaptic adhesion molecules. Collybistin, a member of the Dbl family of GDP/GTP exchange factors, regulates this recruitment process, functioning as a switch that is activated at sites of Neuroligin 2 accumulation, leading to the tethering of the Gephyrin scaffold to the postsynaptic membrane and allowing the subsequent clustering of receptors at sites of transmitter release. The critical role of Collybistin in the development of synaptic inhibition is well documented from studies on mutant mice and a pathological mutation associated with hyperekplexia and epilepsy in humans. Nonetheless, the mechanistic events involving Collybistin and its homologues that underlie the formation of the inhibitory postsynapse are largely unknown. In this study we performed a structure-function analysis on Collybistin, which led to the proposal of two crucial molecular mechanisms; a conformational switch induced by protein-protein interactions and membrane targeting via protein-lipid interactions. Further validation of these data will allow the formulation of a coherent molecular model of the assembly of inhibitory synapses.

Presynaptic mitochondria modulate the functional properties of individual Schaffer collateral boutons

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The properties of evoked transmitter release from the presynaptic sites of a single neuron can be very heterogeneous but the underlying mechanisms are only poorly understood. Synaptic boutons vary in size, shape, and content of subcellular structures. One example is the differential distribution of mitochondria in hippocampal boutons: In *stratum radiatum* only a subset of glutamatergic Schaffer collateral (SC) boutons is occupied by mitochondria. Next to their role in generating ATP, mitochondria are known to be capable of accumulating as well as releasing Ca^{2+}. Mitochondria are actively transported along axons; since both ATP and Ca^{2+} strongly modulate neurotransmitter release, selective localization of this organelle could therefore present a novel mechanism of presynaptic plasticity. However, conventional pharmacological and genetic manipulations can only globally modulate mitochondrial function or axonal localization. Up to now it therefore is unclear if mitochondrial presence directly modulates neurotransmission at these synapses in a bouton autonomous way.

Here we use two-photon imaging to monitor evoked Ca^{2+} changes and vesicle cycling in distal boutons of CA3 pyramidal neurons in cultured hippocampal brain slices. We assess functional presynaptic properties with respect to structural features like mitochondrial occupation, bouton volume, and total amount of synaptic vesicles. To this end, we image CA3 neurons selectively transfected with mitochondrial markers in combination with genetic indicators of vesicle cycling and cytosolic as well as mitochondrial Ca^{2+} concentration. Electrical stimulation of transfected neurons enables us then to asses the functional properties of a large number of individual boutons and presynaptic mitochondria of single identified neurons.

We found that stimulation with trains of action potentials (AP) evokes highly heterogeneous Ca^{2+} responses in SC boutons of individual axons. This heterogeneity could partly be explained by differential docking of mitochondria to SC boutons: Varicosities with mitochondria showed prominently depressed stimulus-evoked Ca^{2+} changes that became evident early during stimulation. Pharmacological inhibition of mitochondrial function increased the evoked Ca^{2+} responses exclusively at boutons containing mitochondria, suggesting bouton-autonomous regulation of Ca^{2+} by mitochondrial presence. To assess if presynaptic mitochondria take up Ca^{2+} during AP firing, we designed a new genetic indicator of mitochondrial Ca^{2+} (mito-GCaMP2) suitable for functional two-photon imaging of presynaptic mitochondria. We found that Ca^{2+} uptake in putatively individual presynaptic SC mitochondria is surprisingly fast. On average, mitochondria docked to SC boutons took up clearly detectable amounts of Ca^{2+} during the first 4 APs of train stimulation at 30Hz.

Genetically encoded reporters of presynaptic activity exploit the pH change that is associated with the release of synaptic vesicles (SV) and report it as a change in fluorescence intensity. While these probes have been successfully applied *in vitro*, they are of limited use in intact tissue where the detected fluorescence intensity is strongly dependent on the depth of the synapse. To assess parameters of SV cycling with respect to mitochondrial occupation *in situ* we therefore constructed the ratiometric dual color indicator Ratio-pHluorin (synaptophysin-pHluorin-tdimer2). Combined with a photoactivatable mitochondrial marker (mitoPaGFP) we were able to show that the fraction of vesicles released at SC boutons during a train of 200 APs was highly variable (10 - 100%) in boutons without mitochondria, but was limited to 10-50% if mitochondria were present.

In summary, we show that mitochondrial presence is likely to change the transmission characteristics of individual SC boutons. We speculate that under physiological conditions, the calcium buffering capacity of mitochondria prevents exhaustion of the SV pool during periods of intense presynaptic activity.

Is CK2ß-deficiency in muscle fibers the cause for mitochondrial myopathies ?

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Motoneurons release the heparansulfate proteoglycan agrin and thereby activate the musclespecific receptor tyrosine kinase (MuSK), which is the main organizer of subsynaptic specializations at the neuromuscular junction. Recently, we showed that (1) the protein kinase CK2 interacts with the intracellular region of MuSK; (2) the CK2 protein is enriched and co-localized with MuSK at postsynaptic specializations; (3) CK2-mediated phosphorylation of serine residues within a specific MuSK epitope, named the kinase insert, regulates acetylcholine receptor (AChR) clustering; (4) muscle-specific CK2ß knockout mice develop a myasthenic phenotype due to impaired muscle endplate structure and function (see Genes Dev 20(13):1800–1816, 2006). Here, we investigated if CK2ß-deficiency in muscle fibers causes additionally myopathies. To this end, we measured transcript amounts of the subunits CK2a and CK2ß and determined holoenzyme CK2 activity in 34 muscle biopsies of human patients with different muscle pathologies. These data suggest that CK2ß-deficiency in muscle fibers might be the cause for human mitochondrial myopathies or LGMDs. In order to understand how CK2ß-deficiency causes myopathies, we characterized murine CK2ß-deficient muscles by histological, histochemical, and functional analysis. These data point to an impaired oxidative metabolism in CK2ß-deficient muscle fibers.

Fenestration of the calyx of Held during development occurs sequentially along the tonotopic axis and is influenced by afferent activity

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The calyx of Held is a type of giant presynaptic terminal in the medial nucleus of the trapezoid body (MNTB) in the mammalian brainstem, which specializes in the processing of auditory information with very high temporal fidelity. This high fidelity is achieved through a number of specializations, many of which are only established during the first 2-3 postnatal weeks. One of these maturation processes is the fenestration of the presynaptic terminal, the calyx of Held, which converts from a cup-shaped morphology just before the onset of hearing into a highly fenestrated morphology typical for the mature synapse. The higher degree of fenestration provides additional diffusional exits for neurotransmitter out of the synaptic cleft, with the result that synaptic currents decay faster and with higher temporal precision.

We investigated the fenestration process in Mongolian gerbils (*Meriones unguiculatus*) by comparing calyx morphology at several ages during early postnatal development. Calyces were labeled with a fluorescent tracer injected into brainstem explants and individually reconstructed in three dimensions from confocal image stacks. At P10, two days before hearing onset, virtually all calyces were completely cup-shaped and unfenestrated. However, at P14, two days after hearing onset, the fenestration process was in full progress, with some synapses being further developed than others. More importantly, at this age synapses located in the medial part of the MNTB showed a higher degree of fenestration than synapses located in the lateral part of the MNTB in the same sections. This finding suggests that there is a developmental gradient of calyx morphology along the tonotopic axis of the MNTB, which runs mediolateral as well, i.e. neurons in the medial part of the MNTB process high frequency sound information, while neurons in the lateral part process low-frequency sound information.

Removal of afferent nerve activity before the onset of hearing prevents the establishment of the developmental gradient and thus afferent activity appears to play a substantial role in early postnatal development of the calyx of Held.

We furthermore investigated possible differences in transmitter (glutamate) clearance capability along the mentioned morphological gradient. We tested whether clearance at P14 is accomplished faster at more highly fenestrated calyces, located medially within the MNTB, compared to rather closed calyces located in the lateral part of the MNTB.

Immunohistochemical stainings suggest that glial processes containing the glutamate transporters GLAST and GLT1 occupy the newly created windows in the calyx and thus augment the fast reuptake of neurotransmitter. Physiological consequences of this faster clearance include a faster decay time course of synaptic currents as well as a lower amount of residual current accumulating during the processing of repeated activity such as high frequency stimulus trains.

Small GTPases Alter Synaptic Plasticity and Function

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At synapses, neuronal cells communicate with their target cells and transduce presynaptic signals into the release of neurotransmitters. To meet the developmental and functional needs of accurate neurotransmission, synapses are extensively remodeled (synaptic plasticity); malfunctioning neurotransmitter release and impaired synaptic plasticity cause learning disabilities and neurological disorders. The goal of this study is to understand the intracellular membrane trafficking routes within the synapse. Each particular membrane trafficking step is controlled by a small GTPase of the rab family. At least 60 human rabs have been identified and Zhang et al. (Genetics, 2007) found 33 *Drosophila* homologs. For non-neuronal cells it is well known how vesicles with a variety of cargos pass endosomal intermediates and which small rab GTPases guide their routes. For axonal terminals and neurotransmitter-containing vesicles the scenario is different and much less is described; neurons are highly specialized cells and long axons separate their presynaptic terminals from the cell body where the endoplasmatic reticulum and the Golgi are located. Thus, SVs refill and recycle locally without passing the endosomal route of their cell bodies. However, recent studies show that a marker protein for the early endosomal intermediate, the small GTPase rab5, is present at the axonal terminal suggesting that at least a subset of SVs fuses with the early endosome. Synaptic endosomes may further function in sorting and recycling of additional synaptic components and synaptic differentiation. We address whether further endosomal intermediates localize to the synapse and participate in SV recycling and use small rab GTPases as endosomal marker proteins. The neuromuscular junction of *Drosophila* larvae serves as a glutamatergic model synapse. Within this system, we express genetically modified rab GTPases neuronally. We prove with immunohistochemical and functional methods whether an altered function of rabs changes synaptic growth and neurotransmission. Our preliminary results implicate that a subgroup of rab GTPases which to date had been only described as endosomal components localizes to the axonal terminal. Our functional studies suggest that certain rab GTPases and possibly also endosomal intermediates are crucial for proper synaptic transmission. Additional biochemical investigations will reveal how rab GTPases may control synaptic membrane transport. Further *in vivo* imaging studies will show whether synaptic vesicles indeed recycle through synaptic endosomal compartments.

An RNAi knockdown approach to elucidate the role of Mover – a vertebrate-specific presynaptic protein specifically associated with synaptic vesicles

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Neurotransmitter release relies on a highly organized cycle of regulated exocytosis, endocytosis and intracellular trafficking of synaptic vesicles. This synaptic vesicle life cycle is controlled by a) cytosolic and cytoskeletal proteins, b) a set of cytomatrix proteins localized at sites of neurotransmitter release, and c) synaptic vesicle associated proteins. In a previous study, we identified Mover (also known as SVAP30/tprgl) as a vertebrate-specific presynaptic protein and putative binding partner of the presynaptic cytomatrix protein Bassoon. Here, we set out to further characterise Mover using biochemical and immunocytochemical approaches on brain tissue as well as RNAi knock down in cultured hippocampal neurons.

Membrane floatation experiments and immunoisolation of synaptic vesicles using Mover specific antibodies revealed that Mover is a membrane-associated protein. Mover is specifically associated with synaptic vesicles, and Mover antibodies can be used to immunoisolate synaptic vesicles. These results are consistent with mass spectrometry analyses performed by Burre *et al. 2006,* in which Mover/SVAP30 peptides were detected in immunoisolated synaptic vesicles. Immunhistochemical analysis of various CNS regions show the enrichment of Mover in different types of inhibitory and excitatory presynaptic terminals while Mover is virtually absent from inhibitory terminals in the CA3 region of the hippocampus. We utilised lentiviral-based shRNA interference technology to specifically silence Mover expression in hippocampal cultures. Mover-depleted neurons show no obvious morphological differences compared to control cells. Analysis of alterations in the synaptic vesicle cycle using FM-dyes shows that neither the reduction nor the overexpression of Mover results in changes in the total recycling pool size or FM-dye release kinetics. These experiments suggest that Mover is not involved in basic synaptic transmission – a process that is evolutionary conserved from yeast to mammals. As a vertebrate-specific protein, Mover might be involved in vertebrate-specific features of synaptic function.

Spatial range of GABAergic synaptic plasticity in hippocampal slice cultures

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Plasticity mechanisms such as LTP and LTD are thought to endow neural networks with the capacity to store and process information. To ensure normal network function a certain operating range of neural activity has to be maintained by means of homeostatic plasticity mechanisms. The balance between excitation and inhibition in a network is crucial for information processing and stability. Previous studies have suggested that excitation and inhibition are regulated interdependently. Here we ask at what spatial scale this regulation occurs (i.e. between groups of neurons or even within single dendrites?).

Although several forms of plasticity are known for glutamatergic synapses, the extent of GABAergic plasticity remains obscure. Using hippocampal slice cultures from GAD65 mice, our group has recently shown that GABAergic axons are capable of forming new synaptic contacts at a timescale of several hours. These contacts occur through formation of new GABAergic boutons at pre-existing axon-dendrite crossings.

We started by examining whether GABAergic bouton turnover and density is affected by neural activity. Activity in hippocampal slice cultures was blocked by bath application of TTX (Na^+ channel blocker) or enhanced through bicuculline ($GABA_A$ receptor antagonist). GFP-expressing GABAergic axons were imaged with a two-photon microscope for four hours at 30 min intervals, and analyzed for bouton turnover and density. Boutons were grouped into the categories stable, new, lost, and transient for analysis purposes. Activity manipulations that had started just before the imaging period (“acute”) increased the proportion of stable boutons at the expense of transient boutons, both in TTX and bicuculline treated cultures. Longer treatments (48 hours prior to imaging, “chronic”) with either TTX or bicuculline, however, did not affect bouton turnover or density. This indicates that GABAergic bouton turnover is affected by activity within several hours. Although TTX and bicuculline manipulate network activity in opposite directions, they had similar effects on bouton turnover. We are currently investigating possible explanations (e.g. GABA receptor signaling).

We intend to examine the spatial range of the observed GABAergic plasticity. Currently, we are reducing the area of activity manipulation using local superfusion. In the future we plan to use viral infection of single neurons with light gated ion channels, and/or local glutamate uncaging to test activity-dependent GABAergic plasticity on even smaller scales.

TRPV1 stimulation suppresses LTP in mice lateral amygdala

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The amygdala is an almond-shaped structure in the medial temporal lobe. As part of the limbic system, the amygdala plays a key role in emotionality, the emotional evaluation of sensory stimuli, emotional learning and memory, as well as affective disorders, including anxiety and depression. The amygdala has been shown to exhibit a high degree of plasticity in various models of long-term synaptic and behavioural modification, including long-term potentiation (LTP). Pain has a strong emotional component and persistant pain is significantly associated with depression and anxiety disorders. Whereas a key role of the central nucleus of the amygdala has been established in integration of nociceptive information, the concept of the lateral nucleus (LA) as an important contributor to pain and its emotional component is still emerging.

The transient receptor potential vanilloid 1 (TRPV1) is a calcium-permeable cation channel responsible for binding capsaicin, the pungent chemical found in chilli peppers. In the peripheral nervous system, TRPV1 is well recognized as a polymodal signal detector that is activated by painful stimuli.

In this study we show for the first time that capsaicin, an agonist of TRPV1 dose-dependently suppresses LTP in the lateral nucleus of the amygdala. In adult male mice brain slices, plasticity changes in the LA were induced by high frequency stimulation of external capsule fibers. Basal transmission was not affected by capsaicin (1 – 10 µM). The effect of capsaicin on the magnitude of LTP could be blocked by the specific antagonist capsazepine. L-NAME, an unspecific inhibitor of nitric oxid synthetase (NOS) blocked the inhibitory effect of capsaicin on LA-LTP. The capsaicin-induced effect on LA-LTP was also missed in nNOS deficient mice. We suggest that the TRPV1 receptor may be involved in pain processing in higher brain structures, such as the amygdala. Our results also suggest that TRPV1 proteins in the amygdala (and putative endogenous vanilloids as the endocannabinoid anandamide) may be involved in the amygdala control of learning mechanisms and affective disorders.

Glutamate receptor mobility is linked to learning and is dependent on n-cofilin mediated actin filament dynamics

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Neuronal plasticity is the key process involved in learning, memory and modulation of complex behaviour. Dynamic changes in the postsynaptic compartment, mainly the morphology of dendritic spines, are thought to be the basis of plasticity and rapid remodelling of the actin cytoskeleton was shown to be essential for these changes. Here we demonstrate that the actin filament depolymerizing protein n-cofilin links synaptic actin filament dynamics to postsynaptic physiology through a novel mechanism. We show that n-cofilin modifies connectivity of neurons as well as the strength of postsynaptic transmission by controlling the diffusion of extrasynaptic excitatory AMPA receptors. N-cofilin mutant mice exhibit specific deficits in learning, while short term memory and presynaptic activities are preserved. These findings demonstrate *in vivo* the requirement and specificity of actin filament dynamics in synaptic receptor recruitment and learning.

Protein Synthesis and Degradation Regulate Activity-dependent Presynaptic Structural Plasticity

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Activity-dependent plasticity of synaptic structures has been intensely studied in recent years. While de novo protein synthesis is known to be important for synaptic plasticity, little is known about the role of protein synthesis and degradation for the structural plasticity that accompanies activity-dependent changes in synaptic strength. Here, we set out to investigate whether and how protein synthesis and degradation regulate the activity-dependent dynamics of presynaptic boutons, whose turnover is significantly increased by the induction of long-term depression (LTD) by low-frequency stimulation (LFS), as we have recently demonstrated.

We combined two-photon time lapse imaging with electrophysiological recordings in organotypic hippocampal slice cultures, allowing us to study LTD-associated changes in presynaptic structures. We report that pharmacologically blocking either protein synthesis or proteasome-dependent protein degradation abolishes the increase in turnover of presynaptic boutons caused by LTD induction, while leaving the turnover during unstimulated conditions unaffected. Our data suggest that protein synthesis and degradation are specifically required for activity-driven changes in hippocampal synaptic circuitry.

Transient metabolic failure induced by short-term hypoxia results in a reversible suppression of memory consolidation-related hippocampal network oscillations in vitro

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Hippocampal sharp wave-ripple complexes (SPW-Rs) have been hypothesized to play a pivotal role in the process of memory consolidation in vivo. As previously shown, the induction of hippocampal SPW-Rs in vitro is closely associated to the induction of long-term potentiation (LTP) and a reorganization of the neuronal network within the CA3 region. Here, we used the in vitro paradigm of SPW-R network oscillations both in the area CA3 and CA1 of the adult rat hippocampus to test for the effects of transient epochs of hypoxia on stimulus-induced SPW-Rs. We found that such network oscillations were suddenly and reversibly suppressed by transient hypoxic epochs, which lasted for either three or six minutes. During recovery we observed that the expression of SPW-Rs was fully reversible in area CA3 while a partial recovery of SPW-R activity was observed in the CA1 region particularly when hypoxic epochs were repeatedly applied. Notably, we found that during early phases of hypoxia, SPW-Rs were suddenly suppressed after less than 2 minutes during 20 percent oxygenation before any changes in the membrane properties could be detected in simultaneously recorded CA3 pyramidal cells. In addition, we show that the hypoxia-mediated suppression of SPW-Rs could be mimicked by the application of rotenone (1 µM). Due to the hypothesis that for the long-term storage of information, hippocampal SPW-Rs are required during the transfer of information into the neocortex, and based on our recent results, we suggest that a transient hypoxia-induced metabolic failure within hippocampal networks results in a reversible suppression of SPW-Rs, and thus might be causally involved in the hypoxia-induced amnesia observed in vivo.

Trans-fissural propagation of stimulus-induced gamma network oscillations between the dentate gyrus and area CA1 in the hippocampus of adult rat in vitro

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Within the hippocampal formation slow (~40 Hz) and fast (70-100 Hz) gamma oscillations can be induced. Here, we analyzed stimulus-induced gamma oscillations both in the dentate gyrus (DG) and in area CA1 in slices of adult rat hippocampus. In both areas short trains of high frequency stimulation (HFS) applied at 100 Hz induced 1 – 3 s long periods of synchronized oscillations, which initially were >70 Hz and then subsequently declined to near 30 Hz. We tested for spread of locally induced gamma activity and found the DG induced network oscillations to spread to both areas, CA3 and CA1, while CA1-induced gamma oscillations only spread to DG but not back into the CA3 network. Removal of both area CA3 and the subiculum did not prevent any oscillation spread from DG to area CA1 and vice versa. On the other side, cuts performed along the hippocampal fissure invariably interrupted such a spread of network activity. Further detailed analyses of the observed spread showed that the initial part of high frequency gamma oscillations remained almost restricted to the stimulation site, each while subsequent low frequency gamma epochs propagated from DG to CA1 and vice versa. Intracellular recordings revealed that during early phases, high frequency gamma oscillations were associated with local neuronal firing while during the late phase of oscillatory activity, this was only occasionally the case. Notably, when gamma oscillations were analyzed remote from stimulation site, pronounced membrane potential oscillations (MPOs) were noted in the CA1 region, which rarely resulted in phase synchronous neuronal firing in recorded principal cells. To further determine which neurons were involved in the synchronization between both areas, dextrane amines were injected into the DG resulting in a retrograde staining of neurones within area CA1. Stained neurons showed a distributed axonal arbour as revealed by biocytine stainings sometimes reaching into the hilus. Recordings from such identified neurones showed MPOs in the low gamma frequency band when depolarized to membrane potentials near threshold for action potential (AP) generation. Together, we conclude that input specific activation leads to high frequency gamma oscillations restricted to the site of activation while subsequent, low gamma frequency network oscillations do spread within the hippocampal formation in a short cut manner, orthogonally oriented to the classical trisynaptic excitatory loop. We suggest that interneurons, via bridging the hippocampal fissure, are substantially involved in processing an inter-region synchronization.

A role of testosterone in hippocampal dentate gyrus'synaptic plasticity and spatial learning in male rats

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Effects of adrenal steroids on learning, memory formation and hippocampal synaptic plasticity are well documented. However, modulatory influences of gonadal steroids on these parameters are known but less well investigated. Therefore, our study aimed at asking the question whether the status of gonadal and adrenal hormones changes during a spatial learning paradigm by using the holeboard maze and whether hippocampal synaptic plasticity is affected by these changes. The formation of a reference memory during this task has been shown to modulate long-term potentiation (LTP), a widely accepted cellular model for memory formation. Animals underwent one to three training sessions. Blood and male rat brain tissue samples from the prefrontal cortex (PFC) and the hippocampus (HIPP) were taken at the end of each session. Corticosterone, an adrenal stress hormone, reached higher levels in the last training session in all three tested samples, while estradiol levels in the brain tissues were reduced in the second session. In addition, the concentration of testosterone correlated with behavioural data indicating memory formation.
At the cellular level, the application of 1µg testosterone proprionate icv 30 minutes before induction of early-LTP revealed opposite effects on field excitatory postsynaptic potentials (fEPSP) and population spike amplitudes (PSA). While the PSA tended to decrease faster than controls, the fEPSP stayed highly potentiated over the first 8 hours of the recording period.

We could show a role for the sex hormone testosterone in behavioral performance and in dentate gyrus synaptic and neuronal plasticity, providing a link between effects of gonadal steroids on memory formation at the cellular and the systemic level.

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Hippocampal CA1-LTP and reinforcement of an early-LTP by stimulation of the ventral tegmental area in freely moving rats

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It has been shown that the prolonged maintenance of hippocampal long-term potentiation (LTP) requires heterosynaptic events taking place during its induction. Recent studies have shown that a transient early-LTP in the dentate gyrus can be reinforced into a long-lasting LTP when modulatory brain structures were stimulated within a distinct time window around its induction. Such a reinforced LTP in the dentate gyrus is dependent on heterosynaptic function - like activation of ß-adrenergic and/or muscarinergic receptors - and protein synthesis.

The aim of our studies is to investigate now, whether there are similar mechanisms of early-LTP reinforcement in the hippocampal CA1 region. Recent data from our laboratory have shown that an electrically induced late-LTP in CA1 of freely moving rats is dependent on protein synthesis. Comparing these findings with results in vitro, which showed the requirement of heterosynaptic mechanisms in maintaining late-LTP in the CA1, we suggested that long-lasting forms of LTP in both hippocampal regions could share similar properties. The concrete question arose whether a transient form of LTP in CA1 can be reinforced by activation of heterosynaptic events such as by direct electrical stimulation of modulatory brain structures carrying other transmitters than glutamate. One of these modulatory inputs to the CA1 is the ventral tegmental area (VTA), a heterogeneous group of dopaminergic cells and a major component of the mesolimbic dopamine system.

To study the role of VTA-activation on early-LTP in the CA1 we implanted chronically electrodes into the contralateral CA3 for stimulation and a bipolar recording electrode into the CA1 to record simultaneously both, the field-EPSP and the population spike in their loci of generation in freely moving rats. This method is now well established in our laboratory. In addition to the stimulation electrode in the contralateral hippocampus, a second stimulation electrode was implanted into the ipsilateral VTA. We could shown, that high-frequency stimulation of the VTA 15 min after induction of an early-LTP in CA1 reinforced this normally transient form of LTP into a prolonged LTP. It is of interest, whether this reinforced LTP is indeed dependent on dopaminergic transmission and whether it requires protein synthesis dependent, thus resembling late-LTP.

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Depotentiation of early-LTP in the hippocampal CA1 region in freely moving rats by mild swim stress

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Stress is a well-documented and potent modulator of learning and memory. The timing and nature of the stressful event on a particular memory phase is thought to be crucial and predictive for the outcome of the interaction of stress with memory function. Long-term potentiation (LTP), a widely accepted cellular model of memory formation, can be affected by a brief episode of swim stress. Thus, a protein synthesis transient early-LTP can be reinforced into a protein synthesis-dependent late-LTP in the dentate gyrus by swim stress, when applied 15 min after tetanization (Korz and Frey, 2003). Taking into account the region specificity in the processing and encoding of distinct spatial and temporal information within hippocampus it was now interesting to study the potential role of the CA1 region. Electrophysiological recordings of field potentials in freely moving rats were used in the present study to investigate the effect of the same stressful event on the course of early-LTP in CA1 region. We found that swim stress alone resulted in a short (less than 2h) depression of baseline values. In contrast to the LTP-reinforcing effect of swim stress in the dentate gyrus, here, swim stress depotentiated an electrically induced early-LTP if the swim protocol was given within a short time window of up to 1 hour after tetanization. Thus, the effect of the stress paradigm on hippocampal functional plasticity events appeared to be subregion-specific.

Short-term plasticity at the embryonic *Drosophila* neuromuscular junction

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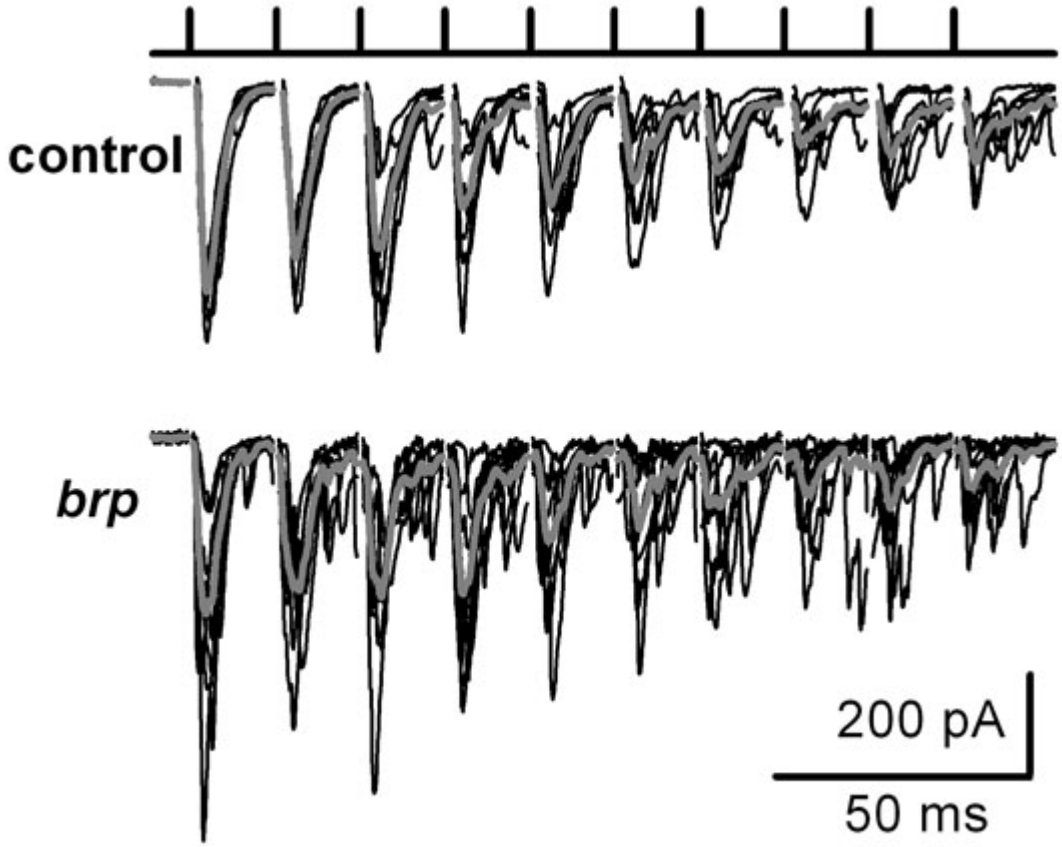
The function of the active zone (AZ) critically determines the characteristics of short-term plasticity (STP) and in turn the computational functions of neural circuits. However, the function and the development of AZs are poorly understood. The *Drosophila* AZ-protein Bruchpilot (BRP), whose N-terminal half encodes the *Drosophila* CAST homologue, is required for establishing proximity between Ca^{2+} channels and vesicles to allow efficient transmitter release at the glutamatergic larval *Drosophila* neuromuscular junction (NMJ; Wagh et al., 2006; Kittel et al., 2006). Here, the function of BRP was investigated at NMJs of morphologically staged (visibly mature) late *Drosophila* embryos. Based on immunohistochemical stainings with the monoclonal anti-body Nc82 against BRP in combination with anti-HRP nerve stainings and differential interference contrast images the number of Nc82 labelled AZ on the ventral longitudinal muscle 6 (VLM6) was estimate to be 17 ± 6 (n = 7, mean ± SD) in control animals. As expected, in *brp* mutants no Nc82-label was detectable. To functionally characterize BRP in embryonic NMJs, whole-cell patch clamp recordings were made from VLM6. The capacitance of the VLM6 was not significantly different in *brp* mutants (16.6 ± 4.1 pF, n = 14) and control animals (14.7 ± 6.1 pF, n = 15). Stimulation of the corresponding segmental nerve at a frequency of 0.2 Hz evoked similar EPSC peak amplitudes in *brp* mutants and control animals. Trains of 10 stimuli at 60 Hz induced synaptic depression to an on average similar degree in *brp* mutants and control animals (n = 5 each, see two illustrated experiments, consecutive trials in black, corresponding average in grey, stimulation artefacts removed). Thus, in contrast to the reduced EPSC amplitude and increased degree of facilitation found in *brp* mutant larvae, a weaker phenotype was found in *brp* mutant embryos. In conclusion, these data indicate a differential function of BRP during synaptic maturation.


control
brp
200 pA
50 ms

Role of the actin network for synaptic tagging and late-LTP in hippocampal CA1 neurons

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Long-term potentiation (LTP) and long-term depression (LTD) are cellular models underlying learning and memory formation. Like memory formation LTP and LTD can be separated into a protein synthesis-independent phase (early -LTP/-LTD) and a protein synthesis-dependent late phase (late-LTP/-LTD). Both, late-LTP and late-LTD in the CA1 region of the rat hippocampal slice in vitro are characterized by heterosynaptic requirements during induction as well as by processes of synaptic tagging. The latter describes how early-LTP can be transformed into late-LTP (Frey & Morris, 1997). Here, we present data investigating the role of the actin network during LTP and specifically, its role for synaptic tagging by using structurally different actin polymerization inhibitors, such as latrunculin A and cytochalasin D. Our results suggest that the polymerization of the actin network is important for the maintenance of late-LTP. In addition to maintaining late-LTP, the polymerization of actin network is required for processes of synaptic tagging. The actin assembly inhibitors prevented the reinforcement of early-LTP into a late-LTP in a synaptic input S2 even if late-LTP was induced in an independent synaptic input S1. Under normal conditions (without drug application) late-LTP in S1 would have been caused the transformation of early- into late-LTP in S2. We suggest that actin is involved in mediating the setting of the synaptic tag machinery, which is required to capture plasticity-related proteins being essential for the maintenance of LTP.

Dopaminergic blockade within the nucleus accumbens core impairs hippocampal dentate gyrus long-term potentiation and spatial learning

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Hippocampal long-term potentiation (LTP) is a long-lasting increase in synaptic efficacy after brief high-frequency stimulation of afferent fibers and is considered to be a cellular model of learning and memory. It has been shown that stimulation of other brain regions like the prefrontal cortex (PFC), ventral tegmental area (VTA), locus coeruleus (LC) or basolateral amygdala (BLA) can influence or modulate hippocampal LTP; however, less is known of the role of the nucleus accumbens (NAc). The NAc is the central component of the basal ganglia that is positioned to integrate signals arising from limbic and cortical areas and to modulate goal-directed behavior, reward mechanisms and learning. Major afferents to the NAc include prefrontal association cortices, BLA and dopaminergic neurons located in the VTA, which are connected via the mesolimbic pathway. Electrical stimulation of the NAc core and shell region has been shown to modulate LTP in the dentate gyrus. This study investigates the effects of D1/D5 dopamine receptor blockade in NAc core on LTP and modulation of holeboard learning in the dentate gyrus. Treated animals show a depression in baseline transmission and reduced LTP after weak tetanic stimulation of the perforant path compared to controls. Similarly, also memory consolidation during a spatial learning experiments was impaired in animals which were treated with D1/D5-receptor blockers within the NAc. . Because, there is no direct dopaminergic connection between NAc and dentate gyrus, the effects must be based on indirect mechanisms, most likely also involving glutamatergic and GABAergic pathways.

Demonstration of mammalian ependymin-related proteins (MERPs) in the cerebellum, hippocampus and neocortical areas of the adult mouse brain by in situ-hybridisation and immunohistochemical staining.

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Brain specific fish ependymins are functionally involved in CNS regeneration and exhibit increased transcription and translation during memory consolidation. These proteins are synthesized in and secreted from meningeal fibroblasts; they are redistributed via the CSF and in part recovered at synaptic membranes of those pyramidal cells (type I neurones) that are involved in the acquisition of the learning paradigm. Cloning of mammalian genomes revealed one primate and two murine genes, respectively, coding for mammalian ependymin-related proteins (MERPs) and exhibiting around 30% homology to the fish ependymins.

By Northern blotting *MERPs* have been demonstrated in various tissues, in particular in neoplastic cells of the intestine and in several brain regions (Apostolopoulos et al. 2001). We have shown by reverse transcriptase PCR that *MERP1*- and *MERP2*-mRNAs increase in the mouse brain during postnatal development (day 1 to 20). Furthermore, expression of *MERP2*-mRNA was enhanced after a spatial learning task, i.e. training to find a hidden platform in a Morris water maze (Schneider and Schmidt 2006).

Here, we analysed the expression of *MERP2* by in situ-hybridisation of brain sections from adult, male C57/Bl6J mice using ^{35}S-a-dATP end-labelling of anti-sense probes. Signals were low in the brain stem and in fibre tracts and intermediate in the thalamus and most neocortical areas. High concentrations of *MERP2*-mRNA were measured in the meninx, in the cellular layer of the cerebellum and in the hippocampus (CA1 - CA3). Particularly strong labelling was detected in the dentate gyrus and the cerebellum. Furthermore, hippocampal neurones derived from neonatal mice and a neuroblastoma cell line (NS20Y) expressed *MERP1*- and *MERP2*-mRNA in culture.

A similar distribution of the MERP proteins was inferred from immunohistochemical staining using antibodies raised against fish ependymins. Fluorescence microscopy revealed labelling of granule cells of the dentate gyrus and at somata of pyramidal cells throughout regions CA1 - CA3, in particular at the axon hillock in addition to signals at ependymal cells and epithelial cells of the chorioid plexus.

Apostolopoulos, J., Sparrow, R.L., McLeod, J.L., Collier, F.M., Darcy, P.K., Slater, H.R., Ngu, C., Gregorio-King, C.C., and Kirkland, M.A. (2001): Identification and characterization of a novel family of mammalian ependymin-related proteins (MERPs) in hematopoietic, nonhematopoietic, and malignant tissues. *DNA Cell Biol.,* 20, 625-635.

Schneider, S., and Schmidt, R. (2006): Immunohistochemical localisation of mammalian ependymin-related proteins (MERPs) in the postnatally developing mouse brain and investigation of a possible involvement in memory formation. *Proceed. 5th Forum Europ. Neurosci.,* p. 325.

The role of BDNF during lesion induced facilitation of LTP in the visual cortex

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Brain derived neurotrophic factor (BDNF) promotes the formation, maturation and stabilization of synapses in the central nervous system. BDNF has been shown to mediate processes of synaptic long-term potentiation (LTP) in several regions of the brain. Under pathophysiological conditions several studies have disclosed elevated levels of BDNF in the vicinity of the injury. However, the function of the BDNF protein during brain injury and recovery is not fully understood. Here we studied a potential contribution of BDNF to injury-induced functional reorganization and plasticity in the visual cortex of mice with chronically reduced levels of BDNF. Heterozygous mice, which partially lack the BDNF coding gene (BDNF +/-), were anaesthetized with chloralhydrate and lidocain at the age of 21 days. The scull was drilled above the cortical surface, and focal laser lesions were induced in vivo in the visual cortex. After a survival time of 1-7 days post-injury the animals were anaesthetized with ether and decapitated. The brains were transferred into ice-cold ACSF and slices of 350µm in diameter were cut by use of a vibratome. As expected, sham-operated BDNF (+/-) mice failed to express any long-term potentiation (LTP) of extracellular field-potentials in cortical layers II/III after electrical stimulation in Layer IV in slices of the visual cortex (n=7). In contrast, a reliable LTP could be induced in slices of lesion treated BDNF (+/-) mice at 2-6 days post-injury. Surprisingly, the level of BDNF, as measured by the expression of m-RNA for BDNF in the vicinity of the lesion, was significantly reduced 24h post-injury in both, transgenic and wild-type animals ($p<0.05$). Further whole-cell patch clamp recordings from pyramidal neurons in layers II/III in slices from BDNF (+/-) mice revealed a lesion induced strong reduction in the strength of basal GABAergic inhibition as shown by the impaired frequency ($p=0.0006$) and amplitude ($p=0.02$) of miniature inhibitory postsynaptic currents (IPSCs, n=12). In contrast, the basal excitatory synaptic transmission shown by recordings of mEPSCs (frequency: $p=0.008$; amplitude: $p=0.08$, n=12) was less affected by the lesion as compared to sham-operated controls (n=10). These data suggest (1.) that the observed lesion-induced rescue of LTP in BDNF (+/-) mice is not mediated through increased levels of BDNF. Instead, our data indicate (2.) that the focal lesion primarily impairs the strength of cortical GABAergic inhibition in BDNF (+/-) mice, while the function of the excitatory synaptic transmission is less affected. This imbalance of synaptic changes might be one key mechanisms to rescue LTP in the visual cortex of BDNF (+/-) mice.

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Influence of Nucleus Accumbens Core or Shell Stimulation on Early Long-term Potentiation in the Dentate Gyrus of freely moving rats

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The nucleus accumbens is an integral part of the ventral striatum and is composed of two regions: core and shell which has been related to reward motivated behaviour. In the last years our group have characterized the influence of several brain structures modulating synaptic plasticity in the dentate gyrus (DG). In a previous study it was shown that the stimulation of the core 15 minutes before stimulating the perforant pathway (PP) blocked the induction of LTP, while the stimulation of the shell facilitated it in anesthetized rats. The objective of the present study was to examine the effects of nucleus accumbens core or shell stimulation on the time course of early long-term potentiation (E-LTP) in the dentate gyrus of freely moving Wistar rats. We stimulated the nucleus accumbens core or shell 15 minutes after stimulation of the granular cells of the dentate gyrus via the PP using a weak tetanus (3x15 impulses at 200 Hz), to induce an E-LTP in the DG. The stimulation of core or shell did not significantly modify neither the amplitude nor the duration of DG-LTP. In a first set of control experiments we investigated if stimulation of the nucleus accumbens core or shell alone -without tetanus to the PP - would have an effect on baseline values after stimulating the DG. The results for these control experiments indicated that stimulation of the nucleus accumbens core or shell had only a slight, and statistically not significant, depressing effect. LTP induction in the DG by a weak tetanus applied at the PP was also not statistically significant influenced by the presence of an electrode either in the core or shell of the accumbens. Histological evaluations were performed after the end of the experiments and the animals with the wrong electrode location in nucleus accumbens as well as dentate gyrus and perforant pathway were discarded. In summary, nucleus accumbens stimulation after the induction of LTP seems to have no effect on the time course and late phases of the potentiation in the DG. Future experiments are planned to further characterize in freely moving animals, the effect pre-tetanus stimulation of the accumbens core or shell.

Expression of new CPEB1 and CPEB2 splice isoforms in hippocampal neurons

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Cytoplasmic polyadenylation element binding proteins (CPEBs) are translational regulators expressed in mouse brain. We have previously shown that all four CPEB family members known were transcribed in the hippocampus. In the present study, we further characterized CPEB1 and CPEB2 expression in the mouse central nervous system. By RT-PCR, we identified 3 splice variants of CPEB1 which differed in the RRM1 motif of the RNA binding domain. Alternative splicing led to a different spacing of conserved residues within RRM1. In electrophoretic mobility shift experiments, all variants showed binding to the CamKIIalpha 3'UTR containing two CPEs. In situ hybridization revealed a differential expression of the splice variants in principal cells of the mouse hippocampus. By RT-PCR of mouse brain cDNA, we found four CPEB2 splice isoforms. Single cell RT-PCR of harvested cytoplasm following electrophysiological characterization of CA1 pyramidal neurons showed a complex pattern of CPEB2 expression, revealing a drop of CPEB2 expression during postnatal development and heterogeneity of splice isoform distribution within individual hippocampal neurons of the same slice preparation. We have raised and characterized antibodies directed to CPEB2 and verified their specificity in astrocytoma cells transfected with various CPEB isoforms. Western blot analyses with affinity-purified antibodies showed expression of CPEB2 protein in cortex, hippocampus and cerebellum. In electrophoretic mobility shift experiments, we found CPEB2 binds to CPEs of the CamKIIalpha 3'UTR similar to CPEB1. Functional studies in cultured cells and transgenic mice are underway.

Neuromodulatory effects of norepinephrine on stimulus-induced sharp wave-ripple complexes (SPW-Rs) in the adult rat hippocampus *in vitro*

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Sharp waves-ripple complexes (SPW-Rs), which have been described in the rat hippocampus *in vivo*, are thought to be substantially involved in learning and memory by mediating the off-line processing and susequent transfer of declarative information from the hippocampus into the cortical mantle in a process of memory consolidation. As previously reported, in hippocampal slices the induction of SPW-Rs is associated to the induction of long-term potentiation (LTP) induced by repeated high frequency stimulation (HFS). Here, we employed the *in vitro* paradigm of hippocampal SPW-Rs to investigate the effects of norepinephrine (NE, 50-100 µM) on both the induction and expression of such network oscillations. We found that in the presence of both 50 and 100 µM of NE the stimulus induction of SPW-Rs was prevented while during subsequent washout, SPW-Rs were generated in the CA3 region and propagated into the area CA1 without any further stimulation. In addition, when NE was applied on established SPW-Rs, such activity was found to be reversibly blocked in both area CA3 and CA1. Interestingly, following washout, we observed a significant increase in both the incidence and amplitude of SPW-Rs. In more detail, we found that the unspecific b adrenoreceptor agonist isoproterenol (2 µM) as well as the b1 agonist dobutamine (100 µM) significantly enhanced SPW-R activity. In contrast, SPW-Rs were significantly reduced by both a1 and a2 adrenoceptor agonists methoxamine (100 µM), phenylephrine (100 µM) and clonidine (100 µM), respectively. Moreover, when the unspecific a adrenoreceptor anatgonist phentolamine (100 µM) was administrated 30 min before NE was then co-applied, we observed that both the SPW-R incidence and amplitude were increased. In addition, the co-application of the more specific a1 antagonist prazosine (100 µM) resulted in a prevention of the previously observed suppression of SPW-Rs altogether suggesting a b adrenoreceptor-mediated effect. Intracellular recordings obtained from both CA3 or CA1 pyramidal cells simultaneously recorded during SPW-R network activity revealed that the blocking effect of NE was accompanied by a pronounced hyperpolarization of about 5-10 mV in the majority of recorded neurons in both regions. Together, our data indicate that hippocampal SPW-R activity is effectively modulated by NE where the suppressive effects are mediated by a adrenoreceptors, and the facilitation of SPW-Rs is b1-mediated. Based on our results that the SPW-R induction was facilitated in presence of isoproterenol, and that dobutamine augmented SPW-R activity, we suggest that the theshold for the induction of LTP and thus that of SPW-Rs within the hippocampus might be modulated by the activation of b1 adrenoreceptors.

Enhanced cortical plasticity of horizontal connections in the vicinity of focal laser lesions in the visual cortex

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Focal injuries in the CNS are accompanied by processes of cortical reorganization that partially compensate the functional loss. One suggested process, which may underlie this functional recovery is an enlargement of receptive field sizes in neurons surrounding the lesion. This increase has been observed for example during in-vivo recordings in a model of focal ibotenic acid lesion in cat visual cortex (Eysel UT , Schweigart G, Mittmann T et al, Restor Neurol Neurosci 15 2 - 3: 153-64, 1999). We postulate that the increase in receptive field size is associated with LTP-like mechanisms, which could be enhanced in the surround of cortical lesions. To test this, we established an ex-vivo, in-vitro model of focal laser lesions in rat visual cortex. By use of this model we have previously shown a significantly enlarged long-term potentiation (LTP) of ascending fibers onto layer II/III neurons in acute slices of lesion treated animals after a survival time of 2 to 6 days. However, so far nothing is known about potential changes in LTP of horizontal connections in the visual cortex post-lesion. Thus, we used the same animal model and performed whole-cell patch-clamp recordings in pyramidal neurons located in layers II/III of the visual cortex from acute slices taken from lesion treated rats and sham-operated control animals. LTP was induced by theta-burst stimulation of horizontal fibers located in layers II/III. Recordings were made in the vicinity of focal laser-lesions and in sham-operated controls up to one week following the laser surgery. The data revealed an increased LTP from inputs of these horizontal connections (sham operated: 121,76 ± 14,35% n= 8, lesion treated: 174,92 ± 19,60% n= 10). To investigate the cellular and molecular mechanisms underlying this lesion-induced metaplasticity, we studied the functional pre- and post-synaptic properties of excitatory synapses. Here we disclosed an increased release probability for glutamate, as shown by the increased success rate of EPSCs in response to a minimal synaptic stimulation (sham operated: 0,4916 ± 0,02 n= 16, lesion treated: 0,58 ± 0,03 n= 19). Furthermore, we recorded a prolonged decay time constant of NMDA receptor mediated EPSCs post lesion (sham operated: 148,94 ± 12,81 n= 10, lesion treated: 178,84 ± 11,90 n= 12), which indicated changes in the postsynaptic subunit composition of NMDA receptors. The inhibitory GABAergic system was also affected by the lesion as shown by a reduction of mIPSCs both in amplitude and frequency.

These data suggest that the lesion provokes a reversal of molecular and biophysical cellular properties towards a more juvenile state, in which excitability is increased, inhibition reduced, and synaptic plasticity facilitated. The resulting functional changes might be useful for improving post-lesion rehabilitation.

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Dendritic compartimentalization determines synaptic plasticity in sensory and associatve spines of the anterior piriform cortex

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The piriform cortex receives olfactory information from the olfactory bulb via axons of mitral and tufted cells organized in the lateral olfactory tract (LOT). These projecting axons terminate in layer Ia on the distal apical dendrites of layer II/III pyramidal cells. In layer Ib, associative connections terminate on proximal apical dendrites. This organization of inputs results in a clear morphological and electrophysiological separation of layer Ia and Ib. In adult animals, long-term potentiation (LTP) of pyramidal cells leads to a smaller increase in synaptic strength in layer Ia compared to layer Ib. During LTP-induction, backpropagating action potentials (bAPs) transmit the information that the neuron fired an action potential to synapses on the dendritic tree, thereby linking neuronal input and output. The amplitude of dendritic calcium signals associated with bAPs at different distances from the soma is a good approximation of the degree of bAP-mediated depolarization. To study the intrinsic properties of action potential backpropagation with respect to the subdivison into a proximal associative and a distal sensory compartment of the apical dendrite, calcium imaging of single bAPs and high-frequency bursts of bAPs was performed. We used a Nipkow-Disc-based fast confocal scanning system and the calcium indicators Oregon Green BAPTA-1 and Oregon GreenBAPTA-6F. A smaller amplitude of the calcium transient evoked by single bAPs could be observed when comparing layer Ia to layer Ib. During high-frequency bursts, there was a sublinear addition of the calcium signal amplitude in layer Ia and layer Ib, still resulting in a smaller amplitude in the distal sensory layer Ia compared to layer 1b. Based on thesefindings, we analysed how the transmission of AP-firing to the dendritic tree via bAPs following presynaptic firing normally resulting in strengthening of synaptic contacts determines spine Ca2+ signals and synaptic plasticity in the piriform cortex.

Protein synthesis and prolonged (late)-LTP in a hippocampal CA1 "two-input-in-vivo-model" as the precondition for "synaptic tagging"-experiments in the intact rat

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In earlier studies we had shown that synaptic input specificity - after the induction of a late long-term potentiation which requires protein synthesis (late-LTP) - is achieved by processes of 'synaptic tagging'. Tetanization or LTP-induction can set a transient 'synaptic tag' at the activated synapses which can capture plasticity-related proteins (PRPs) synthesized synapse-non-specifically in dendritic branches or the somata. Thus, only those synapses with a 'tag' set are able to form late-LTP by capturing the PRPs. The original experiments were performed in hippocampal slices in vitro. Until now, it remained unclear, if 'synaptic tagging' can also be directly described in the CA1 region of the intact, freely moving animal.
We have therefore developed a technique which allows us to induce distinct forms of LTP ipsilaterally by specifically stimulating glutamatergic hippocampal structures contralaterally in the intact, freely moving rat. First, we could show that high-frequency stimulation of the contralateral CA1 or CA3 with a stimulation intensity of 30% (of max. I/O-values) resulted in input-specific LTP in ipsilateral CA1-neurons in freely moving animals (Hassan et al. 2006). We are now determine if these prolonged LTPs do also dependent on protein synthesis as a pre-requisite for "late-LTP" and 'synaptic tagging'. First results revealed, that a prolonged LTP induced by tetanization of the contralateral CA1 or CA3 with a stimulation intensity of 40% (of max. I/O-values) was partially affected by the reversible protein synthesis inhibitor anisomycin). However, this prolonged LTP was more effectively prevented if the irreversible protein synthesis blocker emetine was used instead of anisomycin.
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Role of NogoA in regulating activity-dependent synaptic plasticity in the mature mouse hippocampus

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The nervous system is characterized by an intricate balance of plasticity and stability allowing the acquisition, storage and clearance of memories in a dynamic and highly controlled manner. Changes in the connectivity of neurons - synaptic plasticity - regulate the fine-tuning of neuronal networks during CNS development and adult learning. Synaptic plasticity includes functional and structural changes at neurons. Both changes occur in a 'positive' (synapse strengthening, dendritic spine growth) and in a 'negative' way (synapse weakening, dendritic spine loss). Strong evidence indicates that neuronal activity influences the morphology and function of neuronal networks; however the cellular and molecular mechanisms translating activity into long-lasting structural and functional changes remain largely unknown.
NogoA has been identified as an inhibitor of neurite outgrowth and sprouting in the injured CNS. Despite the wealth of data involving NogoA in preventing CNS repair after lesion, few studies address the crucial question of the physiological role of NogoA in the mature brain. The neurite growth inhibition and growth cone collapse are due to two distinct extracellular regions: the 66-residue loop (Nogo 66), common to all Nogo transcripts and the NogoA amino sequence Δ20, specific for NogoA. Nogo66 signals via a receptor complex containing NgR, $p75^{NTR}$ and Lingo1. NogoA is expressed not only in myelin but also in some neuronal populations both during development and adulthood. Interestingly, NogoA expression in mature neurons is maintained in areas of high plasticity (e.g. the hippocampus).
We first analyzed whether a genetic deletion of NogoA may interfere with activity-dependent synaptic plasticity at the Schaffer collaterals in the mouse hippocampus. We report a mild phenotype in NogoA knockout *versus* wild type mice. Specifically, we show a slight, increase in LTP as well as a minor impairment in LTD. However, an acute blockade of NogoA function, by specific blocking antibodies induces a highly significant increase in LTP suggesting that surface NogoA is indeed involved in modulating activity-dependent synaptic plasticity in the mature mouse hippocampus.
Further experiments will first confirm the NogoA specificity by means of gain of function experiments, applying the active Δ20 fragment, and will clarify the signalling pathway of this NogoA function, by blocking single components of the Nogo receptor complex. Finally, we will also investigate possible structural changes occurring concomitantly to the reported functional changes induced by NogoA neutralization. (Supported by the DFG, Az. ZA554/2-1).

Formation, secretion and redistribution of the glycoprotein Ependymin and its functional localisation in an ultra-structural study of goldfish brain

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Fish Ependymins are brain specific glycoproteins constituting 14 % of the protein content in the extra cellular fluid of the goldfish brain. Homologues are found in invertebrates (echinoderms), amphibians and mammals. Ependymins comprise the HNK-1 carbohydrate epitope characteristic of many cell adhesion molecules. Arranged as monomers, dimers and polymers with a Ca^{2+} and Zn^{2+} binding capacity, they exhibit several microheterogenities in the monomer amino acid sequence. Ependymin concentrations were found to increase during CNS regeneration and after learning-events.

In situ hybridisation by anti-sense probes against Ependymin mRNA demonstrates exclusive synthesis in the leptomeninx of fish brains. Ultra-structural analysis reveals formation in fibroblasts and storage in specialised cellular extensions. After secretion into the extra cellular fluid, Ependymins are recovered at distinct layers of the Tectum opticum. Immuno-gold labelling is concentrated in extra cellular inclusions within the uppermost tectal layer (Stratum marginale), at neuronal cell membranes in the deeper Stratum fibrosum et griseum superficiale (SFGS) and at cells surrounding blood vessels. In addition, immuno-fluorescence studies show strong labelling of apical dendrites of type I neurons in the tectal SFGS that obtain synaptic input from myelinated retinal ganglion cell axons and from unmyelinated marginal fibres of the longitudinal torus. Optic fibres form synapses with the medial and proximal parts of the apical dendritic shafts.

Goldfish were trained on a shuttle-box to avoid electric shocks administered after a conditioning light signal. *In situ* hybridisations revealed a marked increase in the level of Ependymin mRNA in the goldfish (and zebra fish) leptomeninx three hours after learning of the active avoidance response. Intracerebroventricular injections of anti-Ependymin antibodies interfere with memory consolidation during a defined time-frame after avoidance learning that corresponds to the time-course of Ependymin synthesis and secretion. Anti-Ependymin antibodies, however, neither affect learning nor memory retrieval.

The prominent immuno-gold labelling described here in the immediate proximity of postsynaptic densities suggests that the redistributed adhesion molecules may – during memory consolidation after learning - structurally modify synapses on neurones involved in the preceding acquisition in order to improve their functional efficacy for future use.

Learning head-centered representations by temporal invariance learning.

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Movement planning based on visual information requires a transformation from a retina-centered into a head- or body-centered frame of reference. It has been shown that such transformations can be achieved via modulatory gain field interactions [1,2], as observed in the parietal cortex of monkeys [3]. We investigated whether gain field properties underlying coordinate transformations in parietal cortex can be learned by a biologically plausible neural network. We employed a model network of spiking neurons that learns invariant representations based on spatio-temporal stimulus correlations [4]. The model consists of a three-stage network of leaky integrate-and-fire neurons with biologically realistic conductances. The network has two input layers, corresponding to neurons representing the retinal image and neurons representing the direction of gaze. These inputs are represented in the map layer via excitatory or modulatory connections, respectively, that exhibit Hebbian-like spike-timing dependent plasticity (STDP). Neurons within the map layer are connected via short range lateral excitatory connections and unspecific lateral inhibition. We trained the network with stimuli corresponding to typical viewing situations when a visual scene is explored by saccadic eye movements, with gaze direction changing on a faster time scale than object positions in space. After learning, each neuron in the map layer was selective for a small subset of the stimulus space, with excitatory and modulatory connections adapted to achieve a topographic map of the inputs. Neurons in the output layer with a localized receptive field in the map layer were selective for positions in head-centered space, invariant with changes in retinal image due to changes in gaze direction. Our results show that coordinate transformations via gain fields can be learned in a biologically plausible way due to spatio-temporal correlations between visual and eye position signals under natural viewing conditions.

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Analysis of Dendritic Spine Plasticity with 2-Photon Glutamate Uncaging and 2-Photon Imaging

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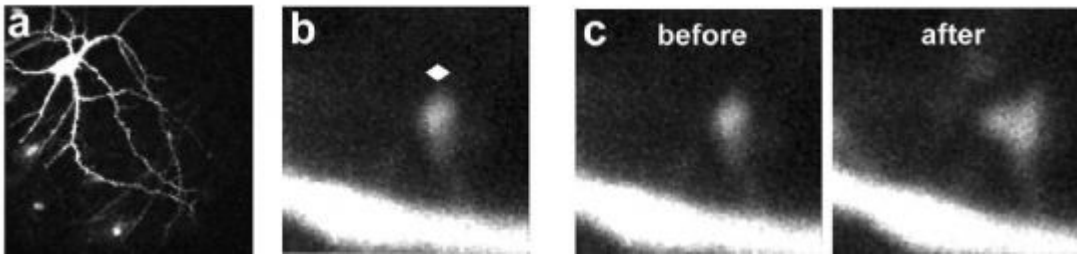
The anatomical architecture of cortical circuits provides the physical framework for sensory processing, generation of behavior, learning, memory and cognition. About 80% of cortical neurons are spiny and excitatory, and form 85% of cortical synapses. The majorityof these glutamatergic synapses are located on dendritic spines. Spines have a small head connected by a thin neck to the parent dendrite. Spine head size appears to be indicative of synaptic strength. Current models attribute a dual role to spines: i) Functionally, spines contain molecular signaling complexes, which control synaptic strength in response to synaptic activity. ii) Structurally, spines are considered to serve as substrate for synapse formation by bridging the distance between axons and dendrites of synaptic partners. However, spine synapses are found only at a small fraction (< 30%) of the sites, where axonal and dendritic arbors are less than the average spine length apart. Thus, whereas the anatomical organization of axonal and dendritic branches provides the basic layout of neuronal circuits, the establishment of specific connections by formation of spine synapses can be considered to program such circuits for performing particular neural computations. Therefore to understand neuronal circuit function and plasticity it is important to understand the processes controlling spine formation, elimination, stability and plasticity.

To explore the processes involved in spine synapse stability and plasticity, we implemented over the last year a microscope for combining 2-photon imaging, 2-photon glutamate uncaging and electrophysiology. This involved custom hardware design and software development. 2-photon glutamate uncaging allows to directly stimulate individual identified spines, in contrast to other, e.g. electrical, stimulation techniques. This has the advantage that plasticity can be induced at particular spines, and their properties be probed directly before and after plasticity induction. In addition it has been shown that changes in postsynaptic strength during plasticity are accompanied by an increase in spine volume (Matzusaki et al., Nature, 2004). Thus, synaptic plasticity can be monitored in a non invasive manner by recording spine morphology of fluorescently labeled neurons (Fig. 1). By performing dual color imaging, spine morphology can be simultaneously imaged with fluorescently labeled postsynaptic proteins, to analyze protein translocation and accumulation during spine plasticity.

Figure 1: Morphological spine plasticity induced with 2-photon glutamate uncaging. (a) GFP expressing CA1 pyramidal cell in slice culture. (b) Dendritic spine with location of the uncaging stimulus (diamond). (c) Spine before (left) and after (right) 30 glutamate uncaging stimuli (0.5 Hz; duration 4 ms; 25 mW in objective back focal plane; 4 mM Ca^{2+}; 0 mM Mg^{2+}).



Interaction between short-term facilitation and depression at the calyx of Held

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Repetitive stimulation of the calyx of Held synapse in slices typically results in a decrease of synaptic strength, so that this synapse has been classified as a "depressing" synapse. *In vivo*, auditory brainstem neurons fire continuously at elevated rates (tens of Hertz), even in the absence of sound, and sound stimuli lead to a further increase in firing frequency. Therefore, classical stimulation protocols that have been employed to probe short-term plasticity – high-frequency trains that are applied after relatively long pauses (~ 10 - 40 sec) – might be inappropriate to capture the full range of short-term dynamics of these auditory synapses. Here, we investigated short-term plasticity of the calyx of Held with a protocol designed to comprise the basic characteristics of the synapse's physiological sound-evoked stimulus pattern: a 'low'-frequency (20 Hz) 'conditioning' train, which was instantly followed by a high-frequency (200 Hz) train. As expected, EPSCs showed depression during the 20 Hz conditioning train. Surprisingly, synaptic transmission was strongly (~ twofold) facilitated during the onset of 200 Hz trains that were conditioned by preceding 20 Hz trains. Direct presynaptic whole-cell recordings and Ca^{2+} imaging showed that this facilitation was not mediated by Ca^{2+}-current facilitation, and that facilitation depended on the build-up of residual Ca^{2+} during the first few stimuli of the high-frequency train. The conditioning 20 Hz train depleted the readily-releasable vesicle pool by ~ 50 %. Interestingly, we found that the release kinetics of the remaining pool of fast-releasable vesicles (FRP) was notably slowed (by about 1.5 fold). This implies that a decrease in the release probability (*p*) of FRP vesicles contributes to synaptic depression. Preliminary results obtained from presynaptic Ca^{2+} uncaging experiments also revealed a slowing of the release kinetics of FRP vesicles, indicating that the reduced *p* is caused by an intrinsic mechanism. We hypothesize that vesicles with a reduced intrinsic *p* are more prone to the effects of short-term facilitation, and therefore cause a marked transient overshoot in synaptic strength during the onset of a high-frequency trains under near-physiological conditions.

NRG1-ErbB4 signaling modulates synaptic function in the mature cortex

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The human Neuregulin-1 (NRG1) gene, which encodes a family of neuronal growth factors, has been identified as a susceptibility gene for schizophrenia (SZ). ErbB4, a transmembrane tyrosine kinase, is the predominant NRG1 receptor in neurons of the central nervous system (CNS). Multiple functions of NRG1-ErbB4 signaling have been suggested, including a role in neuronal migration and synaptic plasticity. However, in vivo data for the adult brain are lacking, due to the embryonic lethality of NRG1 and ErbB4 null mutations in mice. Here, we have generated a set of conditional mouse mutants and transgenic mice to study the effect of altered levels of NRG1-ErbB4 signaling on synaptic plasticity and behavior in adult mice. Conditional null mutants lacking NRG1 or ErbB4 in cortical projection neurons beginning at postnatal stages (using a CamKII-Cre driver line) develop normally and exhibit no obvious defects in cortical development. Moreover, cortical expression levels of AMPA and NMDA receptors were unaltered in the absence of NRG1 or ErbB4. However, behavioral and electrophysiological analysis of projection neuron-specific NRG1 mutants at older stages revealed impaired synaptic plasticity, learning and memory. Unexpectedly, the corresponding ErbB4 mutants were largely unaffected. Subsequent expression analysis demonstrated that ErbB4 mRNA is predominantly expressed in parvalbumin expressing interneurons, but absent from calbindin and calretinin expressing cells. In contrast, ErbB4 expression was rarely detected in pyramidal neurons. Using a parvalbumin-Cre driver line, we have generated mice lacking selectively ErbB4 in interneurons, which are currently being analysed. We suggest that NRG1-ErbB4 signaling in the mature brain modulates synaptic functions, most likely acting through an interneuronally expressed ErbB4 receptor. Impaired synaptic plasticity as a consequence of chronic alterations in the level of NRG1-ErbB4 signaling might contribute to the pathophysiology of SZ.

Genetically Encoded Calcium Indicators as a Tool to Dissect Nuclear Calcium Transients Induced by different LTP Stimulation Paradigms in Acute Hippocampal Slices

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The term synaptic plasticity was introduced into neurophysiology to describe the ability of central nervous system synapses to alter their synaptic strength as a response to previous synaptic activity.
The probably best studied form of synaptic plasticity is long-term potentiation (LTP) in the hippocampus where a brief high-frequency afferent activity leads to a long-lasting increase in the strength of synaptic transmission. To induce a persistent form of LTP (L-LTP) which lasts longer than 4 h, 3 -4 tetanic stimulations are needed. Besides mRNA translation, L-LTP requires gene transcription possibly involving activation of cAMP response element (CRE)-driven genes and the CRE-binding protein, CREB.

In neurons the activity of CREB is tightly controlled by Ca^{2+}/Calmodulin-dependent kinases and other signaling pathways. We have previously shown that in hippocampal cultures NMDAR-mediated Ca^{2+} currents trigger nuclear Ca^{2+} transients, and that in AtT20 cells increases in nuclear and cytosolic Ca^{2+} concentrations differentially activate transcription.
In this study we combine stereotaxic injections into the hippocampus and recombinant adeno-associated virus (rAAV)-mediated gene transfer to generate genetically modified rats. Acute hippocampal slices of those animals show a large fraction of infected pyramidal cells and can be analyzed by electrophysiology and imaging techniques.

Using a nuclear localized, genetically encoded Ca^{2+} indicator, we can demonstrate that in acute hippocampal slices L-LTP-inducing stimulation paradigms trigger nuclear calcium transients in CA1 pyramidal cells. The aim of the study is to investigate possible correlations between nuclear Ca^{2+} transients and the maintenance of LTP for different LTP stimulation protocols. In addition, experiments are being designed to study the role of nuclear calcium signals in the maintenance of L-LTP.
Besides, we performed gene chip analysis to get candidate genes which are down or up regulated after LTP induction. We are currently investigating the role of these genes in the maintenance of LTP by using knock-in or knock-down (RNAi) strategies.

Role of TrkB.T1 and p75 neurotrophin receptors in shaping neuronal morphology of hippocampal neurons

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Neurotrophins and their receptors are important modulators of both functional and structural plasticity in the developing and adult nervous system. Remarkably, they have been shown to mediate positive as well as negative changes in synaptic efficacy and structure depending on the type of receptor they bind to – either the Trk receptors or the pan neurotrophin receptor p75.

Whereas numerous studies report the essential role of BDNF and its receptor TrkB in the regulation of hippocampal long-term potentiation (LTP), the p75NTR is known to be involved in synaptic weakening (LTD) (Rösch *et al.* 2005). Furthermore, BDNF signalling via TrkB has been described to promote neurite outgrowth and elongation. In contrast, the over-expression of the p75NTR has been shown to produce a negative effect on dendritic morphology and spine density (Zagrebelsky *et al.* 2005).

But the picture is even more complex as the TrkB receptor exists in three splice variants. While the full length tyrosine kinase is well characterized, the role of the other truncated kinase-lacking isoforms remains so far elusive.

In our study we address the question of whether the ratio of the truncated TrkB.T1 receptor and p75NTR and the potential interaction of these two receptors could play a role in shaping neuronal morphology.

Experiments were done in TrkB.T1 transgenic mice over expressing the splice variant as well as in WT cultures biolistically transfected with p75NTR. Our results indicate that dendritic complexity of CA1 pyramidal neurons is negatively regulated by the over expression of p75NTR as well as by TrkB.T1. However, the over expression of each one of the two receptors modulates the structure of different dendrite compartments – respectively proximal and distal. In addition, spine density is increased in TrkB.T1 over expressing neurons but is reduced in those with elevated p75NTR levels. Remarkably, we could show that a combined over expression of both neurotrophin receptors rescues dendritic morphology as well as spine density to WT levels. Additional experiments using TrkB.T1 deletion mutants in primary embryonic hippocampal cultures revealed that it is the extracellular domain of TrkB.T1 which is necessary to inhibit p75NTR dependent negative alterations in neuronal morphology.

Our findings demonstrate that indeed the ratio of p75NTR and TrkB.T1 plays an important role in modulating dendrite morphology in mature hippocampal neurons. We propose a model where T1 could be considered as acting dominant negative not only on the full length TrkB receptor but in addition on p75NTR. Further experiments will analyze the possible interaction between these two receptors and downstream signalling pathways involved in mediating structural changes.

Critical experimental conditions for spike time dependent plasticity in hippocampal slices

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Long term potentiation (LTP) and long term depression (LTD) are considered as neural substrate for learning and memory and can be induced in different brain regions by various patterns of rhythmic synaptic stimulation. The growth factor BDNF is an important mediator of LTP processes in the brain. In contrast to classical high frequency stimulation for LTP and low frequency stimulation for LTD, spike time dependent changes in synaptic gain can be induced by low-frequency pairing of pre- and postsynaptic action potentials with millisecond fidelity. The net effects of spike time dependent plasticity (STDP) are dependent on precise relative timing of pre- and postsynaptic activation, yielding LTP in case of the postsynaptic spike is following the presynaptic activation, and LTD in case the postsynaptic spike precedes presynaptic transmitter release. However, literature provides various but often contradictory rules for STDP, even for the same synaptic circuit.

In an attempt to establish a reliable STDP protocol in our lab, we systematically analyzed the experimental conditions for successful induction of spike time dependent plasticity rules in the CA1 region of rodent hippocampal slices. To this aim we applied different previously published STDP paradigms (with differences in pairing frequency and pairing patterns) to acutely isolated (P12-P21) or organotypic hippocampal (12 DIV) brain slices of rats and mice, and analyzed experimental conditions that are crucial for successful positive and negative changes in synaptic efficiency. Dialyzed whole cell patch clamp recordings were established, cells were held at a holding potential of -70 mV, and amplitudes of synaptic responses and STDP protocols were measured in the current clamp mode. Basal synaptic properties (e.g., input resistance, paired pulse ratio, initial synaptic amplitude) of recorded neurons were analyzed with respect to subsequent success of the STDP protocol.

Among the experimental parameters evaluated for successful STDP were: composition of the internal recording solution (varying the contents of e.g., ATP, GTP, phosphocreatine, EGTA) as well as composition of the external solution (including different ratios of calcium to magnesium). In acute hippocampal slices we checked for age related differences in effectiveness of the STDP protocols between P12 to P21 *Spraque Dawley* rats. Last, we determined the possible role of different experimental conditions during preparation of acute hippocampal slices (e.g. sucrose based versus reduced Ca^{2+} containing ACSF cutting solution) for the reliability of STDP protocols.

From our experiments we conclude that the efficiency to observe positive and negative changes in synaptic amplitude in response to STDP protocols is highly dependent on details of the synaptic slice preparation and on basal synaptic properties established prior to STDP induction. Thus, comparison of learning rules between different experiments/experimental setups requires careful selection of basal synaptic conditions. In future experiments it will be challenging to find out to which extent plasticity mediators like the neurotrophic factor BDNF contribute to establishing STDP.

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When less is more: Impact of short term plasticity on spike sequence processing

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The precise timing of action potentials (spikes) is essential for information transmission and processing in a variety of neural systems. In particular in the presence of short term synaptic plasticity, each single presynaptic spike has a specific impact on the effect of following presynaptic spikes. Nevertheless, both experimental and theoretical studies have so far focused on characterizing the input-output relation of neurons by average quantities, e.g. the f-I-curve that gives the output firing rate of a neuron in terms of its input firing rate.

Here we investigate the input-output relation of a model neuron with one depressive input synapse on the level of sequences of individual spikes: For arbitrary presynaptic spike sequences we compute a self-consistency equation that determines the invariant joint distribution of membrane potentials and synaptic strengths. Interestingly, even in the strongly stochastic limit of Poisson input spike sequences the output rate deviates from the mean field predictions based on averaged "rate" inputs. In the opposite, deterministic limit of periodic input sequences, a neuron's response frequency **decreases** upon **increasing** the input frequency across specific critical values. We analytically explain this counter-intuitive less-is-more response dynamics and confirm it by numerical simulations. The phenomenon is insensitive to changes in the neuron model and may thus be experimentally testable in biological neurons that receive spiking input over a synapse.

Magnetic Stimulation Induces Long-term Potentiation in Rat Hippocampal Slices

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Recent reports indicate that the exposure of brain tissues to repetitive transcranial magnetic stimulation can induce persistent changes in neuronal activity and influence hippocampal synaptic plasticity, the mechanisms involved in learning and memory. However, the modulation of synaptic efficiency by magnetic stimulation is still unclear. In present study, we investigated the effect of high-frequency magnetic stimulation (HFMS), in vitro, on synaptic transmission rat hippocampal slices. Field excitatory postsynaptic potentials (fEPSPs) were recorded within the CA1 stratum radiatum in response to an electrical stimulation of Schaffer collateral inputs. Following a stable baseline recording, HFMS was delivered at 100 Hz through a circular coil positioned closely over the slices using different paradigms. The intensity of the magnetic stimulus was adjusted to 40-50% of the maximal output of the magnetic stimulator. After HFMS, CA1 fEPSPs were enhanced inducing significant amounts of long-term potentiation (LTP) by using a paradigm consisting of 10 trains of 20 pulses (1 sec apart) with 5 repetitions (interval 10 sec; LTP 142±9% of baseline, n=6) or by using a different paradigm consisting of 3 trains of 100 pulses (interval 20 sec; LTP 129±7% of baseline, n=8). Furthermore, HFMS-induced LTP was prevented by the presence of selective NMDA receptor blocker D-AP5 (50 µM) in the ACSF solution (95±6%, n=6; $p<0.05$).

ERK-phosphorylation decides whether Jacob is a mediator of NMDA-receptor induced plasticity or cell death

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In hippocampal excitatory neurons NR2B-containing subunits of the NMDA-receptor are located at synaptic and extrasynaptic sites and the activation of these receptors has fundamentally different consequences in terms of nuclear gene expression depending upon their localization.While activation of synaptic NR2B receptors induces the expression of cell survival and plasticity genes, activation of extrasynaptic NR2B receptors drives primarily the expression of cell death genes. The underlying mechanisms for this sharp distinction are till now essentially unclear. Previous work has shown that the Jacob's nuclear translocation is instrumental for NMDA-receptor mediated gene expression and the CREB shut off pathway. In this study we show that Jacob is exclusively part of the NR2B but not of the NR2A receptor complex. Most importantly after stimulation of synaptic but not of extrasynaptic NMDA-receptors its nuclear translocation requires ERK activity. Jacob is a ERK binding protein and is phosphorylated by ERK at a Ser180. Importantly, Jacob is phosphorylated at this position only after activation of synaptic NMDA-receptors. Nuclear overexpression of a Jacob mutant that can't be phosphorylated at Ser180 causes destabilization of synapses, simplification of dendrites and gene expression in favour of reduced plasticity and cell survival. The opposite is found with a phospho-mimicking mutant at this crucial position. Of note, this is independent of synaptic activity. Thus, even after blockage of synaptic neurotransmission the cells overexpressing the phospho-mimicking mutant in the nucleus react with a gene expression pattern and subsequent morphological changes that are characteristic for enhanced synaptic strength. In other terms the presence of ERK-phosphorylated Jacob suggests the nucleus that plastic events must have happened at the cells synapses despite these synapses were silent. Hence, the presence of non-phosphorylated Jacob suggests the opposite and is followed by a series of deteriorative events in terms of synaptic integrity that subsequently leads within a few days to neuronal cell death.

High resolution recording of organotypic brain slices with multi-transistor array

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We report on the two-dimensional extracellular recording of evoked field potentials in organotypic brain slices at a spatial resolution of 7.8 µm. The slices from rat hippocampus are cultivated on an extended CMOS chip with 16384 sensor pixels on an array of 1 mm^2. The novel multi-transistor array (MTA) has a reduced noise of 70 μV_{rms} and a higher sampling rate of 6 kHz compared to a previous MTA [1]. Stable signals were recorded over several hours. We stimulated at different locations in CA3 and CA1 with common tungsten electrodes.

Detailed maps of evoked field potentials were recorded: (i) The propagating action potential along the Schaffer Collaterals could be traced due to the high sampling rate and the low noise. (ii) We observed all steps of the trisynaptic circuit from the mossy fibers to the alveus. The recorded pEPSPs had amplitude of 2-3 mV. (iii) Complex patterns due to interneuron contribution were measured. In particular an outward current was recorded at the border of stratum radiatum/lacunosum-moleculare with a sharp onset 4 ms after the pspike in CA1. (iv) We evaluated maps of LTP induced by theta stimulation. Along cornu ammonis the effect was found to be rather homogeneously, whereas the shape of facilitation was modulated across the layers. (v) During the postsynaptic field potential we were able to record action potentials of individual neurons.

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Low-frequency stimulation of the temporoammonic pathway induces heterosynaptic disinhibition in the subiculum

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Joint recruitment of homosynaptic and heterosynaptic mechanisms of synaptic plasticity is essential for stabilization of Hebbian plasticity and the maintenance of memory traces. The subiculum is the principal target of CA1 pyramidal cells. It serves as the final relay of hippocampal output and thus mediates hippocampal-cortical interaction. In addition, the subiculum receives direct input from the entorhinal cortex via the temporoammonic pathway. In the present study, we demonstrate that low-frequency stimulation of the temporoammonic pathway results in a substantial increase of synaptic strength at CA1-subiculum synapses. We provide evidence that this heterosynaptic potentiation is mediated by an NMDA receptor-dependent long-term depression (LTD) of GABAergic inhibition. Hence, the temporoammonic pathway facilitates synaptic transmission at CA1-subiculum synapses by modulation of local inhibition. This mechanism might bear physiological significance for stabilization and processing of mnemonic information at hippocampal output synapses and underpins the functional role of hippocampal-entorhinal interaction in memory formation.

Role of the 5-HT4 Receptor in Morphogenic Signalling in Neurons

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The neurotransmitter serotonin (5-HT) plays a pivotal role in the regulation of multiple events in the CNS. Recently we demonstrated that in mouse hippocampal neurons, activation of endogenous 5-HT7 receptors significantly increased neurite length, whereas stimulation of 5-HT4 receptors led to a decrease in the length and number of neurites. We showed that the 5-HT4 receptor is coupled not only to the heterotrimeric Gs, but also to G13 protein. Activation of this signaling pathway results in RhoA-mediated modulation of gene transcription and in reorganization of the actin cytoskeleton. We also demonstrated that serotonin receptor

5-HT7 can activate heterotrimeric G12 protein, leading to the selective activation of small GTPases RhoA and Cdc42. Agonist-dependent activation of the 5-HT7 receptor induced pronounced filopodia formation via a Cdc42-mediated pathway paralleled by RhoA-dependent cell rounding. (1,2)

This molecular model for 5-HT4 and 5-HT7 receptor mediated signalling provides a link between receptor activation and a subsequent change in morphology. Our recent studies confirmed a direct link between 5-HT7 receptor activation and an increase in synaptogenesis as well as modulations in synaptic plasticity in hippocampal neurons.

As the molecular model suggests an opposing role for the 5-HT4 receptor in morphology we investigate in this study whether 5-HT4 receptor activation leads to changes in synaptogenesis (formation of presynaptic clusters, filopodia, spines) and spontaneous synaptic activity in primary culture of mouse hippocampal neurons. Additionally we study synaptic plasticity looking into changes in long-term potentiation related to the 5-HT4 receptor activity in organotypic cell culture.

Our data suggests that serotonin plays a prominent role in regulating the neuronal cytoarchitecture and synaptic plasticity in addition to its classical role as a neurotransmitter.

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Synapse-specific and compartment-specific excitation of dentate gyrus basket cells.

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The dentate gyrus has key functions in learning and memory processes. It comprises excitatory principal cells (granule cells, [GCs]) and GABAergic inhibitory interneurons. While GCs provide the major route for information processing in this area, interneurons, specifically soma-inhibiting basket cells (BCs) are assumed to determine when and where information can flow through the network. To understand the role of interneurons in this process, we first have to understand how they are activated. In this project we investigate the mechanisms underlying excitation of BCs by two defined excitatory inputs, (i) axons from the granule cell layer (gcl), which target basal dendrites and (ii) axons from layer II neurons of the entorhinal cortex, forming the perforant-path (pp) and targeting apical dendrites of BCs. We have performed whole-cell patch-clamp recordings of EPSCs in BCs in response to extracellular stimulation in the gcl or pp in acute hippocampal slices of rats (32-34 ºC). We have found (1) that gcl-evoked EPSCs are rapid whereas pp-EPSCs are slow (decay time constant 2.6 ± 0.61 ms *versus* 6.2 ± 0.9 ms, respectively). (2) Gcl-EPSCs are mediated by Ca^{2+}-permeable AMPA receptors (AMPAR) and show strong rectification (rectification index = 0.15). In contrast, pp-EPSCs are mediated by Ca^{2+}-impermeable AMPAR and are non-rectifying (rectification index = 0.75). (3) Gcl-inputs are blocked by the Ca^{2+}-AMPAR-blocker philanthotoxin (66.3%), whereas pp-inputs not. Finally, LTP can be induced at gcl- (183.7%) but not at pp-inputs indicating. Finally, LTP is independent on NMDAR suggesting that Ca^{2+}-permeable AMPAR are involved in synaptic plasticity. In summary, our data indicate input-specific segregation of AMPAR subtypes in BCs. This segregation is compartment-specific and will increase the computational role of BCs, allowing transmission of information in a synapse-specific manner.

Recognition, presence and survival of locust central nervous glia in situ and in vitro

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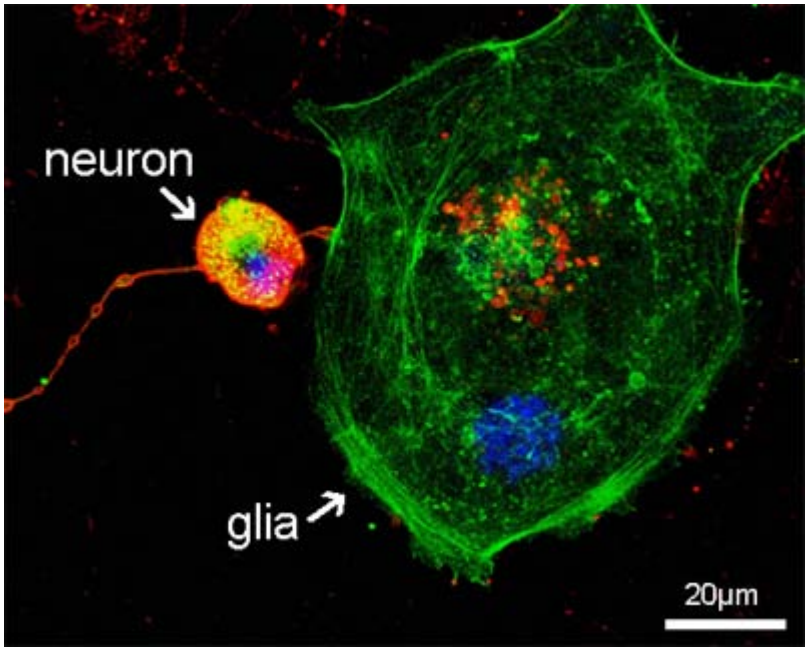
Insect glial cells serve functions for the formation, maintenance and performance of the central nervous system in ways similar to their vertebrate counterparts. Characterization of physiological mechanisms that underlie the roles of glia in invertebrates is largely incomplete, partly due to lack of markers that universally label all types of glia throughout all developmental stages in various species. Studies on primary cell cultures from brains of *Locusta migratoria* demonstrated that the absence of anti-HRP immunoreactivity, which has previously been used to identify glial cells in undissociated brain tissues, can also serve as a reliable glial marker in vitro, but only in combination with a viability test. Since cytoplasmic membranes of cultured cells are prone to degradation as they lose viability, only cells that are both anti-HRP immunonegative and viable should be regarded as glial cells, while the lack of anti-HRP immunoreactivity alone is not sufficient. Cell viability can be assessed by the pattern of nuclear staining with DAPI (4',6-diamidino-2-phenylindole), a convenient, sensitive labelling method that can be used in combination with other immunocytochemical cellular markers. We determined the glia-to-neuron ratio in central brains of 4th stage nymphs of *Locusta migratoria* to be 1:2 both in situ and in dissociated primary cell cultures. Analysis of primary cell cultures revealed a progressive reduction of glial cells and indicated that dead cells detach from the substrate and vanish from the analysis. Such changes in the composition of cell cultures should be considered in future physiological studies on cell cultures from insect nervous systems.



Autocrine cell volume regulation of retinal glial cells: Involvement of voltage-gated calcium and sodium channels

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Gliotransmitters such as glutamate and ATP play an autocrine role in the regulation of the volume of rat retinal glial (Müller) cells. It has been suggested that stimulation of retinal glial cells with vascular endothelial growth factor (VEGF) evokes a calcium-dependent, exocytotic release of glutamate from retinal glial cells. In cell swelling experiments using acutely isolated retinal glial cells of the rat, we investigated the involvement of voltage-gated sodium and calcium channels in the release of glutamate. We found that the inhibitory effect of VEGF on the osmotic swelling of retinal glial cells (which is mediated by a release of glutamate) is prevented in the presence of antagonists of voltage-gated sodium (tetrodotoxin, saxitoxin) and T-type calcium channels (kurtoxin, mibefradil). In contrast, the swelling-inhibitory effect of glutamate (which evokes a release of ATP) remained unaffected in the presence of the blockers. The data suggest that voltage-gated sodium and calcium channels are implicated in the release of glutamate (but not ATP) from retinal glial cells and, therefore, in the autocrine regulation of cellular volume. The results indicate a functional role of voltage-dependent calcium channels in the mediation of the calcium influx for the vesicular release of glutamate from retinal glial cells. The involvement of voltage-dependent sodium channels suggests that rapid fluctuations of the membrane potential underlie the activation of calcium channels.

Spatial Expression of the Glutamate Transporters GLAST and GLT-1 during Postnatal Development of the Mouse Hippocampus

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Glutamate is the major excitatory neurotransmitter in the CNS and plays an important role during brain development by regulating neuronal migration, proliferation, synapse formation and function. Its extracellular concentration is regulated by the action of specific high-affinity transporters, which are located on neuronal and glial plasma membranes. To date, five transporters, termed excitatory amino acid transporters 1-5 (EAAT 1-5), have been cloned. They are electrogenic and are mainly driven by the electrochemical gradient of sodium. Glial cells predominantly express EAAT1 (GLAST) and EAAT2 (GLT-1). Their activation results in a fast decline in the glutamate concentration in the synaptic cleft and shapes the time course of synaptic conductance. In the present study we examined the spatial distribution of GLAST and GLT-1 in the mouse hippocampus at three stages during the early postnatal development (P3-5, P10-15, and P20-25) by employing comparative immunohistotochemistry. In parallel, hippocampal sections were labelled for glial acidic fibrillary protein (GFAP) to identify astroglia.

At P3, GLAST expression was arranged in a dense ribbon along the pyramidal cell layer and the granule cell layer of the dentate gyrus. Between P10 and P25, the labelling pattern changed into a dense and diffuse punctated immunoflourescence of cellular structures surrounding neuronal somata throughout the hippocampus. Simultaneous counterstaining for GFAP revealed the first distinct surface labelling of astrocytes at P10 in the stratum oriens and the stratum radiatum. At P25, GLAST-immunoreactive astrocytes were found preferentially in the stratum oriens and stratum radiatum and at ectopic, GFAP-negative cells in the stratum lacunosum-moleculare which were sparsely scattered.

GLT-1 immunoreactivity was characterized by a punctate label in the pyramidal and granule cell layer from P3 on, that became only slightly denser during development. At P10, distinguishable GLT-1-positive astrocytes emerged augmented throughout the hippocampus, but excluding the hilar region. At P25, GLT-1 was evenly expressed throughout the hippocampus.

In addition to the labelling of GFAP-positive cells exhibiting mature astroglial morphology, we detected a moderate to strong immunofluorescence of GLAST-positive cells with long radial processes, presumptive radial glial cells, at P3 spanning the pyramidal cell layers. A weaker expression pattern was found in the granule cell layer of the dentate gyrus. At P25, in contrast, no staining of GLAST-positive radial glia in the pyramidal cell layer and a moderate to strong labelling in the granule cell layer of the dentate gyrus was observed. GLT-1-positive radial glia was only present in the granule cell layer of the dentate gyrus, exhibiting an increasing labelling from a weak (P3) to a moderate (P25) staining.

These observations indicate that glial glutamate transporters show distinct and differential changes in expression patterns within the hippocampus during the first three weeks of postnatal development, and, thus, during the maturation of glutamatergic synapses.

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Synaptically-induced intracellular sodium signals in hippocampal astrocytes *in situ*

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Classical astrocytes are in close contact to excitatory synapses and, following activation of metabotropic receptors, respond to glutamate release with intracellular calcium transients. In addition, they express transporters which mediate the uptake of glutamate. Glutamate transport is electrogenic and energized by the inward transport of three sodium ions. In cultured astrocytes, it was demonstrated that application of glutamate or D-aspartate results in an increase in the intracellular sodium concentration (Rose & Ransom, 1996b; Chatton *et al.*, 2000; Voutsinos-Porche *et al.*, 2003), stimulating Na^+/K^+-ATPase, ATP consumption and glucose uptake (Pellerin & Magistretti, 1994; Chatton *et al.*, 2000). While these studies identified sodium elevations in astrocytes as relevant components of physiological signalling cascades in the brain, the occurrence of synaptically-induced sodium transients in the intact tissue has so far only been reported from cerebellar Bergmann glial cells (Kirischuk *et al.*, 2007; Bennay *et al.*, 2008).

In the present study, we analysed if excitatory synaptic activity elicits sodium signals in classical astrocytes by performing quantitative sodium imaging with the fluorescent sodium-sensitive dye SBFI, combined with whole-cell patch-clamp measurements in acute tissue slices of mouse hippocampus. Astrocytes were identified by staining with sulforhodamine 101. SR101-positive astrocytes are characterized by a prominent expression of electrogenic glutamate transport, whereas they largely lack ionotropic glutamate receptors (Kafitz *et al*., 2008). We found that short bursts of Schaffer collateral stimulation (5 – 10 pulses at 50 Hz) evoke sodium transients in the mM range in somata of both CA1 pyramidal neurons and SR101-positive astrocytes. While AMPA receptor activation was necessary for the generation of synaptically-induced sodium transients in neurons, sodium transients in astrocytes were largely reduced by the glutamate uptake blocker TBOA, emphasizing the role of electrogenic glutamate transport in their generation. With low stimulation intensities, glial sodium transients were defined to primary branches and adjacent fine processes and only weakly invaded the soma, indicating the existence of microdomains for sodium signalling in astrocytes. More intense stimulation, in contrast, elicited global sodium transients throughout the entire cell including the soma.

Taken together, our results establish that glutamatergic synaptic transmission in the hippocampus results in either local or global sodium signals in hippocampal astrocytes. These activity-induced intracellular sodium transients might serve as key signals in the coupling of excitatory synaptic activity with glial metabolism and glucose uptake.

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Ammonia inhibits mGluR-mediated calcium signaling in hippocampal astrocytes and neurons *in situ*.

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Glutamate is the most important excitatory transmitter in the brain. Its synaptic release evokes intracellular calcium signals in both neurons and astrocytes which play a central role for many cellular processes among them synaptic plasticity, neuron-glia interaction and regulation of blood flow. Neuronal calcium signals are mediated by activation of ionotropic and metabotropic glutamate receptors (mGluR), as well as by the opening of voltage-dependent calcium channels, while glutamate-induced calcium signals in astrocytes have been mainly attributed to the activation of PLC-coupled mGluR1 and mGluR5. In patients with hepatic encephalopathy (HE), a reduction in excitatory neurotransmission has been reported which was attributed to an increase in brain ammonium (NH_4^+/NH_3) concentration. However, the mechanisms by which NH_4^+/NH_3 affects glutamatergic neurotransmission are not understood in detail. In the present study, we examined whether application of NH_4^+/NH_3 evokes alterations in calcium signaling induced by activation of metabotropic glutamate receptors in neurons and two different subtypes of astrocytes in hippocampal slices.

Intracellular calcium transients were measured by ratiometric imaging of the calcium-sensitive fluorescent dye Fura-2 in CA1 pyramidal neurons and astrocytes in the *stratum radiatum* of acute hippocampal slices from 19-21 postnatal day old mice. Astrocytes were classified using the fluorescent marker SR101, which selectively stains passive astrocytes, while complex astrocytes are spared (Karl Kafitz et al., 2008, J Neurosci Methods 169:84-92). DHPG (3,5-Dihydroxyphenylglycin), a selective agonist for mGluR1 and 5, was applied by focal pressure application into the *stratum radiatum* using a fine micropipette. Under control conditions, application of DHPG (2 mM, 100 ms), induced transient increases in calcium in CA1 neurons and in SR101-positive as well as SR101-negative astrocytes. Peak amplitude and total area of these calcium signals were about four times larger in neurons than in astrocytes. Bath application of the specific mGluR5 antagonist MPEP (2-Methyl-6-(phenylethynyl)pyridine; 25 µM), blocked DHPG-induced calcium increases, suggesting that DHPG primarily acts on mGluR5 to elicit calcium increases in neurons and astrocytes. In contrast, in the presence of the specific mGluR1 antagonist YM-298198 (6-amino-N-cyclohexyl-N,3-dimethylthiazolo[3,2-a]benzimi dazole-2-carboxamide; 1 µM) DHPG continued to induced robust calcium increases. Addition of 5 mM NH_4^+/NH_3 to the bath, in contrast to earlier reports obtained from cultured cortical astrocytes (Christopher Rose et al., 2005, J Biol Chem 280:20937-44), failed to elicit large transient increases in the calcium concentrations in neurons and astrocytes in hippocampal slices. NH_4^+/NH_3, however, reduced calcium transients evoked by DHPG in neurons and SR101-positive astrocytes by more than 50%. DHPG-induced calcium signals in SR101-negative astrocytes, in contrast, were virtually unaltered by NH_4^+/NH_3.

Taken together, our results demonstrate that NH_4^+/NH_3 inhibits intracellular calcium signaling via metabotropic glutamate receptors in neurons and passive astrocytes. This inhibition may contribute to the deficits in glutamate transmission observed during HE.

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Characterization of synaptically-evoked calcium transients in different subtypes of hippocampal astrocytes

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It is well known that astrocytes, both *in vitro* and *in vivo*, respond to synaptic activity with transient increases in their intracellular calcium concentration. These calcium increases can result in the release of gliotransmitters by astrocytes which influence a multitude of processes such as blood flow, neuronal development, and synaptic transmission (Agulhon et al., 2008). In the hippocampus, two astrocyte populations have been characterized based on their electrophysiological properties: cells exhibiting a linear IV-relationship and those with a more complex current pattern. These two types of astrocytes can be distinguished by labeling with the fluorescent dye sulforhodamine 101 (SR101, Kafitz et al., 2008), which selectively stains astrocytes with a linear IV-relationship. Both astrocyte subtypes express metabotropic glutamate receptors linked to phospholipase C and IP_3 signaling cascades. In addition, SR101-negative cells express calcium-permeable AMPA-type glutamate receptors. Based on these differences, the kinetics and mechanisms of calcium signaling in response to synaptic activity might differ in the two types of glial cells. In the present project, we therefore characterized synaptically-evoked calcium transients in SR101-positive as compared to SR101-negative hippocampal astrocytes.

To this end, we performed widefield and high resolution 2-photon calcium imaging in combination with whole-cell patch-clamp and field potential recordings in acute hippocampal slices of juvenile mice. Previous studies reported intrinsic calcium oscillations, occurring in the absence of neuronal activity, in the majority of astrocytes (Parri et al., 2001). Likewise, we found spontaneous oscillations occurring in the absence of electrical stimulation in the majority of SR101-positive astrocytes. These oscillations were observed at varying amplitudes and frequencies. SR101-negative astrocytes, in contrast, only rarely participated in the spontaneous oscillations. Short burst Schaffer collateral stimulation (3 pulses, 50 Hz) reliably elicited calcium transients in both SR101-positive and SR101-negative astrocytes. These calcium transients displayed different types of kinetics and spatial properties in different cells: In some astrocytes global calcium transients traveling in a wave-like manner through all primary astrocytic processes, including the cell body, were observed. In other cells calcium transients occurred locally. In this case they were restricted to cellular subdomains within small astrocytic processes and did not invade the cell body.

Taken together, our results indicate that basic calcium signaling properties vary between SR101-positive and -negative astrocytes, implying that these two astrocyte subtypes contribute differently to calcium-mediated glia-neuron-interaction.

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GABA transport-mediated calcium signaling in olfactory bulb astrocytes

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We studied the mechanism of GABA-induced signaling in astrocytes of olfactory bulb slices using confocal Ca^{2+} imaging and 2-photon Na^{+} imaging. GABA evoked Ca^{2+} transients and Na^{+} transients in astrocytes that persisted in the presence of $GABA_A$ and $GABA_B$ receptor antagonists, but were greatly reduced by inhibition of GABA uptake by SNAP 5114. GABA uptake-mediated Na^{+} rises might reduce Na^{+}/Ca^{2+} exchange, thereby leading to intracellular Ca^{2+} transients. To test the effect of reduced Na^{+}/Ca^{2+} exchange on Ca^{2+} signaling, we used the Na^{+}/Ca^{2+} exchange inhibitor KB-R7943. Application of KB-R7943 mimicked GABA-induced Ca^{2+} signaling. Withdrawal of external Ca^{2+} entirely suppressed GABA-induced Ca^{2+} transients, and depletion of intracellular Ca^{2+} stores with cyclopiazonic acid reduced the Ca^{2+} transients by approximately 90%. This indicates that the Ca^{2+} transients depend on external Ca^{2+}, but are mainly mediated by intracellular Ca^{2+} release, in line with Ca^{2+}-induced Ca^{2+} release. Neither activation nor inhibition of ryanodine receptors affected basal Ca^{2+} or GABA-induced Ca^{2+} transients, whereas the $InsP_3$ receptor blocker 2-APB inhibited the Ca^{2+} transients. The results suggest a novel mechanism of GABAergic signaling, composed of GABA uptake-mediated Na^{+} rises that reduce Na^{+}/Ca^{2+} exchange efficacy, thereby leading to a small Ca^{2+} increase sufficient to trigger Ca^{2+}-induced Ca^{2+} release via $InsP_3$ receptors.

Vesicular release of glutamate and ATP along axons accounts for neuron-glia communication in the mouse olfactory bulb

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Interactions between neurons and glial cells are crucial not only for pathfinding axons, but also for the formation of proper synaptic connections and networks. Different pathways for communication between neuronal and glial processes are known, a rather unexplored one is the extrasynaptic vesicular release of transmitter by axons eliciting receptor-mediated activation of glial cells.

We examined the nature of interaction between olfactory receptor neuron axons and a specialized glial cell type, termed olfactory ensheathing cell (OEC), by monitoring calcium signals in OECs of the olfactory bulb in postnatal mice. OECs respond to glutamate and ATP with cytosolic calcium increases mediated by mGluR1 and P2Y1 receptors. Electrical stimulation of ensheathed axons evoked calcium increases in OECs that could be inhibited by blockers of these two receptor subtypes, suggesting glutamate and ATP release along olfactory receptor axons. Vesicle-associated proteins such as VGLUT2 and synaptophysin were identified in olfactory receptor axons by means of antibody stainings. Electrical stimulation of olfactory receptor axons expressing the vesicle fusion marker synapto-pHluorin resulted in a calcium-dependent increase in synapto-pHluorin fluorescence, indicating the fusion of synaptic vesicles along the axons. In addition, suppressing vesicular release mechanisms with bafilomycin A1 and botolinum toxin also abolished stimulation-induced calcium transients in OECs. Ultrastructural studies of the olfactory nerve indicated the presence of vesicles within axons adjacent to OECs. Hence, our results suggest a pathway, in which axonal activity leads to vesicular release of both glutamate and ATP and thereby triggers calcium signals in OECs via P2Y1 and mGluR1 receptors. The expression of the receptors, as studied by calcium imaging and antibody staining, seems to be regulated developmentally in a manner that during initial formation of the olfactory map the expression of the receptors is strong and decreases to a lower level in the adult olfactory system.

The 473 epitope influences axon growth and survival of cultured embryonic motoneurons

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Mechanisms controlling neuronal survival and regeneration play an important role during development, after birth and under lesion conditions. Isolated embryonic mouse motoneurons (E13) have been a useful tool for studying such basic mechanisms. These embryonic motoneurons in culture depend on extracellular Matrix (ECM) molecules which potent mediators of survival, axonal growth and guidance in the CNS and in vitro, exhibiting either attractive or repellent guidance cues. ECM proteoglycans and glycoproteins were identified as components of the glial scar acting as a growth barrier for regenerating axons. We used cultured embryonic mouse motoneurons to investigate axon growth effects of glial derived matrix molecules in culture. Matrix produced by the glial derived cell lines A7, Neu7 and oli-neu, primary astrocytes as well as the immortalized Schwann cell line IMS32 was used as a substrate for primary motoneuron cultures. The results indicate that the DSD-1 epitope, present on chondroitin sulfate proteoglycans plays an important role as axon growth promoting cue.

Deletion of aquaporin-4 induces osmotic swelling in retinal Müller cells

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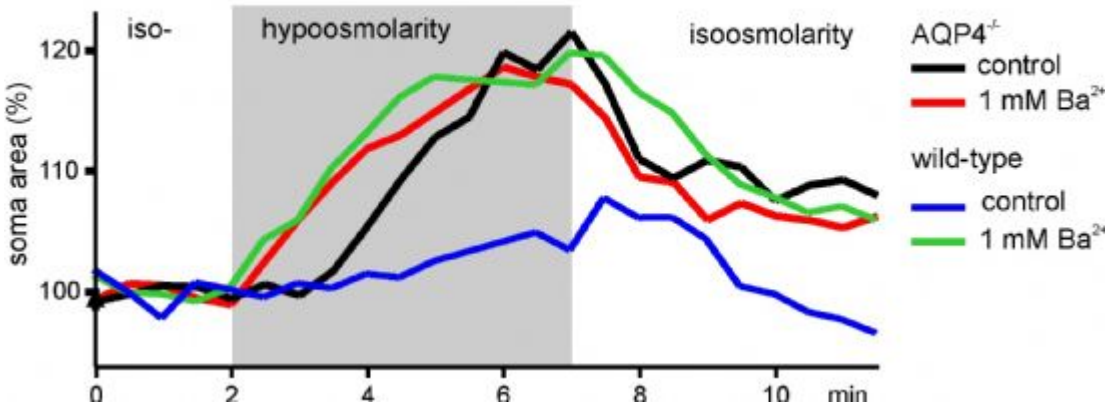
Retinal Müller glial cells control the osmotic and ionic homeostasis of the retina mainly by transcellular K^+ and water fluxes which are facilitated by weakly inwardly rectifying Kir4.1 channels and aquaporin-4 (AQP4) water channels. In addition to the spatial buffering of K^+ ions, the volume regulation of the extracellular space is a critical parameter in the regulation of the neuronal activity. Kir channel-mediated K^+ currents are supposed to be associated with water movements due to osmotic reasons. Morphological studies revealed that Kir4.1 and AQP4 channel proteins are normally colocalized in distinct membrane domains, mainly at glial processes enwrapping blood vessels and in the endfoot membrane contacting the vitreous body. Phenotype studies of AQP4-deficient ($AQP4^{-/-}$) mice resulted in the hypothesis that AQP4 and Kir4.1 in Müller cells interact functionally. However, a recent study (Ruiz-Ederra et al., 2007, J Biol Chem 282:21866) demonstrated that Kir channel function remains unaltered in $AQP4^{-/-}$ mice. The aim of the present study was to determine whether a deficiency in AQP4 is associated with alterations in the swelling properties of Müller cells and, thus, whether AQP4 is implicated in the homeostasis of the cellular volume under varying osmotic conditions. To investigate the ability of Müller cells for volume regulation, we incubate retinal slices with the fluorescent dye Mitotracker Orange which is selectively taken up by Müller cells. Thus, the size of stained Müller cell somata can be observed microscopically. When the extracellular osmolarity is reduced to 60%, Müller cells in control tissue keep the volume of their cell bodies constant. Blocking Kir currents with Ba^{2+} ions causes a swelling of Müller cell somata in the hypotonic solution. $AQP4^{-/-}$ mice in a CD1 background were used for immunohistochemical stainings, patch-clamp recordings and swelling experiments. Deletion of AQP4 does not alter the retinal distribution of Kir4.1 and AQP1 immunoreactivities. Whole-cell recordings of isolated Müller cells displayed no significant differences in membrane currents, potential and capacitance between Müller cells from $AQP4^{-/-}$ and wild-type mice, suggesting that lack of AQP4 does not modify electrophysiological properties of Müller cells. Application of a hypotonic solution caused an increase in the volume of Müller cell somata from $AQP4^{-/-}$ mice. As expected, cells from wild-type animals did not swell under the same conditions. In the presence of Ba^{2+}, a swelling could be induced in both groups. The swelling of Müller cells from $AQP4^{-/-}$ mice could be significantly reduced by several pharmacological means: the selective inhibitor of PLA_2 activation, 4-bromophenacyl bromide, the cyclooxygenase inhibitor, indomethacin, the reducing agent, dithiothreitol, and by replacing extracellular Na^+ ions. Moreover, activation of metabotropic glutamate, $P2Y_1$ and adenosine A1 receptors inhibited Müller cell swelling by activation of a complex signalling cascade. The data indicate that glial cells in retinae of $AQP4^{-/-}$ animals are more sensitive to osmotic stress than glial cells in wild-type retinae. Müller cells of $AQP4^{-/-}$ animals are restricted in their ability to regulate their volume in response to alterations in the transmembrane osmotic gradient.

Time-dependent alteration of the soma area of Müller cells in retinal slices under hypotonic conditions



Acute osmotic swelling of retinal glial (Müller) cells evoked by glutamine - implications for hepatic retinopathy

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Müller glial cells play a crucial role in the regulation of ion and water homeostasis in the retina. Müller cells are the only type of retinal cells which are capable to detoxify ammonium due the activity of the enzyme glutamine synthetase which is specifically expressed in Müller cells and catalyses the reaction from glutamate and ammonium to glutamine. In hepatic retinopathy, a disease of patients with chronic liver diseases associated with elevated blood ammonia levels, Müller cells become gliotic and display swollen cell nuclei. This may suggest a disturbance of Müller cell-mediated ion and water regulation, and may contribute to the development of retinal edema which further impairs retinal function. The aim of the present study was to determine whether increased extracellular glutamine levels (which are present in neural tissues of patients with hepatic failure because of the activity of the glutamine synthetase) may alter the volume regulation of Müller cells. We recorded the alterations in the size of Müller cell somata during superfusion of acutely isolated slices of the rat retina with a hypoosmolar solution (60% of control osmolarity). Hypotonic extracellular solution mimics the osmotic gradient between the extra- and intracellular milieu which occurs in pathologically altered retinas. Müller cells in slices of control retinas did not display alterations in the size of their somata under osmotic stress conditions. Perfusion with extracellular glutamine (0,01-10 mM) in a hypotonic solution resulted in a swelling of Müller cell bodies in a concentration-dependent manner. Administration of inhibitors of inflammatory mediators, inhibitors of oxidative/nitrosative stress, as well as sodium-free extracellular solution, reduced significantly the swelling of Müller cells. The results suggest that an increase in the level of glutamine as occurring in hepatic retinopathy may induce the formation of inflammatory factors under osmotic stress conditions. Inflammatory mediators evoke oxidative/nitrosative stress as a major factor of the development of cytotoxic edema of Müller cells.

Post mortem activity of microglia in the mouse spinal cord

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Microglia (MG) are the macrophages of the CNS. In the unaffected nervous tissue they show distinct ramification and a high motility of their extensions, thus, continuously surveying the integrity of their microenvironment. Upon local CNS injury processes of the surrounding microglia immediately (within min) turn to the direction of the lesion. Subsequently (after 60 to 90 min), degenerating axons are engulfed and digested by phagocytosing MG. In addition, numerous MG of the surrounding neuropile migrate towards the injury site and clear the cellular debris.

Since MG are also active in hypoxic, stroke tissue, we investigated how long MG can stay functional without blood supply, i.e. for how long keep MG their responsiveness to injuries within dead neuronal tissue.

We used time-lapse 2-photon laser-scanning microscopy (2P-LSM) to study microglial reactions in the spinal cord of double-transgenic mice (CX3CR1-EGFP/Thy1-EYFP) mice with green fluorescent MG and yellow fluorescent neurones. Spontaneous MG activity and the MG responsiveness to injury was investigated first *in situ in vivo* (controls) and then *post mortem* (*p.m.*), i.e. after cardiac and respiratory arrest in the progressively degrading nervous tissue.

During the first hour *p.m*. spontaneous MG activity and early Mg reaction to laser-evoked local injuries were almost unchanged. However, axons started developing irregular swellings. Two to three hours *p.m*. the movement of MG processes towards laser lesions still existed, but less pronounced. Some MG cells even retracted their processes again. The axons showed increased fragmentation. Four to five hours *p.m*. MG still respond, but only weakly to a lesion. Axons and surrounding neuropile display increasing deterioration. After 5 hours *p.m*. MG lost their responsiveness, but some spontaneous activity of the MG processes remained.

These observations demonstrate that MG can survive long lasting anoxia and metabolic undersupply for several hours. We hypothesize that the *p.m.* degradation of the nervous tissue induces a progressive and uniform distribution of MG chemoattractants such as ATP or NO. Since chemical gradients are necessary for directed MG responses, MG lose their response to local injuries and transform into phagocytozing MG with ameboid-like shape. However, for quite some time *p.m.* freshly induced lesions are obviously still able to produce a certain NO or ATP gradient which allows at least for an initial chemoattractive MG reaction.

Transport and metabolism of fluorescent glucose in cerebellar slices

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Glucose is an essential energy requirement to maintain brain metabolism. It is believed that the supply of energy is adjusted to energy demand in the brain, and that different cell types may have their individual glucose handling. In a previous study, the uptake of glucose was studied in real-time using fluorescent tracers and confocal microscopy in brain cells in culture[1]. Here, we have adapted this technique to acute slices prepared from rat cerebellum studied with multiphoton microscopy. The transport of the fluorescent glucose analogs (N-7-nitrobenz-2-oxa-1, 3-diazol-4-yl-amino)-deoxy-D-glucose, 2-NBDG and 6-NBDG, was 3-4 fold faster in the molecular layer of the cerebellar cortex than in layer of Purkinje cell somata and of granule cells. After washout of the free dye, the majority of the 2-NBDG appeared to be phosphorylated in Bergmann glia. This was confirmed by counterstaining with sulforhodamine 101, which is preferentially taken up by glial cells. The recovery of fluorescence after locally defined photobleaching showed that phosphorylated 2-NBDG can diffuse horizontally across the molecular layer, presumably through gap junctions between Bergmann glial cells. The dye calcein, which is not taken up by livingcells, served as control for diffusion kinetics of dye in the extracellular spaces and in the experimental chamber. Our results suggest that in acute cerebellar slices, the glucose transport capacity and glycolytic rate of Bergmann glia are considerably higher than those of Purkinje cells. Since the cerebellum, like other parts of the brain, is largely fueled by glucose, and as Purkinje neurons are estimated to spend significantly more energy than Bergmann glia, these results suggest substantial shuttling of energy-rich metabolites, for example lactate and/or pyruvate via monocarboxylate transporters, between glial cells and neurons.

[1]Loaiza, A., Porras, O.H. & Barros, L.F. (2003) *J Neurosci,* **23,** 7337-7342.

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Nucleotide-mediated signaling in sustentacular cells in the olfactory epithelium

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Extracellular nucleotides are important signaling molecules that mediate various biological effects via cell-surface receptors termed purinergic receptors. It has previously been shown that ATP activates sustentacular cells (SCs) of the olfactory epithelium (OE) of larval *Xenopus laevis*. Here we characterized the ATP-induced responses of SCs using functional calcium imaging. We were able to show that ATP elicits intracellular calcium waves in SCs. The waves initiate in the apical part of the SCs and propagate towards their endfeet in the basal part of the OE (wave velocity 17.10 ± 1.02 µm/s). Furthermore, we defined the purinergic receptor subtype(s) involved in the ATP-induced responses. ATP evoked increases in $[Ca^{2+}]_i$ in both the presence and absence of extracellular calcium. Depletion of the intracellular calcium stores abolished the $[Ca^{2+}]_i$ responses. The above results together with the determined order of potency of the used purinergic agonists and antagonists implicate an involvement of $P2Y_2$/$P2Y_4$-like receptors. Thus, our findings suggest that the release of nucleotides in the OE could stimulate a distinctive spatiotemporal pattern of $[Ca^{2+}]_i$ increases in SCs. This allows to speculate about a novel form of intraepithelial communication. The physiological role of purinergic receptors in SCs of larval *Xenopus laevis* remains to be determined, but our findings suggest to view the OE as a tissue and to start to concentrate on possible functional interactions between the different cell types in the OE.

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MITOCHONDRIAL DIVERSITY AND CORRELATED DYm OSCILLATIONS AS REVEALED BY ONE- AND MULTIPHOTON IMAGING

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Mitochondria even on the subcellular level display an enormous diversity of shape, cellular localization and probably also their functional determination. Glial cells of the rat hippocampus contain clearly separated mitochondrial structures allowing a detailed analysis of their functional diversity. The application of the mitochondrial potential (DYm) marker JC-1 and the use an optical image splitter device enabled real time microfluorimetric recordings of the activity of highly (red fluorescing) or less polarized (green fluorescing) mitochondria. Both types of mitochondria were present within a single cell. Mitochondrial density and polarization were found to be higher in the perinuclear region as compared to the cellular periphery. Challenging mitochondria by the application of 1 mM cyanide, 2 mM azide, 1 mM glutamate or 50 mM K^+ showed that the expected shift from red to green fluorescence was quite heterogeneous among the mitochondrial population. Especially perinuclear mitochondria responded with pronounced depolarizations and also recovered more readily. Frequent rhythmic changes in DYm appeared both in single mitochondria or synchronized in clusters consisting of multiple mitochondria. These synchronized DYm oscillations were most obvious in perinuclear mitochondrial clusters, but surprisingly also spatially separated mitochondria showed such synchronized spontaneous DYm oscillations. They persisted upon withdrawal of extracellular Ca^{2+}, but were antagonized by dantrolene. Fluo-3 recordings in Ca^{2+}-free ACSF revealed localized cytosolic Ca^{2+} sparks suggesting Ca^{2+} release from the endoplasmic reticulum to underlie the synchronized DYm oscillations. In conclusion, we obtained evidence for a functional heterogeneity of mitochondria in glial cells, a less vulnerable mitochondrial population around the nucleus as well as spatially confined functional interactions of mitochondria and the endoplasmic reticulum.

Tenascin C Controls Oligodendrocyte Differentiation by Activation of Distinct Signalling Pathways

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Oligodendrocytes are the myelin-forming cells of the central nervous system (CNS). The differentiation of oligodendrocytes evolves in steps from bipolar progenitors to immature oligodendrocytes bearing multiple processes and ends with membrane sheath-bearing mature oligodendrocytes. This process coincides with the upregulation of myelin gene expression. The individual steps of oligodendrocyte lineage progression are tightly regulated by intrinsic and extrinsic factors with regard to proliferation, differentiation and survival. We investigated the impact of extracellular matrix (ECM) components as spatiotemporal cues for oligodendrocyte development. We have previously shown that astrocyte-derived Tenascin C (Tnc) is a major functional component of astrocyte-derived ECM, which inhibits the expression of myelin basic protein (MBP) in oligodendrocytes. Moreover, Tnc presented as a purified glycoprotein also delayed oligodendrocyte differentiation regarding the expression of MBP, but also with respect to process elaboration and the formation of myelin membranes. On the molecular level, we could reveal that Tnc-induced reduction of RhoA activation was accountable for impaired membrane formation, but not for the delay in MBP-expression. Here, we investigated relevant receptors and the signalling mechanisms that are elicited by Tnc and that mediate delayed MBP-expression. In a candidate approach, we investigated whether molecules that are known to be involved in oligodendrocyte differentiation also partake in Tnc-signalling. These candidates include growth factors, membrane receptors and intracellular signalling molecules, which were analyzed in terms of subcellular localization, direct protein-protein interactions, and phosphorylation in response to Tnc.
Our results will provide novel insights into the complexity of cellular environments and may lead to a better understanding of oligodendrocyte behaviour during development and disease.

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The NAD^+/NADH redox state of astrocytes: impact on signal processing and gene expression.

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The metabolism of nicotinamide adenine dinucleotides has recently attracted increasing attention due to its central position in cellular metabolism and signal regulation. Essential metabolic pathways (e.g. glycolysis, Krebs cycle) are coupled to the mitochondrial respiratory chain and thereby to energy production via the NAD^+/NADH-redox state. In the brain, astrocytes are important cells providing metabolic support to neurons as well as crucially contributing to brain signaling. The astrocytic and neuronal energy metabolism is coupled via the lactate shuttle involving the NAD^+/NADH-redox state via the reaction of lactate dehydrogenase in both types of cells. Furthermore, evidence has been presented that the NAD^+/NADH-redox state is also involved in regulation of cellular functions like enzymatic activities and gene expression. Here we show that the NAD^+/NADH redox state is indeed an important regulatory node for astrocytic signaling. Using an enzymatic assay for NAD^+ and NADH as well as taking advantage of the endogenous fluorescence of NAD(P)H we monitored the NAD^+/NADH-redox state of cultured cortical astrocytes after metabolic insults or stimulation by neurotransmitters. In parallel, we analyzed the impact of the redox state on a classical astroglial signaling paradigm, the intercellular calcium-wave. We show that some neurotransmitters like biogenic amines increased NAD(P)H-fluorescence and also accelerated calcium waves. Using pharmacological tools we provide evidence that the increase in NADH observed is directly and causally linked to the acceleration of calcium waves, which is in addition dependent on gap junctional coupling. We conclude that at the NAD^+/NADH redox state metabolic and signaling information is integrated in astrocytes, thereby most likely contributing to fine tuned participation of astrocytes to neuronal activity and functional states of the brain.

Cell adhesion and structural plasticity of astrocytes: focus on vinculin

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The interaction between astrocytes and other brain cells fulfils important functions in physiology, pathophysiology and regeneration of the brain. Astrocytes are essential for the structural and metabolic support of neurons and contribute to signal processing. A prerequisite for this functional interaction are close and tightly controlled structural contacts. Astrocytic processes are not static but rather characterised by dynamic morphological changes. However, the mechanism and function of this structural plasticity remains unclear. The formation and retraction of filopodia and lamellipodia is regulated by a dynamic interplay between cell adhesion proteins and the actin cytoskeleton. To allow co-ordinated interactions between the actin cytoskeleton and the cellular environment, the formation of tightly regulated cell contacts sites, cell-cell or cell-extracellular matrix adhesions (focal adhesions), are required. Among the many proteins that constitute the interface between adhesion receptors and the actin cytoskeleton, vinculin is a key player, which strongly affects the structural and functional link of central adhesion receptors, such as integrins and cadherins. To study vinculin function in astrocytes, we first analysed the composition of contact sites *in vitro* as well as their regulation. In primary cortical astrocytes, vinculin localized to conventional cell-matrix contacts (focal adhesions) and cadherin-positive cell-cell contacts of a distinct morphology. We used time-lapse video microscopy to characterise the dynamics of astroglial cells. The different modes of actin-based motility we observed in culture were similar to our results in acutely isolated brain slices. Expression of a vinculin mutant deficient in lipid-binding reduced the spontaneous motility of cultured astrocytes, indicating an involvement of vinculin in the control of astroglial motility. Furthermore, we analysed the localisation of vinculin in mouse brain slices by immunohistochemistry and confocal microscopy and visualized vinculin in the astrocytic soma as well as in the processes, where it revealed a punctuate pattern reminiscent of focal adhesions. Astrocytic motility *in situ* was investigated by using two-photon laser scanning microscopy to image astrocytes and neurons in acutely isolated brain slices prepared from transgenic TgN(hGFAP-EGFP) or TgN(Thy1.2-EYFP)-mice confirming previous results that astrocytes *in situ* form and retract filopodial and lamellipodial structures. To address the underlying mechanisms of structural plasticity we finally generated an astrocyte-specific conditional and inducible knockout of vinculin. This mouse model allows a spatial (astrocyte-specific) and a temporal (time of injection of the inducer tamoxifen) control of DNA-recombination, and thereby deletion of vinculin. First analyses indicate that in this mouse line, vinculin can be successfully deleted in astrocytes, allowing us to investigate the contribution of vinculin regulation to the control of astroglial morphology and motility *in situ*.

Activation of Microglia in the Retina evoked by varied Stimuli

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Microglia are the resident immune cells of the nervous system. Within the retina, resting microglia cells are located in the inner retinal layers. In virtually every retinal disease microglia are activated and take then an active part in the pathological process. In spite of first models for examination of microglial functions, the interaction of microglia in retinal diseases is not yet well understood. To optimize therapeutic interventions a better knowledge of microglia functionality and regulation is required in pathologically altered retina as well as in healthy tissue. One aim of the present study was the characterisation of microglia activation after partial surgical vitrectomy in the rabbit eye. Furthermore, a viable model to characterize and influence microglial activation in the guinea pig eye via pharmacological intervention was established.
During partial vitrectomy of the rabbit eye a part of the vitreous body was replaced by balanced salt solution (BSS) or sodium hyaluronate. The retinae were fixed after 2 or 7 days and stained with the microglia marker *Griffonia-simplicifolia*-agglutinin (GSA) and the proliferation marker *anti*-Ki67. The total number and the number of Ki67-positive microglia cells were significantly increased two days after partial vitrectomy compared to control. After one week the proliferation decreased strongly, only few (BSS) or no (sodium hyaluronate) cells were still positive for Ki67 in retinae from vitrectomized eyes. The total number of microglia cells was similar to that in control retinae one week after treatment. Thus, the surgical procedure itself seems to induce solely a transient activation of microglial cells.
To further investigate and characterize microglial reaction, intravitreal application of LPS was used as activation stimulus. We aimed at a selective manipulation of microglia using a microglia-specific immunotoxin. The immunotoxin has been shown to selectively induce apoptosis in microglia cells *in vitro*. At varying time points after injection the retinae were fixed and stained with the microglia markers, GSA and Iba 1. Three days after the injection of LPS the number of microglia cells doubled whereas after seven days the number of cells was increased threefold. After 14 days the number of microglial cells decreased considerably. It can be concluded that the LPS-induced microglial activation is also a transient process. The combined injection of LPS with an anti-mitosis-cocktail caused a decreased number of activated microglia compared to LPS injection after seven days. Thus, microglia reaction can be influenced pharmacologically. Intravitreal application of the immunotoxin induced a strong microglial reaction and atypical morphological alterations which may refer to dystrophic changes.
Both *in vivo*-models are suitable to investigate processes of microglia activation and proliferation and to study the influence of microglia cells on neuronal degeneration. The LPS-model offers a valuable possibility to characterize the effect of pharmacological interventions. The immunotoxin might be a useful tool for inducing artificial, dystrophic modifications in microglial functionality.

Pre-ischemic, but not post-ischemic Nogo-A deactivation aggravates neuronal injury after middle cerebral artery occlusion in mice: Implication of Rac1 and RhoA pathways

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Nogo-A is an oligodendroglial neurite outgrowth inhibitor, the deactivation of which enhances brain plasticity and functional recovery in animal models of ischemic stroke and spinal cord trauma. As such, clinical trials are presently on the way evaluating neutralizing Nogo-A antibodies in human stroke patients. In view that previous studies were performed in permanent stroke models, we here examined the effect of Nogo-A deactivation after transient focal cerebral ischemia, induced by 30 min middle cerebral artery (MCA) occlusion. In Nogo-$A^{-/-}$ mice we show that abrogation of this growth inhibitor goes along with an elevated mortality, exacerbated motor deficits and increased neuronal injury particularly in the ischemia-vulnerable striatum. Deactivation of Nogo-A with a neutralizing antibody (11C7) that was infused into the lateral ventricle or ischemic striatum increased neuronal injury, but not neurological deficits, when Nogo-A blockade was initiated 24 hours prior to MCA occlusion, but not after the stroke. Nogo-A inhibits neurite outgrowth mainly by activating RhoA and deactivating Rac1, two members of Rho GTPases family. The main molecular function of the (11C7) antibody is inhibiting RhoA/Rho-Kinase (ROCK) signalling pathway permitting neurite outgrowth. We investigated Rac1 activation using Pull-down assays, and we found out Rac1 was excessively activated in Nogo-$A^{-/-}$ mice and in mice when Nogo-A blockade was initiated 24 hours prior to MCA occlusion, this activation was followed by an activation of stress signals (p38MAPK and SAPK/JNK). Moreover, decreased ROCK activity led to an increase in PTEN activity, which negatively regulates Akt/PKB and ERK1/2 survival pathways and stabilising p53 protein.

Our findings showed that early deactivation of RhoA creates an intracellular stress conditions in the acute phase of stroke, and even before, which activate many signalling pathways involved in enhancing neuronal death, and contributing in aggravating injury.

Firstly we conclude that Nogo-A could have a physiological role in maintaining at some level cell survival.

And secondly, clinical trials should be aware of potential injury effects of growth-promoting strategies, and more investigations might be needed to understand the molecular mechanisms of Nogo-A activity. Thus, Nogo-A antibodies should not be used in the very acute stroke phase.

Investigating the expression and function of CPEB proteins in astrocytes

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Cytoplasmic polyadenylation element binding proteins (CPEBs) are a family of translational regulators expressed in brain. They bind to mRNAs which contain the cytoplasmic polyadenylation element (CPE) and control protein synthesis. Using transgenic mice expressing a dominant negative CPEB in forebrain neurons we previously showed a role for CPEBs in synaptic plasticity, learning and memory. We now ask the question whether CPEBs arerelevant also in astroglia. CPEBs (2, 3 and 4) were found to be expressed in distinct astroglial subpopulations of mouse hippocampus, by immunofluorescence, single cell RT-PCR and nonradioactive in-situ hybridisation (ISH). Their expression varied with respect to different astroglial subpopulations (GluT and GluR cells) of the mouse hippocampus. In addition, all these CPEB transcripts (2, 3 and 4) were found to be expressed in rat primary astrocyte cultures. The presence of CPEBs in astrocytes could be explained by their possible translational regulation of different mRNAs present in astroglial cells, such as GLT1 and connexins (Cx43 and Cx30). By reporter gene assays in cultured astrocytes, we are testing this hypothesis. To study the role of CPEBs in astrocytes, we are generating transgenic mice expressing CPEB3 and a dominant negative CPEB in astrocytes under GFAP promoter control (tetO-CPEB3EGFP; GFAP-tTA and tetO-DNCPEB; GFAP-tTA mice, respectively).

Comparison of Cx43 knock-in reporter mice to investigate translational regulation of Cx43 in the central nervous system

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The 3' untranslated region of connexin43 (Cx43), the major astrocytic connexin in the mouse hippocampus, contains binding sites for microRNAs miR-1 and miR-206. In addition, bioinformatics indicate the presence of binding sites for Cytoplasmic Polyadenylation Element Binding Proteins (CPEBs), translational regulators expressed in mouse brain. Two different lines of transgenic mice are available which express reporter genes instead of Cx43: Cx43del mice express lacZ instead of Cx43 and the corresponding mRNA lacks the endogenous 3' UTR with its microRNA binding sites (MRBs) and CPEB recognition sequences (CPEs). Therefore, translational regulation of lacZ derived mRNA should not occur via these mechanisms. By contrast, Cx43ki-ECFP mice express the Enhanced Cyan Fluorescent Protein (ECFP) instead of Cx43 and the corresponding mRNA contains the endogenous 3' untranslated region with its MRBs and CPEs and thus translational regulation might be operational for the ECFP reporter. We performed a detailed comparative analysis of reporter gene expression in the two mouse lines by double immunofluorescence staining for reporter proteins and cell type specific marker proteins (GFAP, S100beta, NG2, CNPase, endothelial marker proteins and microglial markers). Our rationale was that any discrepancy between lacZ expression and ECFP expression might be indicative of translational regulation.
Preliminary findings show expression of lacZ, but not of ECFP, in endothelial cells. Quantitative analyses for other cell types are underway.

How does proteoglycan deficiency affect the mouse brain?

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Chondroitin sulfate proteoglycans like brevican and neurocan are important constituents of brain extracellular matrix. Together with link proteins, tenascins and hyaluronic acid they form a macromolecular meshwork of perineuronal nets, which surround and insulate nerve cells and synaptic contact sites. Neurocan is expressed already during early mouse brain development with a peak around birth, but brevican expression increases during postnatal brain maturation. Investigations with knock out mice deficient for one or both proteins show multiple changes. These mice are viable, fertile, and have no obvious deficits in general performance. But they exhibit aberrant properties in hippocampus morphology, amount and distribution of the extracellular matrix components, long-term depression, in vivo apparent diffusion coefficient and diffusion tensor imaging. For example the loss of neurocan leads to a dysplastic phenotype of the CA1 pyramidal cell layer in the early development of single and double knock out mice. These data point to a complex role of extracellular matrix proteoglycans for structural, biophysical and plastic properties of the brain.

ECM and Synaptogenesis: Monitoring the Impact of Extracellular Matrix on Synapse Formation in Hippocampal Neurons

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Synapses are specialized cell-cell contact sites between nerve cells. These structures are surrounded by glial cells and mediate a rapid efficient transmission of signals between neurons. Previous investigations have shown that astrocytes and astrocyte-derived extracellular matrix components are important for formation, maintenance and function of synapses in the CNS. To examine the influence of soluble astroglial ECM-components on synapse formation we established a transwell co-culture assay for E18 rat hippocampal neurons and glial cells. Furthermore we developed a technique that permits the semi-automated quantitative determination of the number of colocalized pre- and postsynaptic proteins per neuron (synaptic puncta). We could show that astrocytes support the survival of embryonic hippocampal neurons and the formation of structurally intact synapses, as documented by the co-localisation of bassoon- and proSAP1-positive puncta. The development of synapses was paralleled by the emergence of perineuronal net (PNN) like structures that contained the 473HD-epitope, the ECM molecules brevican tenascin-C and hyaluronic acid. Our present studies focus on maturation of synapses in the presence or absence of enzymes, that degrade ECM components like hyaluronic acid or chondroitinase ABC. Hyaluronic acid as well as the chondroitin-sulfate proteoglycans are highly expressed in brain tissue and therefore likely to be also effector molecules with regard to synapse formation.

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Astrocytes communicate with the calyx of Held synapse

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The calyx of Held (CoH) synapse is a giant nerve terminal that serves as a model system to analyze basic mechanisms of synaptic transmission. Astrocyte processes are in close contact to both, pre and postsynaptic membranes as revealed by electonmicroscopic analysis of identified astrocytic compartments. To study mechanisms of neuron glia interaction in the medial nucleus of the trapezoid body, we recorded from astrocytes and principal neurons in acute slices from 8-10 day old mice. We observed spontaneous slow inward currents in the principal neurons with low frequency which are distinct in kinetics from spontaneoeous postsynaptic events. These currents were sensitive to the NMDA receptor antagonist D-APV + MK-801 and were abolished when a closely aposed astrocyte was dialyzed with BAPTA via the patch pipette. We assume that we interfere with Ca^{2+} signalling in the astrocytic network since dye loading revealed that the astrocytes in the medial nucleus of the trapezoid body form a syncytium. In turn, neuronal activity also triggered responses in astrocytes: repetitive electrical stimulation of the midline, a paradigm to stimulate the calyx, triggers a frequency dependent slow inward current in the astrocytes. Thus, there are distinct pathways of communication between astrocytes and the calyx synapse.

Cocultures of rodent olfactory ensheathing cells (OEC) and olfactory receptor neurons (ORN): synthesis of ciliary neurotrophic factor (CNTF) and neurite growth

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ORN are the only neurons situated in a surface epithelium and are thus directly exposed to the environment. Toxic or infectious insults can lead to gradual or massive ORN degeneration. A loss of odor perception is prevented by continuous generation of new ORN from neuronal precursors in the olfactory epithelium. Immature ORN extend their axons through the fila olfactoria into the olfactory bulb. OEC are specialized glial cells of the fila which have been shown to promote axonal outgrowth in vivo and in vitro. Production of neurotrophic factors in OEC is thought to be influential in the axon growth promoting properties of OEC. CNTF is constitutively highly expressed in OEC in vivo. We have devised an ORN/OEC coculture system to study the role of CNTF for neuronal regeneration in vitro.

In previous investigations, we found that OEC isolated from both neonatal rats and wildtype mice and cultured according to established, slightly modified methods, showed increasing homogeneity as documented by the expression of OEC markers (p75 neurotrophin receptor, S100ß, GFAP), but ceased to produce CNTF with prolongued culture duration. Coculturing of late passage (=3) OEC with neurons increased the percentage of CNTF-immunoreactive(ir) OEC ~1.7fold (rats) and ~1.9fold (mice) if neurons were grown in direct contact with the OEC. For this study, ORN and OEC were isolated from neonatal rats, and wildtype and CNTF-deficient mice, and ORN were cultured over night on passage 3 OEC. All ORN displayed typical bipolar morphology and immunoreactivity for neuron specific tubulin, but were essentially negative for olfactory marker protein, a protein produced in mature ORN. CNTF immunoreactivity (ir) was not observed in rat or wildtype mouse ORN in cocultures. Coculturing of ORN from $CNTF^{-/-}$ mice on wildtype OEC led to a similar increase in the percentage of CNTF-ir OEC as observed for wildtype ORN/OEC cocultures (~1.8fold). ORN from both wildtype and CNTF-deficient mice displayed significantly enhanced neurite growth if cultured on CNTF-deficient compared to wildtype OEC. The increases in the length of the longest neurite and in the total length of all neurites amounted to approximately 30-40%, regardless of ORN genotype. Preliminary results of experiments in which CNTF antibodies were applied to rat ORN/OEC cocultures showed slight but recognizable increases in neurite length after this treatment.

The findings show that, unexpectedly, coculturing of ORN on CNTF-producing OEC leads to reduced neurite growth compared to coculturing on CNTF-deficient OEC. Preliminary results indicate that the effect may be mediated by CNTF in the medium. Further analyses are needed to examine the mechanisms underlying the observed effects.

Activity-dependent currents recorded from astrocytes in the respiratory network

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Breathing requires a complex pattern of neuronal activity resulting in alternating contraction and relaxation of respiratory muscles. An important part of the respiratory network is located in the pre-Bötzinger complex (preBötC), which can be functionally isolated in a slice preparation. In this "breathing" slice the respiratory neurons continue to generate respiratory burst activity. While activity of respiratory neurons has been analyzed intensively, the discovery of specific functions of glial cells, e.g. astrocytes, in the respiratory network is still at its beginnings. In the preBötC the majority of astrocytes show a high potassium conductance, this indicates that they importantly contribute to potassium buffering. Furthermore astrocytes express neurotransmitter transporters. Here we analyzed the behavior of astrocytes in the preBötC during ongoing respiratory activity.

We performed whole cell recordings from identified eGFP-labeled astrocytes in the preBötC using TgN(hGFAP-eGFP) mice. Approximately 10 % of recorded astrocytes showed some degree of inward current (I_{resp}) in phase with the preBötC field potential bursts.

After blockade of synaptic inhibition by strychnine (10 µM) and bicuculline (10 µM) the amplitude of I_{resp} increased. Furthermore, the inward current did not reverse within a membrane potential range from -80 to +20 mV, indicating that I_{resp} is not mediated by ionotropic transmitter receptors. Additional application of the glutamate uptake inhibitor L-*threo*-beta-hydroxyaspartate acid (THA) reduced the amplitude of I_{resp}. These data suggest that glutamate transporter mediated glutamate uptake occurs during ongoing respiratory activity. However, we can not exclude that a major part of the measured current is due to extracellular accumulation of potassium.

Functional ex vivo analysis of mouse microglia reveals developmental profiles in responses to Toll-like receptor (TLR) stimulation from birth to adulthood

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Microglial cells, like other macrophages, can commit to diverse reactive phenotypes in order to adapt and alter responses to challenges and environmental conditions. Their activities may also change during development and CNS tissue maturation. We established a technique to isolate microglia from mouse brain at various time points into adulthood and to keep cells in culture for one month. Cells were analyzed for functional properties such as induction of cytokines and chemokines in response to stimulation of TLR1/2, TLR4 and TLR6/2 with microbial and putative endogenous agonists. While the functional readouts of the cultures remained stable during the time in vitro, marked differences were observed between the time points (days after birth) at which cells were prepared from the brains. Profiles of functional properties over the postnatal period (compared to the situation of cells prepared at birth, P0) revealed, for example, drastic but temporary decreases or increases in inducible amounts of the proinflammatory cytokine TNFa and neutrophil-attracting chemokine CXCL1. Similar profiles were obtained with microglia of Iba1-GFP-transgenic and C57BL/6 wildtype mice. The characteristic time courses suggest that microglial properties were not simply overwritten by the culture conditions. The courses rather indicate dramatic reorganization of the TLR expression/signalling in microglia as the surrounding tissue matures by shaping its neuronal circuitry and forming myelin sheets. Data are presented and alternatively discussed as to systemic versus CNS-specific factors and mechanisms which could instruct and underlie the microglial behaviour. Applications to the study of regional differences and intrapopulational heterogeneity of microglia are shown as well. Taken together, this method could complement in vivo and acute ex vivo studies by allowing for cell-specific experimental manipulations and response monitoring. Supported by the DFG (SFB/TRR43).

Contribution of extracellular matrix (ECM) molecules to synaptogenesis and synaptic plasticity: studies in the quadruple knock out mice

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The central nervous extracellular matrix (ECM) is known to have strong impact on developmental processes such as migration, proliferation and differentiation of neural precursors and axon growth and guidance. Furthermore, evidence has been provided that the ECM is also involved in synaptogenesis, maintenance of the synapse and synaptic plasticity. This effect is probably mediated by astrocytes which enwrap the site of synaptic contact, giving rise to a tripartite synapse. To investigate the impact of the ECM in developmental processes, we investigate the quadruple knock-out mice, that lacks the four ECM components tenascin C, tenascin R, brevican and neurocan. To assess the influence of astrocyte derived ECM molecules, we have established an indirect neuron astrocyte co-culture assay where cells from mice hippocampus can benefit from astrocyte-derived soluble factors. The advantage of the assay is that cells from wildtype (wt) and knock-out (ko) mice can be co-cultivated in various combinations. The cells can be cultured for at least 21 days and synaptogenesis can be quantified via a semi-automatic immuncytochemistry-based read out. Beside the development of CNS synapses we paid particular attention to the development of cortical neurons in the knock-out mice, to the distribution of inhibitory interneurons and to the structure and development of the barrel cortex. With regard to synaptogenesis and synaptic plasticity, the indirect co-culture system was used to monitor the effects of distinct treatments, e.g. to investigate the influence of typical and atypical antipsychotic drugs.

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Quantitative proteomic analysis of astrocytic secretion

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High-resolution quantitative mass spectrometry was used to analyse in vitro secretion of rat hippocampal astrocytes. The supernatants of astrocytes labelled with stable 'heavy' lysine and arginine isotopes (SILAC) could be quantitatively compared to supernatants from 'light' or 'medium' labelled astrocytes under various conditions. In addition, SILAC labelled proteins could be distinguished from unlabeled proteins in the culture medium/serum. We first assessed the relative extra– to intracellular abundance of more than 2000 proteins and re-analysed these proteins with the web-based tools SignalP and SecretomeP for in silico identification of secreted proteins. With these tools, several hundred proteins produced positive hits and most of them displayed high extra-to intracellular ratios as expected. Control experiments with either BrefeldinA or low temperatures to block secretion confirmed our results. Functional, quantitative analysis of secretion in the absence or presence of physiologically relevant hippocampal neurons compared to 'foreign' cerebellar granule neurons provided insight to astrocyte-derived extracellular guidance cues for neurites and migrating neurons. We found several proteins whose extracellular abundance specifically increased in the presence of hippocampal neurons while decreasing with granule neurons and vive versa. This comprehensive 'secretome' data set should aid the understanding of the astrocytic involvement in brain development, regeneration, and neurodegenerative diseases.

Functional interaction of trout myelin protein heterologously expressed in a mammalian oligodendroglial cell line

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Myelin is a multilamellar, spirally wrapped extension of the glial plasma membrane that is essential for fast impulse conduction along axons. It is produced by two specialized types of glial cells, oligodendrocytes in the central nervous system (CNS) and Schwann cells in the peripheral nervous system (PNS). The structure and molecular composition of myelin is unique. In contrast to most plasma membranes, myelin is highly enriched in lipids with 70% of dry weight and exhibits a restricted array of proteins, most of which are exclusively found in myelin (Baumann and Pham-Dinh, 2000; Kramer et al., 2001). Myelin proteins provide the molecular basis for the extremely high adhesion forces between adjacent membrane lamellae. In all vertebrates, the major myelin proteins fall into two categories, on the one side a set of positively charged low-molecular weight proteins called myelin basic proteins (MBP) which are believed to provide adhesion of myelin membrane leaflets at the cytoplasmatic interface (Shine et al., 1992). Furthermore the highly hydrophobic tetraspan proteolipid protein (PLP) accomplished the extracellular membrane apposition in the CNS and the glycoprotein myelin protein zero (P_0) in the PNS. In the CNS of phylogenetically older species such as bony fish a remarkable divergent protein composition is observed (Waehneldt et al., 1986), in which two P_0-like glycoproteins (IP1 and IP2) act as major extracellular adhesion molecules substituting PLP (Lanwert and Jeserich, 2001). Apart from MBP in the cytoplasmatic leaflets, an additional major protein component is encountered in bony fish myelin, termed 36K after its apparent molecular weight (Waehneldt et al., 1984).

For a better understanding of the molecular events underlying the myelination process an integrative approach considering mutual interaction of the major myelin protein constituents is indispensable. Heterologous expression studies provide an effective instrument for investigating the functional properties and trafficking of myelin proteins in living cells. Different fluorescence reporter constructs of trout myelin proteins were expressed in a rat oligodendroglial cell line (OLN-93, Richter-Landsberg and Heinrich 1996) which is expected to provide a more natural cellular environment. These constructs were comparatively analyzed by confocal laser scanning microscopy. They exhibited a diverse intracellular trafficking and membrane targeting within these cells. In case of 36K and MBP a striking reorganization of cellular structures occurs. Additionally, these fusion proteins colocalized with the actin cytoskeleton on the edge of the membrane sheet. Thus, 36K and MBP may be involved in binding actin to the membrane at these sites where membrane sheets extension occurs. This colocalization raises the question if there is a direct interaction of 36K and MBP with actin or is there an influence of factors for example small G-proteins which are involved in the reorganisation of cells or the actin cytoskeleton. These first hints can be verified in future by inhibitor studies and biochemical approaches. Potential interactions between the various myelin proteins are currently studied by co-transfection experiments. Preliminary results indicate a potential interaction of IP2 and MBP. Further transfection experiments with the other myelin proteins and co-immunoprecipitation studies are being pursued.

Impaired Adult Neurogenesis in Mice with reduced Cx43 Expression

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Background and purpose: Connexin43 (Cx43) is the main gap junction protein followed by Cx30. Besides their common function in astrocyte coupling, gap junctions have been further revealed to be important for cell proliferation, migration and differentiation during CNS development. A diminished Cx43 expression in cell culture models simulating developmental processes caused a reorganization of purinergic receptors accompanied with an impaired propagation of intercellular calcium waves. Furthermore cell proliferation, migration and differentiation were shown to be altered. Neurogenesis is an ongoing process in selected regions of the adult brain. The aim of the study was to determine if reduced astrocytic gap junctional coupling, as a consequence of loss of Cx43 and Cx30, influences the expression of P2Y1 and P2Y2 and thereby impairs proliferation of neural progenitor cells in the subgranular zone (SGZ) and granule cell layer (GCL) of the adult hippocampus. **Methods:** Adult mice with a conditional knock out of Cx43 driven by Cre expression under the human GFAP promoter (Cx43KO; Theis et al., 2003, The Journal of Neuroscience 23(3):766-776) and mice with complete disruption of astrocytic coupling created by crossing conditional Cx43-deficient mice with conventional Cx30 knockout (DKO) were investigated using qPCR, western blot and immunohistochemistry. C57/Bl6 and Cx43fl/fl mice served as controls. **Results:** All genetic modified mice (Cx43fl/fl, Cx43KO, Cx30KO and DKO) exhibited a significant reduction of Cx43 transcript and protein level. Insertion of loxP sites (Cx43fl/fl) already dramatically decreased Cx43 transcripts to about 30% and protein level to 20% as compared to C57/Bl6. hGFAP-cre-mediated inactivation of Cx43 resulted in a further significant reduction of Cx43 transcripts in Cx43KO and DKO mice to 5%. Cell proliferation rate in the SGZ and the GCL decreased to about 50% in all genetic modified mice. Ablation of Cx30 did not influence the proliferation rate. Phenotypical characterization of newborn cells was performed using different cell markers. Two weeks after BrdU labeling more than 70% of all newborn cells were doublecortin positive with no differences among all investigated genotypes and GFAP positive cells were found to be less than 10%. The proportion of NeuN positive cells was almost 20% with exception of Cx43fl/fl mice where a significant reduced number of neuronal cells (10%) was revealed. Expression of P2Y1 and P2Y2 mRNA did not significantly vary between the different genotypes. **Conclusions:** The impaired proliferation rate (50%) in all tested mice (Cx43fl/fl, Cx43KO, Cx30KO and DKO) is probably due to a strong downregulation of Cx43 proteins which is already reduced to 20% in Cx43fl/fl mice. We assume that a further downregulation to about 5% in mice with hGFAP-cre-mediated inactivation of Cx43 has no further influence on the proliferation of newborn cells. For the first time we could show an impact of Cx43 but not Cx30 expression on adult neurogenesis. Stable expression of P2Y1 and P2Y2 *in vivo* implicates another mechanism of Cx43 mediated effects on cell proliferation as shown in previous *in vitro* studies during development.

Activin A enhances NO release from microglial cells stimulated with Toll-like receptor agonists

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Objective: Activin A, a member of the TGF-beta family of growth and differentiation factors, is a multifunctional cytokine with roles in the immune system and the inflammatory response. Activin A levels are elevated in the cerebrospinal fluid of patients with meningitis. Microglial cells, the major constituents of innate immunity within the brain, express Toll-like receptors (TLRs) recognising exogenous and endogenous ligands. Upon stimulation with agonists of TLR2, TLR4, and TLR9, primary mouse microglial cells become activated and release nitric oxide (NO), a variety of cytokines, and also activin A, suggesting that microglial cells are a source of elevated activin A levels during central nervous system (CNS) infections. Both pro- and anti-inflammatory effects of activin A have been observed *in vitro*. We investigated whether activin A influences NO release from microglial cells stimulated with agonists of TLR2, TLR4, and TLR9.

Methods: Primary mouse microglial cells (75000 cells/well, 96-well plates) were kept in DMEM + 10 % FCS + penicillin/streptomycin either in presence or absence of activin A (10 µM). 24 hours after addition of activin A, cells were stimulated with the TLR2 agonist Pam3CSK4 (P3C), the TLR4 agonist endotoxin (LPS), and the TLR9 agonist cytosin-guanosin oligodesoxynucleotide 1668 (CpG) in the presence of interferon-γ (IFN-γ; 100 U/ml) for 48 hours, control cultures were treated with IFN-γ only. Activin A was present throughout the whole experiment. Concentrations of the TLR agonists evoking about 50 % of the maximum NO release (100 %, achieved by treatment with LPS 1 µg/ml) were used: 0.01 µg/ml P3C, 0.0003 µg/ml LPS, and 0.1 µg/ml CpG. NO release was quantified using the Griess reaction. Viability of microglial cells was determined using the WST-1 Cell Proliferation Reagent. Means ± standard deviations of NO release in % of maximum NO release are presented (n = 9 per group, three independent experiments). Data were analysed by one-way ANOVA followed by Bonferroni´s multiple comparison test. $P < 0.05$ was considered statistically significant.

Results: Activin A did not influence NO release from control microglial cells [10.03 ± 2.90 % (IFN-γ only) versus 9.13 ± 2.69 % (IFN-γ + activin A); $p > 0.05$]. However, pre-treatment with 10 µM activin A significantly enhanced NO release from microglial cells upon stimulation with P3C [60.16 ± 4.54 % (P3C alone) versus 76.84 ± 5.58 % (P3C + activin A); $p < 0.001$], LPS [50.60 ± 4.59 % (LPS alone) versus 64.15 ± 11.07 % (LPS + activin A); $p < 0.001$], and CpG [44.48 + 4.14 % (CpG alone) versus 57.17 ± 8.90 % (CpG + activin A); $p < 0.001$] for 48 hours. Viability of microglial cells was not affected by treatment with activin A, the different TLR agonists, or their combination with activin A.

Conclusions: Pre-treatment with activin A enhances NO release from microglial cells activated by agonists of the principal TLRs involved in the recognition of bacteria. These findings provide further evidence for a role of activin A in the innate immune response and suggest that activin A acts as an pro-inflammatory modulator during infection and inflammatory processes in the CNS.

Activated complement products C3a and C5a stimulate phagocytosis of *Escherichia coli* DH5a by murine microglial cells

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Objectives: Infections caused by *Escherichia coli* often lead to long-term sequelae and high mortality in infants, immunocompromised and elderly patients. Microglial cells can recognize invasive pathogens through Toll-like receptors (TLR) and elicit an immune response to clear the infectious agents. Hence, bacterial phagocytosis by microglia during infections contributes to the resistance of the brain. We hypothesized that complement may stimulate microglia thereby increasing their ability to phagocytose bacteria.

Methods: Primary cultures of mouse microglia were exposed to activated complement products C3a (1 mg/ml) and C5a (10 ng/ml) alone or in combination with the TLR2 agonist tripalmitoyl-S-glyceryl-cysteine (Pam_3Cys; 0.1 mg/ml) for 24h. After stimulation, cultures were challenged with *E. coli* DH5a at a ratio of 100 bacteria per cell. Phagocytosis was left to proceed for 90 and 180 min at 37ºC. After washing, the microglial cultures were incubated in medium containing gentamicin (200 mg/ml) for 1 h to kill extracellular bacteria. Thereafter, cells were washed and lysed with distilled water. Viable intracellular bacteria were enumerated by quantitative plating of serial 10-fold dilutions. ANOVA test (followed by Bonferroni's multiple comparisons test) was performed to analyse differences in phagocytosed bacteria between groups (n =12); *P*-values <0.05 were considered statistically significant.

Results: Unstimulated microglia ingested bacteria at a low rate. After 90 min, cells stimulated with either C3a or C5a alone showed comparable bacterial uptake to that observed in the unstimulated group. After 180 min, cells primed with either C3a or C5a phagocytosed higher amounts of bacteria than unstimulated cells. Co-stimulation with Pam_3Cys and C3a significantly increased the number of phagocytosed bacteria by microglial cells compared to unstimulated group (P <0.001) and cells pre-treated with C3a (P <0.01) after 90 and 180 min. Microglia pre-treated with the combination of Pam_3Cys and C5a ingested higher amounts of bacteria than unstimulated group (P <0.01 at 90 and 180 min) and cells primed with C5a (P <0.001 at 90 min).

Conclusion: Stimulation of microglial cells with activated complement compounds C3a and C5a alone or in combination with a TLR2 agonist increases their ability to phagocytose *E. coli*.

Molecular composition of perineuronal nets

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Somata and dendrites of central nervous system neurons are surrounded by specialized extracellular matrix structures, the perineuronal nets (PNN). PNN are important regulators of neuronal and synaptic functions.So far it is known that secreted proteoglycans and glycoproteins are expressed by neurons as well as glial cells. They are bound to the core component of PNN, hyaluronic acid, and thus build up that dense meshwork structure. However, knowledge about the exact structural and molecular composition is still sparse.

In order to study the biochemical composition of PNN we prepared peri-synaptic PNN-like structures from synaptosomes of rat brain, identified through the appearance of brevican, a highly abundant proteoglycan component of the PNN and performed gel filtration.

We could show that brevican-containing complexes have a molecular size of up to 1.5 MDa, measured by size exclusion chromatography.

To identify the molecular composition of such high MW PNN structures we used mass spectrometry and iTRAQ for brevican-binding proteins as a control we used lysates of synaptosomes prepared from brevican knock-out mice. We performed further biochemical interaction studies and cell biological approaches with a number of selected candidates from the obtained list of putative interaction partners. Thus, we could confirm known binding partners like tenascin-R, but also identify novel molecules like contactin-1 and pleiotrophin. Knowledge about the composition will lead to a better understanding how the cell surface and the PNN communicate.

Glia-neuron interaction during hippocampal epileptiform activity

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The intrahippocampal kainate model for Mesial Temporal Lobe Epilepsy (MTLE) in mice reproduces histological and functional changes of human MTLE, including cell loss in CA3 and CA1, granule cell dispersion and mossy fiber sprouting. In both human MTLE and the mouse model changes of the glia network were reported. Extent and structural reorganization of the glia network, as well as details of glia-neuron interaction in seizure initiation, however, are currently not known.
We recorded disinhibited hippocampal slices (bicuculline) from untreated (control-slices) and kainate injected mice (KA-slices) using microelectrode arrays (MEA). In KA-slices taken close to the injection site, EA could not be induced, whereas in distal KA-slices EA was readily elicited but the coherence of EA within hippocampal areas was lower than in control-slices (Häussler et al., SfN, 2007). To estimate contributions of the network size to EA induction we compared slices of 400 µm and 600 µm thickness with respect to inter-event-intervals (IEI), spatio-temporal structure, coherence and frequency spectrum of EA within hippocampal subregions. In thin KA-slices IEI were shorter than in thick slices, indicating a more stable balance of excitation and inhibition within the larger network. IEI distributions were, however, comparable in thin and thick control-slices.
In control-slices and KA-slices, EA could typically first be seen in CA1 or CA3, consisting of a low frequency LFP with spike activity superimposed. Activity in granule cell layer and hilus followed with approx. 5 – 10 ms delay. In most cases, EA was accompanied by large-amplitude oscillations =200 Hz, which were most prominent in the dentate gyrus.
To identify glia-neuron interaction during the initiation of epileptiform activity (EA) we pharmacologically attenuated the effect of glia-derived glutamate by blocking NR2B receptors (Ifenprodil). Preliminary results indicate that attenuation of glia-neuron interaction in KA-slices did not affect the IEIs, amplitude or shape of EAs, but decreased coherence within the dentate gyrus. In contrast, in control-slices coherence increased or did not change. This suggests a synchronizing, pro-epileptic effect of glia-neuron interaction in the epileptic tissue, possibly required for the initiation and/or maintenance of spontaneous seizures in the epileptic brain. In contrast, in the healthy tissue, glia-neuron interaction might have a less pronounced role in synchronizing neuronal activity or might even help to prevent EA by desynchronizing neuronal activity.

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Effects of tyrphostin AG126 on Toll-like receptor (TLR)-activated microglia

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Microglia are the immunocompetent cells within the central nervous system which are also involved in the detection and clearance of pathogens. Using pattern recognition receptors like the Toll-like receptors (TLRs), microglia detect bacterial and viral structures. Activation of diverse TLRs can lead to proinflammatory responses. Microglial activation is generally a beneficial reaction aiming at the protection of endangered CNS structures and functions. However, overshooting or wrongly adapted release of inflammatory mediators by microglia seems to be associated with detrimental outcomes. In the present work, we investigated the anti-inflammatory properties of tyrphostin AG126. Tyrphostins represent a class of synthetic protein tyrosine kinase (PTK) inhibitors with the potential to selectively interfere with substrate tyrosine phosphorylation. AG126 has been reported to attenuate inflammatory processes in various *in vivo* and *in vitro* settings. We demonstrated that AG126 effectively suppresses the cyto- and chemokine release as triggered by several TLR agonists, whereas in TLR4-stimulated microglia AG126 has a more modulatory effect on the release pattern. Focusing on microglia, we analysed putatively involved signaling pathways and potential AG126-sensitive targets. To identify AG126-affected signaling components we analysed the activation of TLR signaling pathways, such as mitogen-activated kinases (MAPK) and NFkB, in mouse wild type microglia as well as in microglia deficient for TLR4, MyD88 and TRIF. Apparently, the target associates with but does not integrate into the MyD88-dependent route. Besides the evidence for a TLR-associated AG126-targeted PTK candidate, four alternative modes of action were addressed. Basically, our observations indicate a critical role of the tyrphostin AG126-sensitive target in the signaling of multiple TLRs in microglia. Supported by the Niedersachsen-Israeli Research Cooperation.

No evidence for spiking properties in NG2 glia of the mouse cerebellar white matter.

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Glial cells expressing the proteoglycan NG2 were discovered more than 20 years ago. They are regarded as a third macroglial cell type, although their physiological role remains yet unknown. So far, after recording their physiological properties in slice preparations, their cellular identification was based on immunodetection of the NG2 antigen. Transgenic mice generated by non-homologous recombination (GFAP-EGFP, CNP-EGFP, NG2-DsRED) provided the advantage of cell-type recognition prior to electrophysiological recording and were used extensively to study glial cells. NG2-glia cells express voltage-gated sodium and potassium channels, neurotransmitters receptors and receive synaptic inputs. Recently, it has even been proposed that NG2-glia could generate action potentials in the cerebellar white matter of young rats.
Here, we took advantage of new genetically modified mice with fluorescently labelled NG2 cells. The yellow fluorescent protein EYFP was placed directly into the NG2 locus by homologous recombination. These mice have the advantage that EYFP expression is controlled by all regulatory elements of the NG2 gene and are, therefore, the best possible model to characterize the physiological properties of NG2-glia.

Our results obtained from acutely isolated cerebellar slices of the early postnatal mouse brain demonstrate that the fluorescent NG2-glial cells can be segregated in two distinct populations according to their electrophysiological properties. One NG2-glia population expresses voltage gated sodium and potassium channels and receive synaptic inputs, while the other population possesses passive membrane properties and is devoid of synaptic inputs. In none of the recorded cells of both populations action potentials could be observed or evoked, neither in the white matter nor in the other regions of the cerebellum. In addition to functional synaptic inputs, intimate structural contacts between NG2-glia and neighbouring interneurons were observed. The precise nature and the role of these interactions in the maturation of the cerebellum remain to be determined.

Long-term, multi-cellular, time-lapse imaging analysis of spinal cord injury in vivo

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The cellular mechanisms of spinal cord injuries are still poorly understood. This is particularly true for the glial scar and the time course of its formation with coordinated cellular reactions in relation to axonal de/- and regeneration.

To visualize axons and glial cells in the dorsal columns of the lumbar spinal cord, we used triple-transgenic mice (TgN(Thy1-EYFP)xTgN(GFAP-ECFP)xTgH(CX3CR1-EGFP)) in which axons, astrocytes and microglial cells are labelled by yellow, cyan and green fluorescent proteins, respectively. The same mice were imaged before and after lesioning at the day of injury and at subsequent days for up to two months. The combination of multi-cellular labeling with multiple-time-point imaging allowed for the first time to explore unambiguously the spatio-temporal relationship between the cellular responses in spinal cord injury.

In practice, we anaesthetized mice with isoflurane and N2O. The depth of anesthesia was assessed by monitoring pinch withdrawal, breathing rate and rectal temperature, which was maintained between 36C and 38C with a heating pad.

For accessing the spinal cord, we removed the connective tissue between the vertebral arches L1 and L2 until the intact dura matter. After imaging, the mice received analgesics and were sutured for subsequent imaging.

In vivo imaging was performed using a custom-made two-photon laser scanning microscope. We excited simultaneously ECFP, EGFP and EYFP and projected emitted light into four channels for spectral unmixing. Standardised lesions were applied by a Titanium-Sapphire laser focussed for a few seconds to a region of 20 m, resulting in a lesion diameter of 20 to 40 m. Care was taken to keep the dura matter intact.

To separate ECFP, EGFP and EYFP signals spectral unmixing was performed with our program based on least-squared error algorithm and giving the confidence interval of the estimates. The reference spectra were measured for each stack of images by selecting a few regions containing only one dye (typically a soma for microglia and astrocytes and axons for neurons.) and their validity was verified by checking the similarity of the measured spectra.

In the first minutes after injury, microglia sent processes toward the lesion as previously reported in the cortex. Within the next 24 hours numerous microglial cells with an ameboid shape accumulated and stayed at the lesion site for about a few weeks depending on the lesion size. Dissected axons were partially retracted from the lesion site and - some distal endbulbs were removed by microglia. Spontaneous regeneration could not be observed. Interestingly, neighboring astrocytes did not respond earlier than two days after injury. They slowly became activated as seen by the formation of thicker processes and enhanced expression of ECFP. Their reaction became evident after a week when microglial reaction started to cease. Both glial cells reactions did not only differ in time, but also spatially in as much as microglia filled the direct site of injury and astrocytes formed a cellular wall around the microglia.

Our analysis of acute glial reaction in the spinal white matter suggests that different time profiles of both glial cell types contributing to scar formation have to be taken into account when novel therapeutic approaches will be developed for treating spinal cord injuries.

AQP4, Nestin and GFAP expression in striatal and midbrain mouse astrocytes *in vitro*

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Astrocytes play an important role in the development and plasticity of the mammalian brain[1]. Proliferation of astrocytes occurs during embryonic development but also postnatal, during puberty[2] and in astrogliosis[3]. We recently showed that dopamine (DA) decreases proliferation in striatal mouse astrocytes *in vitro*[4]. Due to the fact that proliferation involves– among other things – a change in cell volume, which normally comes along with water movement across the membrane, we hypothesized that water channels are involved in astrocyte proliferation. Aquaporins (AQPs) are a family of water-channel proteins and AQP4 represents the predominant water channel in the mammalian brain, where it is found in glia cells[5]. We showed that DA down-regulates AQP4 expression and that blocking of AQP4 function reduces the proliferation in striatal mouse astrocytes *in vitro*[4]. Although we therefore concluded that DA modulates proliferation via regulation of AQP4 expression, we could not identify the target cells for the DA effect. Since astrocytic cultures are very heterogeneous[6] with respect to the expression of different markers we investigated striatal and midbrain astrocytes for the expression of glial acidic fibrillary protein (GFAP), Nestin and AQP4 under control conditions and after treatment with DA (10^{-4}M). GFAP and Nestin are both members of the intermediate filament (IF) protein family. GFAP the main component of the astrocytic IF network is typically used as an astrocyte marker while Nestin is only expressed in cultured and reactive astrocytes[7]. Immunochytochemistry with antibodies specific for GFAP, Nestin and AQP4 was done and stained cells were counted using a Zeiss Axioplan2 microscope. The number of positively stained cells was expressed as a percentage of the total cells.

DA-treated cell cultures contained around 21-24% less cells in total than unstimulated cells. In both brain regions ~50% of all cells were GFAP+, 70-80% were Nestin+ and 60-70 % were AQP4+. DA treatment did not have any effect on these distributions. In striatal astrocyte cultures ~80% of the Nestin+ cells co-stained for AQP4 and ~60% of the GFAP+ cells co-stained for AQP4. Compared to this, midbrain astrocyte cultures showed a higher co-expression of Nestin+/AQP4+ and GFAP+/AQP4+. In striatal astrocyte cultures we observed a slight reduction in Nestin+/AQP4+ cells after DA treatment while GFAP+/AQP4+ cells slightly increased.

Our data demonstrate that not all striatal and midbrain astrocytes *in vitro* express AQP4. Further we conclude that Nestin+/AQP+ cells represent these astrocytes that are capable of proliferation and susceptible for DA while GFAP+/AQP4+ cells represent mature astrocytes insusceptible for DA.

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Temporally controlled ablation of AMPA-type glutamate receptors in Bergmann glia

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Cerebellar Bergmann glial cells possess arborized processes which irregularly extend lamellae toward synapses on Purkinje-cell spines formed either by parallel or climbing fibers. The glial appendages do not only seal the synaptic cleft, they also constitute microdomains for signalling pathways. On these membranes, Bergmann glia expresses highly localized either glutamate transporters (GLAST close to the synaptic cleft) or AMPA-type glutamate receptors (GluR A and GluR D close to the shaft of the presynaptic terminal). While the role of GLAST in controlling extracellular glutamate levels is rather obvious, we have only a limited knowledge of glial AMPA receptor function.

To investigate the particular impact of glial transmitter receptors *in vivo*, we generated a transgenic mouse line in which the tamoxifen-sensitive Cre recombinase fusion protein CreERT2 is expressed under the astroglia-specific human GFAP promoter. Such transgenic mice were further crossbred with genetically modified mice in which exons 11 of the GluR A and GluR D genes, respectively, were flanked by loxP sites.

Here, we demonstrate that *in vivo* GluR A and D can be ablated from Bergmann glial processes using the cre/loxP system in an inducible and timely controlled manner. Efficient tamoxifen injection protocols have been established to enhance genetic recombination. The astroglia-specific deletion of GluR A and D reveals ultrastructural changes in the cerebellum in which the Bergmann glial lamellae retract from Purkinje-cell synapses and Purkinje-cell dendrites show enlarged or swollen dendrites. The physiological and behavioural consequences of these ultrastructural alterations within the network of the cerebellum have to be determined yet and will elucidate whether astrocytes play a functional role as an element of communication within the central nervous system, and therewith substantiating the physiological impact of the neuron-glia interaction.

Microglial contribution to neurodegeneration in the SOD1 (G93A) mouse model for ALS – a 2P-LSM study *in vivo*

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Amyotrophic lateral sclerosis (ALS) is an adult-onset neurological disorder, characterized by progressive loss of upper and lower motor neurons and degeneration of pyramidal tracts. Dominant mutations in the gene encoding the enzyme superoxide dismutase (SOD1) are the most frequent cause of inherited ALS. Transgenic mice expressing various ALS-linked mutations in SOD1 recapitulate the fatal paralysis seen in human patients. The SOD1-mediated toxicity is shown to be non-cell-autonomous deriving not only from motor neurons but also from neighboring glia. In particular, microglia and astrocytes substantially contribute to motor neuron death and disease progression. The impact of neuroinflammation to disease progression is still a matter of debate.

We used transgenic SOD1 G93A mice with fluorescently labeled neurons and microglial cells and performed single axon dissections to the lateral columns under in vivo conditions. Time-lapse 2P-LSM imaging was then applied to analyze the rapid microglia-upper motor axon interaction during ALS disease course. First, we observed a continuous change of microglial morphology towards an ameboid-like shape from preclinical to advanced stages of the disease indicating ongoing inflammatory activity. Application of laser-mediated axon dissection induced a significantly increased response of microglia towards the lesion in presymptomatic stages compared to control animals. However, this response becomes significantly reduced in advanced symptomatic and end-stage animals coinciding with morphological transformation of microglial cells.

Our study on the SOD1 model for ALS provides substantial in vivo evidence that microglial inflammation can be divided into two distinct phases: i) microglia that are highly overactive in preclinical stages and ii) morphologically activated microglia that loose their directed response towards lesioned tissue in advanced clinical stages. During disease course, aberrant microglial reaction may promote motor neuron death and disease progression.

Deformation-based morphometry revealed cerebellar volume alterations in rats with cortical dysplasia.

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Deformation-based morphometry (DBM) is a fully automatic intensity-based method to analyze regional volume changes in the whole brain using magnet resonace imaging (MRI). This technique works without user bias or *a priori* defined ROIs and is based on the estimation of deformation fields using a general multivariate statistical approach. Deformations are obtained by a nonlinear registration routine transforming a reference brain (template) onto another brain (object). Deformation fields are calculated for each subject and differences between groups displayed as morphometric changes. T2-weighted MRI-sequences were perfomed with a 3-Tesla-Scanner. Our work included development of a reference template for rats and applying volume measurements on Paxinos's atlas „The rat brain".

Pilot experiments were performed on young and aged wistar rats (3 vs. 26 month) with cortical dysplasia to examine brain volume changes through lifetime. We revealed compensational cerebellar volume increase and enhanced global cortical atrophy in aged lesioned rats. Volume alterations came along with loss of functional capabilities examined in behavioural testing with ladder rung walking test.

Defective sorting of L1 missense mutations in the endoplasmatic reticulum

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Gene mutations in the cell adhesion molecule L1 are responsible for the x-linked L1 syndrome that leads to abnormal brain development. We here studied disease-associated L1 mutations that affect extracellular immunoglobulin-like or fibronectin type III domains of L1. We show that these mutations cause endoplasmatic reticulum (ER) retention and reduced L1 cell surface expression. Despite pronounced ER accumulation, no induction of ER stress or cell death was observed following overexpression of L1 mutations in the neuronal NSC34 cell line. Interestingly, we identified wildtype L1 as a cargo of COPII-vesicles, as evidenced by its ER retention after inhibition of COPII-vesicle formation by mutant Sar1-H79G. Immunofluorescence analysis of cells expressing wildtype L1 and Sar1-H79G further indicates that L1 accumulates at ER exit sites. In contrast, no such co-localization was found for L1 missense mutations. These results indicate that L1 cell surface expression depends on COPII-vesicle formation. They further suggest that L1 missense mutations interfere with L1 sorting to ER exit sites and subsequent capture into COPII-vesicles. We propose that ER-associated sorting defects represent an important pathological mechanism for the x-linked L1 syndrome.

Is there an impact of neuronal Ras activity on Rett Syndrome?

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Rett Syndrome (RTT) is a progressive neurodevelopmental disorder caused by mutations in the X-linked gene encoding methyl-CpG-binding 2 (MeCP2) (1). Loss of function as well as overexpression of MeCP2 cause a variety of neuropsychiatric disorders. Recent studies show that MeCP2 can act as activator and repressor of transcription (2). One of the MeCP2 target genes, the BDNF (brain derived neurotrophic factor) promotor III, becomes activated after neuronal activity leading to MeCP2 phosphorylation and BDNF synthesis (3,4). A lack of MeCP2 protein results in missing activity-dependent BDNF signaling (5). This leads to a number of severe symptoms in the brain like a decreased brain volume, decreased number of synapses and a loss of experience-dependent plasticity. Loss of MeCP2 leads also to a shortened lifespan, underweight and autistic features (5). It has been previously described that BDNF signaling occurs mainly through binding and activation of the TrkB-receptor resulting in the transient increase in relative levels of the signaling competent GTP-bound Ras versus the inactive GDP-bound Ras conformation. It has been shown previously, that in synRas transgenic mice expressing constitutively active human Ha-Ras under direction of the synapsin I promotor Ras activity is able to mimic various effects of neurotrophins such as those of BDNF (7). These neurotrophin-like effects by Ras activation include increased brain volume due to neuronal hypertrophy, increase in the dimension of dendritic trees, development of an increased number of spines and synapses and protection from injury-induced neuronal cell death (6,8). Previously, introduction of a BDNF over-expressing transgene into MeCP2 knock out mice led to a partial recovery of the adverse phenotype (9). Here we investigate the possible involvement of the master-signaling protein Ras in the etiology of RTT syndroms.

By cross-breeding of MeCP2-KO mice with synRas transgenic mice we will analyse if permanent elevation of Ras activity in differentiated neurons could counteract the effects resulting from limiting amounts of BDNF or other unknown trophic signals in MeCP2 mice.

At first we characterized the phenotype of the MeCP2-KO mice and we confirmed that these mice were underweighted and smaller in body size (5). Our preliminary results show that Ras activity is decreased in various brain regions of MeCP2-KO mice. Moreover, we find an altered anxiety behaviour of MeCP2-KO mice and a reduced capacity of motor-coordination. Taking together, genetic modification of MeCP2 protein may influence Ras activity pointing to its implication in Rett Syndrome.

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Proteolytic Processing of Reelin is Altered by Epileptic Activity in Rat Hippocampal Slice Cultures

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The glycoprotein reelin, synthesized and released from Cajal-Retzius cells, controls the formation of cortical layers during development and maintains lamination in the adult brain. Decreased levels of reelin lead to a migration defect of dentate granule cells in temporal lobe epilepsy (TLE) patients and in a mouse TLE model (Haas et al., 2002; Heinrich et al., 2006). However, not only absolute reelin levels, but also proteolytic processing giving rise to several reelin isoforms is important for the biological activity of reelin. With the concept that pathological processing of reelin may contribute to the malpositioning of dentate granule cells in TLE, we mimicked epileptic conditions by kainate (KA) treatment of organotypic slice cultures from rat hippocampus.

As a prerequisite, we investigated whether reelin-synthesizing neurons survive KA treatment. Immunocytochemistry for reelin revealed that the number of reelin-immunolabeled cells was not substantially altered in hippocampal slice cultures after exposure to KA for 24h. In addition, double immunolabeling for reelin and c-fos, a marker of neuronal activation, demonstrated that KA strongly induced c-fos expression in dentate granule cells, but only in a small subpopulation of reelin-immunolabeled neurons. However, when reelin levels and isoforms were studied by quantitative Western blot analysis in extracts from tissue and supernatants, we found that KA treatment caused an accumulation of high molecular weight reelin (400 and 320 kDa) in tissue samples, whereas in supernatants the levels of the smaller 180 kDa fragment were decreased. This KA effect could be mimicked by incubation with protease inhibitors, indicating that KA alters the extracellular processing of reelin. While inhibition of plasminogen activators caused an increase in the 400 kDa reelin fragment, blockade of metalloproteases (MMPs) by GM 6001 resulted in the accumulation of the 320 kDa reelin isoform. Taken together, our results suggest that epileptic conditions influence the biological activity of reelin without directly affecting reelin-synthesizing cells. Instead, proteolysis of reelin appears to be impaired by overexcitation resulting in a functional inactivation of secreted reelin which may contribute to the migration defect in TLE patients. (Supported by the DFG, SFB TR3).

Metabolic and structural changes in the rat brain after transient occlusion of the anterior cerebral artery

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Little is known about metabolic and structural changes in the brain occurring months and years after ischemic lesions, and how those changes are related to early events after stroke. We followed changes in cerebral blood flow, metabolic activity, and brain structure for one year in a rat model of anterior cerebral artery (ACA) occlusion.

The distal ACA (rostral and dorsal from the corpus callosum) was occluded in eight Lister hooded rats (*Rattus norvegicus*) by stereotactic injection of 150 pmol endothelin-1 (ET-1) which transiently blocks cerebral blood flow by vasoconstriction. Positron emission tomography (PET) with ^{15}O-H_2O was performed before and 45 min after ET-1 injection to evaluate acute changes in cerebral blood flow. The tracer ^{18}F-fluorodeoxyglucose (FDG) was used before and 1-2 h, 28, 90, and 360 days after ET-1 injection to assess alterations in basic metabolic activity. T2-weighted magnetic resonance imaging (T2-MRI) was performed before and 24 h, 28, 90, and 360 days after ET-1 injection to delineate the oedema and resulting cysts produced by degradation of affected tissue. Two animals were sham operated, and underwent the same imaging protocol.

H_2O-PET at the day of occlusion revealed a significant reduction of cerebral blood flow in areas of the ACA territory, including olfactory bulb, septum, prefrontal and cingulate cortical areas. Profile analysis of FDG-PET showed that metabolic activity in the olfactory bulb was reduced by 20 % at the first day of occlusion, but showed a partial recovery after 28 days which remained stable over one year. In the cingulate cortex initial metabolic reduction was less than 10 %, but activity was further reduced after 28 days, and stabilized at a reduction of 20 % by 90 days after occlusion.

T2-MRI at 24 hours showed an oedematous region strongly overlapping with the area of reduced blood supply. The oedema had disappeared after 28 days, but tissue shrinkage and small cysts could be observed throughout the remainder of the study. Over the course of one year, the ventricles progressively enlarged in both the lesioned and the control animals. In three of the eight occluded rats, oedema developed in the piriform region at the first day, but was not accompanied by reduction of cerebral blood flow. The piriform lesions transformed into large cysts connected to the ventricular system.

Correlation analysis of lesion sizes revealed a correlation between H_2O-PET deficits and lesion size assessed by T2-MRI at all time points. H_2O- and FDG-PET were not correlated at the time of the lesion, but at day 28 a correlation could be established. In the piriform region, however, H_2O-PET was not correlated to FDG-PET or T2-MRI and may therefore represent a lesion that develops secondarily in a non-ischemic region.

We conclude that in this model of transient ischemia, primary and secondary lesions occur, and that metabolic changes possibly representing further degenerative processes or also recovery of function take place at least until 90 days after stroke.

Transcriptomic Analysis of Schizophrenia-Related Brain Regions of Neuregulin-1 Deficient Mice

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Schizophrenia is a devastating disorder of the brain, characterized by positive symptoms like hallucinations and delusions and negative symptoms like affective flattening, social withdrawal and thought disturbances. Many subtle structural and functional alterations have been found in the schizophrenic brain, the most robust of which are losses of neuropil in supragranular layers of the dorsolateral prefrontal cortex (dlPFC) and volume reductions of corresponding thalamic association nuclei. Lacking signs of neurodegeneration, these deficits are supposed to be of developmental origin.

There is a strong but complex genetic contribution to the etiology of schizophrenia. Among the susceptibility genes, Neuregulin-1 (Nrg-1) is particularly suggestive due to its genetic linkage and its described biological functions in mice (roles in synaptic plasticity, guidance of GABAergic interneuron precursor migration, thalamocortical pathfinding as well as myelination). All of these processes have previously been suggested to be affected in schizophrenic patients.

To obtain a more detailed understanding of the molecular effects of alterations of the Nrg-1 gene dose with respect to the pathophysiology of schizophrenia, we compared the transcriptomes of Nrg-1 hypomorphic mouse brains with that of their wildtype siblings. In particular we investigated the frontomedial infralimbic and prelimbic cortices as distant homologs of the dlPFC and their reciprocally connected nuclei around the anterior thalamic midline as schizophrenia-related regions in contrast to non-affected control regions (somatosensory cortex, ventrobasal thalamus). Developmental timepoints were the stage of midcorticogenesis (beginning formation of upper cortical layers) on embryonic day 15,5 and early postpubertal stage (age of disease manifestation) on postnatal day 28.

With Affymetrix GeneChip Array technology, we observed very few strong, but many subtle changes of gene expression as a consequence of Nrg-1 deficiency. Through our approach, using GeneSpring GX software, we try to identify among the deregulated transcripts functionally related gene sets, which might hint to cellular systems potentially related to the pathomechanism of schizophrenia. We started to verify some that are specifically deregulated in the frontomedial as compared to the "non-affected" somatosensory thalamocortical system by in-situ hybridization and qPCR.

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Cognitive and emotional changes in the behaviour of rats after occlusion of the anterior cerebral artery

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The anterior cerebral artery (ACA) supplies cingulate and frontal cortical regions in both humans and rats. Ischemic lesions in the ACA territory of humans lead to cognitive impairments and reduced incentive drive. In order to investigate functional loss and recovery of function over time, we used a rat model of ACA occlusion (eight male Lister hooded rats and two sham operated controls). ACA occlusion was achieved by stereotactic injection of the vasoconstrictor endothelin-1 (ET-1; 150 pmol in 0.3 µl phosphate buffer) 1.5 mm anterior, 0 mm lateral, and 3.5 mm ventral from bregma.

For analysis of functional outcome, we carried out behavioural tests, which measure decision-making during foraging (food-carrying task), attentional set-shifting (bowl-digging task), spatial working memory (spontaneous alternation in the Y-maze), exploratory behaviour (open field), and anxiety (elevated plus maze). In order to set up a longitudinal study, these tests were carried out before, a week after (early testing phase) and a year after ACA-occlusion (late testing phase). Behavioural testing was accompanied by MRI (T2)- and metabolic (^{18}F-fluorodesoxyglucose-) PET-Scans, and correlation between behavioural differences after lesion and anatomical/metabolic data was analyzed.

Attentional set-shifting behaviour and locomotor activity in the open field did not change in both test phases after lesion. In the open field in contrast, rats showed impairment of homebase-behaviour in the early testing phase whereas this performance recovered considerably in measurements in late testing phase. In early testing phase all ACA lesioned rats carried less food pellets in the food-carrying task and showed jagged movement patterns in video tracking. All these changes remained constant in the late testing phase. In the elevated plus maze, lesioned animals spent in both testing phases more time on the open arms than before occlusion. Arm alternation rate in the spontaneous alternation task decreased from 83 % to chance level in the early measurements but recovered again to 77.6 % in late testing phase a year after occlusion.

We found positive correlations of behavioural changes in the elevated plus maze and of impairments the open field with the size of metabolic reduction (PET) in the anterior cingulate cortex (ACC), prelimbic regions, septum, piriform cortex and the hippocampus. Furthermore, behavioural impairments in the food carrying and bowl-digging task were positively correlated with sizes of structural lesions (MRI) in the ACC, prelimbic areas, and septum, as well as with the amount of ventricular enlargement.

We conclude that ischemic lesions in the ACA-territory of rats as well as in humans lead to cognitive deficits such as impaired decision-making. Rats further showed effects in behavioural performance like impairment of spatial memory and reduction of anxiety. The cognitive impairments and emotional changes in behaviour are accompanied by structural and metabolic losses.

Comparison of the cadherin expression in the cerebral cortex of wild type and reeler mice

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Cadherins, a large family of adhesion molecules, play a role in a wide variety of developmental mechanisms in the mammalian brain, including cell proliferation, cell differentiation, cell-cell recognition, neurite outgrowth and synaptogenesis. Previous studies demonstrated that cadherins are regional markers for functional brain regions and neural circuits (for reviews see Redies, 2000; Takeichi, 2007). In the cerebral cortex, they are markers for cortical regions and layers (Krishna-K. et al., 2008).

The Reeler mutant is a mouse model of cortical development with a highly disorganized cortex. Due to the absence of the Reelin protein, early-born cortical neurons fail to invade the preplate and a so-called "superplate" is formed. As a consequence, late-generated neurons are unable to pass their predecessors in the reeler cortex, supposedly resulting in inverted cortical layers.

In the present study, we used cadherin expression mapping to define the histoarchitecture of the Reeler mutant cortex. By in situ hybridization, we examined the expression of thirteen cadherins in three cortical regions, the cingulate cortex, the motor cortex and the primary somatosensory cortex. In contrast to wild type cortex that exhibits a layer-specific expression of each cadherin, the reeler cortex displays a widespread distribution of cadherin-expressing cells throughout all cortical layers. A comparison between wild type and reeler mice revealed no evidence for an "outside-in" layering in reeler mice. Strikingly, the area-specific expression of the cadherins is preserved in the reeler cortex. We conclude that cortical layering is abrogated in the different cortical areas but cortical regionalization of the neurons is not altered in the reeler cortex.

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Function of BACE1 and Neuregulins in the developing and adult brain

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BACE1 (beta-site APP cleaving enzyme), an aspartyl protease, plays a critical role in the production of amyloid Abeta peptides; insoluble plaques containing Abeta constitute the molecular basis of pathogenesis in Alzheimer's disease. We recently identified the principal physiological function of BACE1 to be the cleavage of Neuregulins, growth and differentiation EGF-like factors that are highly expressed in the peripheral and central nervous systems. Our previous work has shown that cleavage of Neuregulin-1 by BACE1 is required for signaling of axonally-expressed type III Neuregulin-1 to apposing Schwann cells during myelination. Interestingly Neuregulin-1 is a gene linked to schizophrenia and psychosis. We have now generated mice carrying compound mutations in BACE1 and specific isoforms of Neuregulin-1 with the aim of characterizing the functions of BACE1 in Neuregulin-1 signaling in the neonatal and adult mouse brain. The relevance of our findings to schizophrenia and synaptic function in the central nervous system will be discussed.

Fragile X Mental Retardation Protein regulates the levels of select scaffold proteins and glutamate receptor subunits in postsynaptic densities

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Functional absence of the fragile X mental retardation protein (FMRP) causes the fragile X syndrome (FXS), a hereditary form of mental retardation characterized by a change in dendritic spine morphology. FMRP is an RNA-binding protein that has been implicated in the regulation of local protein synthesis at synapses. Here, we have analyzed whether the abundance of main scaffold proteins and neurotransmitter receptor subunits in postsynaptic densities (PSDs) are altered in different brain regions of FMRP deficient mice as compared to wild-type animals. Whereas the levels of several PSD components are unaltered, the concentrations of Shank1 and scaffold proteins of the SAPAP family as well as various glutamate receptor subunits are increased in FMRP knockout mice. With the exception of a slight increase in SAPAP2 mRNA levels in the hippocampus, the concentrations of the transcripts encoding these proteins are not altered. Thus, the loss of FMRP in neurons appears to mainly effect the translation and not stability of particular brain transcripts. Semi-quantitative analysis of RNAs present in FMRP immunoprecipitates shows that in the mouse brain Shank1 mRNAs are associated with FMRP. Luciferase reporter assays performed in primary cortical neurons derived from FMRP deficient and wild-type mice indicate that FMRP interacts with the 3' untranslated region of Shank1 transcripts to down-regulate translation efficiency. As Shank1 is known to control dendritic spine morphology our data suggest that dysregulation of Shank1 synthesis in neurons may significantly contribute to the abnormal spine development and function observed in brains of FXS patients.

Characterisation of dyslamination in focal cortical dysplasia with layer-specific markers

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Focal cortical dysplasia (FCD) is a common cause of pharmaco-resistant focal epilepsy and characterised by a circumscribed area of dyslamination in an otherwise normal cortex. FCD results presumably from disturbances in different stages of cortical development, but the exact pathomechnisms are not known so far.

In this study we used layer-specific markers, interneuron stainings, maturation markers and glial fibrillary acidic protein (GFAP) on the level of *in situ* hybridisation and immunohistochemistry in order to better characterise dyslamination of human FCD, mainly in patients without balloon cells. Specimens deriving from neurosurgically resected tissue in patients with pharmaco-resistant epilepsy were investigated.

We observed a correct sequence of the cortical hexalamination in all specimens. The existence of ectopic neurons in the white matter was a common finding. These were small immature neurons or pyramidal cells from lamina 5/6 and probably from lamina 3, but never granular cells from lamina 4.

FCD in the anterior temporal lobe typically showed small immature neurons predominantly in the pyramidal cell layers, a reduction of neurofilament-positive neurons and of parvalbumin-positive neurons in lamina 4. Severe gliosis was seen in the lower layers of the cortex, especially in patients with additional hippocampal sclerosis. Giant neurofilament-positive neurons, large doublecortin-positive neurons or a reduction of lamina 4 granular neurons have never been observed in this group.

In contrast, FCD in extratemporal areas was highly variable. In this patient group, changes in lamina 4 were the most prominent finding. In several patients a severe reduction of lamina 4 neurons was detectable in areas which normally have a well-developed lamina 4. In these patients and in patients with FCD in physiologically agranular cortical areas (Brodmann areas 4 and 6) large, sometimes doublecortin-positive neurons occurred. Additionally, these patients showed a nearly complete loss of interneuronal markers. This cluster of findings points to an early disturbance in neuronal maturation which may entrain severe interneuronal problems. Another group of extratemporal FCD was characterised by an enlargement of lamina 4. In these patients, a strong increase in interneuron numbers was detectable. These findings were often associated with very large neurofilament-positive neurons and a disturbance in the parallel alignment of the apical dendrites of pyramidal neurons. This cluster points to a later disturbance in migration and cortical organisation.

Taken together, FCD without balloon cells is characterised by a large variety of abnormalities. Although the FCD differs from individuum to individuum, there are several clusters of abnormalities, which may help to find out the time point of the disturbance and possibly to trace sequences of developmental disorganisation.

Developmental Regulation of the Serotonergic System in the Brainstem of Mecp2-Deficiency

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Rett syndrome, a neurodevelopmental disorder caused by mutations in the X-linked gene encoding the transcription factor methyl-CpG-binding protein 2 (MeCP2), is a leading cause of mental retardation in females who also show severe breathing dysfunction like apnoea. The serotonergic system is an important neuromodulator of the respiratory network. Discovering which genes are misregulated in the absence of functional Mecp2 in mice are important steps for understanding the pathogenesis of breathing dysfunction in Rett syndrome and related disorders. We analyzed the expression of the whole serotonergic system in the pre-Bötzinger complex, essential for respiratory rhythm generation, at the RNA level with qPCR at four postnatal development stages (P7, P15, P21, P40) in Mecp2-deficient mice compared to wild type mice. We found a dramatic up-regulation of the serotonin 5B receptor (5-HT_{5B}R) at P40 in Mecp2-ko mouse that was also confirmed at the protein level using a homemade monospecific polyclonal antibody against 5-HT_{5B}R. Overexpression of Mecp2 and 5-HT_{5B}R in neuroblastoma cells showed a collocalization of both proteins in the nucleus. Intraperitonal application of Mecp2 protein normalized 5-HT_{5B}R gene expression suggesting that 5-HT_{5B}R is under the direct control of Mecp2.

Developmental expression of GFAP and S-100B in Fluoxetine treated rats

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Glial fibrillary acidic protein (GFAP) is a marker for astroglial cells which release S-100ß as a neurotrophic factor for the serotonergic system. Changes in astroglia, ß-S100 and the serotonergic system seemed to be associated with major depression and influenced by treatment with antidepressants like selective serotonin reuptake inhibitors (SSRI). Concerning the vulnerability of the developmental brain and the increased prescription of SSRI (i.e. fluoxetine) in children we examined if fluoxetine treatment (5 mg/kg/KG s.c.) for a period of 14 days during early postnatal, pre- and postadolescence life of rats results in short or long-lasting changes of GFAP or ß S-100. We used different methods like real-time PCR, ELISA and immunhistochemistry. Significant changes for both proteins occur dependent on the examined brain region and on the age during treatment.

Reduced life span and behavioural deficits in alpha-synuclein transgenics

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Parkinson`s disease (PD) is a chronic progressive neurodegenerative disease with several motor impairments including resting tremor, bradykinesia, postural instability, gait difficulty as well as sensory deficits and reduced life expectancy. Mutations in the alpha-synuclein (a-syn) and the Parkin-genes are causal for the familial forms of PD. At the Department of Animal Physiology we generated different mono- and double-mutant mouse models: (1) a transgenic line overexpressing the human doubly mutated (A30P, A53T) a-syn under the control of the beta-actin (BA) promoter, (2) one line with a deletion of the exon 3 in the parkin gene (PaKO), and (3) animals carrying both mutations (BAsyn/PaKO), to study and compare the general health, behaviour, olfactory function and the life span of these transgenics.

For that purpose we measured the body weight, quantified the level of urinary 8-hydroxy-2'-deoxyguanosine, performed several motor behavioural tests like rotarod, open field, elevated plus test, hanging wire and footprints and we investigated the response of receptor neurons of the olfactory epithelium after stimulation with odorants by electrophysiology at the age of 3, 6 and 9 months. Already at 3 months of age the a-syn transgenics showed severe motor impairments. Moreover in the open field the BAsyn mice displayed a higher anxiety in comparison to the LM. The analysis of the olfactory function at 3m, however, gives no evidence for odor perception deficits.
Furthermore we analysed the life span of the different mouse lines. Focusing on the maximal age we found statistical significant differences between LM, PaKO, BAsyn and BAsyn/PaKO. We could show that the BAsyn and the BAsyn/PaKO died up to 8 months earlier as the corresponding LM.
Taken together these results indicate an important role for a-syn in PD. Overexpression of a-syn causes motor impairments and a shortened life span which is similar to the situation in PD-patients.

Parkin-knockout mice: focus on mitochondrial alterations

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Mitochondria play a central role in the pathogenesis of Parkinson`s disease (PD). In accordance to PD patients mice carrying an exon3 deletion in the PD-linked gene Parkin (Parkin-knockout; PaKO) develop severe region-, age- and genotype-dependent mitochondrial abnormalities (Stichel *et al.*, 2007). These structural and functional alterations were not only restricted to neurons but affected also glial cells in the adult transgenic mice (Linnartz *et al.*, 2008).
In our present study we investigated the early development (*in situ*) and the metabolic consequences (*in vitro*) of these structural mitochondrial alterations in astrocytes.
Therefore we analysed the mitochondrial ultrastructure in the substantia nigra and the cortex of mice at postnatal day 16. Already at this early age PaKO mice displayed a higher mitochondrial damage than the non-transgenic littermates in both brain regions. Surprisingly all glial cell types exhibited a larger number of impairments than the neurons of the same genotype.
To characterize the metabolism of the glial cells we cultured astrocytes from cortex and mesencephalon of 1-day-old PaKO transgenics (F4 generation) and littermates and analysed their survival and protein expression with/without oxidative stress. Under normal conditions PaKO-astrocytes showed a stronger expression of the lysosomal cysteine protease cathepsin X than the littermates, while the expression of several mitochondrial proteins was unchanged. Treatment with hydrogen peroxide induced cell death in astrocytes, but the death rate was significantly lower in PaKO than in the corresponding littermates. Moreover this treatment led to a significantly stronger increase of PINK1 (PTEN induced putative kinase 1) and Drp1 (dynamin-related protein) in PaKO-astrocytes. The kinase PINK1 acts upstream from parkin and is involved in mitochondrial function. Both proteins, PINK1 and Drp1, trigger mitochondrial fission.
Our studies show that astrocytes are structurally and functionally affected by the Parkin mutation and are indicative of a substantial contribution of glial cells to PD pathogenesis.

Stichel *et al.*, (2007): Mono- and double-mutant mouse models of Parkinson's disease display severe mitochondrial damage. Hum Mol Genet 16(20): 2377-2393.

Linnartz *et al.*, (2008): Astrocytic mitochondria are damaged in transgenic mouse models for Parkinson`s disease. FENS Abstr.218.56, vol.4.

Degeneration of dendrites occurs in a mouse model of Alzheimer's disease which exhibits senile plaques but not in another one producing only intracellular Aß

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The deposition of the amyloid b-protein (Ab) in the brain is one of the hallmarks of Alzheimer's disease (AD). Various studies have indicated that extracellular aggregates of Ab are neurotoxic. A number of reports have raised evidence for a significant role of intracellular Ab in AD. It is not yet clear whether intracellular Ab is responsible for the neurodegenerative effects. To address this question, we have analyzed the frontocentral cortex of two different transgenic mouse models, i.e., APP48/167 and APP23 mice, and littermate controls, in the age group of 15-18 months. The APP48/167 mouse expresses a construct encoding a rat proenkephalin signal sequence followed by Ab_{1-42} driven by the neuron specific Thy-1 promoter. This mouse model produces almost exclusively intracellular Ab_{1-42} in the brain but no extracellular Ab plaques. The second transgenic mouse model, the APP23 mouse overexpresses human APP with the Swedish mutation driven by a Thy1 promoter and produces extracellular Ab-deposits as well as intracellular Ab. By using the DiI tracing technique we analyzed commissural neurons in layer III of the right frontocentral cortex in these mice. We did not observe degeneration of the dendritic tree of commissural neurons in APP48/167 mice compared to littermate controls. On the contrary, APP23 mice exhibited a degeneration of the dendritic tree of highly ramified commissural neurons as previously reported. To support our finding we stained sections with an antibody against the 68 kDa neurofilament subunit. We have found a significant alteration of dendritic morphology in layer II/III pyramidal neurons in APP23 mice but not in APP48/167 mice. The results of this study indicate that intracellular Ab accumulation does not necessarily lead to dendritic tree degeneration. Thus, different mechanisms exist in which Ab alters neurons. Supported by DFG-624/6-1.

Neonatal brainstem is prone to the generation of spreading depression during severe hypoxia

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Spreading depression (SD) resembles a concerted, massive neuronal/glial depolarization propagating within the gray matter. Being associated with cerebropathology such as cerebral ischemia or hemorrhage, epilepsy, and migraine, it is well studied in cortex and hippocampus. Less is known, however, about the susceptibility of the brainstem to hypoxia-induced spreading depression (HSD), which could critically interfere with cardio-respiratory control. In rat brainstem slices severe hypoxia (O_2 withdrawal) triggered HSD episodes within minutes. The sudden extracellular DC potential shift of approximately -20 mV showed the typical profile known from other brain regions and was accompanied by an intrinsic optical signal (IOS). Spatiotemporal IOS analysis revealed that in infant brainstem, HSD was preferably ignited within the spinal trigeminal nucleus and spread out mostly medially invading the hypoglossal nucleus, the nucleus of the solitary tract (NTS), and occasionally also the ventral respiratory group (VRG). In adult brainstem HSD was mostly confined to the NTS; its occurrence was facilitated by hypotonic solutions, but not by glial poisoning or block of GABAergic and glycinergic synapses. The neuronal hypoxic depolarizations underlying the generation of HSD were massive, but incomplete, and the propagation velocity of HSD and the associated extracellular K^+ rise were less marked than in other brain regions. In conclusion, brainstem tissue reliably generates propagating HSD episodes, which may be of interest for basilar-type migraine and brainstem infarcts. The preferred occurrence of HSD in infant brainstem and its propagation into the VRG may be of importance for neonatal brainstem pathology such as sudden infant death syndrome.

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Impairment of cognitive and behavioural performance after temporary reelin knockdown in the mPFC of juvenile or adult rats

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The extracellular matrix glycoprotein reelin is critically involved in the control of embryonic brain development. However, very little is known about reelins possible neurotrophic functions during pubertal maturational processes and its influence on behavioural and cognitive functions in the adult brain. The present study investigates the effects of a temporary reelin knockdown in the rats´ medial prefrontal cortex (mPFC) on prepulse inhibition (PPI) of the acoustic startle response (ASR), spatial working memory, and locomotor activity during puberty or adulthood. Furthermore, western blot analyses were conducted to quantify reelin concentration in the brain. Three groups of pubertal and adult rats received either local mPFC injections of phosphorothioate-modified antisense oligonucleotides (2.0nmol/µl), missense oligonucleotides (2.0nmol/µl) or vehicle, respectively. Behavioural testing of the pubertal group started one week after the injection period to evaluate reelins function during maturational processes. In contrast, behavioural testing of the adult group was conducted during injection days to investigate reelins acute impact on behavioural and cognitive functions. Reelin knockdown during puberty or adulthood induced a significant disruption of PPI compared to missense oligonucleotides or vehicle treated animals. Additionally, it was found that blocking of reelin translation during puberty caused a significant disruption of spatial working memory in the T-maze delayed alternation task. Interestingly, adult treated animals were less active. Western blot analyses showed a distinct and highly sensitive reelin knockdown in the mPFC in both, juvenile and adult rats. Taken together, these results indicate that a temporary peripubertal reelin knockdown in the mPFC leads to an impairment of working memory and sensorimotor gating. Thus, reelin in the mPFC seems to be essential to assure its neurotrophic functions during pubertal maturational processes, which are crucial for subsequent cognitive and behavioural aspects in adulthood. However, a depletion of reelin during adulthood produced a distinct impairment of sensorimotor gating and general activity, but does not affect spatial working memory. Therefore, we conclude that reelin in the mPFC may play an essential role for behavioural aspects in the mature brain but does not seem to be involved in synaptic plasticity and cognitive processes during adulthood.

Intracellular Aß correlates with neuron loss in Alzheimer's disease

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Over the past years, many transgenic mouse models accumulating beta amyloid (Aß) plaques have been generated, however, most of them show no or very little neuron loss. Recently, intracellular Aß accumulation was identified in mouse models and is thought to cause transport disturbances that could lead to neuron death. Corroborating the intracellular Aß theory, the APP/PS1KI mice accumulate large amounts of intracellular Aß in the CA1 region suffering from neuron loss. The present study investigated the impact of intracellular Aß accumulation on neuron loss in the cholinergic system of the APP/PS1KI mouse model. ChAT-positive neurons of motor nuclei were found to accumulate intracellular Aß abundantly, whereas no intracellular accumulation was found in either forebrain or pons complexes or in caudate putamen. Stereological quantification revealed loss of ChAT positive neurons in APP/PS1KI mice only in the motor nuclei accumulating intracellular Aß.

Another study was designed to more specifically investigate the toxic effect of intracellular Aß versus extracellular plaque Aß on neuronal survival. The thalamus and the frontal cortex of the APP/PS1KI mouse model were chosen for stereological quantification representing regions with plaques only (thalamus) or plaques as well as intraneuronal Aß (frontal cortex). Strikingly, a loss of neurons was found only in the frontal cortex at the age of 6 months coinciding with decreased intraneuronal immunoreactivity. These studies support the intracellular Aß hypothesis and suggest that plaques have no effect on neuron death, whereas accumulation of intraneuronal Aß is an early and transient pathological event leading to neuron loss in AD.

Gene expression analysis of axonal outgrowth factors in a neonate model of Parkinson's disease

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Ventral mesencephalic (VM) cells from embryonic day E14 transplanted into the substantia nigra (SN) of the neonate 6-OHDA model of Parkinson's disease (PD) are able to innervate the striatum (STR) provided that transplantation is performed until postnatal day P12. Mechanisms and molecules involved in neonatal axonal outgrowth have been previously described but their role in transplant fibre outgrowth remains unknown.

Gene expression patterns of the axonal outgrowth inhibitors NogoA and Semaphorin3A (Sema3A) were analysed in the neonatal 6-OHDA model of PD. Neonate rats (P1) received bilateral intraventricular injections of 6-OHDA and were sacrificed on 8, 10, 12, 15 and 18 days post-birth. A combined method of *in situ* hybridisation and double fluorescent immunohistochemistry was performed to analyse gene expression in glia along the medial forebrain bundle (MFB) in 6-OHDA lesioned and control animals.

NogoA gene expression profiles were neither different between control and lesioned animals, nor across different postnatal days (P8-P18). Reduced expression in the SN was due to the bilateral lesion. The results suggest that NogoA gene expression is stable throughout early postnatal development and altered by 6-OHDA lesion. Conversely, gene expression of Sema3A was increased in the STR in P15 lesioned brains compared to controls. Increased Sema3A expression was not detected at an earlier timepoint (P8-P12), suggesting a potential inhibitory effect on graft fibre outgrowth during later neonatal development.

Future analysis will focus on preventing inhibition of axonal outgrowth to promote the reconstruction of the nigrostriatal pathway. The results may further our knowledge on long-distance connectivity of transplanted neurons in PD patients and enhance the development of neurorestorative strategies.

Dopamine-dependent dyskinesia after grafting of serotonin neurons in relation to the proportion of grafted dopamine cells

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Transplantation of fetal dopamine neurons in patients with Parkinson´s disease has given inconsistent results: while some patients showed profound reduction of motor deficits, others showed only minor improvements. In addition, while L-DOPA-induced dyskinesia (LID) was usually reduced in most of the patients, some of the patients showed a new type of involuntary movements later termed graft-induced dyskinesia (GID). As one of the reasons for the development of GID the composition of the grafts has been discussed since grafts of fetal ventral mesencephalon contain large numbers of serotonin neurons in addition to the dopamine neurons. In previous studies in the rat Parkinson model it has been shown that dopamine grafts reduce LID but may induce GID, while serotonin neurons can worsen LID. In particular, serotonin neurons caused a detrimental effect when the number of dopamine neurons within the graft or the number of remaining dopamine neurons within the substantia nigra of the host was very low. Therefore, the aim of the present study was to further characterize the changes of LID and GID with respect to different proportions of dopamine and serotonin neurons within a graft. Sprague Dawley rats received unilateral complete dopamine-denervating lesions by injection of 6-hydroxydopamine into the medial forebrain bundle. The severity of the lesion was evaluated using amphetamine-induced rotation and performance in spontaneous motor behaviors (cylinder and corridor tests). Animals then received daily injections of L-DOPA (6mg/kg) and the peripheral decarboxylase inhibitor benserazide (10mg/kg) for 4 weeks and LIDs were evaluated according to a rat dyskinesia scale. Animals then received intrastriatal grafts of fetal dopamine and serotonin cells. The ratio of dopamine versus serotonin cells in the different experimental groups was 2:1, 1:1, 1:2, 1:4. The expression of LID and GID will be evaluated at 3, 6, 10 and14 weeks post-grafting. In addition, graft-induced changes in amphetamine-induced rotation and spontaneous motor behaviors will be characterized. We expect that we will be able to determine the ratio between dopamine and serotonin cells that will give good motor benefit with little risk for the development of dopamine-dependent dyskinesia.

DIAZOXIDE INCREASES THE NUMBER OF MITOCHONDRIA IN NEURITES AND CHANGES MITOCHONDRIAL TRAFFICKING

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Mitochondria are the major source of ATP production and their correct delivery to sites of high energy demand within a neuron should be important for many cellular functions. However, the mechanisms that regulate mitochondrial trafficking are not completely understood. Here we investigated effects of K^+ channel modulation on mitochondrial number and trafficking in neurites of cortical neurons. To monitor mitochondria cultures were transfected with mitochondrially-targeted eYFP. Co-expression of cytosolic targeted CFP was used to visualize neurite morphology. Mitochondrial movement was calculated as movement events/pixel.

Pretreatment with diazoxide, a K^+ channel opener, increased mitochondrial movements compared to vehicle treated control cultures (control: 100 ± 32%, diazoxide: 154 ± 50 %, both n= 11, $p < 0.001$) . Furthermore, we found an increased the number of mitochondria in neurites of diazoxide pretreated cultures (control: 8.2 ± 2.3, n= 9; diazoxide: 13.9 ± 2.9, n= 13, $p < 0.001$, given as number of mitochondria per 100µm neurite length). In contrast, 5-hydroxydecanoic acid (5-HD, 100 µM), a mitoK_{ATP} antogonist, reduced mitochondrial movement.

We have shown that toxic concentrations of glutamate (30 µM) reduced mitochondrial movement and altered mitochondrial morphology (Rintoul et al, 2003). Pretreatment with diazoxide protected against glutamate induced toxicity and improved the recovery of glutamate induced changes in mitochondrial movement and morphology.

These results suggests that diazoxide increases the number of mitochondria in neurites and changes mitochondrial trafficking. Supported by NIH grant #NS41299 and Michael J. Fox Foundation.

Nucleation-dependent aggregation of Aß is required for neuronal cell death

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The deposition of Aß in the brain is one of the pathological indicators of Alzheimer`s disease. Normally Aß is produced as a soluble metabolic product of the amyloid precursor protein (APP). In case of Alzheimer`s disease, Aß aggregates and accumulates in the brain as insoluble senile plaques. The major constituent found in the core of senile plaques are Aß fibrils, which are frequently described to be neurotoxic. However, recent findings suggest a particular role of soluble Aß species, so called oligomers, as they were shown to disturb long-term potentiation (LTP) of synaptic activity in the hippocampus and to cause neuronal cell death.
First, we reproduced the LTP disruption by Aß (1-40) and Aß (1-42) oligomers in hippocampal slices from mouse and rat, but surprisingly, we could not provoke cell death in neuronal single cell culture as well as organotypic hippocampal slices (OHC) with these oligomers. Similarly, application of Aß monomers to single cells and OHC did not cause cell death too. In contrast, if we induce Aß fibril formation by application of an aggregation seed, tremendous neuronal cell death could be observed in both culture models.

These results indicate that Aß fibril formation is essentially required to provoke neuronal cell death and that can be accelerated with nucleation-dependent aggregation of the Aß peptide. Moreover, it seems that Aß oligomers rather cause more subtle alterations, like an inhibition of plasticity, than a fast neuronal cell death. We plan to analyse and compare the potentially different signal cascades, which are activated by distinct Aß aggregation forms.

Death-associated protein-kinase is activated in oxygen-glucose-deprivation induced cell death in organotypic hippocampal slice culture

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The death-associated protein-kinase (DAP-kinase) is a calcium calmodulin-regulated serine/threonine protein kinase consisting of several domains with various functions. Beside the kinase domain and the calmodulin regulatory domain, the protein contains of 8 ankyrin repeats, a cytoskeleton binding region, 2 potential P-loop motifs and a death domain. DAP-kinase, which is crucially involved in the induction of early apoptotic pathways, is negatively regulated by autophosphorylation on serine 308 in the calcium calmodulin regulatory domain. In order to investigate the mechanism of neurodegeneration following ischemia we study the role of DAPK in organotypical slice culture model. Our study shows that after oxygen glucose deprivation (OGD) the DAP-kinase becomes rapidly dephosphorylated and activated. The neuroprotective NMDA receptor antagonist MK-801 inhibits the dephosphorylation of DAP-kinase after OGD. Our data indicate that the DAP-kinase is one of the mechanisms activated by the excessive glutamate release during ischemia via the NMDA receptor and that DAP-kinase activation maybe a major cause for the subsequent delayed neuronal death.

ENHANCED HYPOXIA SENSITIVITY IN HIPPOCAMPAL SLICES FROM A MOUSE MODEL OF RETT SYNDROME

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Rett syndrome is a neurodevelopmental disorder caused by mutations in the X-chromosomal *MECP2* gene encoding for the transcriptional regulator methyl CpG binding protein 2 (MeCP2). Rett patients suffer from episodic respiratory irregularities and reduced arterial oxygen levels. To elucidate whether such frequent hypoxic episodes induce adaptation/preconditioning of the hypoxia-vulnerable hippocampal network, we analyzed its hypoxic responses in adult Rett mice. The occurrence of hypoxia-induced spreading depression (HSD) – an experimental model for ischemic stroke – was hastened in *Mecp2*$^{-/y}$ males. The extracellular K^+ rise during HSD was attenuated in *Mecp2*$^{-/y}$ males and the input resistance of CA1 pyramidal neurons decreased less before HSD onset. CA1 pyramidal neurons were reduced in size and more densely packed, but the degree of cell swelling during HSD was unaffected. The intrinsic optical signal and the propagation of HSD were similar among the different genotypes. Basal synaptic function was intact, but *Mecp2*$^{-/y}$ males showed reduced paired-pulse facilitation, higher field potential/fiber volley ratios but not an increased susceptibility to seizures. Synaptic failure during hypoxia was complete in all genotypes and the final degree of posthypoxic synaptic recovery was indistinguishable. Cellular ATP content was normal in *Mecp2*$^{-/y}$ males, but their hematocrit was increased as was HIF1a expression throughout the brain. This is the first study showing that in Rett syndrome, the susceptibility of telencephalic neuronal networks to hypoxia is increased. The underlying molecular mechanisms apparently involve disturbed K^+ channel function. The increased hypoxia susceptibility may potentially contribute to the mental impairment and cognitive dysfunction and to the vulnerability of male Rett patients who are either not viable or severely disabled.

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Repetitive sensory stimulation training in stroke

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Somatosensory input is crucial for tactile perception and sensorimotor performance, and for improving performance through neuroplasticity mechanisms. Effective rehabilitation following stroke consists of training and practicing and strongly depends on somatosensory input. In patients with stroke, somatosensory deficits are usually associated with slower recovery of motor function (1). In healthy subjects on the other hand electric somatosensory stimulation was shown to result in reorganization of motor and somatosensory cortices (2). Thereby stimulation led to increases in fMRI activity in primary somatosensory (S1) and motor (M1) cortices (2). Direct connections between S1 and M1 could provide the anatomic substrate for the influence of somatosensory stimulation on motor cortices. Based on these findings the effects of peripheral somatosensory stimulation on motor recovery after stroke are subject of current research (1, 3).

As an alternative to standard training methods, our group developed a passive stimulation protocol called tactile coactivation (CA), which enforces cortical reorganization in parallel to improvement of tactile and sensorimotor performance (4-6). The unique advantage of the coactivation is its passive nature, i.e. it does not require the active cooperation of the subject.
Here we introduce an electrical coactivation paradigm substituting standard physiotherapy in subacute stroke patients suffering from middle cerebral artery infarction. For coactivation, all fingers of the affected arm were electrically stimulated (20 Hz bursts with 5 sec interburst intervals) for 45 min per session. During two weeks of therapeutic treatment the coactivation was applied for a total of 7.5 h per patient. Tactile, haptic and fine motor performance of all patients was assessed prior to treatment, during treatment, directly after treatment and in a follow-up measurement 3 months after treatment.
Assessment of tactile, haptic and fine motor performance directly after the treatment revealed significant improvements in all tasks tested: Touch thresholds were reduced, tactile acuity was increased, haptic form recognition was improved and the time to perform the Moberg pickup test and the nine-hole peg-board test was reduced. Remarkably, even after the end of the therapeutic treatment the improvement progressed, as it could be demonstrated in the follow-up measurements.

Conclusion: Somatosensory coactivation applied to a paretic limb can improve sensorimotor performance in patients with subacute stroke, supporting the proposal that in combination with training protocols electric somatosensory coactivation may enhance the benefit of neurorehabilitative interventions.

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Gene expression changes in brain and testis of Atxn3 ko mice

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Spinocerebellar ataxia type 3 (Machado-Joseph-disease; SCA3/MJD) is the most common autosomal dominantly inherited ataxia. We performed a comprehensive DNA microarray analysis of previously generated Atxn3 knock-out mice and compared gene expression changes in brain and peripheral tissues. Further, we explored whether the lack of the deubiquitinating function of Atxn3 resulted in altered brain protein levels.

357 genes showed significant changes in mRNA expression levels in Atxn3 ko brain.In contrast, only 34 transcripts were regulated in testis. None of these genes showed significant expression changes in both tissues.

Five genes, Prkar2B, Usp3, Sirt2, Trf and Rbmp14 were confirmed in independent qRT-PCR experiments and two of the identified proteins (Sirt2 and Rad23B) were confirmed by immunohistochemistry. However, no specific substrate of Atxn3 has emerged from the analyses so far, indicating a redundantly encoded function of Atxn3.

Spectrally resolved recordings of the intrinsic optical signal in rat hippocampal slices during severe hypoxia

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Severe hypoxia gives rise to a concerted massive depolarization of neurons and glial cells which is being referred to as hypoxia-induced spreading depression (HSD). The electrical changes of HSD are accompanied by a so-called intrinsic optical signal (IOS) which is due to changes in light scattering within the tissue. The IOS can be monitored by recording either light reflectance or transmittance in the tissue of interest. Even though the very mechanisms underlying the generation of these optical signals are still to be identified, the IOS is often used as a non-invasive tool to visualize the site of HSD ignition, its propagation within brain tissue and its spatiotemporal profile. Usually white light is used to illuminate the tissue and to measure the changes in either reflectance or transmittance. During HSD tissue reflectance noticeably increases (transmittance decreases) thereby identifying those areas of the brain tissue undergoing HSD. Taking advantage of a polychromatic, fast-switching light source we have now performed spectrally resolved recordings of the IOS associated with HSD in acute rat hippocampal tissue slices. Instead of taking single images, a full spectrum was recorded every 15 s, ranging from 320-680 nm (20 nm steps, 20 ms exposure time). Displaying this complex data set as surface plot revealed clearly distinct profiles for tissue reflectance under normoxic conditions, early hypoxia, the phase of HSD, and the reoxygenation. Early during hypoxia a noticeable (15-20%) decrease in light reflectance was observed within the entire spectrum that was most pronounced around 380 nm and 500 nm. As soon as HSD was ignited tissue reflectance increased by up to 30%, with the most pronounced changes occurring at 400 nm and 480 nm. At a wavelength of 440 nm hardly any changes were observed, which may suggest a massive absorption band in that range. Upon reoxygenation these changes fully recovered within 2-3 min. Ion substitution experiments and drug treatment caused characteristic changes in this complex reflectance profile. In Ca^{2+} free solutions the IOS was intensified over the entire wavelength range and its recovery upon reoxygenation was incomplete. Replacing extracellular Cl^- by methylsulfate abolished the increase in tissue reflectance, especially in the range beyond 440 nm, and a pronounced decrease in tissue reflectance occurred within the entire spectrum. Inducing HSD by 1 mM cyanide distorted the optical signature and during HSD an irreversible increase of tissue reflectance developed in the range of 340-400 nm. Hypotonic solutions increased, whereas hypertonic solutions decreased the intensity of the IOS. The pronounced changes in the low wavelength range (380 nm and 480 nm) and the characteristic modulation induced by cyanide suggest that mitochondrial metabolism and changes in the redox couples NAD/NADH and FAD/$FADH_2$ contribute to the characteristic optical signature of HSD.>

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Autoantibodies and circulating immune complexes in the plasma of Alzheimer's disease patients

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It has previously been shown in several forms of cancer, characterized by increased or altered production of a certain physiological protein, that circulating immune complexes of that protein with IgM can be used as a diagnostic biomarker.

Considering that Alzheimer's disease is also characterized by an abnormal production of Aß, a physiological protein also present in the blood, its potential as a biomarker has been investigated. IgM-Aß complexes concentration has been measured in 90 plasma samples from patients with Alzheimer's disease, individuals with mild cognitive impairment, patients with Lewis body disease or fronto-temporal dementia patients and healthy controls of matched age and sex.

To characterize circulating Aß, plasma samples from 5 AD patients and 5 HC subjects were pooled and subjected to gel-filtration analysis. Fractions collected from the column were tested for the presence of Aß. A strong reactivity was observed in the fractions eluting at high molecular weight (>500 KDa) and 150 KDa (IgM fraction) (Fig.1). The high molecular weight fraction, containing both IgMs and immune complexes, together with the IgG fraction, the albumin fraction (50 KDa) and the small molecular weights fraction containing monomeric Aß have been further characterized.

The identity of the fractions has been validated by ELISA and Western blot. In the same fractions the presence of Aß has been assessed with antibodies directed to different Aß epitopes. A high reactivity, especially to antibodies against Aß oligomers, has been found in both the IgM and IgG fractions, but not in the albumin one.

The possible diagnostic value of Aß-immune complexes has been evaluated through a series of screenings on all plasma samples. Two dedicated ELISA assays have been developed to quantify the circulating Aß-IgM complexes and Aß oligomers-IgM complexes, since that is the Aß form predominantly present in the IgM fractions.

Screening of plasma samples by two assays showed no statistically significant differences between the means of the studied groups. However, by observing the Receiver Operator Characteristic (ROC) curve it is possible to notice that the two assays have a certain diagnostic power. Therefore, it is possible to set a suitable cut-off level to obtain the best combination of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). These data are the first evidence of the occurrence of IgM complexes with different Aß forms in the blood of patients affected by Alzheimer's disease. The comparison of immune-complexes level between patients and healthy controls didn't show any significant difference. However, by choosing an appropriate cut-off level these preliminary tests showed either a discrete sensitivity, in the case of Aß-IgM, and a discrete specificity, in the case of Aß oligomers-IgM, suggesting that further studies, and the combination of different tests, may lead to improved results.

The influence of pellet density on the graft-induced functional recovery in a skilled paw-reaching test in the rodent unilateral 6-OHDA Parkinson's disease model

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The Staircase test (Montoya et al, 1991) was established to measure side-specific deficits in skilled paw reaching in rats. The apparatus consists of two staircases located one on either side of a central plinth. Food pellets are positioned onto each of the 6 steps of the staircases allowing rats to reach down either side of a plinth to grasp, lift and retrieve food pellets from the steps of the staircase. The number of pellets retrieved, or moved, provides a quantifiable measure of the distance and efficiency of reaching skill. The aim of this study is to investigate the sensitivity of the test to detect functional recovery of grafted PD rats based on the number of pellets placed in each step.
53 Sprague Dawley rats were trained in the staircase boxes. Ten animals compose the control group and 43 were unilaterally 6-OHDA lesioned and after a month half of the animals were transplanted with a total of 400,000 ventral mescenchephalic rat E14 derived cells. Two and four weeks after lesion and transplantation, animals were tested for drug induced rotations. Transplanted and lesion animals were equally divided into two groups. Each group was tested in the staircase under one of the following conditions: i) HIGH: 6 stairwells baited with 10 pellets each (6x10); ii) LOW: 6 stairwells baited with 2 pellets each (6x2). The first round of paw reaching was a free choice test, had duration of 25 days and began five weeks after the grafting. Two weeks after the completion of the first paw reaching round the animals were tested again by a forced choice paw reaching test. Two weeks after this the animals were tested by crossing over the conditions between the groups. Survival and integration of the grafted neurons were assessed by immunohistochemical analysis. This is an ongoing study and the evaluation staircase test will be presented at the conference.

The type of amyloid b-protein (Ab) generation determines the phenotype of Ab-pathology in different mouse models of Alzheimer's disease.

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The deposition of the amyloid b-protein (Ab) in the brain is one of the hallmarks of Alzheimer's disease (AD). Recent work has shown that not only extracellular but also intracellular Ab is involved in the pathogenesis of Alzheimer's disease. Although it is well known that aggregated forms of Ab are responsible for toxic effects it is not yet clear whether intracellular Ab is also responsible for the neurodegenerative effects. To address this question we have analyzed the frontocentral cortex of the APP48/167 mouse at different ages. These mice express a construct encoding a rat proenkephalin signal sequence followed by Ab_{1-42} driven by the neuron specific Thy-1 promoter which enables them to produce almost exclusively intracellular Ab_{1-42}. Immunohistochemical stainings with anti Ab_{42} antibodies revealed no plaques in the brain of these mice at the age of 3 as well as of 18 months. However, three major types of Ab containing lesions were observed: 1. Ab-grains in the cortex, i.e., roundish Ab-positive grain-like structures in the neuropil; 2. somatic granules in the perikaryon of neurons; and 3. dendritic threads. Double staining with anti-microtubulus associated protein MAP-2 and anti-Ab confirmed the neuronal localization of the somatic granules and the dendritic threads. Ultrastructurally, somatic granules appeared as lysosomal organelles positive for Ab in the cytoplasm of neurons. Although all three types of lesions were seen in 3 and 18 months old APP48/167 mice the Ab-positive material appeared more prominent in layers II, III and V in the younger animals whereas older animals showed a more disperse distribution. The number of neurons in the fronto-central cortex as well as their layer-specific distribution in APP48/167 mice was not different from that in wild-type controls. However, a significant loss of neurons was observed in the hippocampal subfield CA1 of APP48/167 mice. In summary, the APP48/167 mouse model exhibits a different pattern of Ab-lesions than transgenic mice overexpressing APP and allows the analysis of the effects of intracellular Ab. Supported by DFG 624/6-1

L-glutamine induces apoptosis in microglia

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Under physiological conditions, astrocytes take up L-glutamate from the synaptic cleft, metabolize it to L-glutamine and give it back to neurons, where L-glutamine is metabolized to L-glutamate and stored in neurotransmitter vesicles. However, liver failure enhances L-glutamine and ammonia globally in the brain. The Trojan horse hypothesis of L-glutamine toxicity predicts that intramitochondrial hydrolysis of L-glutamine elevates ammonia locally and promotes mitochondrial dysfunction.

In the present study, we describe that exposure of primary microglia as well as of the microglial cell-line, BV-2, to L-glutamine (7 mM) induced chromatin condensation and formation of crescent-like structures in the nucleus. Furthermore, L-glutamine promoted increase in annexin-V labelling, cell shrinkage (apoptotic volume decrease), cell fragmentation, and formation of apoptotic bodies. Blockade of the phosphate-activated glutaminase with 6-diazo-5-oxo-L-norleucine (DON) suppressed chromatin condensation and annexin-V labelling in L-glutamine-exposed cells. Complementary, blockade of the glutamine synthetase with L-methionine sulfoximine (MSO) suppressed chromatin condensation and annexin-V labelling in ammonia-exposed cells. L-glutamine and ammonia enhanced production of reactive oxygen species, detected with CM-H_2DCFDA. Apoptosis, induced by L-glutamine, was suppressed either by the radical scavenger a-tocopherol, or by the NOS-inhibitor, L-NMMA. Cyclosporine A, a ligand of the permeability transition pore complex component, cyclophilin D, prevented L-glutamine-induced apoptosis. Furthermore, inhibition of caspase-9 activity with Z-LEHD-FMK prevented L-glutamine-induced apoptosis.

Our findings indicate that hydrolysis of L-glutamine and, accordingly, accumulation of ammonia in mitochondria trigger the intrinsic pathway of apoptosis, characterized by mitochondrial dysfunction, depolarization of the mitochondrial membrane potential, release of mitochondrial intermembrane proteins, and activation of caspase-9, which activates caspase-3.

Application of Parkinsonian toxins in the mouse retina

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Toxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 6-hydroxydopamine (6-OHDA), or rotenone have been used to induce degeneration of dopaminergic (DA) neurons in the nigrostriatal pathway and to reproduce pathological characteristics of Parkinson's disease (PD). DA neurons are also present in the retina, and visual impairments in PD patients have been reported. Therefore, we examined the vulnerability of DA amacrine interneurons in the retina against MPTP, 6-OHDA, or rotenone-induced cell death. We intraperitonealy (i.p.) injected mice with MPTP, which induced degeneration of DA neurons in the midbrain. However, no amacrine cell death was detectable in the same mice. HPLC analysis revealed a 9 times lower level of the toxic metabolite of MPTP, MPP^+, in the eye compared with the striatum. Possible reasons for the survival of retinal amacrine cells after systemic MPTP application was a less efficient conversion into toxic MPP^+ in the retina, or a general higher resistance against toxic insults of retinal DA neurons compared with DA neurons in the substantia nigra *pars compacta* (SN*pc*). Therefore, we directly injected high doses of MPP^+, 6-OHDA, or rotenone into the eye. No effect on degeneration of DA amacrine cells in the retina was observed, suggesting different properties and less vulnerability of DA amacrine neurons compared with DA neurons in the midbrain.

TDP-43 in ALS and FTD, a toxic gain-of-function?

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Frontotemporal Lobar Dementia (FTD) and Amyotrophic Lateral Sclerosis (ALS) are fatal human disorders affecting frontotemporal lobar neurons and motor neurons, respectively. So far, no effective cure is available for either disorder and the precise disease mechanisms for the most common forms of FTD and ALS remain elusive. Understanding the mechanisms of neuropathology in FTD and ALS thus remains a top priority and is a prerequisite for rational design of therapeutic approaches. Recent data has shown that TDP-43 aggregates are found in both FTD and ALS. This strongly suggests a common mechanism underlying neuron loss in these diseases.

TDP-43 is a nuclear ribonucleoprotein with DNA/RNA binding properties. It was found to be involved in splicing and transcriptional regulation. In non- diseased indivudals, TDP-43 is located mainly to the nucleus. However with disease, TDP-43 is found in cytoplasmic aggregates. In addition to the disease-linked protein mislocalization, TDP-43 is cleaved at the N-terminus, resulting in a 24-kD fragment. As this fragment is found exclusively in the disease state, it may therefore be a pathogenic species.

We investigated aggregate formation of TDP-43 in HEK293 cells. We show that certain TDP-43 variants display differrent aggregation properties. In addition we show that neuronal expression of human TDP-43 in *Drosophila* causes adult onset neurodegeneration. TDP-43 expressing flies show decline in locomotion ability and a reduction in longevity. This implies a direct pathophysiological link between TDP-43 and ALS/FTD-U. Interestingly, degeneration of photoreceptor neurons was not observed in aged flies. This suggests that different neurons (e.g.motor neurons) are more susceptible to TDP-43 induced neurodegenetration.

Environmental enrichment improves motor abilities but fails to rescue memory functions and neurogenesis in the APP/PS1KI mouse model of Alzheimer's disease

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Background: The APP/PS1KI mice have been shown to perform poorly in motor tests like the string suspension, grip hanging, and swimming tasks in which they clearly show a disturbed swimming pattern and a shorter swimming distance at six months of age. Working memory, assessed by spontaneous alternation behaviour in the Y-maze and the cross-maze, was also impaired. Immunostaining showed that these bigenic animals have a massive intracellular accumulation of Aß peptides in the pyramidal cells of the CA regions of the hippocampus. Stereological investigation revealed a neuronal loss of approximately 50 % in the CA1/2 regions between 2 and 10 months of age and general axonopathy in brain and spinal cord. **Aim:** evaluate the effects of long-term exposure to an enriched environment on the behavioural and histopathological phenotype of APP/PS1KI mice. **Methods:** Behavioural phenotyping, immunohistochemistry, stereology. **Results:** An environmental enrichment combining social interactions, physical activity, and exploration of new objects between the age of 2 and 6 months improved the motor abilities, expressed by a significant amelioration both in the string suspension and grip hanging tasks (respectively $p<0,01$ and $p<0,05$), compared to age-matched untrained APP/PS1KI mice. Stereology revealed that enrichment had no effect on the neuron number in the pyramidal cell layer of the CA1 region, which still shows a difference of approximately 40 % between APP/PS1KI and wild-type animals. The enriched environment failed to restore the neurogenesis and didn't result in any improvement of the working memory. Interestingly, Ki67-positive cells were found in the sub-granular zone of the dentate gyrus in the double-transgenic animals that spent four months in enriched housing but no signal could be detected in age-control non-enriched double-transgenic animals. The fate of these new cells is being investigated to define whether they contribute to the inflammatory response (glial cells) or to the build-up of new granule neurons. **Conclusions:** Enriched environment has beneficial effects on some motor abilities of APP/PS1KI mice, which is very likely due to a better muscular strength and the use of three dimensional exploration. However, hippocampal dysfunction could not be restored. Enriched housing conditions had no effect on the neuronal loss observed in the CA1 region of the hippocampus and the birth of new cells in the sub-granular zone of the dentate gyrus didn't result in any migrating neuron. Enriched environment has however significant beneficial effects on motor performance and may be translated to some symptoms reported in AD patients.

GDAP1, a protein mutated in hereditary polyneuropathy Charcot-Marie-Tooth disease 4A, protects from oxidative stress

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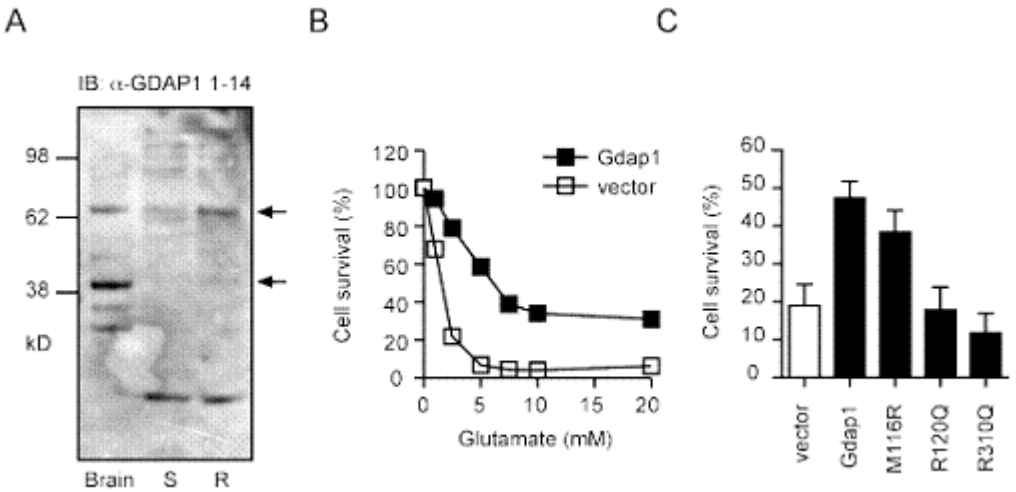
Charcot-Marie-Tooth disease (CMT) is the most frequent inherited polyneuropathy and affects both motor and sensory nerves. It can be grouped into demyelating and axonal forms. Mutations in *GDAP1* (ganglioside-induced differentiation associated protein 1) lead to severe forms of CMT4A, a ressecive, demyelinating form of CMT. GDAP1 is a mitochondrial transmembrane protein that has been shown to play a role in mitochondrial fusion and fission. It features homologies to glutathione S-transferases, which are important enzymes in the detoxification of products of oxidative stress. Oxidative glutamate toxicity in the hippocampal neuronal cell line HT22 serves as a model for oxidative stress-mediated cell death.
Transcriptome analysis of HT22 cells that are resistant to glutamate-mediated oxidative stress revealed an upregulation of several transcripts including *GDAP1*. This upregulation was confirmed on protein level (Figure). Transient overexpression of GDAP1 rescued glutamate-sensitive HT22 cells from glutamate-mediated cell death. When HT22 cells were transiently transfected with pathogenic GDAP1 mutants, this protection was reduced. Morover, the ability of GDAP1 mutants to protect from oxidative stress correlated with their published effect on mitochondrial morphology. GDAP1-overexpressing cells also exhibited an increased amount of glutathione, the most important intracellular antioxidant.
We conclude that GDAP1 is a cytoprotective and possibly antioxidant protein. Therefore, oxidative stress may be involved in the pathology of CMT4A.



Hippocampal ß-amyloid plaques in triple transgenic mice revealed with a novel, fluorescent acetylcholinesterase inhibitor delivered from nanoparticles

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The drastic loss of cholinergic projection neurons in the basal forebrain is a hallmark of Alzheimer's disease (AD), and most frequently applied drugs for the treatment of dementia include inhibitors of the acetylcholine-degrading enzyme, acetylcholinesterase (AChE). This protein is known to act as a ligand of ß-amyloid (Aß) in senile plaques, a neuropathological sign of AD. Recently, new histological methods with luminescent probes were introduced for the further detailed analysis of Aß fibrils in fixed tissue sections (Nilsson et al. 2007, ACS Chem. Biol. 2:553-560).

Here, we present histochemical data on the novel fluorescent, heterodimeric AChE inhibitor PE154 also targeting Aß plaques. The spectroscopic properties of PE154 - a sharp excitation peak with a maximum at 405 nm and an emission maximum at 517 nm - allow for its combined use with immunolabelling based on the bright fluorescent carbocyanines Cy3 and Cy5. Numerous plaques double-stained for PE154 and Aß-immunoreactivity were revealed by confocal laser-scanning microscopy. They were found both in fixed cortical tissue sections from an autoptic case with verified AD and in triple transgenic (TTG) mice with age-dependent ß-amyloidosis and tau hyperphosphorylation, an established animal model for aspects of AD (Oddo et al. 2003, Neuron 39:409-421).

Additionally, we were able to visualize the targeting of Aß-immunopositive plaques *in vivo* three days after injection into the hippocampi of 13-20-months-old TTG mice. Furthermore, PE154 labelled Aß, but not hyperphosphorylated protein tau, in aged TTG mice after intrahippocampal injection of biodegradable core-shell polybutylcyanoacrylate/polystyrene nanoparticles releasing the fluorescent marker *in vivo*.

In conclusion, nanoparticles appear as versatile carriers for AChE inhibitors and other promising drugs for the treatment of AD.

Impaired K^+-channel activity attenuates cyanide-induced hyperpolarization of CA1 pyramidal neurons in Mecp2-deficient mice

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Mecp2-deficiency causes a neurodevelopmental disorder called Rett-syndrome which is characterized by an imbalance between inhibitory and excitatory neurotransmission resulting in hyperexcitability. We observed an increased susceptibility of the $Mecp2^{-/y}$ hippocampal formation to hypoxia-induced spreading depression which in part seems to be due to impaired K^+ channel function. To investigate the underlying mechanisms in more detail, intracellular sharp electrode recordings as well as single-channel recordings were performed in CA1 pyramidal neurons of acute hippocampal tissue slices prepared from adult Mecp2-deficient and wildtype males. Intracellular recordings did not reveal any differences in pyramidal cell resting membrane potential and input resistance between wildtypes and $Mecp2^{-/y}$ males. Chemical anoxia (1 mM NaCN) caused pronounced hyperpolarizations in wildtype neurons (-6 mV) but only mild hyperpolarizations in $Mecp2^{-/y}$ neurons. In accordance, membrane resistance during cyanide-induced metabolic arrest decreased less in Mecp2-deficient hippocampal neurons. As these differences strongly suggest impaired potassium channel function in Mecp2-deficient neurons, further experiments were performed on the single channel level. Recordings in excised inside-out patches of CA1 neurons identified a large conductance (~ 260 pS), BK-type Ca^{2+}-dependent potassium channel. Its basic biophysical properties (single-channel conductance, open-probability, voltage-dependence, Ca^{2+}-dependence) were determined in symmetrical 145 mM K^+-solution and did not differ significantly between wildtype and $Mecp2^{-/y}$ males. Different responses were, however, observed in response to chemical anoxia. In cell-attached patches such metabolic arrest by 1 mM NaCN caused a pronounced activation of various K^+-channels in wildtype which was less intense in $Mecp2^{-/y}$ neurons. Despite inclusion of 200 µM tolbutamide into the pipette solution, an additional intermediate-conductance (~80 pS) K^+-channel, which clearly differed from the BK channel, became activated in cell-attached patches of wildtype and $Mecp2^{-/y}$ neurons. These data suggest that the increased susceptibility to hypoxic spreading depression in $Mecp2^{-/y}$ hippocampal neurons might be due to disturbed activation of different K^+-channels during hypoxia. This results in a dampened hyperpolarization and input resistance decrease, i.e. a less efficient stabilization of the membrane potential and may therefore hasten the onset of hypoxic spreading depression.

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Micro-transplantation approach in a quinolinic acid induced rodent model of Huntington's disease

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Neural transplantation has been demonstrated to be an optional treatment for Huntington's disease (HD). In this study, we investigated the different neuroanatomical and behavioural effects between single- (ST) and multi-tracts (MT) transplants performed in a HD rodent model.
44 female Sprague Dawley rats received unilateral quinolinic acid injections into the left striatum. 10 days after the quinolinic acid lesion the animals were transplanted with a rich GABAergic progenitors single cell suspension (480,000 cells/animal) derived from the striatum of E15 GFP rat embryos. Grafting procedure included 1 tract/2 deposits (ST) or 9 tracts/18 deposits (MT) transplants using a 2µl Hamilton's syringe with capillary. Animals were trained prior to lesion and 4, 8, 12 weeks post transplantation in spontaneous behaviour, skilled forelimb use and apomorphine rotation. Animals were perfused and brain tissue was processed for immunohistochemistry. Graft volume and cell survival will be assessed by stereology.
After lesion all the lesioned rats showed significant functional deficits in all the behavioural tests and apomorphine-induced rotation test. During the whole test course following transplantation ST and MT groups did not show any significant functional recovery in the stair case as well as in the rotation test. Morphological graft analysis is ongoing and all the resource will be presented at the conference.
Skilled forelimb can be used as a sensitive method to evaluate the quinonilic acid induced lesion. Evidence will be provided at the conference if the MT transplants based on micro transplantation approach can offer significant advantages for HD as was shown for Parkinson's disease.

Epileptic seizure-induced changes in fear behaviour and neurophysiological activity in amygdaloid circuits

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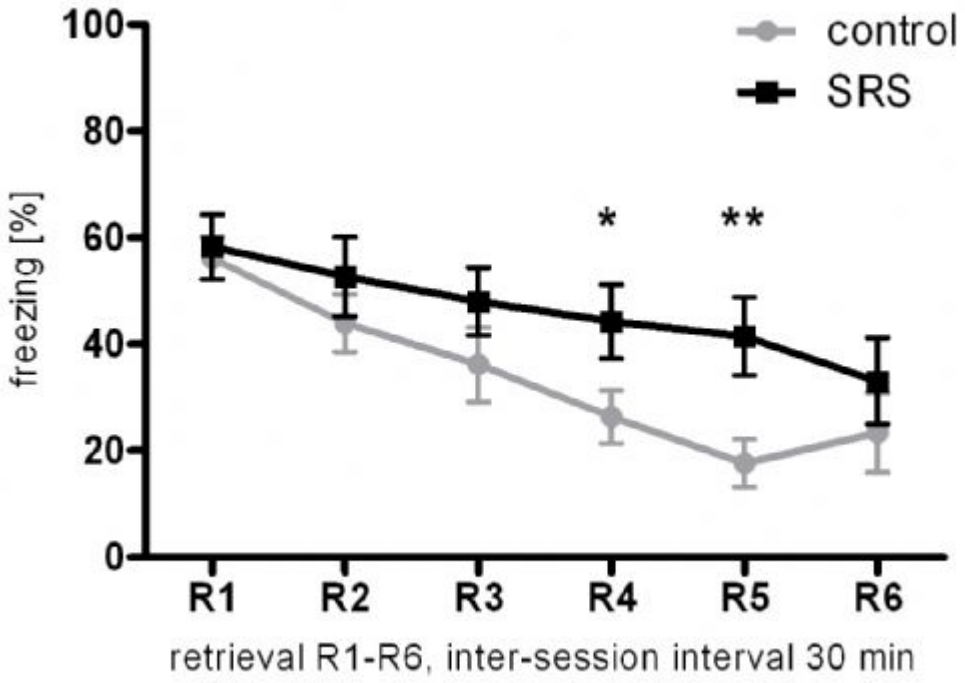
The relationship between epilepsy and anxiety has received much attention. The experience of anxiety reported by patients before or in between the occurrence of temporolimbic seizures has been attributed to activation of the amygdala and/or hippocampus, which are critically involved in both the pathogenesis of temporal lobe epilepsy (TLE) and fear-related behaviour. However, seizure modulated fear and physiological or structural correlates had not been systematically examined, and the underlying basics on network levels remain unclear to date.

Therefore this project was set up to characterize the neurophysiological bases of seizure-related fear in the amygdala-hippocampus-system. The experimental strategy was composed of the following steps: (1) use of the mouse pilocarpine model of TLE, (2) behavioural analyses of anxiety states in the Elevated Plus Maze, Light/Dark avoidance test and Pavlovian fear conditioning, (3) probing neurophysiological activity patterns in amygdalo-hippocampal circuits in freely behaving mice, with particular reference to theta synchronization, which we have shown to correlate with the consolidation of fear memory.

Our results displayed no significant differences in basic anxiety levels (Elevated Plus Maze and Light/Dark-test) comparing mice which developed spontaneous recurrent seizures (SRS) and controls. Furthermore, conditioned fear memory retrieval was not influenced in SRS mice. However, during fear memory extinction, SRS mice showed an extended freezing behaviour (see Figure) and a maintained amygdala-hippocampal theta frequency synchronization compared to controls.

These results indicate specific alterations in conditioned fear behaviour and related neurophysiological activities in the amygdala-hippocampal network contributing to impaired fear memory extinction in mice with temporal lobe epilepsy. Clinically, the non-extinguished fear memories may well contribute to the experience of fear in TLE patients.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Generation of a neuron-specific nonviral gene transfer system *in vivo* – a possible therapeutical approach for neurodegenerative disorders

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Currently, gene therapy is the most promissing therapeutical tool for e.g. genetic diseases and gene defects. Viral vectors based on Lenti- or Adenoviruses are used, predominantly. But due to specific problems in connection with the different applications of viral vectors non-viral vectors become more and more important. Therapeutic gene transfer mediated by non-viral vectors has successfully been applied to various organs including CNS. There might be several advantages of non-viral vectors. They are easy to generate, simple in their construction and potentially safer as viral vectors. There is no risk of uncontrolled replication and their synthesis is less expensive compared to viral vectors (partially because of their easy generation in mammalian cell free systems). Contrary to viral vectors, there is no pre-existing immunity of human against non-viral vectors that could interfere with transfection efficiency and create potential side-effects. Here, we present a therapeutic strategy against neurodegenerative disorders that is based on neuron-specific gene transfer through branched and linear polyethylenimines (PEIs). PEIs are positively charged and condense negatively-charged DNA to sizes below 200 nm, facilitating cell entry and causing endosomal rupture. The degree of branching affects transfection efficiency. PEIs might be particularly promissing for CNS targeting, possessing several advantages: DNA/PEIs are well tolerated when administered to the CNS, where PEIs were found highly enriched even after systemic administration. Cell specifity will be optimized through coupling PEIs to different ligands possessing high affinity to surface receptors of the target cell.

Implicit memory and dopaminergic basal ganglia processes: a new rat model

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Sequential behaviour, a type of procedural behaviour, has been intensively investigated in humans using the so-called serial reaction time task (SRTT), which was first introduced by Nissen and Bullemer (cognitive psychology 19, 1987). In the SRTT subjects have to respond to visual stimuli by key pressing. Decreases in reaction times to sequential – compared to random – stimulus presentation are taken as an indicator of sequential learning.

Human SRTT findings indicate that sequential behaviour is mediated by dopaminergic processes in the basal ganglia. There is evidence that sequential learning is impaired in Parkinson's disease patients (eg: Siegert et.al. Neuropsychology 20(4), 2006). However, the possibilities for research on the underlying neuronal mechanisms of sequential behaviour remain limited in humans.

For a more precise study of the neural structures – especially the basal ganglia and its dopaminergic pathways - involved in sequential behaviour we used an analogous rat model of the human SRTT which was recently developed by Domenger and Schwarting (behav brain res 160; 2005). In the rat SRTT the animals have to respond to visual stimuli by nose poking. Just like in the human SRTT the stimuli are either presented randomly or in a sequential order.

We investigated the behavioral effects of bilateral 6-OHDA lesions on different sites of the basal ganglia, which are commonly used as an animal model of Parkinson's disease. To differentiate between lesion induced effects on the acquisition and on performance of sequential behaviour, the lesions were either placed before or after an intensive training on the SRTT.

Among others, our results show that striatal dopamine is crucial for the acquisition of sequential behaviour.

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Validating the use of BAC-GFP animals as tissue donors in HD graft studies

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Intrastriatal implantation of embryonic striatal tissue has shown long-term benefits in patients with Huntington's disease (HD), albeit limited in scale and restricted to a small number of patients. Grafts in animal models of HD can receive both cortical and dopaminergic afferents, and can extend projections towards their output targets. However, the level of organisation of efferent projections from striatal transplants is less understood. Approximately half of the medial spiny striatal projection neurons (MSN) have dense efferent connections toward the external segment of the globus pallidus (GPe) and make up the "indirect pathway", whilst other striatal neurons send projections on the "direct pathway" toward the pars reticulate of the substantia nigra (SNr) and the entopeduncular nucleus (EP). The two population of MSN are morphologically indistinguishable from each other, are intermingled throughout the striasomes/ matrix compartments of the striatum, but are characterized by different dopamine and muscarinic receptor subtypes and peptides.

The two BAC transgenic lines used in the project are designed to help identify the selective patterns of efferent projections of the MSN, and distinguish between a) the "indirect pathway" where GFP expression is under the control of the D2 promoter, and b) the "direct pathway" where GFP expression is under the control of the M4 promoter. The objectives of the pilot studies were to establish viable breeding colonies from the frozen transgenic embryos, to establish genotyping and immunohistochemical and fluorescent protocols permitting the confirmation and visualisation of the GFP construct and the protein, and to initiate transplantation studies. Preliminary data suggests that tissue from GFP-BAC transgenic animals represent a powerful novel way to study graft-host interaction.

Support: Hereditary Disease Foundation

Early detection of a behavioral phenotype in rats transgenic for Huntington's disease

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Huntington's disease (HD) is an autosomal dominant, neurodegenerative disorder caused by CAG repeat expansion and consecutive polyglutamine expansions in the huntingtin (Htt) gene. The transgenic rat model (tgHD) carrying a truncated human huntingtin fragment of 51 CAG repeats under control of the rat Htt promoter closely resembles the late-onset form of HD exhibiting a slowly progressing behavioral phenotype with emotional disturbance, motor deficits, and cognitive decline. It has been shown that behavioral symptoms precede the appearance of the earliest aggregates at about 6 months of age in this model and can be detected in a very young age.

To further study this early behavioral phenotype in tgHD rats we performed ultrasonic vocalization, acoustic startle response, and prepulse inhibition (PPI) tests in P10-17 pups. To study exploration and risk behavior the novel cage test was performed in pre-weaning 21 days old rats. In this test each animal was allowed to explore a clean macrolone type III cage for five minutes. By video analysis the parameters locomotion (motionless, walk), exploration (free rearing, wall rearing) and risk taking (time spent in the centre of the cage compared to the wall and corner area) were determined.

Exploration and risk taking was greatly altered in pre-weaning transgenic HD rats. Homozygous transgenic male rats exhibit increased free rearing behavior and spent more time in the center and less time in the wall area of the cage compared to wild type rats. Furthermore, transgenic P10 rat pups emitted significantly less ultrasonic calls, which were also of shorter duration. Testing of sensory motor gaiting revealed a loss of prepulse inhibition at day 17 already.

Gene expression profiling and qrtPCR confirmation on P10 pups striata revealed changes in at least 8 behavior-associated genes. Out of them, mRNA levels of the dopamine transporter Slc6a3 were 7-fold upregulated. Additionally, we observed downregulation of mRNA levels of the dopamine receptor D1A. Together, these findings provide evidence for a dopaminergic dysregulation leading to altered behavior in very young tgHD rats.

Characterization of a transgenic rat model for spinocerebellar ataxia type 17 using comprehensive classical and automated phenotyping

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Spinocerebellar ataxia 17 (SCA17) is an autosomal-dominant, late-onset neurodegenerative disorder caused by CAG-triplet expansions and consecutive polyglutamine (polyQ) expansions in the TATA-box-binding protein (TBP), an ubiquitously expressed transcription factor, with unknown pathomechanism.
To further investigate this devastating disease, a rat model transgenic for SCA17 (tgSCA17) was generated, which carries a human truncated cDNA fragment with 64 CAG repeats under the control of the endogenous rat huntingtin promotor. To characterize this new model a comprehensive approach including classical and automated home cage phenotyping was conducted.
For classical behavioral phenotyping, homozygous tgSCA17 rats were repeatedly tested for general health, motor function (accelerod test), sensory motor gaiting (startle response and prepulse inhibition test), and emotionality (social interaction test of anxiety). Furthermore, CNS was screened for neuropathological changes by immunohistochemistry and quantification of polyQ immunoreactive neurons (antibodies 1C2 and N12).
To investigate the general health, physiological parameters such as body weight and body temperature, as well as neurological reflexes and sensory function (visual and hearing abilities, pain perception) were measured twice a month.
TgSCA17 rats revealed a higher bodyweight compared to wild-type littermates being associated with a phenotype composed of disturbances in anxiety-related behavior seen in the social interaction test of anxiety with onset at 6 months of age. A consistent loss of prepulse inhibition was detected from 6 months onwards in the prepulse inhibition test. The automated phenotyping in the PhenoMaster system for rats showed a reduced frequency of rearing behavior starting in month 1. This was associated with bimodal changes in energy metabolism, as young homozygous rats have a decreased respiratory exchange rate (RER=VCO2/VO2), while aged tgSCA17 exhibit an increased RER. Staining of the retinae with 1C2 showed precipitations in photoreceptor cells in the outer nuclear layer and in ganglion cells in 1, 9, 12 and 15 months old tgSCA17. Immunohistochemistry with 1C2 revealed a 12-fold increase in polyQ immunoreactive positive neuronal cells across striatum, nucleus accumbens, and cerebellum in homozygous animals including classical features such as aggregates and dismorphic purkinje cells. This phenotype closely mimics human SCA17.

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Altered phosphorylation but absence of neurodegeneration and no spine loss in a mouse model of tau hyperphosphorylation

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Many mouse lines that develop amyloid beta plaques or tau tangles composed of hyperphosphorylated tau have been developed as models for Alzheimer's disease. However, the role of hyperphosphorylation of tau in the progression of the disease is still unsolved. Here we describe novel transgenic mouse models, expressing a pseudohyperphosphorylated (PHP) variant of the longest human CNS tau isoform (441 aa) and a wildtype version of the same isoform at moderate levels in forebrain neurons. We report that pseudohyperphosphorylation drastically decreases phosphorylation at T205 while other sites (T212, S262) are less or not affected. During aging, phosphorylation at T205 and T212 are increased at wt tau expressing but not in PHP tau expressing animals. Despite the differences in phosphorylation, the subcellular distribution of tau is not affected and mice do not develop highly aggregated states of tau. Wt tau or PHP tau expressing mice do not show any evidence for neurodegeneration neither at young or old age as determined from morphometric measurements of neocortical regions. In addition, spine densities are not decreased on hippocampal and neocortical pyramidal cells that were analyzed in PHP tau transgenic animals. In agreement, no differences in learning and memory are observed. The data indicates that moderate levels of modified tau alone are not sufficient to induce tau aggregation or neurodegeneration in transgenic mice. With our model it becomes possible to differentially study the effects of hyperphosphorylation as one of the hallmarks of Alzheimer's disease at conditions which may prevail in an early preaggregation state of the disease.

In additional experiments the effect of the amount of expressed protein will be tested using homozygous tau expressing transgenic mice. Furthermore the amyloid cascade hypothesis proposes that amyloid beta (Abeta) pathology leads to and induces tau pathology. This will be tested by generating tau/APP double transgenic mice.

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The BAG protein family: Modulators of huntingtin toxicity, aggregation and localisation

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BAG1 is one of 6 known BAG-family members. It delivers chaperone-recognized unfolded substrates to the proteasome for degradation. With regard to Huntington's disease (HD), a sequestration of BAG1 into inclusion bodies along with reduced aggregate formation and neurotoxicity has been reported. These properties are due to an interaction of BAG1 and Siah-1 leading to reduced levels of mutant huntingtin in the nucleus. As the neuroprotective effect of BAG1 proved to be also efficient in vivo, we were interested if other isoforms of BAG1 or members of the BAG protein family would provide similar qualities aiming for new therapeutic targets in neurodegenerative diseases.

Here, we report a general screen of different BAG family members and isoforms regarding their characteristics in different models of huntington's disease. Our data show diverse modulations of huntingtin toxicity, localisation and aggregation and provide novel interesting candidate genes for further pathophysiological exploration

CK2 Dependent Phosphorylation Determines Cellular Distribution and Toxicity of Ataxin-3

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Nuclear localization of the expanded proteins is of critical importance for the pathogenesis of polyglutamine disorders. Here we show that casein kinase 2 (CK2) dependent phosphorylation controls the nuclear localization and aggregation of ataxin-3 (ATXN3), the disease protein in spinocerebellar ataxia type 3 (SCA3). Mutation of S340 and S352 within the 3rdUIM to alanines was sufficient to hinder the nuclear import of ATXN3. Mutation of these sites to aspartate (S340D/S352D) stabilized ATXN3. Activation of CK2 increased, while inhibition of CK2 decreased nuclear ATXN3 and the formation of nuclear aggregates.

Neuronal expression of the polyglutamine-expanded aspartate mutant of ATXN3 in Drosophila, mimicking phosphorylation, reduced lifespan compared to polyglutamine-expanded wildtype ATXN3 and increased the formation of neuronal aggregates, while the corresponding non-phosphorylatable alanine mutant prolonged survival. Cross-breeding with a loss of function mutant of CK2 attenuated the toxicity of polyglutamine-expanded wildtype ATXN3, while a gain of function mutant shortened lifespan in drosophila, indicating a key role of CK2-mediated phosphorylation of ATXN3 in SCA3 pathophysiology.

Proteomics of the striatum, olfactory bulb and substantia nigra of 6-OHDA hemi-lesioned rats

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So far, there exists no data of proteome changes after unilateral 6-OHDA lesions of the substantia nigra by stereotactic injection into the medial forebrain bundle. We are interested in differential changes of functional proteins involved in motoric behavior in the 6-OHDA hemiparkinsonian lesion model. Adult male rats were lesioned in the MFB and lesioning was verified by behavioural testing (apomorphine-induced rotations) after a survival time of 3 months. 6 rats showed a significant increase of rotations per minute. These lesioned animals and 6 untreated controls were transcardially perfused with saline and the olfactory bulb, the striatum and the substantia nigra were dissected.

We used 18 cm, nonlinear IPG-strips (pH 3-10). 6 control probes and 6 lesioned probes were processed at the same time (identical conditions). Gels were stained with colloidal coomassie. Digitization was performed under controlled densitometric conditions at 300 dpi and 12 bit dynamic range. The detection of differentially expressed spots was done by comparing gels using Progenesis. Spot edition and alignment need to be performed semiautomatic due to complex spot distribution of average 1000 spots / gel. 2.5 fold up- and down-regulated spots of lesioned tissues were considered to be differentially expressed if they occur in almost 3 of 6 gels.

Protein identification was performed by MALDI-TOF method and spectrums analysed by using SwissProt and Swall via Mascott.

In the striatum we found 242 differentially expressed proteins. As expected tyrosine hydroylase (Mw 56330, pI 5.74) was downregulated in the striatum of lesioned animals. The olfactory bulb has 120 differentially expressed spots and the substantia nigra 102.

Currently, we are searching functionally relevant proteins that are related to the neurotoxic model of Parkinson disease in the adult rat.

Membrane lipid modification by PUFAs promotes a-synuclein aggregate formation after oxidative stress in OLN oligodendroglial cells

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a-Synuclein (a-syn) is the major building block of cytoplasmic inclusions in neurodegenerative disorders named synucleinopathies, such as Parkinson`s disease, dementia with Lewy bodies and multiple system atrophy (MSA). In MSA glial cytoplasmic inclusions originating in oligodendrocytes are prominent. During disease progression a shift in a-syn solubility is observable, and oxidative modification of a-syn has been linked to neurodegeneration, fibril formation and accumulation in insoluble potentially cytotoxic inclusions. Heat shock proteins (HSPs), tau and polyunsaturated fatty acids (PUFAs) could be detected in these inclusions. It has been suggested that a-syn binds and interacts with PUFAs, which may alter its secondary structure and render a-syn more susceptible to oxidative stress.

To test this hypothesis, OLN-t40 cells, expressing the longest isoform of human tau,

were stably transfected to express the A53T mutation of a-syn, and were subjected to oxidative stress after preincubation with docosahexaenoic acid (DHA). As shown before, DHA treatment (25µM; 72h) caused an enrichment of PUFAs in the plasma membranes of OLN cells. Control cells depict small punctuated thioflavine-S-negative a-syn aggregates. Pretreatment of the cells with DHA followed by oxidative stress, exerted by hydrogen peroxide (100-150µM; 30min), led to an enlargement of the cytoplasmic protein inclusions and to the recruitment of tau and HSPs, including aB-crystallin and ubiquitin. After this treatment, protein aggregates were thioflavine-S-positive, indicating their fibrillary nature. Furthermore, sequential extraction using buffers with increasing solubilization capacity demonstrated a decrease in a-syn solubility and an increase in the amount of a-syn in its oligomeric form.

The results show that modification of the oligodendroglial cell membranes with DHA in combination with oxidative stress led to a decrease in a-syn solubility and to the occurrence of large thioflavine-S-positive inclusions, which was not seen either after DHA application or oxidative stress alone. Hence, an enrichment of PUFAs in the cellular membranes sensitizes oligodenroglial cells against oxidative stress and promotes a-syn aggregate formation, contributing to glial cytoplasmic inclusions as observed in synucleinopathies.

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Nuclear Aggregation of Polyglutamine-expanded Ataxin 3: Toxic Fragments Escape The Cytoplasmic Quality Control

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Expansion of a polymorphic polyglutamine (polyQ) segment beyond ~ 40 residues is the common denominator of polyglutamine diseases. The expanded proteins typically accumulate in large intranuclear inclusions and induce neurodegeneration. However, the mechanisms that determine the subcellular site and rate of inclusion formation are largely unknown. We found that the conserved putative nuclear localization sequence Arg-Lys-Arg-Arg which is retained in a highly aggregation-prone fragment of ataxin 3, did not affect the site and degree of inclusion formation in a cell culture model of spinocerebellar ataxia type 3. Instead, addition of synthetic nuclear export or import signals led to the expected localization of ataxin 3, and determined the subcellular site of aggregate formation. Cytoplasmic expression of poly Q induced a mild heat shock response, while nuclear expression did not. These findings indicate that aggregation-prone fragments derived from expanded ataxin 3 may eventually escape the cytoplasmic quality control resulting in aggregation in the nuclear compartment.

A DROSOPHILA MODEL FOR PARKINSONISM

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Leucine-rich repeat kinase 2 (LRRK2) is a multi-domain protein containing a Ras-homology domain (Ras of complex: Roc) and an effector kinase homology domain (MAPKKK). Mutations in the gene encoding for LRRK2 causes autosomal-dominant Parkinson's disease. Parkinson's disease is characterized by progressive loss of dopaminergic neurons in the brain. Mutations in the Roc domain (R1441C) and Kinase domain (G2019S) have been associated with neural toxicity and cell death (1). Ras is an intracellular protein which cycles between the inactive GDP-bound and active, signaling competent GTP-bound conformation.V12Ha-Ras (Glycine 12 →Valine 12) results in a constitutively active GTP-bound form whereas, dominant negative N17Ha-Ras (Serine 17→Arginine 17) is constitutively inactive. Heumann *et al* 2000 showed that transgenic constitutive activation of Ras significantly attenuated neurotoxin-induced degeneration of dopaminergic neurons in transgenic mouse brain. Dopaminergic SH-SY5Y neuroblastoma cells and mouse primary cortical neurons are used as *in vitro* model to study LRRK2-mediated cell death. Cotransfection of human V12Ha-Ras along with LRRK2 wild-type and mutants rescues the transfected cells. In order to study this effect *in vivo* we have used *Drosophila melanogaster* as a model. We have created knock-in *Drososphila* using GAL4/UAS system to express either human wild-type or mutant LRRK2 in the absence or presence of *Drosophila* Ras1V12 in photoreceptor cells and dopaminergic neurons. Together the cell culture and the *Drosophila* models are used to elucidate a possible protective mechanism towards LRRK2-induced neural pathology.

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The role of microglial CPEB proteins in Temporal Lobe Epilepsy (TLE)

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Cytoplasmic Polyadenylation Element Binding proteins (CPEBs) modulate protein synthesis, thereby controlling synaptic efficacy in neurons. So far several findings confirmed their importance in maintenance of long term potentiation (LTP), learning and memory. As Temporal Lobe Epilepsy (TLE) entails mechanisms similar to the abovementioned processes, CPEBs are likely to play a role in the onset and spread of this disease. Proteins undergoing CPEB-regulated translation include tissue plasminogen activator (tPA), Ca^{2+}/calmodulin dependent protein kinase II-a (CaMKIIa), AMPA receptor GluR2, and many more. Tissue plasminogen activator is an acknowledged player in modulating synaptic plasticity. Furthermore, the microglial expression of this protease has been shown to be critically involved in neurodegeneration following epileptic seizures. Whether CPEB proteins are also crucial for proper microglial cell functioning has not been investigated to date.

By single cell RT-PCR following electrophysiological characterization in acute hippocampal slices, we have observed the presence of CPEBs mRNAs in microglial cells of adult mouse brain. These findings are in line with RT-PCR and immunoblot results, showing abundant CPEB transcript and protein expression in the BV-2 immortalized microglial cell line. To continue the studies of CPEB function in microglia we use CXCR3 EGFP/+ knock-in mice to easily identify microglial cells and separate them by FACS. In cultures of such freshly isolated microglia, we will study their response to various stimulating factors (LPS, chemokines) and compare CPEBs and tPA expression levels in resting and in activated state (RT-PCR, immunoblots, zymography analysis). We will also compare the polyadenylation status of tPA mRNA and the phosphorylation status of CPEB proteins before and after microglial activation. We hope the abovementioned studies will shed a new light on the function of microglial CPEB proteins. We speculate that they are involved in the modulation of microglial tPA secretion, and thereby implicated in neurodegeneration and neuronal cell death associated with temporal lobe epilepsy.

Summary of electrophysiological and neurobehavioural experiments made wth 3-nitropropionic acid on rats, carried out in our laboratory

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3-nitropropionic acid (3-NP) is a model substance used for inducing brain lesions and dysfunctions in animals resemble to Huntington's disease. The underlying mechanisms are succinate dehydrogenase inhibition, resulting in energetic insufficiency, besides glutamatergic and dopaminergic excitotoxicity. These effects are likely to be detectable by electrophysiological and neurobehavioral methods.

Adult male Wistar rats were treated with 10 and 20 mg/kg b. w. 3-NP intraperitoneally in four different timing schemes: immediate (with pre-administration controls), acute (measurements were done 24 h after a single administration), subacute (recordings were made 28 days after 5 consecutive day treatment) and subchronic (tests were done 1 week after 6 consecutive treatments made on every 4th days). Behavioural investigations (open field activity, acoustic startle response without or with prepulse [PPI] stimulus, rota-rod, grip strength test, maze test) were made after the (last) 3-NP administration and before the electrophysiological recordings. In electrophysiological recording spontaneous cortical activity (electrocorticogram, ECoG) and evoked potentials (EPs) were recorded from the primary somatosensory, visual and auditory cortical areas, carried out in urethane anaesthesia. Peripheral evoked activity was studied by stimulating tail nerve. Body and organ weights were also registered in case of subchronic protocol to reveal general toxicological effects

In immediate, acute and subchronic treatments decreased locomotion, increased local activity and immobility was seen at the open-field test probably as a consequence of striatal dopamine level decrease. Positive effects on the startle responses and reduced PPI (because of increase in dopamine release) were clearly seen in the immediate results and slightly after the acute and subchronic experiments. Rota-rod, grip strength and maze tests showed ambiguous results.

3-NP caused lack of energy resulted in a shift in the ECoG to low frequencies observed in the immediate, and partly the acute administration. First over-excitation and then desensitization of glutamate receptors was probably reflected in ECoG changes. The imbalance between excitation and inhibition caused by inhibited glutamate uptake may explain the significant decrease of somatosensory EP duration obtained by frequent stimulation in acute 3-NP treatment. Over a longer period, abnormally high level of glutamate is likely to desensitise receptors, expressed in the latency lengthening of EPs in the subacute protocol. The change of the second:first ratio in the EP amplitudes by paired-pulse stimulation following 3-NP administration reflected probably a kind of disinhibition, similarly to what can be seen in human patients suffering from mitochondrial encephalomyopathy.

Results indicate that behavioural and functional electrophysiological investigations can be suitable for non-invasively modelling, detecting and follow-up not only Huntington's but other neurodegenerative diseases and may be useful in the evaluation of neuroprotective therapies.

Alterations in the dopaminergic system of mice with an alpha synuclein A30P point-mutation in the endogenous genomic locus

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The hallmark of Parkinson's disease (PD) is the loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc), the depletion of striatal dopamine levels, and the presence of intraneuronal cytoplasmatic inclusions, termed “Lewy bodies”. The major component of these protein aggregates is alpha synuclein encoded by the SNCA locus. Specific mutations in the alpha synuclein protein (A53T, A30P) have been found to be associated with familial forms of PD. We addressed the question if mice carrying the same critical amino acid substitutions found in PD patients in their alpha synuclein show a PD like phenotype. Using gene targeting we generated mice with an A30P point-mutation in the endogenous SNCA locus (A30P mice). Since mice already encode at position 53 naturally a threonine (53T), both alterations found in humans where present in these mice. In A30P mice older than 11 months we observed a progressive deficiency in motor performance using an ink and beam walking test. The mice showed also an altered response to a combined amphetamine and reserpine treatment. Moreover, we detected in the striatum of 15 months old A30P mice reduced dopamine level, suggesting significant alterations in the DA system. To investigate the alterations in the DA system of the A30P mice in more details, we started to analyse the A30P mice now immunohistologically. We counted stereologically in 12 month old mice the total number of Nissl and tyrosine hydroxylase (TH) positive neurons. To assess the loss of DA terminals in the striatum, we quantified TH-positive neuronal fibers of A30P and control mice. However, neither a loss of DA neurons in SNpc nor impaired integrity of DA nerve terminals in the striatum could be observed. This suggests that the behavioural phenotype is not caused by nigrostriatal degeneration. To get a better inside of how the phenotype of the A30P mice is established, we currently investigate different brain areas including the spinal cord for alpha synuclein accumulation and other immunohistological alterations. The results of these studies will be presented at the conference. These A30P mice show an age dependent deficiency in their behaviour and in the dopamine levels, alterations also observed in PD patients. Further improvements have to be made to generate a reliable genetic PD model in mice.

High-frequency stimulation of subthalamic nucleus silences excitatory synaptic transmission onto dopaminergic neurons in the substantia nigra pars compacta

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Despite its broad acceptance as a safe and effective surgical therapy for advanced Parkinson´s disease (PD), deep brain stimulation (DBS) has remained enigmatic with respect to its underlying mechanism(s). Because DBS of subthalamic nucleus (STN) mimics the therapeutic effects of STN lesion, it was originally thought that the high frequency (> 100 Hz) necessary to relieve motor symptoms in PD patients causes functional inactivation of STN neurons. However, high-frequency stimulation (HFS) of STN was actually found to *activate* downstream structures, suggesting that HFS uncouples somatic activity, which is inhibited, from axonal output, which is enhanced. In support of the latter, amperometric measurements showed increased striatal dopamine efflux during HFS of STN. This would represent an important novel therapeutic mechanism of DBS, but it is not clear how this effect is brought about. One possible explanation would be that the increased axonal output from STN during HFS exerts a strong synaptic drive on dopaminergic neurons in substantia nigra pars compacta (SNc), which then gives rise to enhanced striatal dopamine release.

To test this hypothesis, we prepared parasagittal brain slices (350 µm thick, juvenile rats) that preserved the synaptic connectivity between STN and SNc. An extracellular stimulation electrode was placed in STN and whole-cell recordings were performed from dopaminergic SNc neurons which were identified by their typical electrophysiologic profile. Neurons were filled with biocytin to determine their morphology and their location within SNc after histological processing. Repetitive stimulation was delivered at 10, 50 or 130 Hz for 10 s each. Whereas 10 Hz stimulation did not significantly affect the mean amplitude of postsynaptic currents (PSCs) averaged over the last second of the stimulation period (82.1 ± 10.6% of control, mean ± SEM, n = 10), stimulation at 50 Hz and 130 Hz reduced PSC amplitude to 47.7 ± 11.6% (n = 10) and to 12.3 ± 7.8% (n = 9), respectively. To determine whether GABAergic mechanisms contributed to HFS-induced synaptic depression, we examined the effect of the GABA(A) receptor antagonist picrotoxin (100 µM) alone or in combination with the GABA(B) receptor antagonist CGP 55845 (2 µM). However, neither drug relieved synaptic depression at 130 Hz (picrotoxin: reduction to 5.0 ± 2.6%, n = 5; picrotoxin and CGP 55845: reduction to 6.4 ± 3.7%, n = 10).

In view of the almost complete disruption of glutamatergic transmission between STN and SNc during repetitive stimulation at therapeutically relevant frequencies, our data strongly argue against the notion that the enhanced output from STN during DBS is capable of driving dopaminergic SNc neurons. As an alternative explanation for the enhanced striatal dopamine efflux accompanying HFS of STN *in vivo*, it seems conceivable that the stimulation electrode produces concomitant activation of dopaminergic fibers of passage dorsomedial to STN, thereby providing a short-cut to increased striatal dopamine release.

Developmentof Anti-HLA antibodies after intrastriatal transplantation of human neuronal foetal cells in Huntington disease patients

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A major impetus for research into the treatment of Huntington's disease (HD) has centered on cell therapy strategies to replace dysfunctional or dying cells. Neural transplantation of human fetal tissue for HD has revived the interest in the immunological status of brain and its response to grafted tissue. The previously held view that the brain was an absolute "immunologically privileged site" allowing indefinite survival without rejection of grafts of cells has proven to be wrong.

Development of Post-transplant circulating anti-human leukocyte antigens antibodies (anti-HLA antibodies) is associated with acute and chronic rejection and decreased graft survival. Antibodies to both HLA class I and class II antigens seem to be detrimental. Twenty patients with genetically proven HD were grafted with human fetal neuroblasts into the right striatum and, after two weeks, in the left striatum. All patients were initially negative to HLA class I and class II antibodies at the time of engraftment. All received immunosuppressive treatment of Cyclosporine, Azathioprin and Prednisolon for twelve months. Three patients developed HLA I and HLA II after discontinuation of immunosuppressive treatment, nine, 11 and 13 months after brain engraftment.

The results show that 6 months protection of neural cell striatal implantation by immunosuppressive agents may not be sufficient, and that immune responses can occur any time later. The results also suggest that continued monitoring of HLA antibodies are necessary for longer periods after engraftment. It is also quite possible that some of the results obtained in past studies with foetal neural transplants may have been biased by an unrecognized immune response to donor cells.

Ataxin-3 interacting transcription factors and implications for disease pathogenesis

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Transcriptional dysregulation is a common feature shared by several polyglutamine (polyQ) diseases. Ataxin-3 (ATXN3), the disease protein in spinocerebellar ataxia type 3 (SCA3) is associated with transcriptional regulation of gene expression. We previously showed that normal ATXN3 binds to specific chromatin regions of target gene promoters and represses transcription by interaction with transcriptional corepressors and deacetylation of core histones bound to gene promoters. ATXN3 thus can function as a component of transcriptional regulatory complexes and interfere with gene transcription.

To identify transcription factors interacting with ATXN3, we performed transcription factor profiling using DNA/protein arrays and ATXN3-containing, immunoprecipitated nuclear protein complexes from native HeLa cells and transgenic cells stably expressing normal ATXN3-Q23 or mutant ATXN3-Q70. Screening of 345 transcription factors revealed 43 transcription factors interacting with normal and/or expanded ATXN3 from HeLa and transgenic cells. Three transcription factors (FOXO4, WT1 and TFIID) were found to interact with ATXN3 in all cell lines. Pulldown-experiments with glutathione S-transferase (GST)-ATXN3-fusion proteins confirmed that endogenous WT1 and FOXO4 but not TBP, component of the TFIID complex, interacts with both normal and expanded ATXN3. Co-immunofluorescence staining of human SCA3 pons sections showed no recruitment of WT1 or FOXO4 to ATXN3-positive inclusions indicating that the protein-protein interaction is not affected by the polyglutamine expansion in ATXN3. However, we found significant changes in the DNA binding properties of endogenous WT1 and FOXO4 in ATXN3Q23 and -Q70 expressing cell lines by electrophoretic mobility shift assays. These findings show that ATXN3 interacts with a variety of different transcription factors. In particular, the interaction of WT1 and FOXO4 with recombinant ATXN3Q23 and ATXN3Q70 suggests that these ATNX3-containing transcription factor complexes may interfere with transcriptional pathways regulated by the identified factors. Further studies using reporter gene assays are currently performed to determine the functional consequences of ATXN3 on gene transcription regulated by these transcription factors.

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On the integration of parahippocampal networks in epileptiform activity

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Mesial temporal lobe epilepsy (MTLE) is the most common classifiable form of focal epilepsies in humans. In most cases, MTLE is accompanied by histological changes within the hippocampal formation, generally summarized as hippocampal sclerosis. Removing the affected parts does not result in a seizure-free outcome in all cases. This suggests that regions beyond the hippocampus proper and dentate gyrus may contribute to initiation and propagation of epileptiform activity (EA).
We use the kainate mouse model, in which EA is observed after focal injections of kainic acid into the hippocampus. This is accompanied by cell loss in CA1 and CA3, granule cell dispersion and mossy fiber sprouting as well as recurrent epileptic seizures, manifest within two weeks after injection. Although the hippocampal network of the injected side is likely responsible for EA generation [1], hippocampal slices taken close to the injection site are unable to generate or sustain EA [2].
As a major source of direct input to the dentate gyrus, CA1 and CA3, the superficial layers of the entorhinal cortex (EC) could play a key role in EA generation. In addition, with its reciprocal connections to the EC and output structure of the CA region, the subiculum is part of a critical excitatory feedback loop.

Hence, we performed multi-site in vivo recordings along the hippocampal septo-temporal axis, the EC and the subiculum to identify the origin and propagation of EA in this animal model. Neuronal degeneration was detectable within the ipsilateral subiculum within one day after injection. Additionally, slight cell loss in the deep layers of the ipsilateral EC was detected after three weeks.
Preliminary results indicate that EA in the hippocampus distal to the injection site precedes proximal EA, whereas the neurons in EC and subiculum fire simultaneously with the septal hippocampus. EA appears to be generated in temporal or adjacent parahippocampal areas instead of parts close to the injection site that showed the most prominent histological changes.

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Altered synaptic plasticity in dorsomedial striatum after status epilepticus

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Temporal lobe epilepsy (TLE) is associated with morphological and functional changes of mesolimbic/hippocampal structures. In patients and animal models of TLE alike, the most conspicuous of these functional alterations is an infringement of cognitive abilities, which in animal models is reflected in reduced spatial learning and up- or downregulation of long-term synaptic plasticity (long-term potentiation; LTP or long term depression, LTD). However, in particular neurochemical investigations suggest that changes also occur in extralimbic structures such as thalamic dorsomedial nuclei and corpus striatum. Since the striatum is instrumental in motor planning and coordination, it is of particular interest whether motor dysfunction sometimes associated with TLE and other forms of epilepsy and clinically reported to bear dystonic or dyskinetic features is indeed due to epilepsy-associated changes of basal ganglia function. We therefore explored this question by analysing long-term potentiation in cocrtico-striatal pathways in brain slices of rats treated with pilocarpine to induce status epilepticus (SE) developing chronic epilepsy after a silent phase of 2-3 weeks. To determine whether chronic development of seizures, or SE itself wield an impact on basal ganglia function, both tissue of animals immediately (3 to 5 days; acute group) after SE, and slices of animals in the chronic phase 4-10 weeks after SE (chronic group) were investigated. We found that in the acute group rats, LTP was not significantly different among all of the three groups (control, sham, i.e. pilocarpine-treated not developing SE, and SE). In the chronic group, however, LTP was significantly enhanced the SE group versus both control and sham preparations. We conclude that the enhancement of cortico-striatal LTP after pilocarpine-induced status epilepticus is a consequence of chronic epilepsy.

Gene therapy tools targeting the central nervous system by viral gene transfer

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Alzheimer's disease (AD) is the most common neurodegenerative disorder of humans with an enormous socio-economic burden on the aging society. The clinical symptoms and the neurodegeneration of AD are caused by accumulation of extracellular plaques and neurofibrillary tangles. Currently, causes of the disease are still unknown and there is neither an effective prevention nor a therapy treatment. To prevent neuronal cell death we want to develop a new gene therapeutic tool, which ensures long-lasting, safe, neuron-specific and regulated transgene expression. Our therapeutic strategy is based on non-integrating (NI) lentiviral vectors, which can regulably express different disease relevant factors. Lentiviral vector mediated gene transfer has useful attributes. These vectors can deliver 8kb – 10kb transgene sequences, they have the ability to infect specific neuronal cells, induce no or low immune response and the normal cell functions are not negative affected. Following entry into target cells, lentiviral vectors stably integrate into the host genome and therefore one potential risk of lentiviral vectors is insertional mutagenesis, where essential genes are disrupted or normally silent promoter/enhancer regions are activated. This safety issues relating to insertional mutagenesis may be avoided by the use of integration-deficient lentiviral vectors. These vectors have been rendered integration-deficient through mutations in the coding sequence of the integrase gene and exist as circular forms in the nucleus without any replication signals. Here, we use such integration-deficient lentiviral vectors to achieve efficient and sustained transgene expression in the adult rodent CNS as a prerequisite for the therapeutic application in the prevention and/or treatment of neurodegenerative disorders. All these aspects create them to powerful tools for gene delivery in the nervous system. Currently, we are focused on the generation of the viral vectors with specific therapeutical genes. The great advantage of this innovative system is the modular constitution. Alternatively, its applicable for other neurodegenerative disorders or non-neurological diseases like AIDS, Cancer, Arteriosclerosis and others.

Two-strep grafting – a new method to enhance cell survival and study graft development in a rat model of Parkinson's disease

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Two-step grafting is a new transplantation approach where a standard amount of cells is divided in half and transplanted in two separate sessions with a certain time interval. Previous studies from our group revealed that two-step grafting provide a significantly higher cell survival rate and graft volume compared to the standard one-step transplantation protocol in the 6-OHDA rat model of Parkinson's disease.

The aim of the present study is to give an insight into the mechanisms causing this interesting effect by altering the amount of cells in the first graft and by evaluating the two grafts independently. To achieve this, we use GFP transgenic Lewis rat embryos as donor tissue to indetify the second graft.

40 6-OHDA-lesioned adult Sprague Dawley rats were divided into 3 two-step grafting groups, each with a time interim of 2, 5 or 9 days between the two transplantation sessions. Each group was sub-divided in two sub-groups receiving either 200,000 (GFP-) + 200,000 (GFP+) cells or 400,000 (GFP-) + 200,000 (GFP+) cells per animal. In order to compare this new protocol with standard procedure transplantation protocol, two standard transplantation groups (n=8) were grafted with the same constellation of cells (200,000/400,000 (GFP-) + 200,000 (GFP+)) in a single operation session.

Transplantation effects were evaluated by drug-induced rotation tests, performed 2, 3 and 6 weeks after the first grafting. In these tests all groups showed significant compensation from the lesion. The animals were sacrificed and processed for immunohistochemistry, eight weeks after transplantation. Stereological evaluation of the transplants is currently in progress and numbers on cell survival, graft volume and fibre density will be provided in the conference.

Preliminary results from the two-stage grafting groups already showed an interesting effect. Only in certain groups with a time interval of 2 or 5 days between the transplantation sessions, formation of vessel-like structures occurred within the second graft (seen in 100% of the rat brains in the high cell number groups and in 60% in the 2 day low cell number group). These could be identified to be graft-derived because they were stained positive for GFP, labelling all cells from the second graft including vessel endothelium. Interestingly, these structures only formed in regions where both grafts overlap. According to the literature, donor-derived vessels were rarely found in graft angiogenesis studies so far and therefore they are considered to play a minor role in the vascularisation process (Baker-Cairns et al. 1996). We will proceed with the histological characterization of this vessel effect and further investigate its possible functional impact on cell survival.

This is an ongoing study and final results will be presented on the conference.

Potential role of the transcriptional co-activator PGC-1a in amyotrophic lateral sclerosis (ALS) - mRNA and protein expression studies in post mortem tissue of ALS patients and in the G93A transgenic ALS mouse model

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Amyotrophic lateral sclerosis (ALS) is a devastating neuron disorder with an average survival of 3-5 years after symptom onset. It manifests in a rapidly progressive paralysis due to the degeneration of motor neurons in primary motor cortex, brain stem and spinal cord. About 90% of cases occur sporadically and 10% are familial. Among the familial cases, 10-20% can be attributed to point mutations in the gene coding for the antioxidant enzyme Cu/Zn superoxide dismutase (SOD1) (Rosen et al., 1993). This discovery led to the generation of mouse models overexpressing common human SOD1 mutations (Gurney et al., 1994). The precise molecular mechanisms leading to motor neuron death have not been fully elucidated, there is, however, ample evidence that oxidative damage by reactive oxygen species (ROS) plays an important role. Mitochondria are the major source of cellular ROS production, which further increases if mitochondria are damaged (Beal, 2005, Hervias et al., 2006). The transcriptional co-activator peroxisome proliferator-activated receptor-γ co-activator 1a (PGC-1a) plays a pivotal role in the regulation of mitochondrial metabolism and biogenesis via activation of transcription factors such as nuclear respiratory factor-1 (NRF-1) (Scarpulla, 2006). Alterations in PGC-1a expression and function have previously been described in models of Huntington's and Alzheimer's disease (Weydt et al., 2006, Shi and Gibson, 2007).

In the present study, we investigated the mRNA and protein expression of PGC-1a, NRF-1 and several genes regulated by these factors in human post mortem tissue of ALS patients as well as in the G93A-ALS mouse model in presymptomatic, early –and late symptomatic stages. Intracellular localization was specified by nuclear and cytoplasmic fractionation. We detected a reduction of PGC-1a and NRF-1 at the mRNA and protein level, in the animal model already detectable before symptom onset. We therefore conclude that PGC-1a could represent an interesting therapeutic target in ALS.

PKG inhibition protects photoreceptors in two mouse models for Retinitis Pigmentosa

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Photoreceptor degeneration in retinitis pigmentosa (RP) is one of the leading causes of hereditary blindness in the developed world. Although causative genetic mutations have been elucidated in many cases, the underlying neuronal degeneration mechanisms are still unknown. Here, we show that activation of cGMP-dependent protein kinase (PKG) hallmarks photoreceptor degeneration in rd1 and rd2 human homologous mouse models. When induced in organotypic, *in vitro* retinae derived from wild-type animals, PKG activity was both necessary and sufficient to trigger cGMP-mediated photoreceptor cell death. Target specific, pharmacological inhibition of PKG activity in both rd1 and rd2 retinae strongly reduced photoreceptor cell death in *in vitro* retinal explants. Likewise, inhibition of PKG *in vivo*, using three different application paradigms, resulted in robust photoreceptor protection in the rd1 retina. These findings suggest a pivotal role for PKG activity in cGMP-mediated photoreceptor degeneration mechanisms. At the same time, they highlight the importance of PKG as a novel target for the pharmacological intervention in RP and related neurodegenerative disorders.

Muscarinic modulation of synaptic transmission and spontaneous activity in area CA1 of hippocampal slices from $Ca_V2.3$-deficient mice, lacking E-/R-type voltage-gated Ca^{2+} channels, and control animals.

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In heterologous expression systems, activation of M2 muscarinic acetylcholine receptors modulates all three members of non-L-type ($Ca_V2.1$ - $Ca_V2.3$) (1), and the $Ca_V1.2$ L-type channel (2). Inhibition of Ca^{2+} currents by M2 receptors is mediated through G-protein coupled pathways, also for $Ca_V2.3$ (3). Only in $Ca_V2.3$ Ca^{2+} channels but not in $Ca_V2.1$ P-/Q- and $Ca_V2.2$ N-type Ca^{2+} channels, persistent activation of M2 receptors causes a Ca^{2+} current increase (4), which is consistent with muscarinic enhancement of R-type Ca^{2+} currents in rat hippocampal CA1 pyramidal neurons (5). This stimulation requires the activation of a PKC pathway (4;5), and may be mediated through the II-III loop of $Ca_V2.3$ (6). As the increase of R-type Ca^{2+} currents in rat hippocampus changes the firing pattern in the theta frequency range (5), we assumed that muscarinic modulation of CA1 synaptic transmission should be different in $Ca_V2.3$-deficient mice, as compared to controls. Therefore, we investigated the effect of carbachol (CCh) on evoked and spontaneous extracellular field potentials in hippocampal slices from $Ca_V2.3$-deficient and control mice. In hippocampal slices from $Ca_V2.3$(+/+)-mice, superfusion of 0.2 µM CCh induced a transient augmentation of CA1 population spike amplitudes to 113 ± 5 % within the first minute (n = 8 slices from 5 animals). In contrast, no increase was observed in slices from $Ca_V2.3$-deficient mice (100 ± 2 %, n = 7 slices from 7 animals; p = 0.037). Further, the pattern of complexity for spontaneous activity after carbachol superfusion (up to 50 µM CCh) was compared between both genotypes. To analyze the derived spike structures more in detail, we are currently using specialized time-frequency analysis with Morlet wavelets.

In conclusion, the specific muscarinic stimulation of $Ca_V2.3$, which was found in heterologous systems (4), and also tested in the rat system (5), is probably underlying the muscarinic stimulation in $Ca_V2.3$ control mice. E-/R-type Ca^{2+} channels containing $Ca_V2.3$ as ion conducting pore may continue to mediate Ca^{2+} influx *in vivo* during steady inhibition of N-type and P/Q-type Ca^{2+} channels by muscarinic receptors, which is important to understand the development of cholinergic-dependent plateau potentials (7) and ictal-type seizure activity (8).

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Spinocerebellar ataxia 2: cellular and molecular action of ataxin-2

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Autosomal dominant spinocerebellar ataxias (SCAs) are a group of genetic disorders characterized by a slow progressing loss of balance and motor coordination. SCA2 is caused by the expansion of a CAG repeat region in the *ATXN2* gene leading to an elongation of a polyglutamine (polyQ) stretch in the gene product ataxin-2. In eukaryotic cells, ataxin-2 appears to regulate the cytoplasmic RNA metabolism. However, its exact physiological function and molecular mode of action are yet unknown. Furthermore, it is unclear why polyQ tract expansion induces neurodegeneration. To better understand both the physiological function of ataxin-2 and the pathogenic action of mutant ataxin-2 we determined whether normal and mutant ataxin-2 differentially regulate translation and/or mRNA abundance, two central regulatory steps in cytoplasmic mRNA metabolism. In addition, we employed affinity chromatography to identify novel ataxin-2 binding partners. Extensive characterization of its cellular interactions is thought to provide further clues to the cellular function of ataxin-2. Moreover, differences in the protein binding patterns of normal and mutant ataxin-2 may help to unravel the pathomechanism underlying SCA2. The final goal of our studies is to provide novel launch points for therapies, which delay or prevent neurodegeneration in carriers of mutant alleles.

The potential of aminoglycoside mediated gene based therapy of Usher syndrome 1C in the retina

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The human Usher syndrome (USH) is the most frequent cause of inherited combined deaf-blindness. It is clinically and genetically heterogeneous, assigned to three clinical USH types. The most severe type is USH1, characterized by profound inner ear defects and *retinitis pigmentosa*. While the auditory deficit can be treated with cochlear implants, so far no effective treatment for the ophthalmic component of USH exists. A nonsense mutation (p.R31X) in *USH1C* leads to a premature translational stop in patients causing the USH1 disease. We investigated the potential of aminoglycoside induced premature stop codon read-through as a possible therapy of USH1. The read-through of the p.R31X mutation by aminoglycosides *in vitro* and *in vivo* was validated. *In vitro* translation assays and analyses of transfected HEK293T cells revealed a read-through of the p.R31X mutation by all aminoglycosides tested. For the first time, we showed a read-through of the nonsense mutation in transfected retinal explants. In addition, we demonstrated differential toxicities of aminoglycosides in the retinal explants and thereby we provide evidence that the pathomechanism underlying the retinal toxicity of aminoglycosides is based on poisonous effects on Müller glia cells. Among all aminoglycosides tested the chemically modified aminoglycoside NB30 revealed the lowest toxicity in the retina. These results place NB30 as a potential new therapeutic agent for USH1C and other genetic conditions.

Comparative analysis of the influence of Refsum disease-associated branched chain fatty acids, pristanic acid and phytanic acid, on cell physiology in neural cells in culture

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The branched-chain fatty acids pristanic acid (2, 6, 10, 14 – tetramethylpentadecanoic acid) and phytanic acid (3, 7, 11, 15 – tetramethylhexadecanoic acid) play an important role in diseases with peroxisomal impairment. Thes diseases are hereditary disorders, like Refsum disease (MIM 266500), Zellwegers syndrome and a-methylacyl-CoA racemase (AMACR) deficiency (MIM 604489). Phytanic acid, whose dietary sources in humans are meat and dairy products, is derived from phytol, the chlorophyll component, and is catabolized in peroxisomes via a-oxidation. The product of this a-oxidation is pristanic acid which is then degraded by three cycles of non-inducible peroxisomal b-oxidation. The accumulation of pristanic acid and phytanic acid seems to be detrimental for neuronal tissue. Several studies analyzing the toxicity of phytanic acid revealed that the toxic activity of phytanic acid is mediated by multiple mitochondrial dysfunctions. However, the action of pristanic acid on brain cells is still completely unknown. The toxic effect of branched fatty acids leading to severe neurological symptoms remains still obscure and has not yet been investigated. Here, we analyzed the toxic activity of pristanic acid and phytanic acid on hippocampal neurons, astrocytes and oligodendrocytes in a mixed hippocampal cell culture. Our studies revealed a massive cell death after incubation with pristanic and phytanic acid. To elucidate the pathogenic mechanism, we investigated the canges in the cytosolic Ca^{2+} concentration and the mitochondrial membrane potential, as well as the generation of reactive oxygen species (ROS). We found that both these fatty acids induced depolarization of mitochondria in situ and increased the intracellular calcium level in all three brain cell types. We found that the Ca^{2+} peak in astrocytes and oligodendrocytes largely depends on intracellular Ca^{2+} stores. Furthermore, we obtained evidence about the mechanism of action of these branched chain fatty acids by using specific inhibitors for intracellular Ca^{2+} signaling pathways (U73122, 2-APB). In addition, pristanic acid (50 µM) induced substantial generation of ROS. Our data provide strong indications that deregulation of Ca^{2+} homoeostasis and mitochondrial dysfunction play a main part in the mechanism of toxicity of pristanic acid and phytanic acid. Our findings suggest that there is a potent toxic activity of pristanic and phytanic acid due to dramatic cell physiological effects. In a comparable study with very long chain fatty acids (VLCFA) we could provide the first evidence for mitochondrial-based cell death mechanisms for neurodegenerative diseases with peroxisomal defects (Hein et al. 2008).

Hein S, Schonfeld P, Kahlert S, Reiser G. 2008. Toxic effects of X-linked adrenoleukodystrophy-associated, very long chain fatty acids on glial cells and neurons from rat hippocampus in culture. Hum Mol Genet 17(12):1750-1761.

Role of CPEBs in development and progression of temporal lobe epilepsy

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Cytoplasmic Polyadenylation Element Binding (CPEB) proteins control activity-induced protein synthesis in the synapto-dendritic compartment. Since four CPEBs are present in the hippocampus, we explored their possible role by expressing in forebrain neurons a dominant negative CPEB protein, rendering CPE containing mRNA non-responsive to synaptic stimulation. Expression of the dominant negative CPEB impaired stimulated protein synthesis of CPEB targets, protein-synthesis dependent aspects of synaptic plasticity, i.e. the late phase of long term potentiation (L-LTP), and spatial reference memory. Like the cellular processes in neurons correlating with learning and memory, the pathological changes in temporal lobe epilepsy (TLE) are characterized by activity-dependent changes in synaptic strength. Epileptiform activity entrains similar mechanisms that lead to LTP, synaptic growth and the rearrangement of synaptic connections. Pathologic manifestations of these mechanisms as the result of epileptic activity are e.g. excitotoxicity, neurodegeneration and mossy fiber sprouting. We therefore set out to study the role of CPEB dependent protein synthesis in the development and progression of TLE. We established a new animal model of TLE (intracortical kainate injection) and determined the severity and duration of seizures by continuous telemetric EEG recording and videomonitoring. The model is characterized by an acute status epilepticus lasting several hours, a latent seizure free period of several days and a chronic phase of recurrent spontaneous seizures. The morphological changes observed closely mimic the condition in human patients presenting with hippocampal sclerosis. We found that mice expressing a dominant negative CPEB experienced a different status epilepticus (SE) compared to littermate controls. The duration and frequency of seizures was significantly decreased during the first 2 hours, and increased in the subsequent period until seizure activity ceased. By contrast, the frequency of spontaneous seizures was increased significantly in the chronic phase. It is at present unclear, however, whether the increased frequency of spontaneous seizures is due to CPEB impairment in the chronic phase or due to the changed SE. We now will use the tet System for expression of the dominant negative CPEB and therefore can suppress transgene expression by administration of doxycycline during SE. In this way, we can ensure an SE comparable to control animals. During the latent period following SE, doxycycline is withdrawn and the expression of the dominant negative CPEB is turned on. In this way, we now are investigating the role of CPEBs selectively during the chronic phase of spontaneous recurrent seizures.

JNK proteins at adult rat brain mitochondria: dynamic changes of isoform presence and activity following ischemia

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The functional specificity of JNK enzymes is essentially determined by the context-dependent formation of signalosoms which regulate not only the activation of JNKs but also the intracellular mobilisation and translocation to organelles. This highly coordinated temporo-spatial activation of JNK isoforms finally determines the assocation with the substrates and the individual functional profile of the 10 JNK isoforms (reviewed by Waetzig and Herdegen, *TiPS* 2005 and *Prog. Neurobiol.* 2006). Recently we have shown for the first time, that JNK2 isoforms translocated to mitochondria and nucleus in PC12 cells following 6-OH-dopamine with subsequent release of cytochrome c and induction of Bim, whereas the constitutive mitochondrial JNK1 presence declined without any degenerative effects.
Here we have analysed the localisation and activation pattern of JNK isoforms in the mitochondria fraction from adult rat brain homogenates following transient middle cerebral artery occlusion (MCAo). The pattern of mitochondrial JNKs substantially differed from that of the cytoplasmic or nuclear pool. In untreated rats, basal activity was driven by JNK1 and to a less extent by JNK3. Following MCAo, the presence of JNK1 strongly declined, JNK2 decreased following a transient raise, whereas JNK3 increased and thereby did account for most of the JNK activity. Localisation and activation of MKK4 and MKK7 and their complexes with JNKs revealed alterations, too. The roles of JNKs for mitochondrial respiratory functions are under current investigation.
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Prevention of non-native disulphide bridges formation in tau protein without the use of reducing agent

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Tau protein belongs to protein family called intrinsically disordered proteins (IDP's). Solution-exposed cysteines of IDPs are highly reactive and therefore reducing agents are frequently used during their preparation to prevent formation of nonnative disulfides. However these agents (e.g. dithiothreitol or ß-mercaptoethanol) are toxic to cells (1, 2) and may interfere with subsequent experiments. Therefore we have developed a method, which protects reactive thiols of purified recombinant protein tau using inert argon atmosphere, making the reducing agents unneccessary. Tau isoforms prepared by this procedure are suitable for investigation of toxicity of their monomeric forms in rat cerebral neurons.

We have expressed and isolated recombinant tau proteins from bacteria. Purification using ion-exchange chromatography was followed by size-exclusion chromatography (3). DTT used during purification procedure was removed by desalting into buffer saturated with argon. These protein preparations did not contain any oligomers even after long term storage whereas proteins desalted without the use of argon contained up to 70% of dimers.

In conclusion we have developed a simple purification method for preparation of tau proteins overcoming the risk of non-native disulfide bridges formation. The method can be successfully employed for preparation of other proteins containing reactive thiols.

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BAG1 mediated neuroprotection in *in vivo* and *in vitro* models of Parkinson's disease

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Recent findings concerning the molecular mechanisms of Parkinson's disease (PD) identified a number of causes for neuronal demise like protein misfolding and aggregation, mitochondrial dysfunction and the impairment of protein degradation pathways. In this context a-synuclein misfolding has been proposed as key initiator event in the pathogenesis of PD. Upon misfolding a-synuclein can self associate, forming oligomers, fibrils and Lewy bodies, the pathological hallmark of PD. In our current understanding oligomeric a-synuclein intermediates are the toxic a-synuclein species, making chaperone induction a promising target as treatment strategy in PD. In this study we investigated the effects of the co-chaperone BAG1, which has been shown to mediate neuroprotection in different neurodegeneration models by increasing Hsp70 foldase activity. Coexpression of BAG1 in the SH-SY5Y neuroblasoma cell line resulted in a significant reduction of a-synuclein wildtype and mutant a-synuclein induced cell death. On a molecular level BAG1 overexpression did ameliorate a-synuclein oligomer and aggregate formation *in vitro*. Furthermore BAG1 overexpression in the substantia nigra of BL6 mice by AAV-mediated gene transfer showed a trend towards a protective effect in the MPTP *in vivo* model of PD. We conclude that BAG1 is a promising neuroprotective target and that further elucidation of BAG1-mediated chaperone induction may contribute to the development of successful drug therapies for aggregopathies like PD.

Molecular pathology of the motoneuron disease Spinal Muscular Atrophy

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Spinal muscular atrophy (SMA) is a neurodegenerative disease accompanied by a selective death of spinal motor neurons. Children suffering from the severe form of SMA (SMA type I) die within their first years of their life. Currently, no therapy is available.
SMA is caused by mutations or deletions of the *survial of motor neuron 1* (*SMN1*) gene. Although SMN is expressed ubiquitously, exclusively motoneurons degenerate related to SMN1 defects. SMN acts as an assembly protein for RNA-protein complexes in the nucleus. However, previously published data of our group demonstrated that SMN plays a additional role in actin dynamics in axons and growth cones during neuritogenesis (van Bergeijk et al., 2007). Suppression of endogenous SMN protein levels by siRNA decreased significantly growth of neurites in PC12 cells, whereas cells overexpressing SMN displayed increased lengths of neurites. Neurite outgrowth is associated with changes of the actin cytoskeleton. Remarkably, the knock-down of SMN led to a significant change of the G-/F-actin ratio indicating a role of SMN in actin dynamics. The Rho-Kinase (ROCK) pathway is an important modulator of the actin cytoskeleton. Our data suggest that actin-regulating proteins downstream of ROCK are involved in SMN-dependent neuritogenesis defects. This pathway could be a suitable target for pharmacological treatment.
Further investigations include analyses of changes of the ROCK pathway in SMA cell culture (PC12 and NSC34) and SMA mouse model. Moreover we want to characterize the interaction between SMN and the ROCK pathway member Profilin IIa, the putative link between SMN and the ROCK pathway.

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Pharmacological modification of ATP- dependent microglial activation in the disease model of ALS

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Microglia as brain immune cells, contribute to the physiological homeostasis in the CNS. Acute or chronic damage of neuronal tissue leads to microglial activation which is a multistep process involving morphological changes, release of pro-inflammatory substances, proliferation and migration to the affected sites of the brain. The purines ATP and ADP as soluble factors are implicated as candidates for the chemotactic attraction of microglia by activating ionotropic P2X and metabotropic P2Y receptors.
Earlier experiments on transgenic mice carrying the human mSOD1 gene presented evidence that microglia cells significantly contribute to the later phase of disease encompassing the progression to complete paralysis.
Here, we studied the purinergic influence on microglia motility and migration in the disease model of ALS. We used time-lapse 2-photon laser-scanning microscopy on spinal cord slices of $SOD1^{G93A}$ mice with EGFP-labelled microglia cells to capture the microglial motility under baseline, ATP-activation and P2-receptor inhibited conditions.
Already under saline conditions, an increased number of mSOD-microglia as well as morphologically transformed microglia were observed in slices of clinically affected animals. This corresponds to microglia overactivation in the SOD1 model of ALS in their presumed resting state. Furthermore, baseline motility of mSOD-microglial branches was significantly increased compared to controls. Upon superperfusion with ATP, numerous microglial branches but not cell bodies extended to the slice surfaceindicating a rapid response to chemotactic stimuli. This ATP-effect was blocked in controls by P2X7-receptor antagonist BBG and the P2X4-receptor antagonist TNP-ATP while in mSOD1-microglia P2-receptor inhibition was only partially effective. These results indicate that microglial overactivation in the SOD1 model is triggered by external ATP but can only incompletely inhibited by P2 receptor antagonists. We suggest thatthe overexpression of the P2X-receptors contribute to the disease progression by triggering inflammatory cascades.
Understanding the impact of altered microglial receptor expression together with motility and migration properties in mSOD-mice may lead to new therapeutic approaches with pharmacological modification of microglia.

Selective drug resistance in immature rat temporal cortex

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We have recently reported[1] that in organotypic hippocampal slice cultures tonic-clonic seizure like events (SLEs) induced by 4-aminopyridine (4-AP) or low magnesium are refractory to standard antiepileptic drugs (AEDs). Drug refractory SLEs could be a property of immature temporal cortex and as such transferred to slice cultures which are explanted from 6-10 days old rats. In the present report, we have investigated this issue in acute horizontal hippocampal-entorhinal cortex slices which were prepared from Wistar rats between 4-10 days old (P4-10group), and 14-18 days old (P14-18group). Slices were perfused with prewarmed (35-36^{o}C) and oxygenated (95%O_2/5%CO_2) ACSF in an interface chamber. The $[K^+]_o$ concentrations were measured with a double barreled K^+-selective/reference glass microelectrode in the pyramidal cell layer in CA3 and in layer 5 of medial entorhinal cortex (ECm), the reference electrode recording the DC-potential and population spikes. Tonic-clonic SLEs were induced by adding 4-AP (100 µM) to the ACSF. The effects of standard AEDs phenytoin (PHT), carbamazepine (CBZ), valproic acid (VPA) and phenobarbital (PHB) on ongoing seizure activity were tested. SLEs were considered blocked if they did not appear during the last 20 minutes of a one hour AED application.

The effects of AEDs were dependent on age, region and drug concentrations. In the P4-10group PHT or CBZ at 100 µM completely blocked the tonic periods of SLEs in CA3 and ECm in all slices, whereas clonic periods were blocked by PHT 100 µM in CA3 in only 13% and in ECm in 58% of slices, and by CBZ 100 µM in CA3 in 63% and in ECm in 100% of slices. In the P14-18group these proportions increased with PHT to 60% in CA3 and to 100% in ECm, and with CBZ to 100% in both CA3 and ECm. In the P14-18group 40 µM PHT blocked SLEs in CA3 in only 17% and in ECm in 80% slices, whereas 50µM CBZ blocked SLEs in CA3 in 72% and in ECm in 100% of slices. Thus CBZ and PHT blocked the tonic but not the clonic periods of SLEs in the ECm and less so in CA3. This pharmacoresistance was more pronounced in CA3 and in the P4-10group. In contrast to PHT and CBZ which failed to block clonic periods, VPA and PHB failed to block dependent on age and region both tonic and clonic periods of SLEs. Thus, proportions of slices in which SLEs were completely blocked with 2mM VPA in the P4-10group were 0% in CA3 and 33% in ECm, and in the P14-18group 50% in CA3 and 83% in ECm. In the P4-10group 200µM PHB blocked SLEs in CA3 in 13% and in ECm in 100% of the slices; proportions in the P14-18group were 43% in CA3 and again 100% in ECm.

We conclude that SLEs in the temporal cortex, in particular in CA3 and less so in the ECm, are drug refractory during the first postnatal week which indicates differences in mechanism of SLEs between both structures. We hypothesize that $GABA_A$ receptor activation, which induces depolarization/excitatory effects during the first postnatal week, contributes to seizure susceptibility and to the transient pharmacoresistance in the early postnatal temporal cortex.

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Innate-adaptive immune cross-talk in a mouse model of Parkinson's disease

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In the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) mouse model of Parkinson's disease, several observations suggest that activation of microglia and microglia-differentiated blood-derived monocytes may be directly involved in dopaminergic (DAergic) neuronal degeneration in the substantia nigra (SN). On the other hand, results on lymphocytic invasion in the murine MPTP model are contradictory. Analyses of direct cross-talk between cells of the adaptive and innate immune system in the nigrostriatal system in the MPTP model is limited. Using different MPTP application modes the kinetics of microglia activation and lymphocyte infiltration into mouse SN after MPTP treatment were determined. Through retrograde tracer-labeling of DAergic neurons a subpopulation of Iba1+ microglia/macrophages were identified as phagocytes of debris of degenerating DAergic neurons. These phagocytes expressed molecules like MHCII or CD86, and were additionally LAMP2+ suggesting lysosomal activity. CD3+/CD25+ T-lymphocytes infiltrating the SN were mainly in direct contact to activated microglia which expressed MHCII. B-lymphocytes did not infiltrate into the SN after MPTP. Our data give evidence that primary microglia/macrophage-derived phagocytes may process and present antigen from degenerating DAergic neurons to adaptive immune cells in the MPTP-lesioned SN.

Perineuronal nets are largely unaffected in Alzheimer model Tg2576 mice

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The participation of key extracellular matrix components, such as aggrecan, in human neuropathology is basically unidentified. We investigated perineuronal nets (PNs) in relation to neurodegeneration and activation of glial cells using immunocytochemical staining of aggrecan, detection of hyaluronan, as well as lectin binding in a transgenic mouse (Tg2576) model of Alzheimer's disease. The formation of amyloid plaques in the cerebral cortex occurred independently of the area-specific distribution of PNs. However, the structural appearance of PNs was affected in advanced stages of plaque pathology. The neuron-free core of amyloid plaques was nearly devoid of aggrecan and hyaluronan. Rudimental PNs persisted around malformed parvalbumin-immunoreactive interneurons close to reactive microglia, astrocytic profiles and dystrophic neurites in the coronal zone of plaques. In contrast, PNs remained unaffected in the large marginal zone, characterized by a dense meshwork of reactive, galactose-expressing astrocytic processes. We conclude that aggrecan-based extracellular matrix is destroyed after death of neurons in the core of amyloid plaques, but withstand selective breakdown in peripheral territories affected by chronical activation of astrocytes.

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Biochemical and genetic analysis of Parkinsons disease-associated proteins, molecular transporters, and stress response proteins in *C. elegans* models of manganism

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Parkinson's disease (PD) and manganism are characterized by motor deficits and damage to the substantia nigra, and dopamine or its metabolites are believed to contribute to both disorders. Proteins in which mutations have been linked with familial Parkinson's disease have also been proposed to contribute to the pathogenesis. In these studies we show that manganese (Mn) causes DA neuron cell death in *C. elegans*, and most of the known PD-associated orthologues, as well as specific glutathione transferases and heat shock proteins, are significantly upregulated following a brief exposure to Mn. Interestingly, a putative mortalin orthologue, a mitochondria-associated heat shock protein, has a 3.5-6-fold decrease in expression relative to controls, consistent with changes observed in PD brains. DA neuron vulnerability to Mn is partially dependent on the divalent metal transporter-1 (DMT-1) orthologues Smf-1-3, since knockdown or deletion of the putative Mn transporter partially protects against the neurodegeneration. Furthermore, expression of the smf genes following Mn exposure is dramatically increased in young animals relative to controls, consistent with its putative role in the toxicity. Gene expression levels of the vesicular monoamine transporter (VMAT) and dopamine transporter (DAT) are also significantly increased following Mn exposure, possibly due to changes in whole animal DA concentrations. DDAT animals are still sensitive to Mn, indicating a DAT-independent mechanism of neurotoxicity. Finally, Mn amplifies a-synuclein induced DA neuron cell death, consistent with its putative role in a-synculein fibril formation. We will also present preliminary studies involving the expression and localization of Mn-induced proteins, as well as results from a novel genome-wide screen to identify mediators of the Mn induced neurotoxicity. Overall these studies suggest that *C. elegans* is a powerful model system to explore the molecular basis of DA neuron vulnerability in human manganism.

Temporal expression of markers for revascularization in the injured rat spinal cord.

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Every year, several million people worldwide suffer from traumatic spinal cord injury leading to permanent paralysis. Acute spinal cord injury (SCI) initiates a series of cellular and molecular events in the injured tissue leading to further damage in the surrounding area. The progression of the secondary damage deteriorates neurological function, and its prevention is of key importance for later regeneration and healing responses (Hausmann, 2003). One of these events is the disruption of spinal cord blood flow and the onset of ischemia (Imperato-Kalmar et al., 1997). Support of regeneration by formation of new blood vessels would provide nutrients to the cells and allow the infiltration of inflammatory cells to implement the cleaning of the lesion cavity. The regenerative capacity of the nervous tissue vasculature has been observed (Beggs et al., 1979; Casella et al., 2002), but the mechanisms are not yet fully understood. Bone marrow-derived endothelial progenitor cells (EPCs) have the potential to participate in the neovascularization of many adult tissues. Therefore, we hypothesize that EPCs from the bone marrow may be recruited for the neovascularization of the spinal cord after a compression injury. Spinal cord injury (SCI) was performed in rats by clip compression at T8-T9. RNA was extracted from spinal cord segments containing the injury site (5 mm) dissected out after various time points (from 2 hrs to 4 weeks after SCI). Complementary DNA was synthesized and quantitative RT-PCR was performed with specific primers for the stem cell markers CD133, CD34, CXCR4, for pro-angiogenic factors SDF-1, and HGF. Immunohistological staining was done to visualize blood vessels (using RECA-1 and laminin antibodies) and endothelial precursor cells (using CD133 antibody) in spinal cord tissue sections. Whereas CD133 expression decreased from 6 hr until 3 days post-SCI, the expression of other markers (CD34, CXCR4, HGF) increased in the injury site after 2 or 3 days, or after 3 weeks for SDF-1. Immunofluorescent staining of the spinal cord sections confirmed the decrease in CD133+ cells in the lesion site and in the surrounding area 2 days post-SCI, and showed an increase in the number and in the complexity of CD133+-blood vessels after 2 and 4 weeks following SCI. These results indicate that the expression of factors involved in the recruitment of bone marrow derived EPCs is actively stimulated in the injured spinal cord some days to weeks after SCI, and that these cells may participate to the building of new functional blood vessels.

ischemic preconditioning attenuateS mitochondrial apoptosis Induced by global brain ischemia.

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Apoptosis is an evolutionarily conserved process critical to development of CNS and, when dysregulated, causes inappropriate neuronal cell death. Global ischemia is a neuronal insult that induces delayed cell death with many features of mitochondrial apoptosis. Ischemic preconditioning affords robust protection of CA1 neurons against a subsequent severe ischemic challenge. The molecular mechanisms underlying ischemic tolerance are unclear.

Here we show that global brain ischemia, induced by permanent occlusion of vertebral arteries and temporal occlusion of carotid arteries for 15 minutes, induces translocation of p53 to mitochondria observed in hippocampus but not in cerebral cortex as well as genomic DNA fragmentation observed predominantly in CA1 layer of hippocampal formation. In addition, Fluro-Jade C positive degenerating cells were observed in vulnerable CA1 layer of rat hippocampus.

Rats were preconditioned by 5 minutes of sub-lethal ischemia and 2 days later 15 minutes of lethal ischemia was induced. IPC abolished completely ischemia-induced translocation of p53 to mitochondria. With respect to apoptosis execution, significant protective effect of IPC on ischemia-induced DNA fragmentation was observed as well. Neuroprotective effect of IPC was documented by significant reduction of Fluoro-Jade C positive cells in CA1 layer of hippocampal formation. Our results indicate that both initiation and execution of mitochondrial apoptosis induced by global brain ischemia are almost completely abolished by IPC.

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Expression of two-pore domain potassium channel TASK2 is altered in T lymphocte subsets of multiple sclerosis patients

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Recently it could be demonstrated for the first time that two-pore domain potassium channels (K_2P) are functionally expressed on human $CD3^+$ T lymphocytes. Inhibition of these channels, namely the family members TWIK-related acid-sensitive potassium channel1 (TASK1) and TASK3, resulted in silencing of T cell effector functions.

Here we show the expression of the alkaline-activated K_2P channel TASK2 on human T lymphocytes. Clofilium, quinidine or extracellular acidification led to reduction of whole cell outward current and blockade of TASK2 resulted in a decreased production of proinflammatory cytokines and proliferation rates. Analyzing different T cell subsets we found high TASK2 expression levels in human $CD4^+$, $CD8^+$ and $CD4^+/CD25^+$ T cells while only low levels were found in NK or B cells on mRNA and protein level. CD3/CD28 bead stimulation of $CD4^+$ and $CD8^+$ T cells resulted in a significant and time-dependent up-regulation of TASK2 channel expression. Illustrating the pathophysiological relevance of these findings we found a significant up-regulation of TASK2 in $CD4^+$ and $CD8^+$ T cells in relapsing-remitting multiple sclerosis (RRMS) patients during acute relapses as well as higher expression levels on $CD8^+$ T cells of RRMS patients during clinically stable disease course. Furthermore, TASK2 positive T cells could be found in inflammatory plaques from autopsy material of MS patients and encephalitogenic T lymphocytes in cerebral spinal fluid showed higher expression than T lymphocytes in the peripheral blood supporting TASK2 channels as interesting molecular target in MS pathogenesis and therapy.

Neuroprotective effects of the survival promoting peptide Y-P30

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Y-P30 is a polypeptide produced by peripheral blood mononuclear cells (PBMC) of the maternal immune system during pregnancy. The peptide passes the blood-placenta barrier and accumulates in neurons of the developing infant brain, where it enhances survival of thalamic neurons and displays neuritogenic activities. Interestingly, expression of the peptide factor can be induced after optic nerve crush in adult rats. To further address its potential role in brain injury we followed up its expression after brain ischemia (filament model in rats) and tested its neuroprotective potential in the oxygen-glucose-deprivation (OGD) model with hippocampal slices as an assay for ischemia. Y-P30 gene expression in PBMC's was surprisingly low after experimental brain ischemia as compared to optic nerve crush. However, Y-P30 conferred significant neuroprotection when the peptide was added at concentrations of 200nM and 2µM to the medium of hippocampal slice cultures two hours before starting the deprivation of oxygen and glucose. A modest but still significant neuroprotective effect was found when the peptide was applied two hours after injury to the medium. Y-P30 has been shown to built up larger oligomers which might hinder passage through the culture membranes. To further enhance the peptides neuroprotective potential we therefore applied Y-P30 directly on top of the hippocampal slice. This administration regime let to the most robust neuroprotection even at very low doses.

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Novel ligands of the mitochondrial Translocator Protein (TSPO) as neuroprotective agents

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The mitochondrial 18 kDa Translocator Protein (TSPO), also known as the peripheral-type benzodiazepine receptor (PBR), is abundant in various peripheral organs and is also expressed in the CNS. TSPO reportedly is involved in various neuropathological conditions, including neurodegenerative diseases and brain trauma. Using standard techniques, we have developed high affinity ligands for the TSPO to block its apoptotic function. Using the classical TSPO specific ligand, [^{3}H]PK 11195, we determined the inhibition constant (K_i) values of our novel compounds. *In vitro*, using the SH-SY5Y neuroblastoma cell line, we tested the anti-apoptotic properties of our novel ligands. *In vivo* we tested their potential neuroprotective effects against neurodegeneration caused by systemic injections of kainic acid in rats. Compounds of the quinazoline family showed low K_i values in competition studies with [^{3}H]PK 11195. Compounds of the phthalazine and quinoxaline families exhibited lower affinity. The presence of an amide moiety attached via an oxygen molecule to the central carbocycle exhibited relatively high affinity for TSPO. Furthermore, the size of the alkyl groups (methyl – propyl) linked to this amide moiety correlated positively with the binding and anti-apoptotic properties of these compounds. In addition, side chains of the freely rotating carbocycle also contributed to affinity and anti-apoptotic properties. Our novel TSPO ligand, MGV-1, reduced apoptosis *in vitro* and was also neuroprotective *in vivo*, in a dose dependent manner. Our results show that TSPO ligands can be designed specifically for neuroprotective properties.

Retinoic acid affects the expression of the pro-inflammatory cytokine IL-1ß in astrocyte primary cultures.

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Astrocytes contribute to inflammatory reactions in the central nervous system. The pro-inflammatory cytokine interleukin 1 beta (IL-1ß), which plays an important role in neurodegenerative disorders, is expressed by astrocytes [Dong and Benveniste *GLIA* 2001]. Recent studies show that the transcriptional regulator retinoic acid (RA) reduces the immune response of astrocytes, e.g. prostaglandin synthesis [Kampmann et al., *J. Neurochemistry* 2008, Xu and Drew, *J Neuroimmunol.* 2006]. We have investigated the effect of RA treatment on IL-1ß expression and release from astrocytes stimulated with lipopolysaccharide (LPS).

Astrocyte primary cultures prepared from cortex and midbrain of neonatal mice received a 12 hrs treatment with all-*trans* RA (0.1 µM). Subsequently, the cells were exposed to LPS (100 ng/ml) and simultaneously to all-*trans* RA (0.01, 0.1, 1 µM) for 1 hr, 6 hrs, 12 hrs, 24 hrs, 48 hrs or 72 hrs. IL-1ß mRNA levels were measured with quantitative real time -PCR. The release of IL-1ß into the cell culture medium was determined with ELISA.

We found that RA was able to reduce mRNA levels as well as IL-1ß protein release after LPS exposure. This may be due to changes in the promoter structure of the IL-1ß gene, which is currently under investigation with the Chromatin Accessibility by real-time PCR (CHART) -Assay.

CD8+ lymphocyte-mediated injury of CNS neurons: relevance of granzyme B and perforin for acute electrophysiological consequences and long-term neurotoxicity

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Objectives: Cytotoxic T lymphocytes (CTL) are considered as important effector cells contributing to CNS damage in neuroinflammatory disorders. We here challenge the mechanisms how CD8+ T cells injure neurons and how major histocompatibility complex (MHC) class I restricted, cell-contact-dependent interaction of CTL with neuronal cells is determined. Specific emphasis is placed on the interdependency for perforin and granzyme B in immune-neuronal interactions.
Methods: We used hippocampal neuronal cells in combination with T-cell receptor (TCR) transgenic CD8 T cells (OT-I) as a paradigm for MHC class I restricted immune-neuronal interaction. Cocultures were analyzed by time-lapse video-microscopy and two-photon microscopy in conjunction with immunohistochemistry, electrophysiological recordings and molecularbiological assays.
Results: Time-lapse video-microscopy showed that CTL kill MHC class I-induced neurons fast and depending on cell-cell contact as well as antigen (appr. 30% over 4 hours). Real-time electrophysiological recordings of basal neuronal parameters upon direct contact with CD8+ effector T cells revealed immediate changes in membrane capacitance and resistance (appr. 60% decrease; = 10 minutes), which occurred only in the context of the appropriate trimolecular complex. With a similar kinetic and MHC restriction, neurons showed a rapid influx of Ca2+ upon CTL interaction. Assuming that the observed effects most likely reflect the consequences of a TCR-dependent pore-formation via the granzyme/perforin pathway, we next studied the effects of OT-I cells deficient for granzyme B or perforin. Interestingly, quantification of neuronal killing by time-lapse videography revealed that CTL deficient for granzyme B or perforin maintain the same neurotoxicity, albeit migratory velocity was significantly slowed in the absence of granzyme B. Single-cell recordings however showed, that perforin deficient T cells had lost their capability to induce neuronal membrane changes or Ca2+ influx, while these immediate effects were maintained in the absence of granzyme B.
Conclusion: CTL-mediated injury of MHC class I induced neuronal cells is a fast, antigen and cell-cell contact dependent process. CD8+ effector cells immediately affect basal cellular parameters and induce Ca2+ influx in a MHC I restricted way. While acute electrophysiological consequences of immune-neuronal interaction require perforin, perforin or granzyme B are redundant for long-term neurotoxicity.

A novo specific 5-HT_{2B}-receptorantagonist for the prophylactic treatment of migraine

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The activation of the 5-HT_{2B}- receptor is one of many triggers of migraine. Many antagonists (methysergide and pizotifene) specific to the 5-HT_{2B} receptor have been successfully used as prophylactic anti-migraines. Despite their anti-migraine effect both drugs have shown lower efficacy and higher side effect.
In order to find a better medication we have started search a new compound that has specific inhibition of the activity of the 5-HT_{2B} receptor over the 5-HT_{2A} and 5-HT_{2C}. In a screening we have found a new substance BF-1 that has shown high affinity to 5-HT_{2B} receptor and antagonist function on the cell culture level. Its efficacy on the 5-HT_{2B} receptor is 100 time higher than the effect on the 5-HT_{2A} and 5-HT_{2C}-receptor.
For the investigation of the anti-migraine efficacy of BF-1, we first established an guinea pig model of migraine. Through the activation of the 5-HT_{2B} receptor by mCPP, a partial 5-HT_{2B}-receptoragoist, in the dura mater is resulting in a payout of inflammatory mediators from the nerve trigeminus leads. By inflammatory mediators it comes to a vasodilatation and a plasma protein extravasation (PPE). This PPE can be quantified by the passage of Evans Blue (EB) into the dura mater tissue which can be measured photometric. This EB-PPE has been used as indicator for the migraine attack This mCPP induced EB-PPE could be abolished by the established anti-migraine drugs (methysergide and pizotifene) that confirmed the EB-PPE as a migraine indicator in this animal model. This animal model were also verified by using both established anti-migraine drugs (methysergide and pizotifen), and it could inhibit the PPE can be observed.

Similar to the methysergide and pizotifene, the substance BF-1 is able to block the mCPP induced EB-PPE with a very high potency that is much higher than both established anti-migraine drugs.

Collateral damage of CNS neurons during an acute oligodendrocyte-directed attack by $CD8^+$ and $CD4^+$ T cells

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Inflammatory T cells are critical to the initiation, progression and regulation of autoimmune pathology in the CNS. The pathology of Multiple Sclerosis (MS), a progressively chronic debilitating neurological disorder of young adults, shows inflammatory demyelination combined with axonal damage. Both CD4+ and CD8+ T lymphocytes can be found in acute and chronic demyelinating lesions and are so proposed to mediate demyelination and axonal damage. However, how immune cells (CD4+ and CD8+) contribute to oligodendrocyte (ODC) death and neuronal damage in CNS inflammation is poorly understood.

We addressed the mechanism of acute neuronal damage by oligodendrocyte-directed cytotoxic CD8+ and CD4+ T cells using the following approach: TCR-transgenic CD8+ T cells directed against ovalbumin (OT-I) and TCR-transgenic CD4+ T cells recognizing MOG35-55 peptide respectively were transferred into acute living brain slices from mice selectively expressing ovalbumin as a cytosolic neo-self-antigen in oligodendrocytes (ODC-OVA mice) or rather C57Bl/6 mice. Interactions between T cells and CNS structures were visualized and quantified, kinetics and mechanisms of cell death were assessed by multicolour stainings of cellular markers of ODC together with a marker of early apoptosis. Different CNS regions were investigated (cortex and hippocampus) and followed for a time interval of 6 hours.

We demonstrate here that transferred prestimulated CD8+ and CD4+ T cells not only preferentially accumulate in myelinated areas, but also induce apoptosis of ODC after 6 hours exposure, which was not observed under any control conditions. This suggests that primed T cells recognizing their cognate antigen can acutely kill ODCs. Interestingly, parallel to the oligodendroglial death we also found significantly increased numbers of apoptotic neurons in ODC-OVA slices exposed to OT-I and MOG-specific CD4+ T cells than under control conditions. Of note, no apoptosis was ever detected in astrocytes.To unravel the molecular mechanism of simultaneous apoptosis of ODCs and neurons by activated cytotoxic CD8+ T cells we used OT-I cells deficient in either perforin or granzyme B. In the absence of perforin but not granzyme B numbers of apoptotic ODCs as well as neurons were significantly reduced. This implies that apoptosis of ODC as well as neurons is in part mediated by perforin and that granzyme-dependent killing does not substantially contribute to acute CNS-tissue damage.

In summary our results indicate that neuronal damage can be very well the result of "collateral damage" by T cells targeting antigen-presenting oligodendrocytes.

HUNTINGTON'S DISEASE RELATED MITOCHONDRIAL TOXINS AFFECT THE IMMUNOLOGICAL PROFILE OF MICROGLIAL CELLS TOWARDS A REDUCED ALTERNATIVE ACTIVATION

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Huntington Disease (HD) is the most common autosomal dominant inherited adult-onset neurodegenerative disease. HD related mutations are ubiquitously expressed and associated with mitochondrial dysfunction. HD shares many salient clinical and pathological features, like mitochondrial dysfunctions, with major sporadic neurodegenerative diseases, Alzheimer´s disease, Parkinson´disease and amyotrophic lateral sclerosis. This suggests that similar pathogenic mechanisms are involved. Inflammation in form of activated microglial cells (MG) is an early hallmark of neurodegenerative disease. MG are the resident macrophage in the central nervous system and are distributed in large non-overlapping regions throughout the brain and spine. MG activation shows mainly two different responses: 1) The classic activation leading to inflammation and neurotoxicity. 2) The alternative activation is linked to anti-inflammatory responses, which can protect brain tissue. The type of MG activation is stimulus-determined. The metabolic and immunological profiles of macrophages are interdependent. Classic activation is associated with glycolysis and oxygen independent, while the alternative pathway is linked to fatty acid oxidation.

Our hypothesis is that mitochondrial dysfunction in MG change their immunological profile with detrimental consequences for the diseased brain.

We incubated primary mouse microglia with the mitochondrial toxins 3-Nitropropionic acid (3-NP) and malonate and investigated their immunological profile. 3-NP and Malonate induce HD like degeneration in animals and affect not only neurons but also microglia. We stimulated microglial cells with mitochondrial toxins, IL-4 and/or LPS. Cytokines (TNF-alpha, IL1-beta, IL6 and IGF-1) were quantified in the supernatant. Antiinflammatory Arginase activity was quantified with Thioarginine and DTNB.

Mitochondrial toxins affected the amount of cytokines and arginase activity induced by the alternative activation.

This suggests that changes of the mitochondrial metabolism that are linked to HD reduce the alternative response of microglial cells exacerbating neuroinflammation. Since neuroinflammation is a remarkably early event not only in HD but also in sporadic neurodegenerative diseases, to reconstitute the alternative microglia response might be a therapeutical strategy. This could lead to prevention of disease progression and a hence better clinical outcome.

Retinoic acid reduces inflammatory chemokine production by astrocytes *in vitro*

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The transcriptional activator retinoic acid (RA) is involved in the physiological processes after injury in the peripheral and central nervous system [Mey et al., *Eur. J. Neurosci*. 21 (2005)]. Spinal cord contusion injury caused intracellular translocation of RAR and RXR in neurons, microglia and astrocytes near the lesion site [Schrage et al., *Eur. J. Neurosci*. 23 (2006)]. In primary astrocyte cultures *all-trans* RA reduced mRNA and protein expression of the prostaglandin synthesizing enzyme COX-2 and thereby diminished the synthesis of PGE_2 [Kampmann et al., J. Neurochem, (2008)]. The RXR agonist 9-*cis* RA decreased the production of NO and TNF-a production, without affecting IL-1ß, IL-6 and MCP-1 secretion [Xu and Drew J. Neuroimmunol. (2006)].

Based on these findings we hypothesized that activation of the RA signaling pathway may be an endogenous protective regulatory signal to reduce inflammation after injury. To test this hypothesis, astrocyte primary cultures, prepared from cortex of perinatal mice, were challenged with lipopolysaccharides (LPS, 100 ng/ml) and simultaneously treated with *all-trans* RA (10 nM, 100 nM, or 1 µM) for 30 min, 1 h, 6, 12, 24, 48, or 72 hrs. Chemokine/cytokine expression and release were determined with quantitative RT-PCR and ELISA. To investigate indirect anti-inflammatory effects of RA cells were also pretreated with RA and subsequently exposed to LPS + RA. RA reduced the release of specific pro-inflammatory mediators including chemokines CCL2 (MCP-1), CCL3 (MIP-1a), CCL5 (RANTES), CXCL1 (KC), CXCL2 (MIP-2), however had no effect on NO release. On the mRNA level, RA was also able to reduce the expression of CCL2, CCL3, CCL5, CXCL1, and CXCL2. Pretreatment with RA caused a stronger reduction of LPS effects than simultaneous application. These results indicate that the astrocytes may be a physiological target of endogenous RA signals after CNS injury.

A novel class of immunosuppressive compounds ameliorates experimental autoimmune neuritis.

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Acridine-iminodibenzyl chimeric compounds were previously introduced as a class of cholesterol-redistributing substances with antiprion and anti-inflammatory effects. Administration of the lead compound quinpramine to mice with experimental autoimmune neuritis, an animal model of human Guillain-Barré syndrome (GBS), significantly ameliorated disease. Quinpramine treatment decreased the number of inflammatory infiltrates in the inflamed nerve in comparison to controls. In cell culture experiments quinpramine reduced MHC class II expression on antigen presenting cells. Our data suggest that quinpramine may influence the immunological synapse by modulating cholesterol distribution and as such represents an immunoregulatory drug that is an interesting candidate pharmaceutical for immune mediated diseases of the peripheral nervous system.

Is the voltage-dependent anion channel 1 (VDAC-1) involved in Ha-Ras-mediated neuronal protection?

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The small GTPase Ras is a key regulator of different signal transduction pathways which influence cell growth, differentiation and apoptosis. To investigate the possible role of H-ras in the brain a synRas mouse model was created in which constitutively activated Val12Ha-ras is expressed in neurons of hippocampus and cortex. One of the phenotypic changes of the synRas mice is protection against lesion-induced neuronal degeneration (Heumann et al., J Cell Biol 151, 1537-48 2000). Proteome studies of cortex and hippocampus of the synRas mice showed alterations in the expression level of several energy metabolism proteins, including decrease of voltage-dependent anion channel 1 (VDAC-1) by 70 %. VDAC-1 is located in the outer mitochondrial membrane (mt-VDAC) and in the plasma membrane (pl-VDAC) in mice as a result of alternative splicing. The mt-VDAC together with the adenine-nucleotide translocase and cyclophilin-D forms the mitochondrial permeability transition pore which releases cytochrome c during apoptosis. It was previously described that pl-VDAC is involved in apoptosis which is prevented in neurons by extracellular application of anti-VDAC antibodies. The aim of this project was to investigate if changes of VDAC-1 expression could be involved in the mechanism of neuroprotection in synRas mice. Real-time PCR analysis revealed a decrease for pl-VDAC-1 mRNA in hippocampus and cortex of adult synRas mice whereas the mt-VDAC-1 mRNA level in hippocampus and cortex showed no difference between wild type and synRas mice. This decrease of pl-VDAC-1 mRNA was replicated in primary cortical cultures of synRas mice. Degeneration of PC12 cells, induced by 6-OHDA, was enhanced by overexpression of VDAC-1. This enhancement of cell degeneration was attenuated by co-expression of activated Ras. Further experiments in primary cortical cultures derived from the synRas mice suggest that the activated Ras/MAP kinase pathway influences the ratio of alternative splicing of VDAC-1. Taken together, these results suggest a possible involvement of VDAC-1 in Ras-mediated neuroprotection.

Specific knock-down of RhoA, ROCK2 and LIMK1 promotes neurite outgrowth and axonal regeneration

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Neuronal regeneration within the lesioned central nervous system is hampered by several inhibitory molecules. Many of these inhibitors signal, either directly or indirectly, via the intracellular Rho/ROCK pathway making it an interesting pharmacological target for the regulation of neuronal outgrowth after injury. Several studies have previously shown that pharmacological inhibition of ROCK can promote axonal regeneration. However, it remains unclear, how specific this pharmacological inhibition is and to what extent molecules signalling upstream and downstream of ROCK contribute to this effect.

To evaluate these questions, we generated adeno-associated viral vectors (AAV) for specific downregulation of RhoA, ROCK2 and LIMK1. Immunoblots and immunohistochemistry were used to confirm specific downregulation of the targeted proteins. In primary midbrain dopaminergic neurons (MDN) as well as in primary retinal ganglion cells (RGC) we found a significantly increased mean neurite length after treatment with ROCK2- and LIMK1-shRNA compared to control shRNA treatment. In addition, neurite regeneration after scratch lesion in MDN cultures was also markedly increased.

We evaluated the shRNA-expressing vectors in a rat optic nerve axotomy and crush model in vivo. Intravitreal injections of AAV expressing shRNA targeting RhoA increased the number of surviving RGCs after optic nerve axotomy. In addition, regeneration of RGC axons after optic nerve crush in rats was also significantly increased.

Taken together, we have established a shRNA-based paradigm to quantify the effects of specific downregulation of key inhibitory cascade molecules using AAV vectors. Our results suggest RhoA, ROCK2 and LIMK1 as promising molecular targets regulating neuronal survival and axonal regeneration warranting further investigation in vivo.

Anti-inflammatory but no neuroprotective effect of adjuvant glycerol in experimental meningitis

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Objectives: A clinical study published in October 2007, demonstrated beneficial effects of adjuvant glycerol in children suffering from bacterial meningitis. The study indicates that glycerol significantly reduces severe neurological sequelae. We initiated a study in infant rat pneumococcal meningitis to investigate the mechanisms underlying the beneficial effect of glycerol.
Methods: Eleven days old rats were infected intracisternally with 10 µL saline containing *Streptococcus pneumoniae* (6.15 $\log_{10}$ cfu/ml, n = 34) or mock-infected with the same amount of sterile saline (n = 8). All animals were treated with ceftriaxone (100 mg/kg body weight s.c., n = 42). Then both groups were randomized to receive either 50 µl glycerol p.o. (1.5 mg/kg body weight, n = 21, i.e. 17 infected and 4 mock-infected animals) or an equal amount of carboxymethylcellulose 2% p.o. (n = 21, i.e. 17 infected and 4 mock-infected animals). At 24 h and 40 h after infection, cerebrospinal fluid (CSF) was obtained by intracisternal puncture. At 40 hours after infection, all animals were sacrificed and the brains dissected. Brain weight and water replacement was determined to assess the density of brain tissue as an index for brain edema formation. Subsequently, the brains were immersion fixed in PFA 4% in PBS followed by sucrose 18% in PBS. Brain sections (45 µm thick) were stained using cresyl violet to determine apoptosis in the hippocampal dentate gyrus and the extent of cortical brain damage. CSF was analyzed for myeloperoxidase (MPO) activity and the concentrations of matrix metalloproteinase (MMP) - 2 and MMP-9.
Results: All parameters were statistically tested to compare infected animals receiving glycerol vs. infected animals receiving carboxymethylcellulose. No significant effect was seen on brain density, brain damage, MMP-2 and MMP-9 concentration at 24 hours after infection. The MPO activity at both time points and the MMP concentrations at 40 hours after infection were significantly reduced by glycerol.
Conclusions: In the present study no reduction of brain damage by glycerol was observed. Nevertheless, our study revealed potential mechanisms underlying a neuroprotective effect of glycerol. The reduced activity of MPO at both time points indicates that less inflammatory cells i.e. neutrophils invaded the CSF. A large body of evidence indicates that MMPs contribute to the development of brain injury in bacterial meningitis. Thus, the decrease of MMP-2 and MMP-9 at 40 h after infection suggests a potential neuroprotective effect of adjuvant glycerol, as found in the clinical study, and may be due to its anti-inflammatory effect.

Altered cytokine expression patterns in patients with chronic musculoskeletal pain

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Background:
Chronic musculoskeletal pain is a disorder of poorly understood pathophysiology and is often associated with depression. In recent years, a pathogenic role of altered cytokine profiles has been implicated in depression and in several chronic pain disorders.

Aim:
To analyse cytokine profiles in patients with chronic musculoskeletal pain and the influence of concomitant depression.

Methods:
40 patients with chronic musculoskeletal pain, 20 of whom fulfilled the ACR criteria for the fibromyalgia syndrome (FMS), were enrolled in this study. The patients had a minimum duration of pain of at least 6 months. The control group consisted of healthy volunteers. These volunteers had no pain disorder, infectious illness or mental disorders at the time of study participation.
The depression and the anxiety states were detected with ADS, HADS-D and STAI-G questionnaires.
Messenger RNA (mRNA) of cytokines in peripheral blood was analysed using quantitative real-time polymerase chain reaction (quant. RT-PCR).

Results:
20 of 32 patients who completed the ADS questionnaire showed increased depression values; 12 of them even critically increased values. 30 patients answered the HADS-D questionnaire. 8 of them had critically high anxiety scores. All control subjects had in normal outcomes both questionnaires. Regarding STAI-G, chronic muscle pain patients showed significantly increased anxiety scores in comparison to the control group.
mRNA levels of the pro-inflammatory cytokines IL-1ß and IL-6 were significantly higher in blood from patients with chronic musculoskeletal pain than in healthy controls. The mRNA levels of the anti-inflammatory cytokine IL-4 were significantly decreased in the patient group without FMS, but not in the FMS patients.
We divided the patient group into critically increased ADS-values (depression+) and normal ADS-values (depression-) to evaluate the influence of depression on cytokine levels. The mean IL-1ß mRNA levels were equal in depressive and non-depressive patients. IL-6 levels had a trend to be higher in depressive pain patients than in non-depressive ones (n.s.).

Conclusion:
Patients with chronic musculoskeletal pain have a higher likelihood to suffer from depression and anxiety states than healthy controls. They also have pro-inflammatory cytokine profiles with reduced anti-inflammatory cytokine production, which may be involved in the pathogenesis of pain. The presence of depression did not independently influence the cytokine profiles.

Pro-inflammatory cytokine expression following transient retinal ischemia/reperfusion in the rat eye. Modulation by simvastatin.

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Retinal ischemia is a serious and common clinical problem and leads to visual loss in a number of ocular diseases including acute glaucoma, branch retinal artery occlusion, diabetic retinopathy, and hypertensive vascular disease. During the ischemic cascade, inflammatory mediators are activated to cause secondary neuronal injury through the release of cytokines, phospholipases and chemokines. 3-Hydroxy-3-methylglutaryl-CoA reductase inhibitors (statins) are potent cholesterol-lowering drugs. Recent data suggest that statin therapy has pleiotropic effects, including protection against acute and chronic neurodegeneration following ischemic stroke, and modulation of inflammatory response e.g. by inhibition of leukocyte accumulation and modification of T-cell activation. Statin delivery increases retinal ganglion cell survival following acute retinal ischemia/reperfusion in the rat. The aim of this study was to characterize the pro-inflammatory cytokine expression and the putative statin-mediated immunomodulatory effect during the acute phase following transient retinal ischemia/reperfusion in the rat eye. We used a model of transient global retinal ischemia in the rat by elevation of intraocular pressure. A single dose of Simvastatin (4 or 8 mg/Kg) was delivered subcutaneously 1 h after ischemia/reperfusion. Microglia/macrophages were quantitated by means of immunohistochemistry on retinal slices 3, 6, 12 and 24 h after ischemia. Expression of cytokines was evaluated by means of RT-PCR and ELISA 3, 6, 12 and 24 h after acute retinal ischemia/reperfusion. mRNA expression levels for IL-1b and TNFa were significantly increased 3 h after the lesion, and almost returned to basal levels 24 h after ischemia. Protein expression levels of TNFa followed a similar pattern after the lesion. IL-1b expressing cells were identified as OX42-positive microglia/macrophages. A significant increase in the number of these cells (260% as compared to controls) was observed 3 h after ischemia, concomitant with a change in cell morphology and distribution in retinal layers. Following simvastatin delivery (4 or 8 mg/Kg), mRNA expression levels of the pro-inflammatory cytokine IL-1b were significantly modified 3 and 6 h after ischemia/reperfusion. Statin treatment significantly reduced TNFa protein expression levels 12 h after retinal ischemia/reperfusion. Results of this study show a strong pro-inflammatory response in the early phase after ischemia and support a potential use of statins as therapeutic agents to modulate acute inflammatory response following transient retinal ischemia/reperfusion.

MS-like cerebral inflammatory pathology in mice: A new experimental model in MS research

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Multiple sclerosis is an autoimmune demyelinating disease affecting the brain and spinal cord. This is in contrast to the most widely used animal model, experimental autoimmune encephalomyelitis (EAE), where inflammation is located predominantly in the spinal cord. Our aim was to develop an experimental model where mechanisms and consequences of inflammatory demyelination in the mouse brain could be studied. Therefore, we combined toxin-induced demyelination by the copper chelator cuprizone with MOG35-55 peptide immunization. This approach led to demyelinated brain lesions with infiltration of T-cells and macrophages through an open blood-brain-barrier, thus closely resembling MS pathology. Inflammation led to abundant axonal damage in contrast to cuprizone induced demyelination alone where only few APP-positive axons were detected. In immunized animals, macrophages expressed proinflammatory proteins such as iNOS and S100A9/MRP14 as well the anti-inflammatory protein HO-1. Withdrawing cuprizone from the chow led to rapid remyelination to a similar extent in immunized and non immunized animals. In summary, we present a new model which is particularly suitable to study mechanisms of damage and repair of MS-like lesions in the mouse brain.

Neuro- and gliotoxicity of engineered nanoparticles

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Applications of synthetic nanoparticles are of increasing interest and therefore nanotoxicology is now an emerging research field. Most available toxicology data for these nanomaterials are derived from inhalation studies. Although some studies have shown that nanoparticles can reach the brain via the olfactory nerve there is little known about the interaction of nanoparticles with neurons and glial cells.

The aim of our study is to investigate the acute neuro- and gliotoxicity of engineered nanoparticles to which humans may be exposed in the context of medical and technical applications, during manufacturing processes of tools or by using nanoparticle containing products. We focused on nanoscaled tungsten carbide (WC), tungsten carbide cobalt (WC-Co), diamond nanoparticles as well as modified single-walled carbon nanotubes (SWCNT).

We assessed the toxic effects of the particles on primary rat neuronal and astrocytic cultures. The cells were exposed to well chemical-physically characterized and stable nanoparticle suspensions in a concentration and time dependent manner. A variety of relevant endpoints were examined such as viability, changes of the mitochondrial membrane potential and adhesion of the primary cultures after exposure to nanoparticles. Cellular uptake and distribution of the particles was analysed by electron microscopy and flow cytometry. Furthermore we treated neurons with conditioned media from exposed astrocytes.

The effects of engineered nanoparticles on primary neurons and astrocytes depend on their chemical nature. There was e. g. no response from both cell types to nanoscaled diamond. However the results of our studies indicate that neurons can be affected by SWCNT, WC- and WC-Co-nanoparticles. The most toxic potential elicited nanoscaled cobalt doped tungsten carbide which also led to increased LDH release in astrocytic cultures.

Surprisingly, pure neurons were less sensitive than astrocytic cultures. This may reflect an effect of nanoparticles on proliferation of cultured cells. Conditioned media exerted the most toxic effect. This suggests that astroglial cells may play an important role in nanoparticle mediated nerve cell injury.

Decreased adhesion, viability and mitochondrial membrane potential, as it could be shown for the examined nanomaterials, may have an impact on communication between neurons and signal transduction if the particles reach the brain.

Status epilepticus: Expression of matrix metalloproteinases MMP-9 and MMP-2 in the developing rat brain

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Matrix metalloproteinases (MMPs) play an essential role in tissue repair, cell death and morphogenesis and may constitute therapeutic targets for acute brain injuries. The aim of the present study was to investigate the role of selected MMPs (MMP-2 and MMP-9, gelatinases) in a model of status epilepticus in infant rats.

Seven days old rats received intraperitoneal injections of pilocarpine to induce status epilepticus. After different survival intervals (1 h, 6 h, 12 h, 24 h, 48 h and 72 h) pups were sacrificed, tissue of different brain regions (hippocampus, cortex frontalis / cortex parietalis and thalamus) was isolated and the expression of MMP-2 and MMP-9 mRNA was analysed by real-time PCR. Additionally, brains were fixed and processed for TUNEL-staining, immunohistochemistry and in situ zymography.

In all brain regions analysed we found an increased number of TUNEL-positive cells 24 h after status epilepticus. This rise in apoptosis was most pronounced in cortical areas and the dentate gyrus, whereas we found only few TUNEL-positive cells in thalamic regions. Furthermore, we detected a marked increase in MMP-9 mRNA expression in hippocampal and cortical tissue. Six hours after status epilepticus mRNA levels showed a significant elevation which lasted up to 24 h (cortex:3,1 ± 0,05; hippocampus 6,1 ± 2,1 compared to sham-treated controls). At 48 h the mRNA expression for MMP-9 had declined to control values. No alterations in the level of MMP-2 mRNA were observed.
Gelatinolytic acitivity, monitored by in situ zymography, was more pronounced in brains of animals after status epilepticus than in sham-treated controls.
Our results may suggest a correlation between the activation of MMP-9 and the increased rate of apoptosis detectable after status epilepticus.

These findings may implicate involvement of MMP-9 in the pathophysiology of status epilepticus in the developing rat brain and suggest that MMP-9 may serve as useful therapeutic target to ameliorate cell loss following status epilepticus in children.

Toll-like receptor 4/MyD88 pathway mediates the microglial proinflammatory response to thrombin-associated plasma-derived protein complexes

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Thrombin, the central blood coagulation factor, exerts a multitude of cellular effects through limited proteolysis of protease-activated receptors (PAR). This mechanism is also considered for the induction of proinflammatory mediators in microglia. Challenging this notion, we identified a minor high molecular weight (HMW) protein fraction as the sole and extremely potent carrier of this activity in various thrombin preparations. The HMW fraction contains mature ¾ yet enzymatically inactive ¾ thrombin presumably within a plasma/coagulation protein complex. We show now that microglial activation by thrombinHMW critically depends on Toll-like receptor 4 (TLR4) and associated MyD88 signaling, with additional receptor/signaling pathways providing contributions. On the other hand, PARs, alternative thrombin receptors, proteolytic thrombin activity and functional domains are either not mandatory or even dispensable. Biochemical analyses, mass spectrometry and functional characterization revealed fibronectin as a microglia-stimulating thrombinHMW constituent with TLR4-agonistic capacity. Importantly, bacterial lipopolysaccharide as a common microbial TLR4 agonist can be ruled out as a confounding contaminant. Our findings identify the essential ligand-receptor mechanism underlying the thrombin/PAR-assigned proinflammatory responses in microglia. They may request a careful revision of other cellular activities previously established for thrombin. Most importantly, they indicate a role for TLR4-centered signaling in the activation of microglia by plasma-derived protein complexes upon vascular impairment. This novel mechanism especially supports the emerging concept of coagulation cascade-driven CNS damage in diseases like multiple sclerosis. Supported by DFG.

Delayed Erythropoietin administration promotes neuronal survival and axonal sprouting with an increase in the motor recovery after mild focal cerebral ischemia in mice

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Apart from its hematopoietic function, erythropoietin (Epo) exerts neuroprotective activity upon ischemia of brain, retina and spinal cord. This study investigated how subacute delivery of Epo, starting at 3 days after stroke onset, and continuing for 30 days (1 UI/day or 10 IU/day; via mini osmotic pump), influences neuronal survival, axonal sprouting and neurological function abnormalities in C57Bl6/j mice submitted to 30-min middle cerebral artery occlusion. Histological studies showed that Epo improved neuronal survival, even when applied 3 days after stroke. Cell survival was associated with a long-lasting improvement of motor and coordination deficits, evaluated by the grip strength and RotaRod tests. By injecting anterograde tract tracers (dextrane amines) in the ipsilateral and contralateral cortex, we show that functional recovery was accompanied by decreased survival of ipsilateral fibers, whereas contralateral projections increased in strength under Epo therapy.
Our data support the concept that brain plasticity goes along with coordinated fibers responses both ipsilateral and contralateral to the stroke.

Y-P30 or how does the maternal immune system participates in building up the embryonic brain?

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Y-P30 is a 30 amino acids (aa) peptide that derives from a 110 aa precursor protein containing the antimicrobial peptide dermcidin, which is expressed in sweat glands. In previous studies we showed that Y-P30 is a survival promoting factor for thalamic neurons and stimulates neurite outgrowth potentially via its binding to the midkine pleiotrophin and syndecans. However, despite that the peptide can be immunohistochemically localized in embryonic brain its transcript is not detected. Instead, Y-P30 is produced by the peripheral blood mononuclear cells (PBMC) of the maternal immune system in rodents, passes the placenta and is taken up by neurons during early development. In humans the peptide is also only found during the 29 first weeks of pregnancy in PBMC of pregnant women but not in non-pregnant women or males. Considering that this cell population mainly includes lymphocytes we therefore determined which cell population expresses the survival promoting peptide. Interestingly, peripheral mature T cells but not B cells showed the expression of Y-P30 transcript. Furthermore, none of the pre differentiated state of T cells, neither in the thymus nor in the bone marrow, were positive for Y-P30 transcript expression. Accordingly, T-cell deficient $Rag^{(-/-)}$ mice exhibited no Y-P30 expression in blood during pregnancy in contrast to wild-type controls. To measure peptide concentrations we established a Y-P30 ELISA protocol. With these tools we are now able to determine the molecular factor(s), which induces Y-P30 expression, the transcytosis of the peptide to the infant's brain as well as consequences of the lack of the peptide in pregnancy on brain development.

Increased Inwardly Rectifying Potassium Conductance and Kir2 Channel Expression in Dentate Gyrus Granule Cells in Temporal Lobe Epilepsy

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Temporal lobe epilepsy (TLE) is associated with Ammon's horn sclerosis (AHS) characterized by hippocampal cell death and dentate gyrus granule (DG) cell dispersion (GCD). DG cells survive AHS and have been proposed to be hyperexcitable in TLE. However, their role in seizure activity and the mechanism for their neuroprotection is unclear. Here we studied the passive membrane properties of DG cells, using the intrahippocampal kainate mouse TLE model with unilateral GCD, brain slice patch-clamp recordings, detailed morphological reconstructions and immunocytochemistry to characterize the intrinsic excitability of DG cells in relation to GCD. Our results show that with increasing GCD, DG cells had decreasing input resistances and membrane time constants. In addition, DG cells displayed a shift from distal to proximal spine density, but overall no membrane surface increase. Dispersed DG cells possessed increased K^+ leak conductances around resting potential with a biophysical and pharmacological profile (e.g. high Ba^{2+} sensitivity) most consistent with "classical" inward rectifier K^+ (Irk, Kir2.x) channels. Protein expression of all Kir2.x channels (Kir2.1, Kir.2.2, Kir2.3, Kir2.4) but not Kir3.2 channels was increased in ipsilateral DG cells. We conclude that GCD is associated with an increased leakiness of DG cells due to the upregulation of Kir2 channels. In summary, these results do not argue for an increased excitability of DG cells in the sclerotic focus but rather indicate increased shunting inhibition via reduced input resistance, a potentially neuroprotective mechanism for DG cells in TLE.

Increased leak conductance in dentate gyrus granule cells of temporal lobe epilepsy patients with Ammon's horn sclerosis

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Temporal lobe epilepsy (TLE) is associated with Ammon's horn sclerosis (AHS) characterized by hippocampal cell death and dentate gyrus granule (DG) cell dispersion (GCD). DG cells survive AHS and have been proposed to be hyperexcitable in TLE. Here we studied whether the passive excitability of DG cells correlates with the severity of AHS. We analyzed the passive membrane properties of identified DG cells using patch-clamp recordings in acute tissue slices obtained from TLE surgery. Independent Wyler grading and GCD measurements were used to assess the severity of AHS. The input resistances and membrane time constants of DG cells were reduced in high-grade versus low-grade AHS samples and negatively correlated with the degree of GCD. DG cells possessed large Ba^{2+}-sensitive K^+ conductances at resting potentials. The increased leak conductance, likely mediated by K^+ channels, does not argue for an increased excitability of DG cells but rather points to a neuroprotective mechanism in the sclerotic focus in TLE.

Sustained oligodendroglial recruitment after repetitive cortical inflammatory demyelination

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Multiple sclerosis (MS) research traditionally focused on white matter pathology, but recent studies revealed important involvement of cerebral cortex in MS patients. The most widely used animal model of MS, experimental autoimmune encephalomyelitis (EAE), shows disseminated lesions in the spinal cord white matter but cerebral cortex is only rarely affected. We therefore developed a focal EAE model where demyelinated lesions are targeted to the cerebral cortex resulting in cortical lesions resembling those observed in MS patients. Our objective in the present study was to determine whether repetitive demyelinating cortical episodes may exhaust the intrinsic cortical remyelinating capacity. For this purpose, we induced repeated lesions (each of which simulates a “demyelinating episode”) at fixed intervals and defined location in the cortex. A total of four sequentially cortical demyelinating episodes were induced with intervals of three weeks in between.

Immunohistochemical analysis revealed extensive remyelination even after repeated demyelinating episodes. Remyelination was accompanied by recovery of oligodendrocyte density in previously demyelinated areas. In the course of myelin restoration, proliferation of new oligodendrocytes and oligodendrocyte precursors was quantified. The number of recruited oligodendroglial progenitors was increased in repetitively demylinated cortex.

In summary, our data provide evidence that cerebral cortex displays a highly intrinsic regenerative capacity even after repeated episodes of demyelination.

Cerebral peroxisome proliferator-activated receptors gamma (PPARγ) and the regulation of interleukin-1ß and interleukin-1 receptor antagonist expression after focal cerebral ischemia in rats

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Inflammatory reactions play a crucial role in the progression of neuronal loss and expansion of brain injury after cerebral ischemia. Post-ischemic inflammation is triggered by activation of proinflammatory genes and over-expression of cytokines. Numerous studies have demonstrated the prominent role of interleukin-1ß (IL-1ß) in the initiation of neurodegenerative reactions leading to the neuronal death after ischemic injury. IL-1ß exerts its actions via binding to a specific plasma membrane IL-1 type-I receptor (IL-1RI). A naturally occurring competitive IL-1 receptor antagonist (IL-1ra) antagonises the effects of IL-1ß by occupying the IL-1RI and confers neuroprotection in ischemic or excitotoxic brain injury. PPARγ is known to acts as a negative regulator of macrophage activation and PPARγ ligands inhibit the production of inflammatory cytokines. A number of studies have demonstrated that treatment with PPARγ ligands counteracts the harmful effects of brain ischemia. To study the neuroprotective mechanisms of PPARγ activation, we investigated the role of brain PPARγ in the regulation of IL-1ß, IL-RI and IL-1ra expression after ischemic injury. The selective PPARγ agonist, pioglitazone, (3 nmol/h) or vehicle (controls) were infused intracerebroventricularly (ICV) via osmotic minipumps in male Wistar rats, over a 5-day period before, and 24 or 48 h after transient middle cerebral artery occlusion (MCAO) for 90 min. The effect of pioglitazone on the induction IL-1ß, IL-1RI and IL-1ra in the frontoparietal cortex ipsilateral to the ischemic injury was studied by immunohistochemistry and immunofluorescence staining at two levels of the brain at 24 and 48 h after MCAO. Western blot analysis was used to ascertain the effect of pioglitazone on IL-1ß, IL-1RI and IL-1ra protein levels in the cortical regions at the border to the ischemic core. Cerebral ischemia augmented the production of IL-1ß and, to a lesser extent, IL-1ra. The majority of microglial cells/macrophages and few neurons showed an intense IL-1ß immunoreactivity. IL-1RI and IL-1ra was localised in both, activated microglia/macrophages and neurons. ICV treatment with pioglitazone considerably reduced the expression and the number of cells positively stained for IL-1ß in the peri-focal cortical areas at both time points after MCAO,the induction of the IL-1RI was not affected. In contrast to IL-1ß, pioglitazone augmented the production of IL-1ra. The increase in IL-1ra expression was more pronounced at 48 h after cerebral ischemia. Our data show that the PPARγ ligand, pioglitazone, reduces the over-expression of IL-1ß, which acts pro-inflammatory and promotes neuronal death, but up-regulates IL-1ra, which counteracts the harmful effects of IL-1ß and acts neuroprotective. Our results demonstrate that PPARγ ligands exert their anti-inflammatory activity not only by inhibiting the production of pro-inflammatory cytokines, but also by enhancing the expression of the anti-inflammatory protein, IL-1ra. Both effects considerably contribute to a better recovery from ischemic stroke.

ROLE OF DIFFERENT CTL-EFFECTOR MOLECULES IN DAMAGING THE NEURO-AXONAL UNIT IN VIVO

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The importance of cytotoxic T cells (CTLs) in virus-induced immunopathology is widely acknowledged and also their role as effectors in autoimmune diseases of the CNS has become increasingly recognized. With regard to neurons as a target for CTLs, evidence was provided for antigen-specific interaction between MHC class I restricted CD8+ T cells and neurons in vitro. However, the case for epitope-specific interaction of CTL with neurons in vivo is less well established and remains controversial. We recently established and characterized a new animal model based on sequential infection of lymphocytic choriomeningitis virus (LCMV) that allows the analysis of molecular requirements of CTL neuroaxonal interaction in vivo (Merkler et al., J. Clin. Invest., 2006). Specifically, our studies focused on the qualitative requirements for peptide/MHC-specific interactions of CTLs with neurons in vivo. For this purpose, we generated several LCMV mutants by a reverse genetic approach (rLCMV) to evaluate the contribution of various viral MHC class I epitopes to recognition by CTLs. First experiments indicated that all generated rLCMV mutants were viable and selectively persisted in neurons. Using congenic markers we were able to track CTLs of distinct specificity in situ and analyzed their contribution in CTL-mediated neuronal damage in the CNS. Our results provide important insights into the molecular mechanisms operating at the immunological synapse between CTLs and neurons.

Ageing effect of Trem2 expression after MCAO in mice

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Background: In healthy organisms the balance between pro- and anti-inflammatory reactions is a prerequisite to ensure an appropriate immune response. Ageing is associated with a progressive appearance of immune dysregulation. The extent of the inflammatory reaction following an insult in the central nervous system influences the spatial and temporal expansion of the infarct as well as the regeneration progress especially in elderly. The triggering receptor expressed on myeloid cells-2 (*Trem2*) is involved in the elimination of apoptotic neurons without local inflammation, to maintain brain homeostasis in healthy organisms. The role of *Trem2* during ageing and under ischemic conditions is still unclear. Using qPCR we studied the gene expression pattern of *Trem2* and *Dap12*, coupled to *Trem2*, as well as mediators of inflammation (*Tnfa*, *Ifng* and *Il10*) in mice brain at different age (2, 9, 15 and 24 month) after an transient middle cerebral artery occlusion (MCAO) as an animal model of human stroke. **Results**: Gene expression level of *Trem2* as well as of *Dap12* is increased moderately during ageing *per se*. Two days after MCAO there is a strong increase in gene expression level of both *Trem2* and *Dap12* (3 fold). This up-regulation is further increased (up to 10 fold) seven days after stroke. However, during ageing the enhanced post ischemic expression rate of *Trem2* and *Dap12* declined. The expression rate of *Tnfa* increased during normal ageing as well as following MCAO. The up-regulation of *Tnfa* is attenuated in old mice following MCAO, whereas the overall transcript level is similar to young mice, due to an elevated expression of *Tnfa* during ageing. *Il10* is very low expressed in healthy brains and ageing *per se* shows no considerable effect to *Il10* gene expression. Though, after MCAO *Il10* was up-regulated in young animals but not influenced in elderly. *Ifng*, expressed at a very low level, remain constant during ageing *per se* and seems to be up-regulated at 7d of reperfusion in old mice. **Conclusions:** The increased gene expression of *Trem2* as well as *Dap12* indicates an increase of phagocytic activity of microglia in mice brain during ageing. Thus, we suggest that an attenuated up regulation of *Trem2* after MCAO in elderly is associated with a reduced phagocytic activity and an enhanced inflammatory reaction. In line with that, we show that *Ifng* is up-regualted in the elderly and *Il10* as one of the prototypic anti-inflammatory mediator is up-regulated post ischemia exclusively in young animals. Interestingly in older mice we found an attenuated *Tnfa* response to MCAO. For the first time this study implies a role of *Trem2* after MCAO though further investigations are need to address the mediators of phagocytic activity.

Hemispheric differences, diurnal and stress-induced changes in the morphology of pyramidal neurons in the rat prelimbic cortex

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Pyramidal neurons of the rat medial prefrontal cortex have been shown to react to chronic stress by retracting their apical dendrites and by spine loss. Since the majority of synapses on neocortical neurons are on basilar dendrites we investigated the basal dendritic tree of layer III pyramidal neurons in both hemispheres of the rat prelimbic cortex. Animals were subjected to daily restraint stress for one week (6 hours/day), during either the resting or the activity period. Basilar dendrites and spines of Golgi–Cox-stained neurons in the left and right hemispheres were digitally reconstructed and analyzed separately. We observed the following:

(1) There is an inherent hemispheric asymmetry in control animals during the resting period: The number of spines on proximal basal dendrites is higher in the left than in the right hemisphere.
(2) Basal dendrites in control animals display a diurnal variation: There is more dendritic material during the resting period compared with the activity period.
(3) Chronic stress reduces the length of basal dendrites only in the right prelimbic cortex.
(4) Chronic stress reduces spine density on proximal basal dendrites.
(5) Restraint stress during the activity period has more pronounced effects on stress symptoms such as reduced body weight and increased weight of adrenal glands than restraint stress during the resting period.
In conclusion, our data show time-of-day-dependent variations in basal dendrites of layer III pyramidal neurons in the prelimbic cortex, a region that regulates emotions such as fear. The present results provide supporting evidence for morphological lateralization of prelimbic neurons, as well as for a pronounced reduction in the length of basal dendrites selectively in the right prelimbic cortex in response to stress.

Chronic Restraint Stress Impairs Endocannabinoid Mediated Suppression of GABA Release in the Hippocampus of Rat

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The endocannabinoid system is a widely distributed neuromodulatory system. The presence of the endocannabinoid system in various stress-responsive neural circuits suggests that it may play a role in the regulation of neuroendocrine and behavioral responses to stress. Evidence has been shown that repeated exposure to stressful situations may result in alterations of the endocannabinoid system in limbic structures. However, the underlying cellular mechanisms as well as the functional consequences of these alterations are still to be elucidated. In the present study, we investigated effects of chronic stress exposure on the endocannabinoid mediated activity dependent modulation of GABA release in the hippocampal CA1 region.

Adult male rats were subjected to three weeks of restraint stress after which whole cell patch clamp recordings were performedon the CA1 pyramidal neurons. Since it is known, that specifically the cholecystokinin (CCK)-positive interneurons respond to endocannabinoid signaling we focused endocannabinoid mediated depolarization induced suppression of GABAergic inhibition (DSI) from CCK-positive interneurons. Our results show that chronic stress did not significantly alter the carbachol-evoked GABAergic IPSCs. On the other hand, three weeks of restraint stress resulted in a large reduction in the DSI as compared to control rat, indicating disturbed endocannabinoid signaling. Since DSI is a phenomenon involved in information encoding in the hippocampus, altered endocannabinoid signaling together with altered DSI may contribute to the cognitive changes commonly observed after chronic stress.

Activities of the intracellular signaling protein Ras in differentiated neurons correlate with antidepressant-like behavior in mice

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Brain-derived neurotrophic factor (BDNF) is implicated in clinical depression. Recently, repeated administrations of antidepressants have been shown to enhance the expression of the neurotrophic factor BDNF leading to increased tyrosine phosphorylation of its cognate TrkB receptor. In contrast, stress exposure and depression is associated with down-regulation of BDNF. The Ras-mediated extracellular signal-regulated cascade (ERK) pathway is considered as a major BDNF/TrkB intracellular signalling pathway. To investigate the possible autonomous contribution of the Ras/ERK-pathway on antidepressant activity we utilized a synRas transgenic mouse model expressing constitutively activated human Ha-Ras in differentiated neurons [Heumann et al 2000 J Cell Biol 151: 1537]. The synRas mice show an elevated level of activated Ras and activating phosphorylation levels of $ERK_{1/2}$ in the cortex and hippocampus. This is associated with an increased density of synapses and enhancement of specific types of synaptic long-term potentiation [Arendt et al, 2004 Eur J Neurosci. 19: 2953]. Immunoblotting analysis revealed that chronic fluoxetine administration to wild type mice led to an increased Ras activation followed with subsequent elevation of ERK1/2 phosphorylation thus mimicking the synRas phenotype. Consistently, our results obtained in animal models of depression show an antidepressant-like behavior of the synRas transgenic mice compared to their wild type littermates. Recently, an upregulation of adult neurogenesis in the hippocampus has been proposed by others to be correlated with drugs effective in the treatment of depressions. Interestingly, in the synRas mice neuronal progenitors were down-regulated in spite of persistently high Ras activities in hippocampal neurons (Manns and Heumann 2002 Soc Neurosci abstr No 618.3). Taken together our data suggests, that neuronal Ras activation results in an antidepressant-like behaviour in mice even in the absence of increased hippocampal neurogenesis.

Differential effects of lesions of the anterior cingulate cortex or lesions of the orbitofrontal cortex on extinction, spontaneous recovery and reinstatement of an avoidance response

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Previous studies have shown that inactivation of the anterior cingulate cortex (ACC; Ng et al. 2007) or the orbitofrontal cortex (OFC; Ghods-Sharifi et al., 2008) of rats produced performance deficits in tasks requiring animals to modify their responses according to changes in cue conditions. The present study investigated the role of the ACC and OFC in extinction where a significant cue subsequently attained a meaning opposite to what it initially indicated. Gerbils were trained to jump over a hurdle in a shuttlebox as an avoidance response (the conditioned response; CR) to an auditory stimulus (the conditioned stimulus; CS) that predicted the occurrence of a forthcoming shock (the unconditioned stimulus; US). Eight sessions of 60 training trials each were given before lesions in either the ACC or OFC were done. After a recovery period of one week, another conditioning session was given before the animals were given extinction sessions of 60 trials a day for eight days. Animals were tested for spontaneous recovery (SR) a week after the last extinction session. To test for reinstatement (Re), the gerbils were presented with the US alone the day before the Re test. Results show that: 1) Lesions of the ACC prevented SR and attenuated Re in the lesioned group compared to the sham group. Lack of SR implies that the ACC plays a role in processing temporal context so that the lesion damage disabled the ability of an animal to perceive the week-long gap between the last extinction session and the test for SR. Low rate of CR recovery in the lesioned animals when tested for Re reflects a regulatory role of the ACC in emotional responses. 2) Lesions of the OFC enhanced the recovery of CRs when animals were tested for Re. Enhanced Re in animals with lesions of the OFC suggests an inhibitory influence of an otherwise intact OFC either in the maladaptive expression of a response strategy after US priming; or an inhibitory role in reinstating the internal contextual state incited by the US that when disrupted, results in the enhanced recovery of the CRs. Further, the dissimilar effects of lesions of either the ACC or the OFC seen on the tests for SR and Re reflect differential engagement of these structures between the two phenomena that share the common denominator of being context-dependent.

AMPA receptor subunit 1 (GluR-A) knockout mice model the glutamate hypothesis of depression

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Recent evidence indicates that glutamate homeostasis and neurotransmission are altered in major depressive disorder, but the nature of the disruption and the mechanisms by which it contributes to the syndrome are unclear. Glutamate can act via AMPA, NMDA or metabotropic receptors. Using targeted mutagenesis we demonstrate here that mice with a deletion of the main AMPA receptor subunit GluR-A represent a depression model with good face and construct validity, showing behavioral and neurochemical features of depression also postulated for depressed patients. GluR-A$^{-/-}$ mice display increased learned helplessness, decreased serotonin and norepinephrine levels, and disturbed glutamate homeostasis with increased glutamate levels and increased NMDA receptor expression. These results correspond well with current concepts regarding the role of AMPA and NMDA receptors in depression, postulating that compounds that augment AMPA receptor signaling or that decrease NMDA receptor functions have antidepressant effects. GluR-A$^{-/-}$ mice represent a model to investigate the pathophysiology underlying the depressive phenotype and to identify changes in neural plasticity and resilience evoked by the genetic alterations in glutamatergic function. Furthermore, GluR-A$^{-/-}$ mice may be a valuable tool to study biological mechanisms of AMPA receptor modulators as well as the efficacy of NMDA antagonists in reducing behavioral or biochemical changes that correlate with increased helplessness.

Behavioural and metabolic effects of chronic Cannabidiol and [3-(3-carbamoylphenyl)phenyl] N-cyclohexylcarbamate (URB 597) administration in adult Lister hooded rats (*Rattus norvegicus*)

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Delta-9-tetrahydrocannabinol (Δ^9-THC) and Cannabidiol (CBD) are the most important compounds of *Cannabis sativa*. Δ^9-THC has been identified as the major psychoactive compound and is held responsible for the twofold increase in the relative risk for schizophrenia in adolescent vulnerable cannabis users. In contrast, CBD appears to act non-psychoactively and might be used as a potential antipsychotic substance. In a first clinical trial in schizophrenia we showed that CBD decreases psychotic symptoms and induces fewer side effects compared to the antipsychotic drug amisulpride. By 1H nuclear magnetic resonance spectroscopy we found an elevation of the glucose concentrations in cerebrospinal fluid from acute naive first episode schizophrenic patients that indicates a disturbance of glucose metabolism (Holmes et al., 2006). Otherwise [^{14}C] 2-deoxyglucose imaging refers to an increase of local cerebral glucose use in some brain regions of rats by CBD (Brett et al., 2006).

To investigate more precisely behavioural and metabolic effects of CBD, we conducted an animal study targeting the endocannabinoid system. This system is composed of cannabinoid receptors (CB1, CB2), endocannabinoids (anandamide, 2-arachidonylglycerol), and enzymes that synthesize and degrade (fatty acid amide hydrolase (FAAH), monoacylglycerol lipase) these endogenous ligands.

We compared effects of CBD with URB 597, the selective inhibitor of FAAH. Adult Lister hooded rats were chronically treated with either CBD (n=5), URB 597 (n=5), CBD+URB 597 (n=5) or vehicle (n=4) and underwent behavioural tests and 2-[^{18}F] fluoro-2-deoxyglucose (FDG) µPET analysis. The behavioural tests were analogous to those used to estimate negative symptoms, cognitive dysfunctions and anxiety of schizophrenic patients and measure general activity (open field), object-related working memory (object recognition task), social behaviour (social interaction task), anxiety (elevated plus maze) and sensori-motor gating mechanisms (prepulse inhibition).

In general, chronic administration of the mentioned substances had no behavioural effect on adult Lister hooded rats. None of the substances had a sedative or stimulating effect. Object recognition and the entire social interaction behaviour were not influenced. Just the time spent on non-anogenital investigation of foreign rats was longer after treatment with CBD compared to URB 597 and CBD+URB 597. Furthermore, no alteration in anxiety behaviour was observed and the substances did not influence prepulse inhibition.

The radioactive tracer (FDG) measures relativ metabolic activity in awake, behaving rats. The analysis of the data is still in progress.

This study provides a basis for the upcoming study in which we will analyse possible antipsychotic effects of CBD and URB 597 in an animal model for schizophrenia. In this model schizophrenia-like symptoms are induced by chronically pubertal Δ9-THC treatment.

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Extracellular cortical serotonin and depression-related behaviour in the forced swim test are influenced by interleukin-2

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It is often assumed that cytokines (like interleukins) can influence depression and anxiety. For example, interleukin-2 (IL-2) is suggested to be one factor, which may mediate the behavioural and neurochemical (e.g., serotonergic) features of depression in the brain. In previous studies we have shown in rats that IL-2 mRNA in the striatum and prefrontal cortex are correlated with anxiety-like avoidance behaviour in an elevated plus-maze. Additionally, striatal IL-2 miroinjections affected anxiety-like behaviour in a biphasic way, with anxiogenic-like effects in low and anxiolytic-like effects in high doses.
In a subsequent study presented here, we investigated the impact of systemically (i.p.) injected IL-2 (2.5 µg/kg) on serotonergic (5-HT) and dopaminergic neurotransmission in various cortical areas by in-vivo microdialysis in anaesthetised rats (Exp. 1). Furthermore, we conducted two experiments (Exps. 2,3) to test for delayed and acute behavioural effects of systemic IL-2 (1-5 µg/kg) on depression-related behaviour in a forced swim test (FST). In experiment 2, animals were tested 2 hours after injection, based on the serotonergic time profile obtained in Exp. 1. In experiment 3, rats were tested twice; acutely and 4 hours after injection. The second test was conducted without further treatment to test for possible lasting consequences of the previous IL-2 impact.
The neurochemical results (Exp. 1) revealed that systemic IL-2 continuously reduced extracellular 5-HT levels in the medial prefrontal (-75%), occipital (-70%), and temporal cortices (-45%) in anaesthetised rats. These effects remained stable for at least 3 hours after IL-2 treatment. In contrast, dopamine was moderately reduced only in the medial prefrontal cortex. The functional relevance of these specific neurochemical changes was supported by the subsequent behavioural evaluation (Exp. 2), since IL-2 induces dose-dependent effects on depression-related behaviour in the FST after delayed testing, with a significant increase in immobility with the lower dose (1 µg/kg). The analysis of Exp. 3 will provide further insight into the acute effects of IL-2 on depression-like behaviour and the possibility of IL-2 to potentiate the reaction towards a stressor (FST).
These data further support the hypothesis that IL-2 can affect emotional and motivational behaviour, namely anxiety-related (previous studies) and depression-related behaviour (present data). IL-2 has impressive inhibitory effects on 5-HT neurotransmission and the most pronounced decreases of 5-HT occurred in brain areas, which have previously been shown to be correlated with depressive symptoms.
In conclusion, we suggest that these neurochemical effects could account for the behavioural outcome in the FST (Exp.2), especially since serotonin seems to play a critical role in mood disorders and their respective animal models. Analysis of experiment 3 will provide further insight into the time window, as well as general effects of IL-2 on depressive-like behaviour.

Initial Sensitivity to Cocaine's Stimulant Effects Predicts Distinct Peptide Changes in the Medial Prefrontal Cortex

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Individual differences in sensitivity to the psychomotor stimulant effects of cocaine have been suggested to be predictive of subsequent abuse and addiction in rodent models. We compared outbred, adult male Sprague-Dawley rats of two behavioral phenotypes, Low Cocaine Responders and High Cocaine Responders, which differ in their ability to develop locomotor sensitization and conditioned place preferencefollowing repeated cocaine exposure. While most studies have focused on the role of dopamine or other classical neurotransmitters, we evaluated the role of peptides and small proteins on the acute effects of cocaine in the dorsal striatum (dSTR), nucleus accumbens (NAc), and medial prefrontal cortex (mPFC). We employed mass spectrometric screening for fast and sensitive surveys of peptides/proteins present at detectable levels in the brain regions of interest. Measurements were made at normal physiological conditions and following treatment with an acute cocaine dose of 10 mg/kg that facilitated robust behavioral responses manifested by locomotor hyperactivity. Comparative mass spectrometric profiling assisted by independent statistical analysis revealed meaningful variations among peptides under the influence of acute cocaine without requiring *a priori* knowledge of the identity of relevant peptides. Our data indicate that rats of the Low Cocaine Responders phenotype, which are more prone to cocaine-induced behavioral sensitization, exhibit significant peptide changes in mPFC after acute cocaine treatment. The mPFC is thought to contribute significantly to the cognitive control of goal-directed behaviors, including drug seeking behavior. Our results demonstrate unique distributions of peptides in regions of the brain's reward circuitry, and illustrate the effects of acute cocaine exposure on the detection of a large number of peptides that are potentially modulated by psychostimulants. The support of P30DA018310 through the National Institute on Drug Abuse and support of R03DA019876 are gratefully acknowledged.

Measuring basal and complex behaviors of rats in automated social home cage systems using IntelliCage for rat technology

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Classical phenotyping of rodent models is traditionally assessed with a number of time-consuming test batteries, in which animals are tested individually and experimental procedures are often difficult to standardize. For behavioral testing of mice, the IntelliCage for mice has been successfully applied for mouse phenotyping with fast and efficient test procedures for evaluation of exploratory behavior, diurnal patterns and learning. In order to validate a newly developed IntelliCage for rats (http://www.newbehavior.com; www.ratstream.eu), transgenic huntington rats (tgHD rats) were introduced into the system. This animal model has been classically phenotyped with behavioral abnormalities including motor dysfunction, anxiety and depressive-like behavior and impaired learning abilities. In the IntelliCage for rats, the tgHD rats were tested for general behavior (circadian pattern), drinking behavior, operant and spatial learning.
Two groups of 2-months-old rats, each composed of five tgHD rats and their wildtype (WT) littermates were subjected to the IntelliCage. The IntelliCage is composed of four conventional Typ 4 cages interconnected with each other and each cage is connected to one operant corner. IntelliCage experiments included experimental modules of cage habituation, nosepoke adaptation, operant learning (side discrimination) and place learning. Data were achieved by the IntelliCage software (New Behavior AG). In parallel, all animals underwent classical behavioral testings based on video-tracking including open-field test, novel object recognition and Y-maze (Biobserve AG, Germany).
The first 90 minutes of cage exposure revealed no differences between tgHD and WT rats in exploratory behavior, indicated as latency to visit the first corner and cumulative number of corner entries. During habituation, the IntelliCage reliably monitors number of corner entries, nosepokes and licks, reflecting a circadian pattern but without group differences. All animals showed a circadian pattern with increased visits, nosepokes and licks in the dark phase. Drinking behavior which was assessed by nosepoke duration correlated with licks but without significant effect of the genotype. Examination of the operant task where animals learned to nosepoke at the correct side showed no difference in the performance between tgHD rats and WT, but in the place learning paradigm, WT rats significant learned faster to visit and nosepoke at their correct corner.
Discussion: To conclude, rat behavior could be measured reliably in the novel IntelliCage set-up for rats, which indicates that the system is a suitable tool to measure rat ethological and triggered behaviors. Here evaluated behavioral modules are currently investigated in a longitudinal study for age-related deficits in learning and memory described in tgHD rats previously. Results are correlated with classical testings in the open-field, novel object recognition and Y-maze test.

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Response-contingent changes in dopamine D_1 receptors in the rat prefrontal cortex during cocaine self-administration and its withdrawal

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We examined neuroadaptive changes in dopamine D_1 receptor binding following cocaine self-administration and its withdrawal in the rat brain using a "yoked" procedure and binding (saturation) analyses. Using [^{3}H]SCH 23390, a dopamine D_1 receptor antagonist, we observed a 28% increase in the density (B_{max}) of dopamine D_1 receptors in the prefrontal cortex in rats that actively self-administered cocaine (0.5 mg/kg/infusion during 2-h sessions daily per 12 days), while their affinity (K_d) was not changed. At the same time the dopamine D_1 receptor binding in animals passively receiving cocaine reached the level seen in the "yoked "saline control group. After 10-day withdrawal from self-administered cocaine, the density of dopamine D_1 receptor [^{3}H]SCH 23390 binding was still increased (ca. 20% in comparison to the "yoked" saline control group). In summary, the increases in the dopamine D_1 receptor binding seem to reflect the effects of chronic administration of cocaine *per se* and not the motivated process of reinforced responding. Furthermore, withdrawal from cocaine self-administration evoked changes in the dopamine D_1 receptor binding in several rat brain areas that may indicate neurobiological adaptations.

Altered affective behavior in a model of multiple sclerosis: impact of neurotrophic factors

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Multiple Sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) characterized by inflammation, but also degenerative changes. Besides neurological deficits, many patients also suffer from psychiatric symptoms. In particular, affective disorders such as depression have a high prevalence in MS. Many aspects of MS can be mimicked in the animal model experimental autoimmune encephalomyelitis (EAE) where the importance of CNS derived factors for the disease course was highlighted. Among others, these factors include the neurotrophic cytokine ciliary neurotrophic factor (CNTF) which was identified as a survival factor for neurons and oligodendrocytes. CNTF exerts protective effects in myelin oligodendrocyte glycoprotein (MOG) induced EAE and possibly also influences affectivity and cognition.

Here we investigate behavioral changes in MOG-EAE and in mice with a deficiency for ciliary neurotrophic factor (*CNTF* $^{-/-}$ mice). In the chronic phase of MOG-EAE, mice were subjected to behavioral tests including the light-dark-box, the acoustic startle response (SR) with a pre-pulse inhibition protocol as well as the learned helplessness (LH) paradigm. Similar analyses were performed in young *CNTF* $^{-/-}$ mice at the age of 8-12 weeks. Behavioral data were correlated with the motor performance in a rotarod test and histopathological changes in the entorhinal cortex.

In severe MOG-EAE with paraplegia, the behavioural changes were mainly governed by the motor impairment. Yet in mild to moderate MOG-EAE with gait ataxia only, but significant inflammatory changes in the perihippocampal region, an attenuated anxiety behaviour with a longer time in the bright compartment of the light-dark-box (225 ± 18 s for the MOG-EAE group vs. 128 ± 49 s in the control group, $p = 0.05$) as well as a decreased SR at 120 dB (0.8 ± 0.22 g/body weight in the MOG-EAE group vs. 1.43 ± 0.16. g/body weight in the control group, $p = 0.05$) were observed. In contrast, there was no significant difference in the rotarod performance as compared to ovalbumin immunized controls thereby excluding the motor impairment as explanation for the observed differences. Finally, *CNTF* $^{-/-}$ mice displayed an increased anxiety with a shorter time and fewer visits to the bright compartment of the light-dark-box.

In summary, these data imply that both inflammation in the CNS as well as neurotrophic cytokines like CNTF may impact on the affective behaviour in rodent models of anxiety and depression. These results will be further corroborated in the LH paradigm.

Adult female Wistar rats derived from three different breeders vary in behavior and epileptogenesis in the kindling model of temporal lobe epilepsy

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Purpose: Outbred rats are widely used in biomedical research studies. The genotypic deviation of outbred strains such as Wistar rats might cause a shift in behavior and seizure susceptibility over several generations and between Wistar rats from different breeders. We tested the hypothesis that adult female Wistar rats from three different European breeders vary in their behavior, seizure susceptibility, and epileptogenesis.

Method: Adult female Wistar rats of the breeders Harlan-Winkelmann (Borchen) [HsdCpb:WU], Charles River (Sulzfeld) [Crl:WI(Han)], and Janvier (Le Genest Saint Isle) [RjHan:WI] were kindled by daily electrical stimulation of the right amygdala at 20% above their individual initial after-discharge threshold (ADT). Seizure severity, seizure duration (SD) and after-discharge duration (ADD) were recorded. Before implantation of the kindling electrode, afterwards, and after the kindling process the behavior of the rats was monitored in the open field (OF) and the elevated plus-maze. Additional tests for hyperexcitability have been carried out before and after the kindling process. Naïve rats served as controls.

Results: Preliminary data revealed significant differences in kindling rates between rats of the three breeders. The rats from Charles River had a significantly longer cumulative SD and cumulative ADD until seizure generalization than the Harlan-Winkelmann rats. In other words, they spent more time in focal seizures until generalization occurred. Seizure susceptibility was highest in Janvier rats as observed by their significantly lower initial ADT compared to Harlan-Winkelmann rats. In the behavioral tests the Charles River rats often differed from the rats of the other two breeders. They showed a comparably low locomotor activity and a high anxiety-like behavior. In contrast, the rats of Janvier showed a high locomotor activity and a low anxiety-like behavior. Electrode implantation resulted in an increase of anxiety-like behavior of the Harlan-Winkelmann rats in the OF. The hyperexcitability tests revealed an aversion to lifting of the Charles River rats.

Conclusion: The data support our hypothesis that rats from different breeders show differences in the kindling process and behavior. The Wistar rats from Charles River might have advantages in kindling studies in which delayed seizure generalization is desired and might be interesting for studies investigating anxiolytics. The Wistar rats from Janvier might be interesting for detection of anxiogenic drug effects.

Lithium modifies the architecture of the dentate gyrus by affecting Cajal-Retzius cells in hippocampal slice cultures

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Hippocampal slice cultures are an excellent model to gain insight into the mechanisms that govern the development of hippocampal cell and fiber layers (reviewed in Förster*et al.*, 2006). Most hippocampal dentate granule cells are born early postnatally, and thus migrate postnatally to their final positions. This migration process is controlled by Reelin, a protein which is secreted by Cajal-Retzius cells in the marginal zone of the dentate gyrus. An identified component of the Reelin signalling cascade is the enzyme glycogen synthase kinase (GSK)3 beta which has been shown to act on the cytoskeleton by modulating tau phosphorylation. Activity of GSK3beta is downregulated by Reelin or by application of pharmacological inhibitors, such as Lithium Chloride (LiCl). Lithium is also known as a drug for the treatment of schizophrenia, a neurological disorder that is thought to be causally related to developmental neuronal migration defects. Here, we were interested in the question whether LiCl may interfere with developmental processes in wildtype hippocampal slice cultures, known to be regulated by Reelin signalling. Different concentrations of LiCl were added to the incubation medium and the morphology of slice cultures was analyzed after 6 days in vitro by double immunostaining with antibodies against Reelin (Chemicon) and Prox-1 (Swant) that stains the granule cells specifically. After treatment with high concentrations of LiCl, we observed a loss of the proper arrangement of the dentate granule cell layer, suggesting that Lithium interfered with the proper positioning of dentate granule cells, a process that is known to be regulated by Reelin. Next, we studied the effects of Lithium on the morphology of Reelin-secreting Cajal-Retzius cells by immunostaining them with an antibody against Calretinin (Swant). We found that application of LiCl in the range of therapeutic concentrations induced neurite retraction of Cajal-Retzius cells in a dose-dependent manner. We also investigated by Western-Blotting whether LiCl treatment of cultures affected the Reelin content in the incubation medium. We found that the Reelin content decreased with increasing LiCl concentrations. To confirm this observation, we immunolabeled hippocampal slice cultures for Reelin and also stained them with FluoroJade B® (Chemicon) to visualize necrotic and apoptotic cells. Our results did not provide evidence for toxic effects of LiCl. Finally, to compare the specificity of the observed effects, we performed the experiments by replacing LiCl with Sodium Chloride (NaCl) and, in order to see if the observed effects were mediated by effects on GSK3beta, we replaced LiCl by SB415286 (Sigma-Aldrich), an inhibitor of GSK3beta.

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Cognitive function and emotional behaviour in the rat 6-hydroxydopamine Parkinson model

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In Parkinson's disease (PD), the progressive loss of dopamine (DA) neurons in the substantia nigra leads to disturbed motor function, but cognitive impairment and psychiatric disturbances are increasingly recognized as disabling factors in these patients. Rats with 6-hydroxydopamine (6-OHDA) induced lesions of the nigrostriatal DA system show significant motor impairment reminiscent of PD, and recent studies indicate cognitive impairment in this rat model, as well.

We here aimed to characterize rats with bilateral 6-OHDA lesions in different behavioral paradigms for cognitive function, as well as for motivational and emotional behavior. Retrograde degeneration of the rat nigrostriatal DA system was induced by bilateral striatal injection of 6-OHDA (11 µg in 3 µl vehicle), sham-lesioned rats (controls) were injected with vehicle only. Three weeks after injection behavioral testing started. Rats were first tested for anxiety in an elevated Plus-maze. Thereafter, they were tested for learning and memory in the spatial continuous alternation T-maze task. Finally, rats were tested for motivation in the progressive ratio test (breakpoint: 5 min inactivity) in the Skinner box.

Lesioned rats showed no anxiety related behavior in the elevated Plus-maze. During continuous alternation in the T-maze task rats with 6-OHDA lesions made more errors, indicating disturbed learning and memory. Additionally, the breakpoint of rats with 6-OHDA lesions was reduced compared to controls, indicating disturbed motivation.

We conclude that rats with bilateral 6-OHDA lesions may also be used to investigate the biological basis of cognitive and psychiatric disturbances in PD, and to develop and test new therapeutic strategies for these symptoms ranging from pharmacological treatment to neurosurgical intervention.

Involvement of the endocannabinoid system in differences in emotional behavior and reward sensitivity in three different rat strains

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Cannabis preparations like marijuana and hashish are the most widely used illicit drugs worldwide and appear to have addictive potential in a subset of users. The notion that certain individuals may be predisposed to cannabis dependence is supported by data suggesting that genetic factors partly determine whether cannabis is experienced as euphoric or dysphoric in humans. Also reflecting this genetic determination is that major strain differences have been found in the reinforcing effects of cannabinoids in rats but also for cannabinoid induced Fos immunoreactivity.

In the present study we investigated behavioral differences in adult Wistar, Lewis and Fischer rats regarding emotional behavior and reward sensitivity. All animals were tested for anxiety-related behavior in the open-field, the light/dark emergence test (EMT) and the elevated plus-maze (EPM). Reward sensitivity for a natural reward (sweetened condensed milk, SCM) was measured by voluntary restricted intake, conditioned place preference (CPP) and progressive-ratio testing.

Strain differences were detected for both emotional behavior and reward sensitivity, with the Wistar rat strain showing the lowest anxiety level and the highest sensitivity towards the SCM. In order to link the behavioral results to possible underlying genetic differences in the endocannabinoid system, Western Blots for the cannabinoid CB_1 receptor were performed in all three rat strains in addition.

Neurosciences, Ethics, and Society

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Neuroscience is evolving at a rapid pace, fast producing new knowledge about the (dys-) functions of the brain and the relationship between mental phenomena and neural matter. In the last decades, studies have provided radical insights into the neural underpinnings of perception, memory, language, and the role of action in social interaction. Neuroscience has consequently changed the way we understand ourselves and is providing much potential for theory building as well as medical intervention.

I classify the arising issues with respect to the *aims* they pursue:

1) On the expanding field of neuro-enhancement (i.e. interventions not aimed to respond to medical needs in sustaining or restoring good health):

Advances in psychopharmacology and neurotechnology can take as target potentially any brain mechanism. From very basic neurodegenerative disorders, over cognitive deficits, to processes that influence our emotional system – there seems to be no limit to what can be the target of an intervention. Stimulant drug treatments designed to treat pathologies are increasingly used to treat normal energetic childhood behaviour. Antidepressants are used even in moderate cases of low mood. Other drugs are consumed to promote wakefulness and alertness, or to manipulate social interaction capacities. Moreover, techniques like TMS, DBS, and brain machine interfaces cannot only serve medical purposes but in future might be employed for enhancement as well. The availability of these interventions impacts individual and social life and requires an investigation of a plethora of aspects: Besides safety issues, pressing topics are social pressures, equality of opportunity and distributive justice, as well as questions on the concept of normality, naturalness, personal rights, dignity, and privacy issues.

2) On the developments and impact of monitoring the brain:

I discuss the potentials and limits of brain-imaging technologies and the issues of privacy and responsibility involved. Non-invasive brain-imaging tools, coupled with academic and public fascination with themes such as the quest for the 'neural correlates of moral cognition and madness', or 'fMRI based lie detectors' as well as the enthusiasm about brain-images seem to render its limits almost boundless. Already, neuroscience has altered the way we perceive human agency and responsibility. This has become apparent through the entry of 'evidence' from neuroscience in the courtroom. However, conceptual and technical issues make the use of neuroimaging in legal contexts problematic.

As results from neuroscience enter into social domains, such as the law and health service policy, it is important to analyse the social changes brought about by recent neuroscientific advances. Here, I propose a pragmatist approach to the questions arising. Strictly applying universal principles cannot satisfy the complexity and existential relevance of quandaries arising from progress in the neurosciences. Complex cases require an approach that does justice to the particular individuals in their social contexts and allows them to live a flourishing life. That is why the present approach considers intuitions, experiences, and contextual factors in the process of deliberation, while taking time-tested principles as guidelines. Consequently, society needs an interdisciplinary endeavour working on the ethical, social, and legal implications in response to the interaction of scientific, public, and cultural developments.

Individual anxiety-like trait behaviour affects social interaction behaviour in adult rats

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The social interaction test is widely used as an ethological relevant test to gauge unconditioned social behaviour in neuropharmacological research. During the test of two rats an increase in social interaction is interpreted as anxiolytic-like behaviour, whereas a decrease in social interaction may be indicative of anxiogenic-like behaviour. Commonly, only the behaviour of the focus animal is assessed, but the behaviour of the partner is not analysed. However, it is unknown if social interactions in pairs depend on individual traits. The aim was to analyse if pre-existing traits for anxiety-like behaviour may have effects on dyadic social interactions.

We tested an unselected sample of group-housed male adult Wistar rats for individual avoidance levels in an elevated plus-maze, and divided all rats by median split into those with high (HOA) or low open arm (LOA) time. These subgroups had shown systematic differences in other tests of anxiety before. For the subsequent social interaction test, we paired HOA with HOA, HOA with LOA, or LOA with LOA animals. The selected rats for each dyad were chosen so that A) the differences in open arm behaviour were maximised between HOA versus LOA rats, but balanced between HOA/HOA, and LOA/LOA pairs, and B) without differences across groups between LOA rats and HOA animals, respectively. The social interaction test was performed twice (24 h apart) for 10 min each in a familiar open field (30 lx white light). Analyses comprised anogenital and non-anogenital sniffing, following, crawling over, social evade, grooming each other, and self-grooming.

First, we observed positive correlations for open arm time in repeated plus-maze tests, which is indicative of trait behaviour. Second, the differential pairs showed similar social interaction frequencies. However, analyses of the most frequent behaviour, i.e. non-anogenital sniffing, revealed substantially more sniffing for HOA/HOA dyads on day 1. Moreover, self-grooming as a measure of anxiety was higher in LOA/LOA compared to HOA rats of the other two groups on day 2. Neither body weight nor baseline measures of general activity (number of rearings, horizontal activity) accounted for these differences. In conclusion, we suggest that specific behaviours in the social interaction test may depend on anxiety-like traits, and therefore to take individual behaviour into account when testing social interactions.

Previous evidence has shown differential behavioural reactions in HOA and LOA rats before and after psychopharmacological treatment in other tests. We have also shown that systemic and brain injections with interleukin(IL)-2 can affect emotional and motivational behaviours. Currently, in a follow-up study with the same experimental design we are analysing the effects of systemic IL-2 on differential behaviour in our social interaction test paradigm. Preliminary data showed no effects on body temperature compared to vehicle controls and untreated animals which indicate that expected behavioural outcomes may not be affected by sickness behaviour. Social behaviour is currently analysed and will be presented.

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Behavioural and neurobiological changes in reward sensitivity while pubertal development in rats

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Human data indicate puberty as a highly sensitive period for the initiation of drug use and enhanced consumption of psychoactive substances. Interestingly, studies in humans and rodents could find an increase in reward sensitivity for natural rewards during adolescence. The hedonic value of a reward is mediated by an interaction of the endocannabinoid and opioid system. Both neuropeptide systems underlie intense age-dependent variations. Thus the pubertal increase in reward sensitivity could potentially be mediated by neurobiological alterations of the endocannabinoid system. We evaluated the changes of reward sensitivity for sweetened condensed milk (SCM) in male Wistar rats over the whole pubertal period until adulthood (postnatal day, pd 30 – pd 100). Animals were tested during puberty and adulthood for free consumption of SCM and progressive ratio (PR) performance. Our data reveal an elevation of consumatory behaviour and a higher incentive value of SCM in puberty compared to prepuberty and adulthood. Especially at pd 50 a dramatic increase in SCM consumption and reward motivational was observed, indicating a restriction of the critical time window of enhanced reward sensitivity around this age. This age-specfic increase in reward sensitivity was correlated with changes in the endocannabinoid system by Western Blot analysis for the Cb1 receptor.

Methylphenidate treatment and stress differentially modify gene expression of immediate early genes in the DAT knockout mouse, a mouse model for ADHD

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Dopamine transporter knock-out (DAT-KO) mice display a phenotype typical for Attention-Deficit/Hyperactivity Disorder (ADHD) such as hyperactivity, cognitive impairment and paradoxical calming responses to methylphenidate (MPH) treatment, which is known for its DAT and norepinephrine-transporter blocking characteristics. Symptoms improvement found by DAT-blockers and alteration in DAT levels in the striatum of ADHS-subjects point to the important role of the dopaminergic system in this disorder. Therefore, the DAT-KO mouse model is a useful animal model for studying therapy effects for ADHD.

The aims of this study were to explore the effect of MPH treatment on gene expression in this mouse model and whether the co-factor stress provoked by intra-peritoneal drug application influences the results. As it is shown that c-Fos expression is affected by both, MPH treatment and stress, we primarily focused on expression levels of the two immediate early genes (IEGs) c-Fos and Fra-2. These two IEGs are shown to display different basal and stress-induced expression patterns.

In order to investigate the effects of different MPH treatment paradigms we analyzed the expression of c-Fos and Fra-2 in DAT-KO and WT mice after an acute, a chronic, and a combined acute and chronic MPH treatment. Treatments with saline were used as control. Stress effects were revealed by comparing the expression profiles of animals injected with saline (DAT-KO & WT) to naïve ones. We carried out a quantitative real time-PCR study using RNA from four different brain regions.

Our expression study revealed distinct influences of the DAT genotype, different MPH treatment paradigms as well as stress resulting from drug application on the expression levels of investigated IEGs. In general, the co-factor stress induced an increase in IEGs gene expression comparing to naïve animals that possibly caused masking effects to the MPH treatment. Additionally chronic MPH treatment compared to acute MPH treatment attenuates IEGs expression.

Proteomic approach to synapse proteins putatively involved in the synaptic pathology of schizophrenia

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Schizophrenia is a mental disease which affects approximately 0.5% of the population with a lifetime prevalence of 1%. Although the etiology of schizophrenia is not completely understood, several previous studies suggest that it is a synaptic disease and crucially involves also a hypofunction of NMDA receptor-mediated signaling. In particular, the left dorsolateral prefrontal (DPLFC) is strongly implicated in the pathogenesis of schizophrenia. Two-dimensional gel electrophoresis was employed to compare the synaptic proteomes between schizophrenia (n=10) and control (n=15) post-mortem DPLFC human tissue. Within this proteomic study 41 protein spots with altered levels in the schizophrenia cohort have been identified using MALDI-TOF/TOF analysis. These proteins can be functionally classified into the following groups: energy/metabolism, neurotransmission/signaling, biosynthesis, cell stress/survival, membrane traffic and cytoskeleton/structure. In parallel to this study, a synapse proteomic analysis of cortical tissue of rats with ketamine-induced psychosis was performed revealing 12 proteins with altered levels. In particular, prohibitin was found to be upregulated in both approaches. In addition we have shown that overexpression of prohibitin in rat cortical primary neuronal cultures resembles morphological changes of dendritic spines similar as observed in schizophrenia. Therefore, prohibitin might be a new potential marker for the synaptic pathology of schizophrenia.

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Effects of chronic Cannabidiol and [3-(3-carbamoylphenyl)phenyl] N-cyclohexylcarbamate (URB 597) administration in adult Lister hooded rats (*Rattus norvegicus*) on endocannabinoids and related lipids in different brain regions

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In the pathophysiology of psychiatric disorders the endocannabinoid system seems to play an important role besides the known dopaminergic and serotonergic disturbances. We have shown that cerebrospinal fluid (CSF) from schizophrenic patients contains significantly higher levels of the endocannabinoid anandamide than CSF from healthy volunteers. Moreover, CSF anandamide levels correlate inversely with psychotic symptoms, suggesting that anandamide release in the brain may serve as an adaptive mechanism countering neurotransmitter abnormalities in acute psychoses (Giuffrida et al., 2004). Furthermore in a first clinical trial in schizophrenia the non-psychoactive cannabinoid Cannabidiol (CBD) - important ingredient of *Cannabis sativa* - was already shown to offer antipsychotic effects which were comparable to Amisulpride. It is very likely that in its mode of action the endocannabinoid system and other related lipids are involved.

In previous in-vitro assays we found first evidence that CBD inhibits the fatty acid amide hydrolase (FAAH). This enzyme is mainly responsible for the degradation of anandamide and other related fatty acid ethanolamides. In FAAH expressing rat brain membrane preparations CBD exhibits an IC_{50} of 8,7mM for the inhibition of ^{3}H-AEA hydrolysis. These data are consistent with the results of Bisogno et al.(2001), who found a slightly higher IC_{50} of 27,5mM.

To illuminate the possible mechanism of Cannabidiol as an antipsychotic drug we conducted an animal study which included behavioural and metabolic testings. Adult Lister hooded rats were chronically treated with either CBD, URB 597, the selective and potent inhibitor of FAAH, CBD + URB 597 or vehicle.

To determine the effects of these substances on the endocannabinoid system and structural related endogenous lipids, the brains of the tested animals were removed and dissected in different regions: prefrontal cortex, midbrain, hippocampus, striatum and thalamus. After lipid extraction of these sections with methanol-chloroform the organic layer was further purified by solid phase extraction. Subsequent, the analytes anandamide, the further endocannabinoid 2-arachidonoylglycerol and the two structural related derivatives oleoylethanolamide and palmitoylethanolamide were measured by LC-MS/MS. The ratio of the peak areas of analyte/internal standard was used to interpolate the tissue concentration of the lipid of interest. The isotope dilution method has the advantage that possible losses of these low abundancy compounds during the extraction don't affect their quantification. The analysis of these data is still in progress and will give information if CBD acts primarily like URB 597 as FAAH-Inhibitor or has additional effects which lead to the in humans observed antipsychotic effect.

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Do descending neurons of the Locust *Schistocerca gregaria* respond to polarized light?

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For many animals, the sun is an important directional cue for navigation. In addition to the sun, many insects can also use the polarization pattern of the blue sky for spatial orientation. In the blue sky, electric field vectors (*E*-vectors) are arranged along concentric circles around the sun. In locusts, this polarization pattern is perceived by a specialized dorsal rim area of the compound eye. The polarization vision pathway includes dorsal rim areas of the lamina and medulla, areas in the lobula, the anterior optic tubercle, the lateral accessory lobe, and, finally, the central complex. In the columns of the central complex, *E*-vector tuning is arranged in a compass-like fashion (Heinze and Homberg, 2007, Science 315:995).

We have analyzed polarization sensitivity in neurons descending from the locust brain to thoracic ganglia to understand how polarized light information affects flight motor circuits. Intracellular recordings from the neck connectives showed responses of descending neurons to visual stimuli including polarized light. Several types of descending neurons were sensitive to polarized light. Most of these neurons showed excitations and inhibitions at orthogonal *E*-vector orientations, characteristic for polarization-opponent interneurons (POL neurons). The *E*-vector tuning of these neurons was partly dependent on the turning direction of the polarizer. In addition to polarization sensitivity, the neurons showed phasic and/or tonic responses to unpolarized light and sensitivity to movement stimuli. Our data so far indicate that polarized light signals are combined with other visual inputs in descending neurons. The neurons might serve a role in spatial orientation, but additional roles in flight balance and flight steering are likely as well. Current experiments are aimed at tracing the arborizations of descending POL neurons in the brain and thoracic ganglia. Supported by DFG grant HO 950/18-1.

Enhanced sensitivity to stimulus discontinuities by adaptation of a fly visual motion-sensitive neuron

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The detection of unexpected changes in the sensory input is a major function of the nervous system. In the optic flow during self-motion such stimulus discontinuities are highly relevant, because they reflect not only sudden changes of the locomotor pattern but also, for example, the appearance of obstacles in the environment.

The H1-neuron in the lobula plate of flies responds in a direction-selective way to motion in a large part of the visual field. It gradually reduces its activity during adaptation with long-lasting motion of constant velocity. When a continuous baseline motion is interspersed with discontinuities in the velocity, i.e. sudden, brief velocity increments or decrements, prominent response transients of the neuron are elicited. We analyzed whether these response transients are attenuated during motion adaptation in a similar manner as the response to the continuous baseline stimulus. In accordance with a previous study (Maddess & Laughlin, Proc R Soc Lond B 225:251-275, 1985) we found that the response transients elicited by brief increments or decrements in velocity were in contrast to the response to the baseline stimulus enhanced in the course of motion adaptation. We asked whether this effect of adaptation is restricted to a certain range of baseline velocities and whether sensitivity to discontinuities in stimulus parameters other than velocity are also subject to an adaptation-induced sensitivity enhancement. We found that adaptation enhances the sensitivity to velocity discontinuities, regardless of whether these are superimposed on a low or on a high baseline velocity. Brief discontinuities either in motion direction or in the contrast or the wavelength of a moving grating elicited pronounced response transients. Unlike the declining response to continuous motion, these responses were either enhanced with adaptation or stable in their amplitude.

Our results suggest that motion adaptation can help optic flow processing neurons to maintain or even enhance their sensitivity to sudden discontinuities in diverse stimulus parameters, a property that might contribute to efficient novelty detection by neurons.

Testing Image Matching in Honeybees using Computer Simulations of Landmark Manipulation Experiments

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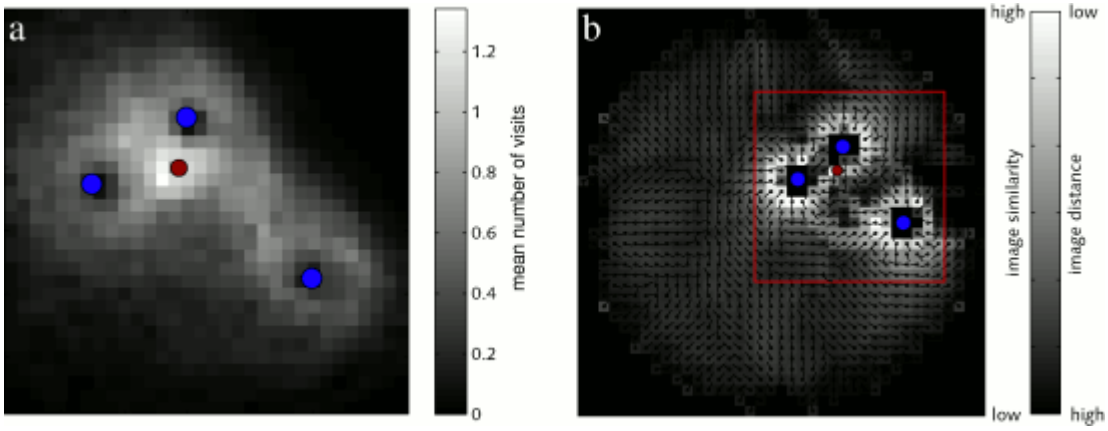
In this study we compare predictions of an image matching hypothesis with flight trajectories of honeybees while returning to a goal position marked by cylindrical landmarks in an indoor flight arena (see poster by Dittmar et al.). Image matching can be seen as a variant of the original 'snapshot model' by Cartwright & Collett (1983). It assumes that the raw or processed image as perceived at the goal location is memorized and, during return, is continually compared to the currently perceived image in order to guide the bee's flight direction.p

Using a computer model of the flight arena we are able to reconstruct the visual input of honeybees during their flights. We compared the spatial distribution of flights with the image similarity between the panoramic image at the feeder and all images in the arena (on a grid with step size of 5 cm), see the figure below for an example. The image matching hypothesis predicts that search density should be correlated with image similarity.

While the search distributions for most landmark manipulation experiments (see poster by Dittmar et al.) are in line with image matching, there are results that are extremely difficult to explain by this hypothesis. For example, when changing the landmark texture from a uniform to the same random texture that was used for the arena walls, thus making it hard to discriminate the landmarks from the background, there was - in contradiction to the corresponding image similarity map - no significant change in the overall flight distribution. Using flow fields computed from rendered image sequences, we show that these experimental findings can be explained by extending the matching scheme to 'matching of flow amplitudes'.



Representation of object motion by tangential cells of blowfly

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Blowflies are able to pass obstacles without collisions. It has been concluded that FD (figure-detection) cells play a role in object detection, since they show a clear preference for objects moving relative to their background [1-3]. A neuronal mechanism proposed to tune one of the FD-cells (FD1) to objects is based on a GABAergic inhibition via the VCH- (ventral centrifugal horizontal) cell [6]. Another set of output cells of the visual system which, in addition, provides input to VCH are the HS- (horizontal system) cells. They have recently been found to provide spatial information during translational motion of the fly between saccadic turns [4-5]. Here we analyse the performance of these major elements of the object detection circuit with semi-natural optic flow which was generated by free-flying flies in a flight arena. We could manipulate the stimuli by inserting objects close to the flight trajectory or by increasing the size of the flight arena. Whereas HS-cells respond strongly to both the motion of background and of nearby objects, the FD1-cell is more sensitive to nearby objects than to the background, in particular, if the background is distant. VCH-cells are more active during background – in contrast to HS-cells especially during saccades - than during object motion. Thus, they reduce the activity of the FD1-cell most during saccades and inhibit it much less when an object is within its receptive field.

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Transformation of receptive field structure and ocular dominance between different stages of the polarization vision pathway in the brain of the locust

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Many animals use the polarization pattern of the blue sky for spatial orientation and navigation. Insects detect the plane of skylight polarization (*E*-vector) with a specialized region of their compound eye, the dorsal rim area. In desert locusts *E*-vector information is transmitted to the optic-lobe neuropils and, via the anterior optic tubercle (AOTu) and lateral accessory lobe, to the central complex (Homberg 2004, Naturwissenschaften 91:199-208). Along the polarization vision pathway, polarization-sensitive (POL) neurons are tuned to particular *E*-vector orientations, which vary widely between individual cells. In addition, neurons show characteristic differences in background activity, relative response amplitude, and bandwidth of *E*-vector tuning.

To gain further insight into the role of the different processing stages in the POL-network, we evaluated the receptive fields and ocular dominance of neurons in the AOTu and central complex through intracellular recordings combined with dye injections. All neurons of the AOTu and one type of tangential input neuron to the lower division of the central body (TL3 neurons) received input from the ipsilateral eye only, whereas all remaining neurons of the central complex had binocular input with identical *E*-vector tuning for ipsilateral, contralateral, and binocular stimulation. Characteristic differences were found in the size and position of receptive fields. Small to medium-size receptive fields of 30-60° diameter were predominant in the AOTu and in input neurons to the central complex, whereas large fields (>90° lateral extension) were typical for the remaining central-complex neurons. In addition, the center of receptive fields changed from eccentric positions in the early processing stages in the AOTu to zenith-centered positions in most central-complex neurons.

The data show that ipsilateral polarization information, transmitted through the AOTu, is integrated in the central complex with information from the contralateral eye. As a consequence, medium-size eccentric receptive fields are transformed to large, zenith-centered receptive fields. The convergence of polarization information from the right and left eye at the input stages of the central complex is a prerequisite to render the neurons of the polarization map in the protocerebral bridge robust against local irregularities of the sky polarization pattern (Heinze and Homberg 2007, Science 315:995-997). Supported by DFG grant HO 950/16-2.

Dendritic integration of local motion signals in motion-sensitive neurons of the fly

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Information about local motion in different parts of the visual field is retinotopically transmitted in the fly visual system and then pooled by a group of large neurons, the lobula plate tangential cells (LPTCs). The local preferred directions of these cells vary across the receptive field in a way characteristic of each LPTC, and many of these cells are thus well suited to distinguish wide-field motion patterns like those generated by self motion of the animal.

Our aim is to examine how incoming signals from local motion-sensitive elements are pooled on the dendritic tree of the cell. Single LPTCs of the blowfly Calliphora were filled with fluorescent dyes to monitor calcium signals in vivo while the animal views motion patterns on an LED array. We made use of conventional wide-field-fluorescence microscopy and of multifocal two-photon laser scanning microscopy (2PLSM), a technique that permits high-resolution imaging while still providing a sufficiently high acquisition rate. By recording the calcium responses of individual areas of the dendrite to different motion directions we are able to resolve putative differences in the direction selectivity of different parts of the cell.

During presentation of drifting gratings in a large part of the visual field typical “calcium response fields” evolve at the dendrites of individual LPTCs (see figure). The spatial patterns of direction selectivity expressed in these “calcium response fields” resemble a dendritic map of local response properties, which have previously been measured by recording axonal voltage responses during presentation of local motion stimuli in different parts of the visual field (Krapp and Hengsternberg 2001). Comparing the “calcium response fields” with these “electrical response fields” provides clues on how the specific response profiles of LPTCs are generated by retinotopic integration of incoming motion signals on the one hand and by non-retinotopic interactions on the other hand. Moreover, potential fine-grained differences between nearby dendritic branches could be indicative of local dendritic sampling of single motion inputs with different preferred directions.

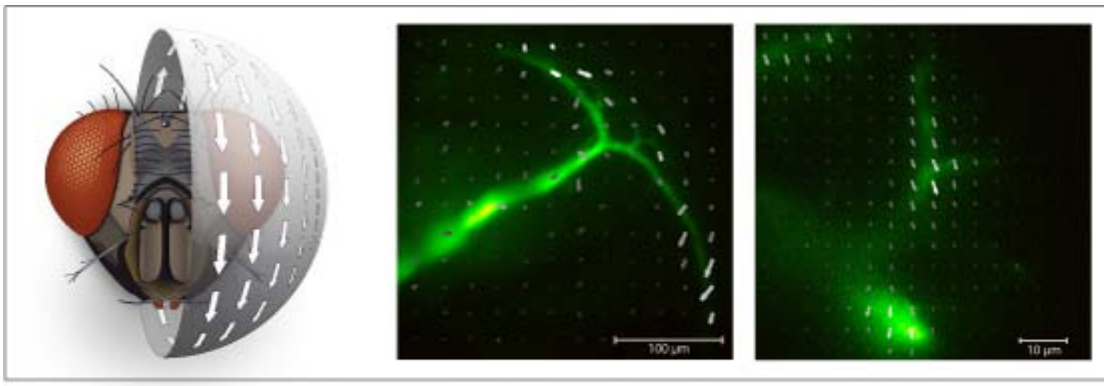

Figure: Schematic representation of a vertical motion pattern on the eye of a fly (left) and arrows representing preferred motion directions of calcium signals measured in different areas of the dendritic tree of a vertical-motion-selective VS-3 cells (middle and right, in different magnifications).

Exploring landmark cues in honeybee navigation

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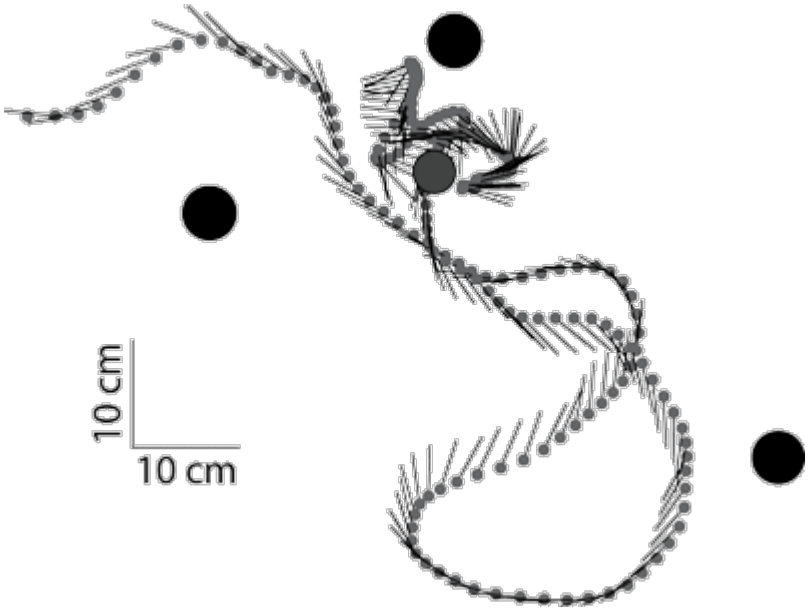
Honeybees learn the spatial location of their food sites and return repeatedly to these places. It is clear that visual landmarks play a prominent role in pinpointing the exact goal location. The final approach seems to be mediated by comparing the current retinal input with a stored retinotopic representation of the landmark constellation around the goal ('snapshot matching'; Cartwright & Collett (1983) J Comp Physiol 151:521-543). Different cues seem to be relevant, such as retinal position, size and colour of the landmarks, but also distance cues. We do not yet know how these cues are extracted from the complex visual input experienced during flight and how these cues are combined in a spatial representation that can be used for effective goal localisation. In this study we explore different landmark cues and their relevance for honeybee navigation.

In an indoor flight-arena we used high-speed cameras to record honeybees approaching a previously learnt food source located between three landmarks placed at different distances from the target. The 3D-flight trajectories and the orientation of the bee's long axis were analysed. An example flight trajectory is shown in the figure below. Grey circles indicate the position and straight lines indicate the orientation of the long axis of the honeybee. By using a virtual 3D-model of the flight arena, we were also able to reconstruct what the bees have seen during their flight.

By introducing unpredictable changes in the visual environment such as the removal of one landmark or changing the texture of all landmarks, we probed the content of the honeybee's spatial memory. These experiments showed that changing the spatial landmark configuration has an effect on the overall flight pattern and on the time required to find the food source whereas we did not find such an effect when we changed the texture of all three landmarks. We then investigated whether honeybees make use of a landmark's texture, if it labels the nearest landmark. Cue-conflicting tests were conducted to examine the role of the spatial configuration and the texture of the landmarks depending on their information content. By means of computer simulations, we investigated whether the snapshot model can predict the actual behaviour of honeybees when introducing such changes (see Poster of Stürzl et al.)



Synchronization of the wing beat cycle of the desert locust *Schistocerca gregaria* by periodic light flashes

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Of those physical or biological systems that are of a periodical nature, many can be synchronized to other periodic systems (Pikovsky et al. 2001, Synchronization – A universal concept in nonlinear sciences, Cambridge University Press). An example is the wing beat cycle of the desert locust *Schistocerca gregaria*. This insect can adopt the frequency of periodic light flashes to its wing beat frequency (Waldron 1968, Z vgl Physiol 57:331-347). Entrainment only occurs if the frequency of the light flashes is close to the normal wing beat frequency of the animal (about 20 Hz). We examined (1) differences in the effect of UV and green light, (2) whether the ocelli or the compound eyes mediate this effect, and (3) the speed and time course of entrainment.

Tethered flying locusts were presented with light flashes in the UV and in the green spectrum. Both stimuli caused synchronization and in both cases the flight cycle had the same phase to the light stimulus. This means that the relationship of a specific wing position during a wing beat and the time of a light flash was always the same. UV light had a stronger effect, and still caused synchronization at 5-80 times lower intensities than green light. To identify the visual system mediating entrainment we tested for synchronization after cutting the optic nerves of the compound eyes, the two lateral ocellar nerves, or the median ocellar nerve. In these experiments only UV light was used. The data show that all of the three ocelli mediate light entrainment of the wing beat. The compound eyes might play an additional minor role. Latencies of the response varied greatly and ranged from the duration of a single wing beat cycle (less than 50 ms) to several seconds. We suggest that this phenomenon is part of the flight stabilization system with respect to the horizon. UV and green light are emitted by the sky and a periodic change in its intensity could be interpreted by the locust as an oscillation around its pitch axis. Supported by AFOSRgrant AOARD 064047 to G. Stange.

How the structure of homing behaviour shapes the responses of motion sensitive neurons in honeybees

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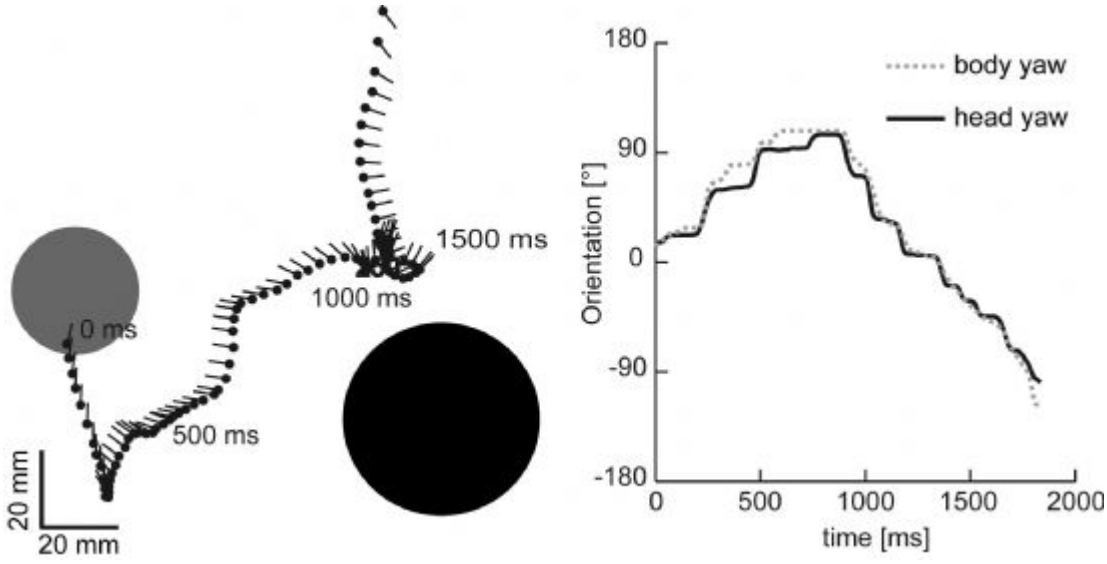
Honeybees and ground-nesting wasps can memorize the spatial location of important places using a visual representation of the goal environment. To understand how they solve this complex task it is necessary to reconstruct the visual input they receive while acquiring a representation of the goal environment.
In this study we aim to understand what aspects of natural optic flow are being sensed by motion sensitive neurons and how optic flow may be used for spatial learning. To identify and critically assess the underlying neural mechanisms we first analyse the bee's behaviour in great temporal and spatial detail. This is important because the insects actively organize the visual information they receive through series of structured movements. On departure from a goal they perform learning flights that generate motion parallax information, which presumably helps them to identify close landmarks.
We used three high-speed cameras to record honeybees approaching and departing from a food source that was located between three landmarks in an indoor flight-arena (see also poster of Dittmar et al.). We measured the 3D-flight trajectory and the bee's body and head orientation off-line. The example trajectory below (left) shows a bee departing from the food source (grey circle). The small black circles indicate the position of the honeybee at 20 ms intervals; straight lines indicate the orientation of the long axis of the bee. The right graph below shows head and body yaw orientation for the same flight plotted over time and reveals a fine structure of the coordinated head and body movements: fast, stereotyped head turns and, thus, gaze changes precede saccadic body turns. Using a computer 3D-model of the flight arena we reconstructed what the bees saw during such flights (see also poster of Stürzl et al.). Image sequences as experienced during learning and return flights were then presented to tethered honeybees while recording the activity of single neurons to analyse under what conditions these neurons reliably detect landmarks. We currently analyse this data to identify visual features that make out a landmark for a honeybee.



Synaptic Plasticity in Visual Pathways in the Brain of the Desert Ant *Cataglyphis fortis*

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The North African Desert, the Sahara, isthe natural habitat of the ant *Cataglyphis fortis.* Well adapted to this harsh terrain, these ants are able to precisely return back to their nest from their daily foraging trips. To accomplish this enormous navigational performance, the ants use a path integration system combining a polarization compass and a proprioceptive odometer in addition to landmark-dependent orientation (Wehner, J Comp Physiol A, 2003) and olfactory cues (Wolf et al. J Comp Physiol A, 2005). Based on an age-dependent polyethism the ants perform these remarkable foraging capabilities for a period of only ~6-7 days after spending the first ~28 days in the dark nest as interior workers. The brief transition to a short, light-exposed life-span presumes that visual pathways express a high degree of plasticity. We explore synaptic plasticity in visual integration centers presumably involved in landmark-processing and neuronal centers along the polarization vision pathway.

We propose that landmark information is projected from the optic lobes to the mushroom bodies (MBs) - sensory integration centers associated with learning and memory. To investigate plastic changes in MB synapses we combined immunolabeling, confocal microscopy and image analyzis tools (Groh et al. PNAS, 2004). The results show that the MB-calyx volume increases during the transition from interior workers to outdoor foragers. This volume increase is correlated with a decrease in the density of synaptic complexes (microglomeruli) in the MB calyx. The results indicate that presynaptic pruning of visual projection neurons and dendritic expansion in MB intrinsic neurons (Kenyon cells) is involved. Current investigations in dark-reared ants are aimed to answer the question to what extent this process is primarily driven by an internal program or triggered by light exposure.

Polarized light is detected via specialized photoreceptors in the dorsal rim area of the compound *eye*that project to special regions in the lamina, medulla and finally to the central brain (Homberg et al. J Comp Physiol A, 2003). Immunolabeling, confocal and electron microscopy as well as ionotophoretic tracer applications were used to analyze neurons and synapses involved in this pathway. As a particular region of interest we focused on potential changes in “giant synapses” within the lateral complex.

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Local and Global Visual Motion Sensitivity in Two Descending Neurons of the Fly

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For a moving animal, optic flow is an important source of information about its ego-motion. In flies, the processing of optic flow is performed in motion sensitive tangential cells in the lobula plate. Among them, cells of the vertical system (VS cells) have receptive fields with similarities to optic flow fields generated during rotations about different body axes [1]. Their output signals are further processed by pre-motor descending neurons feeding into the motor circuit of the fly thoracic ganglion [2, 3, and 4]. How optic flow information from different kind of ego-motion is represented on the level of descending neurons is not yet clear.

Using an LED arena subtending 240 degs of azimuth and 90 degs of elevation, we mapped the receptive fields of DNOVS1 and DNOVS2 by measuring locally the cell's motion sensitivity and preferred direction in different parts of the fly's visual field. Comparing the receptive field of a cell with flow-fields resulting from various kinds of ego-motion one can infer which type of ego-motion the cell should be maximally responsive to. In order to measure this preferred ego-motion independently, we built a virtual cage with regularly tiled walls, ceiling and floor, in which a virtual fly was moved according to the six degrees of freedom as well as rotating it around 36 different axes within the horizontal plane. At every point in time, we projected the environment onto the virtual fly's eye and used the resulting movies subsequently as stimuli displayed to a real fly in the LED arena while recording from descending neurons. The results revealed that DNOVS1 and DNOVS2 have different preferred axes of rotation compatible with their different receptive field structure.

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HS-cells in the visual system of *Drosophila melanogaster* respond selectively to large-field horizontal motion conveyed via the L1 and L2 lamina pathways.

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The combination of physiological recording with genetic manipulation of neuronal function makes *Drosophila* an ideal model organism for studying motion vision. The computations underlying motion vision are well described by the correlation-type motion detector, a model that can account for properties of motion induced behavior as well as for response properties of large field motion sensitive interneurons, the so-called Lobula Plate Tangential Cells (LPTCs). These cells have been extensively studied in large flies like *Calliphora* [1]. However, the neurons presynaptic to them escaped from a rigorous analysis due to their small size. How the motion detector is implemented in the fly optic lobe is a question that can now be tackled in *Drosophila*.

We performed in vivo whole cell recordings [2] from two genetically labeled LPTCs, the HSN and HSE cells, while presenting moving gratings. Both cell-types respond to horizontal motion in a directionally selective way. HSN and HSE are maximally sensitive in the dorsal and equatorial part of the ipsilateral visual field, respectively, corresponding to the position of their dendrites in the lobula plate. In addition, their receptive fields strongly overlap, which can be explained by their overlapping dendritic trees as well as electric coupling between them. Thus, HSE and HSN exhibit similar response properties as their counterparts in *Calliphora*. This characterization of HS-cells will now serve as the basis for studying the circuitry presynaptic to them.

As a first step, we currently try to dissect the functional role of the two lamina monopolar cells L1 and L2 in motion vision. Both cell types are directly postsynaptic to photoreceptors and sense released histamine via histamine-gated chloride channels encoded by the *ort*-gene. In an *ort*-mutant background one can selectively restore either L1 or L2 function by Gal4-driven UAS-*ort* expression [3]. Patch clamp recordings from HS-cells in these flies revealed that motion vision is largely intact and that the two pathways seem to be redundant over a wide range of contrasts.

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Different receptive fields in axon terminals and dendrites underlie robust population coding in blowfly visual interneurons

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In the visual system of the blowfly *Calliphora vicina*, neurons of the vertical system (VS-cells) integrate wide-field motion information from a retinotopic array of local motion detectors. In-vivo calcium imaging reveals two distinct and separate receptive fields in these cells: a dendritic receptive field corresponding to feedforward input from the local motion detectors and an axon-terminal receptive field that additionally incorporates input from neighboring cells mediated by lateral axo-axonal gap junctions. We show that the axon-terminal responses are linear interpolations of the dendritic responses, resulting in a robust population coding of optic flow parameters as predicted by previous modeling studies. Compartmental modeling shows that spatially separating the axonal gap junctions from the conductive load of the dendritic synapses increases the coupling strength of the gap-junctions, making this interpolation possible.

Relating neuronal to behavioural performance: Variability of optomotor responses in the blowfly

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Behavioural responses of an animal to repeated presentation of the same stimulus may vary considerably. Cellular mechanisms underlying sensory transduction as well as signalling in and between nerve cells may cause this variability. Additionally, behavioural responses may depend on the behavioural state of an animal.

Here we analyse the variability of blowfly optomotor head pitch movements. Optomotor responses are thought to counteract an unwanted retinal image slip. The head optomotor responses are monitored by high-speed digital cinematography while the animal is stimulated by a constant velocity visual motion stimulus. Head pitch responses are highly variable. Part of the variability can be attributed to two different states of the animal's optomotor gain. We suggest a central signal to adjust the optomotor gain to the fly's activity state. Gain-modulation does not dramatically affect the signal-to-noise ratio of the optomotor responses.

In addition to the behavioural experiments we examine how much of the variability found at the behavioural level is already present at the output stage of the visual system in the fly's third visual neuropile in so-called tangential cells. A subset of these tangential cells provide the neck motor system with visual motion information for eliciting pitch responses(Milde et al., 1987; Strausfeld et al., 1987)). The neuronal pathway for head pitch is very short. Variability not present at the output level of the visual system can be expected to come into play at the motor neuron level or at the muscles.

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A comparative study of dipteran flight styles and their impact on vision

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Blow- and hoverflies have a distinctly different behaviour even though they belong to the same superfamily Cyclorrapha. We will quantitatively describe the flight strategies of these species on the basis of so-called prototypical movements. This allows us to compare flight strategies and their aftermath on available visual information.

We acquired flight data of the hoverfly *Eristalis tenax* by high-speed cameras (500 frames s^{-1}). Afterwards the three-dimensional trajectories of head and body orientations were reconstructed. From these, the angular and translation velocities were extracted. Based on these data we used clustering algorithms and developed evaluation techniques to extract prototypical movements. We proceeded in the same way with flight data of the blowfly *Calliphora vicina* (data courtesy of Dr. H. van Hateren).

The segmentation of flight behaviour into a sequence of prototypical movements allows us to undertake a Markov analysis of flight behaviour in order to develop a model of the time structure of this behaviour. Such a time structure can be regarded as a probabilistic syntax. On the basis of the model we are able to derive more complex movements, so called superprototypes, as well as similarities and differences in the visual information available during the different flight styles.

Eristalis tenax clearly shows a segregation of rotational and translational head and body movements that is achieved by saccadic turns. The head is stabilized between saccades during flight in much the same way as the blowfly's head (compare e.g. van Hateren and Schilstra, J.Exp.Biol., 1999,202: 1491-1500). The head rotates faster than the body during rotational movements thereby reducing the time of rotational optic flow on the fly's eye. During intersaccadic intervals the head is stabilized against rotational movements of the body, for example body roll during sideward motion.

Despite the before mentioned similarities in both species, their flight style differs with respect to many prototypical movements. Sideward motion plays a much larger role in hoverflies than in blowflies. Hoverflies have the ability to fly stationary or backwards. Blowflies do not exhibit such flight manoeuvres.

Currently it is investigated how the optic flow resulting from the different flight styles in hoverflies and blowflies is reflected in the coding properties of homologous motion sensitive neurons in their visual systems.

Behavioural disambiguation of the responses of motion sensitive neurons

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Flying insects very likely rely on optic flow, the characteristic pattern of retinal image movements which is induced during locomotion, as a source of spatial information about the environment. The neuronal circuitry analysing optic flow is well known in blowflies, and the responses of the output neurons of this pathway (i.e. the lobula plate tangential cells "LPTCs") can be predicted quantitatively by model simulation. However, LPTCs seem to provide visual motion information in a non linear and sketchy fashion. The local motion detectors forming their inputs encode velocity in an ambiguous way and their responses also depend on stimulus components unrelated to velocity such as contrast and spatial statistics of the retinal images.

Here, we systematically compare high-speed video recorded free flight trajectories of blowflies (3D-positions and body orientations) and LPTC responses with respect to their dependence on the spatial statistics of the wall texture in a cylindrical flight arena. We analysed the texture dependence of LPTCs in model simulations and electrophysiological experiments by replaying to the animal what is seen on real and artificial flight trajectories.

On the one hand, flies are able to safely navigate in the differently textured flight setups we tested. The statistical analysis of the flights does not reveal strong texture dependence of most of the analysed flight parameters. Only a weak dependence of the preferred distance to the wall was found. Nevertheless, it is impossible to judge from individual trajectories which texture was present in an experiment.

On the other hand, the responses of LPTCs change qualitatively when tested with stimuli showing identical optic flow fields (equivalent retinal velocities), but different textures. A seemingly small change in the wall texture can invert the differential response situation if LPTCs (HSE, sensitive to horizontal motion) of both brain hemispheres are compared. Model simulations and electrophysiological replay experiments are in good accordance for this result.

Under the assumption that the responses of LPTCs are used to control the animal's orientation behaviour, these findings have interesting consequences for the sensory-motor interface evaluating the LPTC ensemble activity. These circuits must either implement a mechanism that reduces the texture dependence of the LPTC (ensemble) response or extract response components unaffected by the ambiguities inherent to the local motion detectors.

We approach this question by simulation and optimisation of hypothetical sensory-motor interfaces under closed-loop conditions.

Multimodal sensory integration in a fly motoneuron

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In order to control complex flight maneuvers, chasing or landing behavior, flies rely heavily on the computation of optic flow. The processing of optic flow is performed in the third visual neuropil by a set of about 60 lobula plate tangential cells. These motion sensitive tangential cells are well described with respect to their visual response properties [1] and the connectivity amongst them [2]. They have large and complex receptive fields with different preferred directions in different part of their receptive fields matching the optic flow that occurs during various flight maneuvers. However, much less is known about how tangential cells connect to postsynaptic neurons descending to neck muscles or to the motor circuits in the thoracic ganglion. Here we describe the physiology and the connectivity of a neck moto-neuron called VCNM [3]. We find that VCNM is electrically coupled to a subset of HS-cells. In addition, we found that VCNM responds to wind stimuli delivered to the antennae. When we combined wind with motion stimuli, VCNM displayed an interesting supra-linear behavior: whereas wind stimuli and motion stimuli presented alone lead to graded responses in VCNM, the simultaneous stimulation results in the generation of action potentials.

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Light dependent Translocation of the *Drosophila* TRPL Ion Channel to an intracellular Storage Compartment is accomplished by vesicular Transport

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Signaling at the plasma membrane is modulated by up- and down-regulation of signaling proteins. A prominent example for this type of regulation is the*Drosophila* TRPL (TRP-like) ion channel which changes its spatial distribution within the photoreceptor cell depending on the light condition (Bähner *et al,*. 2002 Neuron 34:83-93). In dark-raised flies TRPL is localized in the rhabdomeral photoreceptor membrane and translocates to the cell body upon illumination. Analysis of phototransduction mutants revealed that the internalization of TRPL depends on the activation of the phototransduction cascade and requires Ca^{2+} influx through light-activated TRP channels. However, until now little is known about the transport mechanism underlying TRPL translocation. Here we performed a detailed immunocytochemical study of the localization of TRPL in *Drosophila* photoreceptors after different duration of illumination. Complete internalization of TRPL requires more than six hours of constant illumination, but first changes in the rhabdomeral localization of TRPL can be observed already after five minutes of light exposure. As reported previously (Cronin *et al*.; 2006 J. Cell Sci. 119:2935-2944), the TRPL channel is first transported to the base of the microvilli and to the adjacent stalk membrane. At later time points (2h of illumination) TRPL is detected in vesicles which can best be observed in longitudinal sections. This finding suggests that the transfer of TRPL from the base of the microvilli to the storage compartment is accomplished by vesicular transport. After Six hours of illumination TRPL labeling is observed throughout the cell body except for the nucleus. This clearly confutes the suggestion that the TRPL channel finally ends up in the basolateral membrane of the photoreceptor cell (Cronin *et al*.; 2006 J. Cell Sci. 119:2935-2944). Interestingly, most of the TRPL- containing vesicles are also labeled with antibodies against Rh1 rhodopsin, indicating that TRPL and Rhodopsin are co-transported at least partially by the same vesicular internalization pathway. In conclusion, our data indicate that TRPL is translocated from the base of the microvilli to an intracellular storage compartment by a vesicular transport mechanism that is also used for the internalization of Rhodopsin. It remains to be determined at which stage the transport of Rhodopsin, which is thought to become degraded, and the transport of TRPL to its storage compartment become separated.

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When eyes are *dimmed*: genetic conversion of *Drosophila* photoreceptor synaptic terminals to neuroendocrine terminals

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During development, each neuron in the nervous system must make a decision concerning the phenotype of its neurotransmitter, and how and in which storage vesicle this is packaged prior to release. Some neurons have both classical fast neurotransmitters and a neuromodulator, typically stored in the dense-core vesicles typical of neuropeptide modulators. Many neurons have only a single neurotransmitter and a single class of synaptic vesicle, however. Thus, photoreceptor terminals of the wild-type fly's compound eye contain exclusively many small, clear ~30-nm diameter synaptic vesicles (Meinertzhagen & O'Neil, 1991); these contain histamine (Borycz et al., 2005) which is released by the photoreceptors (Hardie, 1987; Sarthy, 1991). The release occurs at many active zones with presynaptic T-bar ribbons, sites of vesicle exocytosis (Saint Marie & Carlson, 1982). The *Drosophila* Atonal family bHLH gene *dimmed* (*dimm*) normally controls the expression of the secretory peptide phenotype in neuroendocrine cells. When mis-expressed in *Drosophila* photoreceptors, it causes a radical restructuring of the photoreceptor's synaptic phenotype. After *dimm* is mis-expressed exclusively in photoreceptors of the eye by means of the Gal4/UAS system (Brand & Perrimon, 1993), small dark vesicles appear amongst the normal small clear vesicles. These are approximately 35 nm in diameter, only slightly larger than clear vesicles. Their appearance depends on the Gal4 driver and is seen only when *Rh1*-Gal4 is the driver. When the more powerful early driver *GMR*-Gal4 is used, large dense-core vesicles appear, and these have diameters ranging from 40-100 nm (averaging about 60 nm). To examine whether these vesicles might share some features with those in neuroendocrine cells, and to identify their contents, we co-mis-expressed *dimm* with the gene for a neuropeptide. We chose for this purpose the gene *pigment dispersing factor*, *pdf*, which codes for a well characterised neuromodulator of fly circadian clock neurons. When we co-mis-expressed both *dimm* and *pdf* in photoreceptors driven by *GMR*-Gal4, large dense-core vesicles appeared in their terminals, and these were immunoreactive to PDF. T-bar ribbons were moreover lacking. In addition, phenotypically transformed photoreceptors in both *GMR*-Gal4-UAS-*dimm* and *GMR*-Gal4-UAS-*dimm*; UAS-*pdf* flies lacked the light-absorbing rhabdomeres that are typical of photoreceptors. Thus the photoreceptor, still clustered in a normal ommatidium and expressing photoreceptor-specific antigen Chaoptin, as revealed by labelling with mAb 24B10, was otherwise transformed to a neuroendocrine phenotype.

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Plasticity of the intrinsic visuo-motor representation for flight in *Drosophila*

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Experience plays a key role for the acquisition of complex motor skills in running and flight of many vertebrates. The adjustment of eye-hand transfer functions by practice in humans and monkeys is probably one of the most prominent examples of this behaviour. In insects and other invertebrates, by contrast, it has conventionally been assumed that most motor skills are innate fixed-action patterns. Evidence for this view comes from a large body of observations on a wide group of animals including web building by orb spiders, locomotor actions used by walking insects such as stick insects and fruit flies and optomotor responses that stabilize flight heading in tethered and free flight. During the past decades, however, several studies have repeatedly emphasized that these stereotypic behavioural patterns yield some degree of plasticity and are potentially shaped by previous experiences.

To evaluate the significance of previous experience for the efficiency of motor behaviour in an insect, we investigated the flight behaviour of the fruit fly *Drosophila*. We reared flies in chambers in which the animals could freely walk and extend their wings, but could not gain any flight experience. These naïve animals were compared with control flies under both open- and closed-loop tethered flight conditions in a flight simulator as well as in a free-flight arena. We found significant changes in several key parameters for flight in naïve flies such as a reduction in mean aerodynamic force production (-39%) and horizontal speed (-23%) including subtle changes in turning rate in free flight (-48%). Naïve flies produced 27% less yaw torque than the controls in response to a visual stripe rotating in open-loop around the tethered animal in the simulator indicating a flight-dependent adaption of the visuo-motor gain in the control group. Naive animals, moreover, showed a significantly reduced precision of wing motion control that resulted in a reduced ability to maintain straight flight heading. The underlying temporal structure of this learning process and the level at which this plasticity occurs are still unknown. We conclude that previous experience adjusts locomotor fine control and aerial performance in fruit flies allowing the improvement in precision of flight control, although this effect seems to be minor compared to vertebrates such as birds.

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Staring at the Sun - Outdoor Performance of Blowfly Photoreceptors

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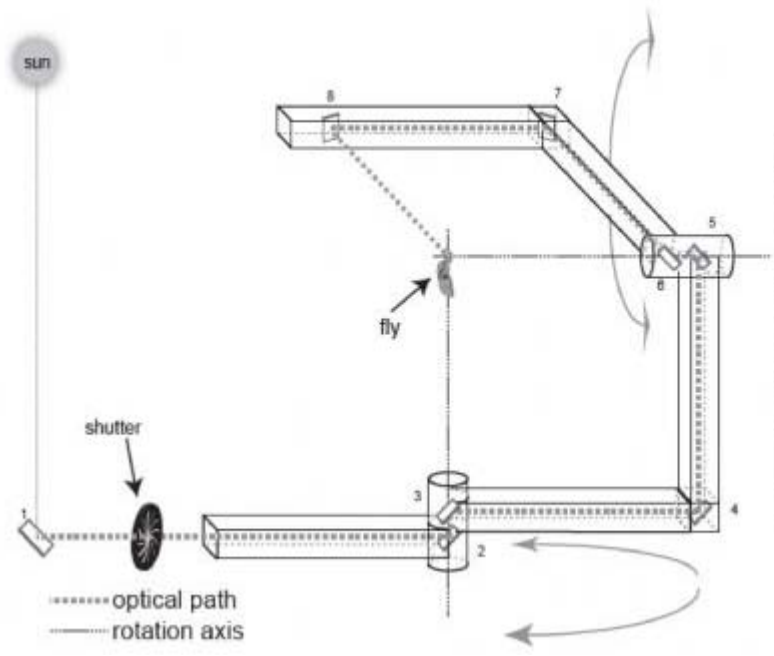
Visual systems face the problem of light intensities varying over a wide range covering several orders of magnitude. Light intensities vary tremendously not only between night and day but also within a sunlit scene. Despite different adaptational mechanisms such as the iris reflex or behavioural mechanisms like squinting the eyes even a short blink into direct sunlight is relatively painful and longer exposure can lead to serious eye damages. Hence, under normal circumstances we guide our gaze not to look at the sun directly. However, in flies cruising around on a sunny day or sitting on a leaf always some ommatidia are directed at the sun due to the fly's panoramic visual field. The question arises how do the fly photoreceptors perform under such high light-intensities.

Here we present intracellular data from blowfly photoreceptors recorded outdoors. The recorded cells were stimulated with sunlight projected into the cell's optical axis by means of a mirror system. We find that fly photoreceptors do neither saturate nor undergo extended photopigment bleaching when looking into sunlight for several seconds. They are thus well able to deal with the extremely high light intensities frequently encountered under natural conditions. We also show that the very bright light leads to a reduction of the cell's input resistance that further speeds up the responses. Hence insects, or at least *Calliphora* photoreceptors, possess powerful adaptation mechanisms that allow them to cope with very high light intensities as occur in the real world.



The mirror system. The mirror system (Spindler and Hoyer mircrobench) resembles a cardan arm construction which projects sunlight via 8 mirrors into the optical axis of the recorded photoreceptor. The light path could be opened using an electronic shutter.

Segmentation of Honeybee Flight Trajectories into Prototypical Movements for Analysing Navigation Behaviour

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Honeybees exhibit extraordinary abilities in navigating within their environment to efficiently relocate the position of previously detected food sources. While performing the task, they rely to a large extent on visual landmarks in the vicinity of the food source. However, it is still not entirely clear, which features of the visual landmarks are exploited, whether active vision strategies are applied to extract the relevant visual information from the environment, and , thereby, how the visual surrounding influences the flight behaviour (see posters of Dittmar et al. and of Stürzl et al.).

For characterising navigation strategies based on flight trajectory data we reduce behavioural complexity by identifying a finite set of basic flight manoeuvres, which we call *prototypical movements.* The assignment of trajectory data to corresponding prototypical movements allows us to segment the flight into meaningful sections. The segmented trajectories constitute the basis for analysing flight behaviour in dependence on the visual context by characterising the temporal sequence as well as the spatially resolved occurrence of individual prototypical movements.

We filmed honeybees using high-speed cameras, while relocating a previously visited food source surrounded by three landmarks. From the images of two calibrated and synchronized cameras the three dimensional position of the bee's body can be reconstructed. The top view, additionally, delivers the orientation of the body long axis, the yaw angle, at any point of time (see Poster of Dittmar et al.). For identifying and distinguishing the prototypical movements based on this four-dimensional trajectory data, we propose the local velocities (yaw rotation as well as forward, sideward and upward translation) at each point of time to be the most relevant characteristic features. A general purpose clustering approach, here the k-means, applied to the amount of occurring velocity data vectors reduces clouds of similar velocity data to a finite set of representatives, the *centroids*. Valid centroids that represent significant structures within the velocity data can be interpreted as prototypical movements.

By applying cluster analysis on behavioural data we are able to objectively and fast determine prototypical movements for varying experimental conditions. Temporal and spatial patterns of the occurrence of prototypical movements allow us to infer how the bee’s navigation strategies depend on the visual context.

Convergence of compound eye and ocellar signals in Lobula Plate Tangential Cells of the blowfly, *Calliphora vicina*

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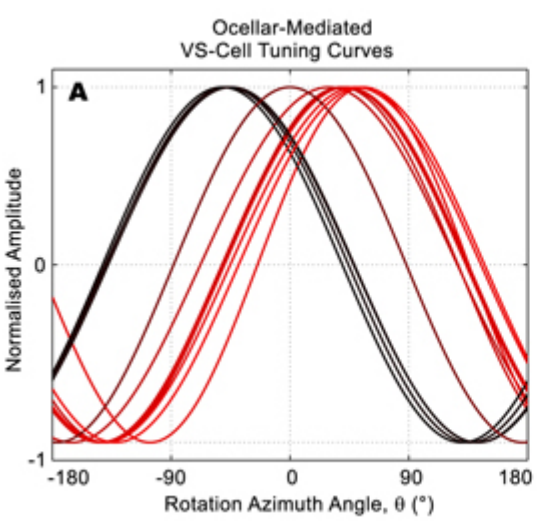
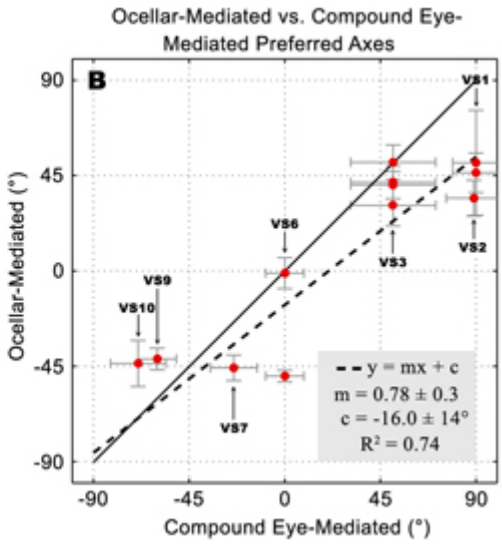
Gaze stabilisation is a fundamental task across phyla that reduces motion blur and maintains a default orientation of retinal images, in order to facilitate visual information processing. Some dipteran flies, such as the blowfly *Calliphora vicina*, have a highly dynamic flight strategy that involves frequent and rapid changes in direction and velocity. Effective gaze stabilisation, therefore, requires fast integration of self-motion information from a variety of visual and mechanosensory mechanisms [Review – Hengstenberg 1991, Rev. Oculomot. Res., Vol. 5].

Two separate visual mechanisms involved rely on the compound eyes and the ocelli, both of which are photoreceptor-based, but differ in their anatomical and functional organization. Consequently, to extract self-motion information along the respective pathways, photoreceptor signals from the compound eyes and the ocelli have to be processed and integrated in different ways. Along the compound eye pathway local directional motion information is computed and selectively integrated by a population of identified visual interneurons, the Lobula Plate Tangential Cells [Reviews - Hausen 1993, Rev. Oculomot. Res., Vol. 5; Taylor & Krapp 2007, Adv. Insect. Physiol., Vol. 2]. A sub-population of LPTCs, the VS cells, is particularly well tuned to encode horizontal rotations, such as pitch and roll. Each VS cell prefers turns of the fly around a specific rotation axis in the horizontal plane [Krapp et al. 1998, J.Neurophysiol., Vol. 79(4)]. Signals of output LPTCs are either directly or indirectly connected to the various motor systems of the fly.

The ocelli are a group of three simple lens eyes, arranged in a triangular formation on the dorsal side of the fly's head. The extended visual fields of the ocelli, in combination with their blurring optics are suited to measure average light intensities over large areas, rather than resolving local image details. Based on the direct comparison of integrated light intensities obtained at separate but overlapping areas in the dorsal visual hemisphere, the ocelli are also suited to signal certain rotations of the fly.

We have previously provided evidence that compound eye and ocellar-mediated self-motion information converges early along the visuo-motor pathway [Parsons et al. 2006, J. Exp. Biol., Vol. 209(22)]. Here we present the results of intracellular recordings from VS cells, using a novel ocellar stimulus that mimics the fly’s rotation around horizontal body axes. Each VS cell shows a preferred rotation axis for ocellar stimulation, as was already shown for compound eye-mediated visual motion stimuli in previous studies (see Figure part A). A comparison of the two sets of preferred rotation axes shows that, rather than being perfectly well matched, the ocellar system indicates horizontal rotations in a much coarser way they the compound eye does. The ocellar-mediated rotation tuning of the VS cells shows a strong bias towards those rotation axes which are intrinsic to the arrangement of the ocelli on the fly’s head (see Figure part B). Our results are discussed in the context of the rotation specificity of neck motor neurons which receive input from the VS cells and control compensatory head movements [Huston and Krapp 2008, PLoS Biol., Vol. 6(7)].


Ocellar-Mediated
VS-Cell Tuning Curves
A
Normalised Amplitude
1
0
-1
-180
-90
0
90
180
Rotation Azimuth Angle, θ (°)


Ocellar-Mediated vs. Compound Eye-
Mediated Preferred Axes
B
VS1
VS6
VS2
VS3
VS9
VS10
VS7
y = mx + c
m = 0.78 ± 0.3
c = -16.0 ± 14°
R² = 0.74
Ocellar-Mediated (°)
90
45
0
-45
-90
-90
-45
0
45
90
Compound Eye-Mediated (°)

Modulation of visual information processing in blowfly lobula plate tangential cells by an octopamine agonist

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Different locomotor states, such as walking or flying, are usually associated with a change in the dynamic range of the sensory inputs. To maintain effective motor control, the operating range of sensory neurons processing information for motor control needs to adapt accordingly. Increasing the operating range of a neuron may be constrained, however, because neural coding is a significant energy cost (Laughlin, 2001). To explore whether sensory neurons adjust their coding of stimuli to different locomotive states, we studied the effects of an octopamine agonist on the response properties to visual motion of two identified direction-selective lobula plate tangential cells in the blowfly.

Octopamine is a neurotransmitter, neuromodulator and neurohormone that induces flight activity in a number of insects, and plays a central role in the initiation and maintenance of flight in flies (Brembs et al., 2007). We used the octopamine agonist chlordimeform (CDM) to induce a "fictive flight" state in fixed flies while recording extracellularly from the spiking V1 and V2 cells, both of which signal specific self-motion components by analysing panoramic visual image shift, optic flow. The receptive fields of the V1 and V2 cells suggests they analyse banked turns and roll rotations respectively. The angular rates of both these rotations are increased in flight compared to walking (Blaj and van Hateren, 2004).

We presented periodic gratings of different orientations to characterise changes in the directional selectivity of the cells. From the responses to multiple trials, we calculated the directional information and response latencies over different time windows. The application of CDM at a concentration of 2.6µM was sufficient to more than double the spontaneous activity of both cell types.

CDM increased the mean depth of the directional tuning curve for both cells, by 43% for the V1 cells and 48% for the V2 cells. The negative signalling range of both cells was significantly increased by the higher spontaneous activities. Compared to the control group, the directional information was 55% and 39% higher in CDM-treated V1 and V2 cells, respectively, when calculated over a time window of 50ms. However, the mean information per spike was reduced, by 11% for the V1 cells and by 21% for the V2 cells over the same time window, because the proportional increases of the spike rates were greater than the increases in the directional information. CDM also reduced the response latency of both cells: by 6% from 23.4ms to 22.0ms for the V1 cells, and by 5% from 21.4ms to 20.5ms for the V2 cells.

These results suggest that octopamine adapts the system to the higher dynamic range of stimuli during flight as opposed to walking, or resting, by increasing the cells' response ranges and by reducingtheir response latencies. In particular, the elevated spontaneous activity of the V1 and V2 cells expands their negative signalling ranges. In this way, the blowfly can save energy in locomotive states with low sensory ranges by reducing neural signalling costs.

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Analysis of the visual motion detection pathway in *Drosophila* with RicinA induced cell ablation.

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The visual system of the fly presents a powerful model system to investigate principles of neural computation. In our studies we focus on a set of large, motion sensitive interneurons in the fly lobula plate, the so-called 'lobula plate tangential cells' (LPTCs) and their role for the optomotor behaviour of the fruit fly Drosophila melanogaster. Previous experiments using genetic, mechanical and laser ablation techniques suggest that these cells indeed provide the input to visually guided course control [1,2,3]. Furthermore, recent electrophysiological studies revealed that LPTC response properties of Drosophila share many characteristics with its optomotor behaviour [4]. To investigate in more detail the functional role of these cells in visual flight control, we use the GAL4/UAS system to genetically ablate LPTCs of the VS and HS type. Our construct (DB331 GAL4; UAS-Δ-stop-Δ-Ricin A, UAS-GFP, hsFLP10) allows us to ablate a variable proportion of these cells by heat-shock triggered expression of Ricin, a powerful protein synthesis blocker leading to cell death. We characterise the behaviour of the heat-shocked flies in both free [5] and tethered flight [6]. Using confocal imaging, we afterwards identify for each individual fly remaining HS and VS cells. Although flies with only a few remaining cells tended not to fly, some flies could nevertheless be tested in free and/or tethered flight. Their behavioural performance is currently being analysed.

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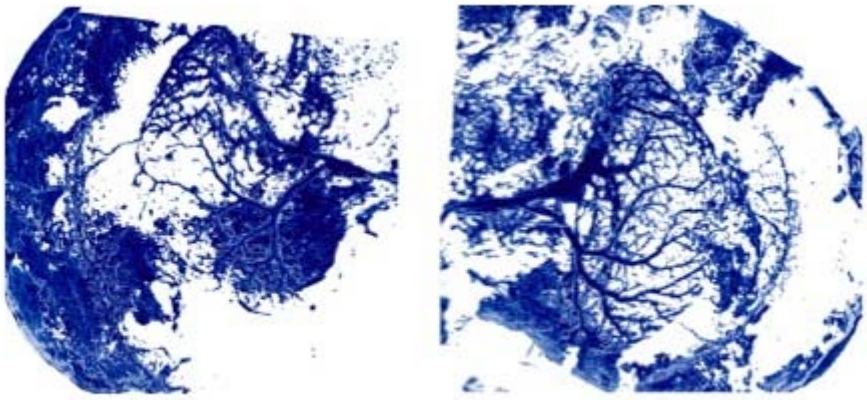

Figure 1: Confocal Imaging Confocal stacks of optical lobes of a heat shocked fly which flied in the wind tunnel. In the left lobe, 2HS cells are still visible, in the right lobe, 2HS and 2 VS cells are visible.

Effects of active head movement on near-range tactile sensing and far-range vision

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Autonomous behaviour such as locomotion in natural terrain requires continuous integration of sensory information from different modalities. In terrestrial locomotion, tactile and visual cues are of particular relevance for the control of heading, speed and foot placement. As yet, the working ranges of tactile and visual sensing differ strongly: Tactile sensing yields near-range cues about local obstacles that may affect the execution of the next step. Vision yields additional far-range cues about distant features that are important for choice of heading, course control and navigation during many steps. An intriguing feature of these two sensory systems is that both of them affect motor behaviour and are affected, themselves, by motor behaviour such as active head movements.

Here we study the effect of active head movements on tactile and visual sampling in walking stick insects (*Carausius morosus*). Unrestrained walking insects were tracked either on a straight walkway (120x4 cm) or in a round arena (r=60 cm) that held three visual landmarks (either black stripes on a white wall, subtending 5° as seen from the centre, or black pillars of 3 cm diameter, placed at 27-51 cm radial distance on the corners of an arbitrary triangle). In the arena, animals were released near the centre and approached one of the three targets. Kinematic analysis was based on tracked retro-reflective markers on various body segments (0.25mm resolution at 50-100 fps). Trial durations were 11-24s on the walkway (n=10; N=5) and 5-30 s in the arena (n=120; N=6). The dependence of choice likelihood for one of three targets on visual eccentricity of targets was tested in 6 animals (n=240; three 2.5° black stripes). Finally, we reconstructed a compound eye of one stick insect from a stack of histological sections. 3D orientation of all 467 ommatidia yielded both the visual field and spatial resolution.

On the walkway, horizontal head movements covered a range of ca. 12° and were strongly coupled to front leg stepping. In the arena, the frequency of rhythmic head movements increased linearly with walking speed, also indicating strong coupling with stepping. The turning points of the head nearly coincided with the end of stance of the ipsilateral front leg. This improved the coupling between antenna and front leg. Head and prothorax movements increased the horizontal working range of the antennae by ca. 30%.

Since insects lack eye movements, only head movements control their gaze. Head movements never stabilized the gaze against thorax rotation. As maximum spatial resolution of the eye is 4° (at 45° azimuth) and declines to >10° toward both ends of the visual field (0-165°), head movements cause an image slip over up to three ommatidia. This may decrease the effect of local visual adaptation. As the eyes are located 3mm distal to the neck, head movements induce lateral translation of the eyes by 0.6mm (equiv. to ca. 20 ommatidial diameters) and associated motion parallax. Prothorax movements further increased these effects. However, neither head nor thorax fixation affected the choice likelihood distribution for alternative targets in the arena, suggesting that head movements do not affect visual scene analysis. Also, active horizontal gaze changes showed no relation to target distance or size, suggesting that the head is not involved in active fixation.

We conclude that active head movements affect tactile near-range sampling more than far-range vision.

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New eyes on visual habituation in locust: an experiment description language for integrative neuroscience

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Neuroscientists have a vast array of instruments at their disposal to examine the function of neural tissue. For example, to investigate visuomotor integration, one can combine *in vivo* intracellular voltage recordings with visual presentations, focal stimulation of nerves and behavioural observation. While this technological diversity holds enormous promise, it introduces its own set of complexities: how does one control these instruments and ensure that they are configured to perform the experiments most likely to shed light on the hypothesis under consideration? We have developed a domain-specific programming language for describing experiments in cellular and sensory neuroscience. This language aims to let scientists with little programming experience focus on creating a specification of what should be done in an experiment and in the subsequent analyses. We can build complex experiments by combining elementary tasks including animations, sounds, extracellular or intracellular recordings and injection of somatic current waveforms. We have written a series of computer programs to execute these experiments on living animals, or on simulated networks of neurons. These programs allow us to directly compare models and experiments by automatically running the same protocol *in vivo* and *in silico*. The results of these trials are stored in a relational database together with the experiment description, information about the animal, and any other available metadata, and are retrieved with a simple custom-built query language.

We have used this new way of conducting experiments to reexamine the response of the descending contralateral movement detector (DCMD) in the locust to looming visual stimuli. The locust is a well-established experimental preparation which permits the study of cross-modal sensorimotor integration. We have measured the kinetics of habituation in the DCMD firing rate response to repeated presentations of similar looming stimuli, and the dependence of this time course on the stimulus amplitude. We have also investigated the influence of auditory stimuli on the visual looming response and the timecourse of its habituation. Finally, we aim to investigate the effect of manipulations of the looming object visual presentation, such as priming portions of the visual field, on the DCMD response. These experiments show how complex multi-modal protocols can be simply defined in an experiment description language and can reliably be carried out repeatedly in a living animal.

A robotic platform to study closed-loop optomotor control in the blowfly

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Flies heavily rely on visual feedback for motor control. They analyze visual wide-field motion - optic flow - to minimize panoramic retinal image shifts when the animal drifts of course due to external forces and to avoid collisions. Optomotor responses to reduce retinal image shifts have previously been shown to be adaptable, with the fly able to establish “novel” sensory-motor configurations: fruit-flies can use either flight motor commands or their front legs differentially to stabilize panoramic pattern motion under closed-loop conditions [4]. In these experiments both motor systems receive visual input from lobula plate tangential cells (LPTCs) which process optic flow parameters related to self-motion (rev.: [1]).

We have developed an experimental closed-loop platform that allows us to study whether the activity of identified LPTCs in the blowfly visual system is sufficient to control optomotor responses and collision avoidance in a mobile robot. As a first step, an immobilized fly is placed in front of two computer monitors such that the monitors are positioned at ±45° azimuth relative to the fly’s longitudinal body axis with each subtending an area of 50° azimuth and 38° elevation. We recorded extracellular spiking activity from the H1 cell which is strongly activated by horizontal back-to-front motion in its extended ipsilateral receptive field. The electrophysiology signal is threshold-detected to obtain unit ms spike pulses which are filtered to compute a smoothed spiking rate estimate and subsequently used to control the robot’s steering. The robot is positioned on a turntable that is surrounded by a vertically oriented grating (contrast – 1, spatial wavelength – 5.5°). Two high-speed video cameras are mounted on the robot. A sinusoidal signal modulates the turntable speed. The rotating turntable generates visual wide-field motion which the cameras sample and transmit to the monitors at 200 fps. Preliminary closed-loop experiments show that the H1 cell activity is sufficient to stabilize the grating motion across its visual field using a simple spiking rate control algorithm.

Our setup operates with a delay time of < 25ms, which is within the range of visually induced responses in the fly, and is 4 times shorter than that reported for similar closed-loop experiments in another model system [2]. In the next set of closed-loop experiment we will explore whether the activity of the H1 cell can also be used to avoid collisions of the robot with any obstacles while moving freely in the lab. The final goal of our study is to mount the fly on the robot and, using a silicon micro recording probe, monitor the activity of several spiking LPTCs including those which have recently been shown to receive input from other modalities [3]. This will allow us to assess the impact of, for instance the ocelli and antennae on the processing of optic flow information in LPTCs while the animal is actually moving in space.

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Reverse engineering speed control in the fruit fly *Drosophila Melanogaster*

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The fruit fly *Drosophila* achieves impressive flight control despite its tiny size. Its 'helicopter-like' control strategy [1] involves the precise control of body posture from subtle changes in wing motion, which consequently lead to the desired changes in flight attitude.
In this research, we focus in particular on ground speed control, which depends on visual and antennal feedbacks as external inputs, as well as proprioceptive mechanical feedback from the halteres for the control of body pitch. In such a complex behavioral context, where multimodal sensory integration is essential, it is challenging to figure out the properties of the system, in which sensory modalities cannot be uncoupled. In order to understand fruit fly's speed control strategy, the interactions between the different sensory inputs need to be explored under free flight conditions.
Experiments were performed in a wind tunnel equipped with a 3D tracking system and a virtual reality display [2]. Speed responses were elicited by stimulating individual flies visually in 'virtual open-loop', with a step increase of retinal slip speed, while the body pitch angle and position of the fly were measured using a high-speed computer vision system (see figure 1A). Unlike previous measurements [3], this paradigm allows measuring transient responses to open-loop visual step inputs, which are essential for a complete characterization of the speed controller.
We applied system identification techniques to reverse engineer flight speed dynamics and neural controllers. This approach allows investigating fundamental questions like multisensory integration, reafference, stability, etc. in a control theory framework.
We characterized pitch-to-speed dynamics and simulated them using a simple linear model. In addition other features like wind speed compensation and visual system computational delay were investigated. This research is also relevant for the design of micro air vehicles (MAVs).

Figure 1: A) Subsampled high-speed recording. Flight trajectories were sampled with 1 kHz and the position as well as body pitch angle (see Inset) extracted. B) Grouped acceleration responses. The flies pitch more nose down (bigger angles in top plot) to reach higher accelerations (from red to black in bottom plot).

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ERG recordings in two phyllostomid bats, *Glossophaga soricina* and *Carollia perspicillata*: light adaptation and action spectra>

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Microchiroptera are well known for their extraordinary capacity for orientation and navigation in absolute darkness by echolocation. Nevertheless, some species have reasonable visual acuity and use vision to find prey and monitor their environment for potential predators. These ethological findings agreed with the anatomical data of the rod dominated microbat retinas. Recent studies showed for a variety of mircrobat species, that they also possess significant cone populations and hence the basis for daylight and color vision. In the present study, in vivo and in vitro electroretinograms (ERGs) were recorded to measure the effects of light adaptationand the action spectra S(λ) in the two phyllostomid species *Glossophaga soricina* and *Carollia perspicillata*.

In general, ERG amplitudes depend on intensity and spectral composition of the stimulus as well as on adaptation level. In the investigated species, the maximal b-wave amplitudes of corneal ERGs were quite small (15 µV – 30 µV). Nevertheless, comparison of sensitivities measured with 550 nm test stimuli under different light adaptation levels indicated that *C. perspicillata* tolerates about tenfold higher adaptation levels than *G. soricina.* These results correlate well with *C. perspicillata's* tolerance for higher ambient light levels during daytime, for example roosting in exposed locations like well-lit caves, hollow trees or under exposed roots of trees

Chromatic stimulation in *C. perspicillata* and *G. soricina* S(λ) showed one major maximum at 360 nm (UV), and two smaller maxima at 450nm (blue) and at 550 nm (green). The S(λ) curve diverged distinctly from the pure rod photopigment absorption spectrum, indicating the additional contribution of cones to the ERG. With intense monochromatic 551 nm (green) or 656 nm (red) background illumination the relative sensitivities in the S(λ) curve dropped significantly for wavelengths >400 nm, whereas UV sensitivity stayed high. This indicates a UV sensitive cone pigment that is not affected by the long-wave bleaching lights. A long-wave sensitive cone pigment which histology shows to be present could not yet be isolated unequivocally in the in vivo ERGs. However, preliminary results of in vitro ERGs recorded from isolated retinae of *C. perspicillata* revealed much larger b-wave amplitudes (300 - 400 µV), because the recording electrode is close to the retina. Hopefully, this will facilitate future experiments to detect the long-wave sensitive cone pigment.

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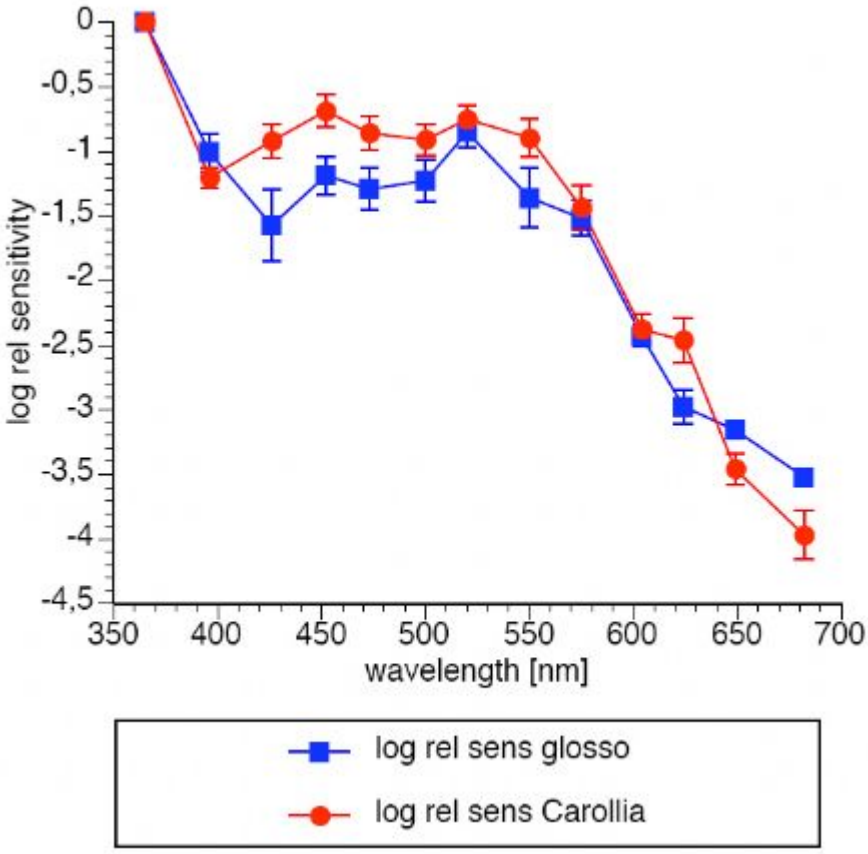
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Effects of presynaptic mutations on a postsynaptic Cacna1s calcium channel co-localized with mGluR6 at mouse photoreceptor ribbon synapses

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Photoreceptor ribbon synapses translate light-dependent changes of membrane potential into graded transmitter release via L-type voltage-dependent calcium channel (VDCC) activity. Functional abnormalities (e.g. a reduced electroretinogram b-wave), arising from mutations of presynaptic proteins such as Bassoon and the VDCCa1 subunit Cacna1f have been attributed to altered transmitter release. To investigate the underlying pathological mechanism, we examined L-type VDCCa1 subtype expression in wild type and mutant mice. Two antisera against Cacna1f, and a Cacna1f mouse mutant (*Cacna1f*ΔEx14-17) were generated. Immunocytochemistry for L-type VDCCa1 subunits and additional synaptic marker proteins was performed in wild type, *Bassoon*ΔEx4-5 and *Cacna1f*ΔEx14-17 mice. Active zone staining at photoreceptor ribbon synapses with a pana1 antibody co-localized with staining for Cacna1f in wild type mouse retina. Similarly, in the *Bassoon*ΔEx4-5 mouse, residual mislocalized staining for pana1 and Cacna1f showed co-localization. Unlike the presynaptic location of Cacna1f and pana1 antibody staining, the skeletal muscle VDCCa1 subunit Cacna1s was present postsynaptically at ON-bipolar cell dendrites, where it co-localized with metabotropic glutamate receptor 6 (mGluR6). Surprisingly, Cacna1s labeling was severely down-regulated in the *Bassoon*ΔEx4-5 and *Cacna1f*ΔEx14-17 mutants. Subsequent analyses revealed severely reduced ON-bipolar cell dendritic expression of the sarcoplasmic reticulum Ca^{2+} ATPase Serca2 in both mouse mutants and of mGluR6 in the *Cacna1f*ΔEx14-17 mutant. In conclusion, presynaptic mutations leading to reduced photoreceptor to bipolar cell signaling are associated with disturbances in protein expression within postsynaptic dendrites. Moreover, detection of Cacna1s and Serca2 in ON-bipolar cell dendrites in wild type animals suggests a putative role in regulation of postsynaptic Ca^{2+} flux.
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Epithelial sodium channels (ENaCs) in the retina and their possible involvement in the pathogenesis of glaucoma

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Glaucoma, the most frequent optic nerve disease, is characterized by an increased intraocular pressure (IOP) leading to ganglion cell death and hence to blindness. Searching for factors involved in the pathogenesis of glaucoma, a previous study showed an upregulation of the epithelial sodium channel subunit a (ENaC a). To test whether ENaCs are involved in the pathogenesis of glaucoma, we generated specific antibodies against the three ENaC subunits a, b and g and examined their distribution and expression with immunocytochemistry and light microscopy in the C57BL/6 wildtype mouse retina, the retina of the DBA/2J mouse - a model system for secondary angle-closure glaucoma - and the retina of the DBA/2JRj mouse, which develops an increased IOP but without ganglion cell death at 2, 6 and 10 months of age.

All three ENaC subunits are present in wildtype retina: ENaC a is diffusely distributed in both plexiform layers, the outer (OPL) and inner (IPL) plexiform layer. ENaC ß is found in two distinct strata in the IPL and as punctate label in the OPL. ENaC γ is present as punctate staining in the photoreceptor layer, the OPL, and the inner nuclear layer (INL). Additionally, somata are labeled in the INL and the ganglion cell layer. So far the comparison of ENaC subunit distribution and expression between wildtype, DBA/2J, and DBA/2JRj retinae (age 2, 6 and 10 months) showed no differences.

Our newly generated anti-ENaC antibodies specifically detect all three ENaC subunits in the mouse retina. Their distribution patterns indicate that the ENaC subunits could form functional channels composed of the subunits a/ß and a/γ. So far our results from immunocytochemistry - comparing ENaC subunit distribution in the three mouse lines at different postnatal ages - do not support a vital involvement of ENaCs in the pathogenesis of glaucoma.

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A novel type of interplexiform amacrine cell in the mouse retina

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Mammalian retinas comprise an enormous variety of interneurons, which are responsible for the processing of photoreceptor signals and their transmission to the retinal output neurons, the ganglion cells. Among these interneurons, amacrine cells represent the largest group, with at least 30 different cell types (Masland, 2001, Nat Neurosci 4(9):877-86) and an extraordinary variety of morphological and functional properties. Most amacrine cell types stratify exclusively in the inner plexiform layer, where they synapse with bipolar, amacrine and/or ganglion cells. However, some amacrine cell types, e.g. the dopaminergic amacrine cell, are interplexiform cells, meaning that they possess additional processes ramifying in the outer plexiform layer. Here, we describe a new, non-dopaminergic interplexiform amacrine cell in the mouse retina.
To study these cells, we used a transgenic mouse mutant that expresses the gene for the enhanced green fluorescent protein (EGFP) in several retinal cell classes, among which a single amacrine cell population is most prominently labeled. Staining for EGFP and different marker proteins showed that these cells are interplexiform: they stratify in stratum S4/5 of the inner plexiform layer and send thin processes to the outer plexiform layer, where they branch. Using intracellular dye injections, we show that these cells are medium-field amacrine cells and are homologously and heterologously coupled to other amacrine cell types by connexin45. Immunostaining revealed that the EGFP-expressing interplexiform cells are GABAergic and express GAT-1, a plasma membrane-bound GABA transporter possibly involved in non-vesicular GABA release in the outer plexiform layer of the retina.
To characterize the light responses of the EGFP-positive interplexiform cells, we performed patch-clamp recordings in mouse retinal slices. Consistent with their stratification in the ON sublamina of the inner plexiform layer, these cells depolarized in response to light ON stimuli and transiently hyperpolarized in response to light OFF. Their responses to chromatic stimulation with green (578 nm) and blue (400 nm) light suggest that these cells receive non-selective cone input, and thus, are not involved in chromatic processing.

In summary, we present here a new type of non-dopaminergic interplexiform cell in the mouse retina with interesting properties; whether its dendrites in the outer plexiform layer are functional and serve to release GABA remains to be seen.

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Pericentrin, a centrosomal protein, identified at the basal-body complex in mammalian photoreceptor cells

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Pericentrin – also known as kendrin - is one of the best-studied mammalian centrosomal proteins. It was first identified as a 220-kDa mouse protein; which was later corrected to a 250-kDa protein referred to as pericentrin A. A larger protein of about 350 kDa with an N-terminal homologous region and a C-terminal unique calmodulin-binding domain was found in humans and called pericentrin B (kendrin). Pericentrin A and B are supposed to be encoded by alternatively spliced transcripts from orthologous genes in mice and humans.

Pericentrin has been demonstrated to mediate microtubule organization by centrosomal anchoring of g-tubulin complexes that nucleate microtubules. It is characterized by coiled-coil domains throughout most of its structure and a PCM (peri-centrosomal-martrix) targeting motif called the PACT domain near its carboxyl terminus. The multiple coiled-coil domains of pericentrin mediate interactions between resident structural lattice proteins of the PCM and a number of regulatory and transient centrosome proteins, including protein kinase A, cytoplasmic dynein and the γ-tubulin complex. The PACT domain targets pericentrin and other PCM proteins, such as the protein kinase A anchoring protein AKAP450, to the centrosome. Jurczyk and co-workers (1) investigated the role of pericentrin in centrosome related structures, the primary cilia. They showed that pericentrin localizes to the base of primary and motile cilia and is involved in cilia development and cilary function in mammalian cells.

In the vertebrate retina, photoreceptor cells are morphologically and functionally arranged in several compartments. The light sensitive photoreceptor outer segment is linked with an inner segment, which contains the typical energy producing and protein synthesizing components of an eukaryotic cell, via a modified, non-motile cilium, termed the connecting cilium. Using the method of laser capture microdissection in combination with RT-PCR techniques, we could show the gene expression of all known pericentrin-isoforms in photoreceptor cells of mouse retina. Furthermore, we found with immunocytochemistry and high-resolution light microscopical analyses that pericentrin and several interacting partners, known from the centrosome, are localized at the basal body and the centriole of the connecting cilium. Here they co-localize with the whole protein transport machinery from the inner to the outer segment.

The presence of pericentrin at the connecting cilium, the site of transport regulation and interaction with transport molecules like IFTs (intraflagellar transport molecules) suggests a role of pericentrin in ciliary transport in photoreceptor cells. Studying pericentrin function may help us to understand the regulation of protein transport in photoreceptor cells and provide new insights into human disorders related to defects in ciliary function.>

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Munc13 knock-in mice define segregated neurotransmitter release sites in the retina

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At chemical synapses, a small number of proteins are known which tightly regulate neurotransmitter release from the presynaptic compartment. Among them, Munc13s are ubiquitously distributed in the brain and are essential for active zone function (Varoqueaux et al., PNAS 99:9037-42, 2002).
Knock-in mice were generated for each of the three Munc13 isoforms (Munc13-1, -2, -3) by introducing a sequence coding for the fluorescent proteins GFP or YFP at their respective genomic loci, resulting in the expression of C-terminally tagged fluorescent proteins at endogenous levels and locations (Kalla et al., J. Neurosci. 26:13054-66, 2006).
We examined the distribution of the Munc13 isoforms in the retina of the knock-in mice during development and in the adult. Labeling for the fluorescent protein was combined with the detection of marker proteins for various populations of retinal neurons and synapses.
In the adult retina, Munc13-1 is present in the inner plexiform layer (IPL) at conventional chemical synapses of amacrine cells. Interestingly, during early postnatal development, Munc13-1 is also found in the outer plexiform layer (OPL) with the photoreceptor ribbon synapses as the main synapse type. With preceding retinal development, however, Munc13-1 labeling decreases in the OPL and is no longer observed around P14, the time when photoreceptor ribbon synapses are fully functional. Of all the Munc13 isoforms, Munc13-2 is most widely expressed among the synapses of the retina. It is present at conventional amacrine cell synapses and at cone and rod bipolar cell ribbon synapses in the IPL and at photoreceptor ribbon synapses in the OPL. This is different to Munc13-3, which seems restricted to the IPL and to conventional chemical synapses of certain populations of amacrine cells. Finally, the localization of the Munc13 fusion proteins in the knock-in retinae was verified and confirmed by immunocytochemical stainings with specific antisera against the Munc13 isoforms.
Our results show a selective and mutually exclusive localization of the Munc13 isoforms at the various synapse types of the retina. The equipment of different retinal synapses with different Munc13 isoforms represents most likely an adaptation to different transmitter release kinetics. Our study demonstrates that the Munc13 knock-in mice are a valuable tool to monitor the various populations of retinal synapses.

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A special kind of reflecting layer: The *Tapetum lucidum* of the elephant nose fish (*Gnathonemus petersii*).

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The retina of the elephant nose fish *Gnathonemus petersii* is a complex organ which is characterized by a very thick layer of photoreceptor segments. Rods and cones are arranged in bundles and the retina is called "grouped retina". Cone outer and inner segments and rod inner segments are localized in the cup-shaped area whereas rod outer segments are situated behind the cup in a part called bouquet. Between cup and bouquet a further part can be found. The so called bottle neck contains invariable rod inner segments. Each bundle is surrounded by 6 retinal pigment epithelium cells (RPE) which form processes towards the inner retina. In contrast to conventional *Tapeta* of other species which can be found behind the retina and the photoreceptors this one is situated amidst the bundles of photoreceptors. The cup-like RPE-derived sheaths display light-reflecting guanine crystals which keep any light within a given bundle. Crystals are organized in four regular layers within each RPE-cell. Between every layer there is an interspace of 145 nm. This organization of crystals is only present in the cup-shaped area of the photoreceptor bundle, but not in the outer part where rod outer segments are located. It results a parabolic reflector which may serve collect the incoming light at the top of the bundle where cone outer segments are situated. Additionally, excessive light is reflected out of the bundle.
Furthermore, this retina possesses a distinct retino motor activity and shows a completely different organization of photoreceptors during dark adaptation. Because of this the reflection during night is changed in "unstructured" and diffuse light.
To measure the reflection of light and dark adapted retina the reflection mode of the laser scanning microscope can be used.

Protocadherin ß16 at AMPA and kainate receptor containing synapses of specific neurons in the outer plexiform layer of adult primate retina

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The largest subfamily of cadherins is comprised by the protocadherins (Pcdh). Most of these cell adhesion molecules are encoded by three gene clusters (a, ß, and γ). Together with the non-clustered Pcdhs (d), ~ 70 Pcdhs have yet been identified, differing mainly in their cytoplasmatic domains. Pcdhs are thought to be key features of cell type specific synapse formation and they potentially provide the basis to form distinct neuronal networks from sets of single neurons in the central nervous system.

Horizontal cell sprouting and the formation of ectopic synapses in the outer retina of mutant mice lacking functional rods and cones

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At the first chemical synapse of the mammalian retina, rod photoreceptors transfer light signals to rod bipolar cells and horizontal cells. After loss of rod photoreceptor function, postsynaptic partners respond by extending sprouting processes into the outer nuclear layer (ONL). In order to study this plasticity in detail and to evaluate how the cone pathway influences this response, we analyzed horizontal cell sprouting in mouse lines with targeted deletions of *CNGA3* and/or *CNGB1*. These genes encode essential subunits of the cone or rod cyclic nucleotide-gated channels, and are indispensable for normal function of the respective photoreceptor class.

We generated mice with non-functional rods and cones by crossbreeding CNGB1$^{-/-}$ mice with CNGA3$^{-/-}$ mice and compared these double knockouts with CNGB1$^{-/-}$ mice that lack rod photoreceptor function. The developmental time course of the outgrowth of horizontal cell processes and the formation of ectopic synapses was studied by immunolabeling horizontal cells with antibodies against calbindin and synaptic ribbons with antibodies against the C-terminal binding protein 2 (CtBP2). Transmission electron microscopy was performed to examine the ultrastructure of the ectopic synapses.

In CNGB1$^{-/-}$ mice horizontal cell sprouting into the ONL was first observed at about one week after eye opening. At the beginning, the horizontal cell outgrowths seemed to have no apparent target. Two to three weeks later, the number of sprouting processes decreased, but the remaining sprouts developed synapse-like contacts at rod cell bodies in the ONL. The appearance of ectopic synapses at the base of rod somata within the ONL strongly suggests the formation of new synapses due to neuronal growth. Qualitatively, horizontal cell sprouting into the ONL was similar in CNGB1 single and CNGA3/B1 double knockouts. Interestingly, the onset of the sprouting response appeared about one week earlier in the double knockout than in CNGB1$^{-/-}$ mice and coincided with the time point of eye opening at postnatal day 12. This suggests that cone photoreceptor function influences the sprouting behavior of the rod photoreceptor's postsynaptic partners. Importantly, no horizontal cell sprouting was observed in knockouts which were kept under constant darkness, suggesting a light-dependent mechanism for this phenomenon.

Electrophysiological Characterization of the Neurons in the Tectum Opticum of the Goldfish Regarding "Color" and "Motion"

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Color vision as well as motion vision in goldfish is very well investigated in behavioral experiments. The retina of goldfish is studied in detail in many neuropharmacological and electrophysiological investigation. On the contrary there are almost no studies on the processing of color and motion information on the level of the tectum opticum, which is considered as the main integration center for the visual processing of fish. In this study we characterized response patterns of neurons in the tectum opticum to color and motion stimuli by means of conventional electrophysiological recordings.

Goldfish *(Carassius auratus)* are anesthetized and paralyzed. The cranium is opened, the tectum uncovered and moisturized with Hickman's solution. The activity of individual cells is recorded extracellularly with conventional methods. The electrodes are filled with a 2% solution CBS in 0,5 M sodium acetate and have a resistance between 2 and 10 MO. For marking the recording site by electrophoresis a current of 2µA was passed through the electrode.

To identify color specific neurons a sequence of 21 different HKS color sheets from the entire color circle (excluding UV) are presented for five seconds each. Each color stimulus is followed by a neutral gray paper sheet. Furthermore a sequence of black, white and gray was presented to examine the response of the cell to variations in brightness. This sequence is repeated at least three times. In order to identify motion sensitive neurons a rotating black and white random dot pattern is used, providing all movement directions.

A variety of cells could already be distinguished with respect to the color, motion and brightness sensitivity: Some cells responded narrowband to red, some broadband to red or they responded to red with inhibition. Some cells responded only to the color blue or only to green, other cells broadband to both blue and green. Extreme narrowband cells, which responded only to one specific HKS paper, were found for the red region only, and never for blue nor green. Another type of cell was inhibited by red and activated by blue. Some cells responded to a change of brightness. Motion sensitive cells were selective for specific motion directions. Cells that were color sensitive were insensitive for motion stimuli and vice versa. In addition, a large spectrum of temporal response patterns was found: There are cells, that responded only one spike at stimulus onset. But most frequently was a tonic response pattern. There are cells, that have a long latency period. Other tectal cells have a comparatively high base activity, which is increased or decreased by different stimuli.

The present results show that cells in the tectum opticum of goldfish have a large variety of response patterns regarding their color, brightness and temporal properties. Furthermore the present results confirm the evidence of a parallel, separate processing of color vision on the one hand and motion vision on the other hand, because all cells responded either color specific or motion specific to the applied stimuli.

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Presynaptic cytomatrix proteins at the photoreceptor ribbon synapse

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Cytomatrix compartments at both sides of the synaptic junctions assure the high accuracy of synaptic transmission. The dense post- and presynaptic protein networks are called postsynaptic density and presynaptic cytomatrix at the active zone (CAZ), respectively. Many of the proteins at the CAZ have been identified but little is known about the function of the proteins.

We use the retinal rod photoreceptor ribbon synapse as a model system to unravel the function and molecular architecture of the CAZ. The rod ribbon synapse is characterized by a prominent cytomatrix structure that lines the active zone in a horseshoe-shaped manner already visible at the light microscopic level. In a mouse mutant deficient for the CAZ protein Bassoon the synaptic ribbon is not anchored to the presynaptic plasma membrane (1) and CAZ proteins at the ribbon synapse can be grouped into synaptic ribbon-associated and arciform density/plasma membrane-associated proteins (2). Bassoon is involved in linking the two compartments. To identify molecular players in that linkage we analyzed mouse mutants for candidate proteins.

CAST, a CAZ protein known to interact with Bassoon in brain synapses, is present at the arciform density, co-immunoprecipitates with Bassoon from retinal extracts and thus is a candidate molecular link to the arciform density. Surprisingly the retinal phenotype of the CAST knock out mouse is not a phenocopy of the Bassoon mutant retinal phenotype but proved to be much milder. It is unlikely that CAST plays a key role in ribbon synapse assembly and maintenance. In contrast, comparison of the Bassoon mutant retina to retinae of three different mouse mutants for the calcium channel subunit Cacna1f suggests a role for Cacna1f in synapse formation and role for Bassoon in Cacna1f localization.

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1) Dick et al., 2003 Neuron 37:775-786 (2) tom Dieck et al., 2005 J Cell Biol 168:825-836

Retina Optics I: Visualization of light propagation through the vertebrate retina

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The retina of the vertebrates has an inverted design in the sense that the light detecting cells are located at the back side of the retina. Therefore the light - the signal - has to pass through several tissue layers before hitting the signal transducing photoreceptor cells. These layers include structures which have sizes on the order of the wavelength of the visible light and this would result in a scattering and reflection of the photons into the tissue. We suppose that the Müller cell of the retina is responsible for the light transport where this glial cell channels the light from the vitreous body to the nuclei of the photoreceptor cells. The Müller cell occupies several features which point to the lightguidance ability: e.g. its strategic position in the path of light through the tissue, its funnel shape, its rareness of highly scattering objects, its refractive index and its endfoot which covers the entire retinal surface. This project investigates the optical properties of the retinal glial cell in its normal tissue by using a single-mode fiber as small radiation source where the external laser light leaving the optical fiber simulates the physiological illumination of one Müller cell endfoot. Finally a laser scanning microscope in detection mode records the scattering of the laser light by its way through the retinal layers. While the retina is moving with respect to the optical fiber there are changes of the beam structure similar to a fiber optic plate. Thus it seems that the Müller cell channels the light to its own photoreceptor unit (see also abstracts Gryga et al., Guck et al., and Peichl et al.).

Retina Optics II. Nuclear architecture of rod photoreceptors adapts to vision in the evolution of mammals

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We have analyzed the chromatin arrangement of rod photoreceptors in a range of mammals to elucidate the role of rod nuclear architecture for vision. In many mammals, the nuclei of rods look different from those of other retinal neurons by conventional light microscopy. In nuclei of most eukaryotic organisms, transcriptionally active and inactive chromatin (euchromatin and heterochromatin) form spatially distinct domains: heterochromatin abuts the nuclear envelope and the nucleolus, while euchromatin localizes in the inner regions of the nucleus. The arrangement of chromatin is pivotal for nuclear functions and highly conserved in evolution, hence modifications of the nuclear structure in rods are of particular interest.

The distribution of euchromatin and heterochromatin was assessed using 3D-FISH with probes for marker DNA sequences (major satellite repeat, LINE L1, SINE B1), and with antibodies against marker histone modifications (H3K4me3, H4K9me3, H3K20me3). We studied the retinae of 40 nocturnal and diurnal species belonging to a wide range of mammalian orders.

In all retinal cells except rods, heterochromatin adjoined the nuclear envelope and the nucleolus, while euchromatin localized in the inner regions of the nucleus (conventional pattern). In contrast, the small rod nuclei of all studied nocturnal species had an inverted pattern with heterochromatin in the center and euchromatin as an outer shell. The rod nuclei of all diurnal species had the conventional pattern and were somewhat larger, irrespective of taxonomic position. Hence rod nuclear architecture correlated strictly with adaptation of the retina to nocturnal and diurnal vision, respectively.

We suggest that the conventional pattern was altered in mammalian rods to facilitate nocturnal vision. Mammalian retinae are inverted, i. e. light has to traverse the retina to reach the photoreceptors. Nocturnal retinae are faced with a dilemma: They have high densities of the sensitive rods and hence thick outer nuclear layers. At the same time they critically depend on efficient light transmission through the retina to make as many of the few photons as possible available to the photoreceptors. Smaller rod nuclei reduce retinal thickness, possibly improving transmission. Modeling of the optical properties further indicates that the inverted nuclei with their dense heterochromatin core also may serve as series of lenses wave-guiding the light to the outer segments (see abstract Guck et al.). Both these features would reduce light loss in the retina (see abstract Agte et al.). Based on the general view that early mammals were nocturnal, we propose that the inverted pattern arose early in mammalian evolution, and that the conventional pattern was re-acquired by mammals that secondarily re-adopted a diurnal life style.

Retina Optics III: Living Optical Elements in the Vertebrate Retina

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While cells are mostly transparent they are phase objects that differ in shape and refractive index. Any image that is projected through layers of cells will normally be distorted by refraction, reflection, and scattering. Strangely, the retina of the vertebrate eye is inverted with respect to its optical function and light must pass through several tissue layers before reaching the light-sensitive photoreceptor cells (PRC), with each photon having a chance of being scattered.

However, there seem to be specific adaptations within the retina on the cellular and subcellular level that have optimized this apparently unfavourable situation. We have recently shown that Müller cells, which are radial glial cells spanning the entire thickness of the retina, act as optical fibers and guide light, which would otherwise be scattered, from the retinal surface to the level of the outer nuclear layer (ONL) (Franze *et al.*, PNAS, 2007). Their parallel arrangement in the retina is reminiscent of fiber-optic plates used for low-distortion image transfer (see abstract Agte *et al.*). After passage through the Müller cells, light still has to traverse the ONL to reach the photoreceptor cell segments for detection.

There also appears to be a specific adaptation of the rod photoreceptor nuclei in the ONL for improved light transmission in nocturnal animals. The strong correlation between an inverted chromatin structure and nocturnal lifestyle has been shown recently (see abstract Peichl *et al.*). We have now investigated in detail the optical consequences of the inversion using quantitative phase microscopy and computer simulations. The chromatin inversion leads to a higher and more homogeneous refractive index in the center of the nuclei, which turns them into collecting lenses and reduces light scatter. Since the nuclei are stacked in columns (see abstract Gryga *et al.*), the series of nuclear microlenses channels light effectively through the ONL. These observations suggest a specific mechanism for an improved light transmission through the ONL, which leads to less scattering and consequently light loss and which could be the physical advantage driving this nuclear inversion in the evolution of mammals adapting to nocturnal vision (see abstract Peichl *et al.*). These findings demonstrate the first nuclear adaptation for an optical function and shed new light on the inverted retina as an optical system. (see abstract Peichl *et al.*). These findings demonstrate the first nuclear adaptation for an optical function and shed new light on the inverted retina as an optical system.

Retina Optics IV. Nuclear Architecture of Rod Photoreceptors in Postnatal Development

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The vertebrate retina (in contrast to that of most invertebrates) is inverted: light must pass all retinal layers before it can be detected by the photoreceptor cells. Light scattering should occur in these layers, and should prevent both high spatial resolution at daylight and high sensitivity in darkness. Recently we have shown that Müller radial glial cells act as light guiding fibers, bypassing the light scattering layers (Franze et al., PNAS 104:8287-8292, 2007; see abstract Agte et al.). However, this light guidance must end at the outer nuclear layer (ONL) where the Müller cell processes split into thin cytoplasmic tongues which certainly cannot act as light guides. Our hypothesis is that the radial rows of photoreceptor cell nuclei serve as 'chains of lenses' bridging the distance between the Müller cell stem processes and the photoreceptor inner segments (see abstract Guck et al.).

To test this hypothesis, we performed immunohistochemistry and laser scanning microscopy on retinal cryosections. This confirmed the sophisticated structure of the outer nuclear layer (ONL) with a distinct columnar arrangement of nuclei. This arrangement exists in all mammalian species examined but differs quantitatively, correlated with diurnal vs. nocturnal life style. The aim of the present study was to elucidate the postnatal development of rod nuclear architecture in five mammalian species, *viz*. mouse, rat (strictly nocturnal), rabbit (nocturnal), guinea pig (crepuscular to diurnal), and pig (diurnal). We found that at birth, the rod nuclei of all species displayed a 'conventional' chromatin pattern. The inverted nuclear architecture of rods in nocturnal species is basically established within four weeks after birth while final fusion of chromocenters needs about two months. During this latter period, the double chromocenters of murine rod nuclei and the double-bodies of dense chromatin in rats (before they fuse to a single one) as well as the bipartite chromocenters of rod nuclei in rabbit and other adult nocturnal mammals (see abstract Peichl et al.) are aligned along the light path. The maturation of rod nuclei in these species was accompanied by changes in their shape; the nuclei were elongated / ellipsoid at birth and assumed a less elongated (rabbit) or even spherical shape (mouse, rat). Importantly, mouse visual functions do not become mature before these changes are completed (Prusky et al., IOVS 45:4611-4616, 2004).

Taken together, our data suggest that (i) formation of small, spherical nuclei with dense chromatin bodies in the center and (ii) establishment of columns of dense chromatin masses are essential for visual functions of nocturnal mammals. Assuming that these nuclei may act as light lenses, these data support the idea that the columns of cell nuclei may complete the transretinal light path, and thus optimize the sensitivity capacities of the retina. Further support comes from visualizing this light path directly on vitalslices illuminated by a thin laser beam (see abstract Agte et al.) and from direct measurements of the optical properties of the nuclei as well as simulations of the light propagation through individual nuclei and the ONL (see abstract Guck et al.).

Darkness-induced effects on rod ribbon synapses in Bassoon mutant mice

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The ribbon synapse is a specialized type of chemical synapse that sustains high and long-lasting rates of neurotransmitter release. Their most prominent active zone organelle is the so called synaptic ribbon (SR), a large electron dense thin plate which is suggested to serve to tether synaptic vesicles and to convey them efficiently to the active zone. Recent studies of Bassoon mutant mice, which lack the central part of the giant 420-kDa presynaptic cytomatrix protein Bassoon (Bsn), showed impaired synaptic transmission and remarkably altered photoreceptor ribbon synapses with *inter alia* most SRs floating freely in the cytoplasm. This observation lead to the obvious conclusion that Bsn is necessarily required to attach the SR to the presynaptic membrane. To gain more information on the synaptic role of Bsn we examined the effect of light- and dark adaptation on the ultrastructure of rod photoreceptor cells of the Bsn mutant (mt). Wild-type (wt) mice exposed to the same light/dark-cycle of 12:12 hrs served as controls.

In the light-adapted mt retina, most SRs in rod terminals were not attached to the presynaptic membrane, lying freely in the cytoplasm often aggregated in so called “SR-fields”. More than 49% of them exhibited spherical forms, compared with only 2% spheres in the wt light-adapted retina. Length measurements revealed clearly that horizontally cut profiles of rod-like SR profiles in the mt retina were significantly shorter (about 50%) compared to wt-SRs. Interestingly, when the mt animals were examined in the dark phase, the appearance of their SRs differed significantly. After dark-adaptation, we could observe a significant increase in the number of attached SR (20% more) and a prominent decrease of spherical SRs (3-fold) in rod ribbon synapses. 3D-reconstructions revealed that all (examined) rod terminals showed at least one SR-plate clearly associated at the presynaptic membrane.

In summary, our results reveal striking photodependent changes in the mt retina after dark-exposure. Association of ribbon material with the plasma membrane in the dark and the potential of ribbon material to change their state seem to be independent of Bassoon. Nonetheless, synaptic active zone architecture is compromised in Bsn mt animals both in the dark- and light adapted state. The contact area of SR and plasma membrane is highly reduced and the percentage of ribbon states is shifted. We conclude that Bsn plays a crucial role in shaping the three dimensional cytomatrix network at the rod photoreceptor active zone.

Chromatic pathways in the mouse retina

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Color vision is wide spread among mammalian species, but color vision research tends to focus on primates, most of which are color 'specialists'. To understand the general principles of retinal chromatic processing it can be advantageous to study non-primate mammals. For instance, mice, which offer the possibility for genetic manipulations, feature dichromatic color vision (*Jacobs et al., 2004, Vis. Res. 44(14):1615-22*) – like most non-primate mammals – based on two cone types, a short wavelength-sensitive (S, 'blue') cone and a middle wavelength-sensitive (M, 'green') cone (absorption peaks: 365 and 511 nm, respectively). It is commonly thought but has not yet been demonstrated that mammals share a retinal circuit that compares S- and M-cone (in primates S- vs. M- and L-cone) responses to generate a 'blue/green' (in primates 'blue/yellow') antagonistic signal.

While color vision is the result of complex computations involving a number of different visual centers in the brain, the necessary separation of chromatic signals into parallel channels begins at the first synapse of the retina. Therefore, we started by characterizing the chromatic properties of different types of bipolar cells.

A custom-built two-photon microscope that uses the objective for both imaging and stimulus delivery to *in-vitro* retina (for details see *Hausselt et al., 2007, PLoS Biol. 5(7):e185*) was modified for dichromatic stimulation. The visual stimulator consists of a reflective miniature LCD that is alternately illuminated by two band pass-filtered LEDs (near-UV: 400BP20; yellow-green: 578BP10) within each frame. We recorded electrical responses from mouse bipolar cells to dichromatic stimulation in retinal slices using the whole-cell patch-clamp technique. The stimuli were generated by sinusoidally modulating the intensity of the 'green' and the 'blue' component of a spot while varying their relative phase. Transgenic mouse lines expressing fluorescent protein in subsets of retinal cells were used to target specific types of bipolar cells.

To analyze the chromatic preference of the different types of bipolar cells we compared their responses to the 'green' and the 'blue' stimulus component to get a characteristic ratio for each recorded cell. Responses from bipolar cells of the same type (so far, we studied types 1, 2, 6, 8 and 9; as defined by *Gosh et al., 2004, J. Comp. Neurol. 469:70-82*) tend to cluster in a scatter plot, suggesting at least four physiological classes, defined by their direct cone input. In addition to non-selective contacting ON- (types 6, 8) and OFF- (type 1) bipolar cells we found an ON-bipolar cell (type 9) that exclusively receives S-cone input and an OFF-bipolar cell (type 2) with reduced S-cone input. This finding is consistent with immunohistochemical data *(Haverkamp et al., 2005, J Neurosci.;25:5438-45,* and *Puller, Haverkamp, personal communication)* and with a simple model that uses the cone spectral sensitivity curves to predict – for a given connectivity – a bipolar cell's chromatic preference.

In conclusion, our data is consistent with the proposed antagonistically organized 'blue/green' circuit as the common basis for dichromatic color vision in mammals.

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Contrast-Dependent Temporal Resolution

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Introduction: Temporal resolution is a fundamental property of the visual system. The ability to code for temporally modulated light depends upon the coding of intensity changes as a function of time, hence contrast. We are now able to behaviourally measure how temporal resolution in goldfish changes with contrast, i.e. modulation depth.

Material and Methods: Temporal resolution of goldfish was investigated with a behavioral, two-alternative forced-choice procedure (Behrend et al, 2007) under photopic illumination conditions (22 cd/m^2).The fish were trained to choose the training stimulus by food reward. This stimulus was a steady white light (50 cd/m^2) and the test stimulus was a flickering equal white light. For flicker frequencies exceeding 20 Hz the test stimulus intensity was reduced by 50% according to Talbot Plateau law. The flicker frequency was varied in the range from 5-50 Hz in steps of 5 Hz. Temporal resolution was measured for modulation depths of 60, 40, 30, 20 and 10%.

Results: The data allow some general statements: a) At 60% modulation depth the behavioral data are highly similar to data obtained with 100% modulation depth (Behrend et al, 2007) except for an approx. 5 Hz reduction of the flicker fusion frequency (FFF). b) With increasing modulation depth the FFF is shifted to higher flicker frequencies. Beside the shift of the FFF, the ability to detect slow flicker frequencies was diminished with decreasing modulation depth, i.e. the total bandwidth of the discriminable frequency range decreases when the modulation depth is diminished. c) The negative slope of the decrement in temporal resolution ability in the high frequency range around the FFF is alike for modulation depths 20-60%. In the high frequency range the reduction of temporal resolution ability has a slope which is independent of the modulation depth, in the low frequency range the slope decreases with 60 and 40% and is the same for 10-30%. For the lowest tested modulation depth (10%) data are not conclusive yet.d) The highest choice frequencies for 20-60% modulation are all at approx. 94% correct choices and at a flicker frequency of 15 Hz.

Discussion: Temporal resolution in goldfish is dependent on the modulation-depth of the light-stimuli. It decreases with diminished modulation depth. At 20% modulation depth it is reduced to a narrow frequency band of about 20 Hz with maximal resolution at about 15 Hz. For 10% modulation depth current data indicate an even more limited temporal resolution.

In contrast to full field motion perception, the highest measured median responses do not vary with modulation depth (contrast). Furthermore, the decrement of temporal resolution for higher and low flicker frequencies indicates a single mechanism for temporal coding with both an upper and a lower threshold and an optimum flicker frequency (at ~15 Hz) which appears invariant to modulation depth. This is in sharp contrast to the modulation dependent full field motion perception in which at 20% modulation depth the behavioral response is reduced by about 50%. Currently pharmacological experiments are in progress to investigate retinal modulation depth dependent temporal resolution.

Experiments on Spatial Depth Perception in Goldfish

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Purpose: The perception of spatial depth is crucial for most animals in a 3-dimensional surrounding. To get depth information from a 2-dimensional retinal image, it has to be analysed in many different ways. Some cues to perceive depth are binocular disparities, motion parallax and accommodation. Although the visual system of the goldfish is intensively studied, it is not known if and how they are able to discriminate distances in spatial depth and which retinal image cues are crucial for depth perception. In a first approach (**a**) we investigated quantitatively the ability of goldfish to discriminate object distances in spatial depth. Additional experiments (**b**) were made on size discrimination without the influence of distance.

Method: a) Six goldfish were trained to discriminate between two equally sized objects. The stimuli consisted of two black disks with 4.5 cm diameter placed in a white surrounding. They were presented at different distances in a two alternative forced choice task. Some goldfish were trained to choose the near disk (training stimulus) while the other (test stimulus) was shown as far from the training stimulus as possible. Five measurements were made with the training stimulus positioned at 5, 7, 10, 12.5 and 17.5 cm. Other goldfish were trained on the distant stimulus (25, 30 and 40 cm) and tested against a near stimulus. The distance between training and test stimulus was decreased until the goldfish were no longer able to choose the training stimulus with 70% of choices. **b)** In the experiments on size discrimination three goldfish were trained on black circles shown on a stationary flat screen, the visual angles of the stimuli corresponding to the visual angles in the first experiment. The diameters were gradually approximated until the goldfish were no longer able to choose the training stimulus.

Results: All the goldfish were perfectly able to learn both discrimination tasks.
a) In each measurement the results are similar for all fish with every training stimulus position: When the distance between the stimuli was diminished, the choice rate remains constant up to a certain point after which it declines rapidly. The smallest inter-stimuli distances with >70% choice rate increase steadily with the increasing distance of the training stimulus, irrespectively of a near or distant training stimulus. **b)** In the experiment on size discrimination the goldfish were able to discriminate the stimuli with considerably smaller differences in visual angles, than would be predicted from the results of the first experiment.

Conclusion: The results show clearly that the discrimination ability of the goldfish decreases with increasing training stimulus distance: the nearer the training stimulus is to the goldfish, the better. The distance dependent decrease of the discrimination ability shows that goldfish do not discriminate the objects through size discrimination by use of the size difference of the retinal image, but that the distance has a great influence on their performance. We assume that accommodation plays an important role in depth discrimination, which we will investigate in further experiments. In addition experiments on visual acuity and size constancy are still in progress.

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optic flow generation and processing in free flight neuroethological insights from the zebra finch

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Fast moving animals need an adequate mechanism to gather information about the environment for navigation and manoeuvring. Interpretation of the optic flow on the retina provides such information from the whole visual field and is fast enough for in time manoeuvring. The movement of the image projected to the retina is generated by self motion. Translational locomotion generates an optic flow that provides information about the three-dimensional composition of the environment, while optic flow experienced during a rotational self motion does not.

We found a saccadic gaze strategy during locomotion of the zebra finch that leads to a segregation of rotations from translational movement. This might be employed as an active behavioural strategy to facilitate extraction of spatial information from the optic flow as is already found for flying insects like the blowfly.

We then used data from the behavioural experiments to generate naturalistic stimuli for electrophysiological experiments. In an animated film the camera moved through a 3D model of the experimental cage based on the trajectory and head orientation data measured during obstacle avoidance or perching in behavioural experiments. The movie was then presented on a high speed panoramic LED monitor during electrophysiological recordings.

Here we present results from behavioural experiments and corresponding response properties of units found in optic flow processing areas of the avian brain.

Electrical Stimulation of The Human Retina With a Wireless Intraocular Retinal Prosthesis

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Electrical stimulation of retinal neurons has been shown to be a feasible way to elicit visual percepts in patients blind from degenerative diseases of the retina. Here we report on stimulation testsperformed during a clinical trial evaluating the safety and efficacy of the wireless intraocular EPIRET3 retinal implant [1, 2].
The implant is a remotely controlled, fully intraocular wireless retinal prosthesis consisting of a receiver and a stimulator module. The stimulator is placed epiretinally on the retina. Data and energy are transmitted via an inductive link from outside the eye to the implant. Six blind retinitis pigmentosa (RP) patients were included in the study.
The implants were successfully implanted and activated, and all subjects reported visual percepts as a result of electrical stimulation with the implant. Stimuli were charge-balanced square current pulses of different durations and current amplitudes. Percepts were described as dots, arcs, or lines of different colours, intensities and orientations, depending on the spatio-temporal stimulation pattern. In four of the subjects, stimulation thresholds were determined quantitatively, with resulting charge densities ranging from 2.2 to 576.8 µC/cm². Stimulation thresholds depended critically on the duration of stimulation pulses. Threshold currents dropped to very low values once pulse durations exceeded a certain value, indicating that the total charge necessary can be reduced by using longer stimulation pulses. For suprathreshold stimuli, perceived brightness and thickness of lines correlated with current amplitude. Subjects were able to discriminate between different stimulus orientations and different stimulus locations.
The results of this clinical trial show that the EPIRET3 system is suitable to elicit visual percepts in blind RP patients with low stimulation strengths. Visual percepts depended on the spatio-temporal profiles of the stimulation patterns, confirming the feasibility of this approach to provide useful artificial vision to patients blind from retinal degenerative diseases.

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[1] Walter P, Mokwa W (2008). Sehen mit kabellosem Retina-Implantat. Spektrum der Wissenschaft, June 2008, pp. 20-22
[2] http://www.bmbf.de/press/2256.php

Norrin is an angiogenic factor that protects against vascular degeneration in retinopathy of prematurity

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Norrin, the protein product of the Norrie disease gene (NDP), is a secreted protein that activates the classical WNT pathway via the frizzled (fzd)-4 receptor. Ndp(y/-) mutant mice that are deficient in norrin show a distinct failure in retinal angiogenesis, and completely lack the deep capillary layers of the retina. In contrast, betaB1-norrin mice with transgenic overexpression of ectopic norrin under control of a lens-specific promoter show an increase in ocular capillaries, while the vascular phenotype of Ndp(y/-) mutant animals is completely rescued in mixed betaB1-norrin/Ndp(y/-) mice (Ohlmann et al., J. Neurosci. 2005).

To analyze, if norrin has direct angiogenic properties, we isolated and purified recombinant norrin in 293-EBNA cells, and studied the effects of recombinant norrin on proliferation, viability, migration and tube formation of human retinal microvascular endothelial cells (HRMEC). In addition, at postnatal day 7 (P7), betaB1-norrin mice with ocular overexpression of norrin and their wildtype littermates were exposed to 75% O_2 for 18 hours or 5 days as mouse model of retinopathy of prematurity. Following O_2 exposure, flat mounts of FITC-dextran-perfused retinas were isolated and vasoobliterated areas were quantified.

Recombinant norrin significantly increased proliferation, viability, migration and tube formation in HRMEC. The effects could be significantly blocked by inhibition of wnt-/beta-catenin signaling with Dickkopf (DKK)-1. Vasoobliterated areas after O_2 exposure for 18 hours or 5 days were significantly smaller in betaB1-norrin mice as compared to wildtype littermates. After O_2 exposure for 5 days, the recapillarisation of vasoobliterated areas was marked increased in betaB1-norrin mice as compared to wild type controls. This effect on recapillarisation could also be significantly deceased by DKK-1.

We conclude that norrin is an angiogenic factor for retinal microvascular endothelial cells with an important role during development and maintenance of retinal capillaries.

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Anatomy and Physiology of the tectum opticum in the weakly electric fish *Gnathonemus petersii*

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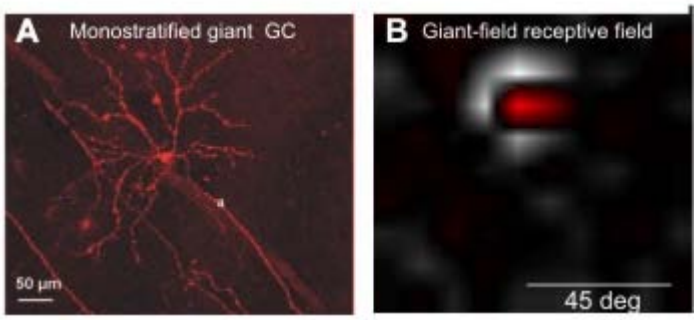
The mormyrid fish *Gnathonemus petersii* possesses an active electric sense – i.e. the ability to generate and perceive its electric organ discharge (EOD) - which is used for orientation, the search of prey and communication. This sense is frequently regarded as an adaptation to live under conditions less favourable for visual orientation; i.e. nocturnal lifestyle and/or muddy water conditions. On the other hand the visual system in *G. petersii* is neither reduced nor of minor specification. Bundled photoreceptors and a light gathering tapetum are features of the visual system which enables it to deal with dim light conditions.

The aim of our study is to first describe the retinal input to the midbrain tectum opticum and then try to understand the neural processing of visual information and possible interactions with other senses.
In accordance to their nocturnal lifestyle the retina of *G. petersii* is highly specialized with hundreds of rods and tens of cones grouped in bundles. These "macro receptors" extend over roughly 50 mikrons and are arranged in a regular hexagonal pattern and each bundle is optically isolated from the others. Information from each bundle is transferred with a strong convergence to a few neurons of the inner retina, i.e. bipolar cells and horizontal cells, and to only three or four ganglion cells. Here we report the stratification and dendritic morphology of these ganglion cells based on anterograde labelling. The density of photoreceptor bundles and ganglion cells is highest in the dorsal part of the retina. Thus, the region of the visual field with the highest spatial resolution in *G. petersii* is directed towards the bottom. Regarding the dendritic tree morphologies nine different subtypes of ganglion cells are discernible, which can be grouped into narrow-field, wide-field or giant-field ganglion cells.

Following the anatomical characterisation, we investigated the physiological properties of visual neurons in the optic tectum. Temporal aspects of both field potentials and single cell recordings show that the visual system is capable to follow frequencies in a range of 1 to roughly 25Hz. In accordance to the three classes of ganglion cells, we find various spatial organisations ranging from receptive fields that cover less than 1 degree visual angle to receptive fields covering visual angles of 40 degrees. Receptive fields typically are concentric and simple, with few centre-surround fields.

First results suggest that the spatial and temporal resolutions of visual neurons are comparatively low, yet better than initially expected. Currently, we investigate how visual and electrical information might be merged at the level of the tectum. It is tempting to speculate that it might be optimised for far field detection of predators.


A
Monostratified giant GC
50 µm
B
Giant-field receptive field
45 deg

Usher Protein MyosinVIIa Expression in the Zebrafish Retina

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MyosinVIIa is part of the USHER-protein network-interactome. Mutation in the human *myosinVIIa* gene causes Usher syndrome 1B which leads to innate deafness, vestibular dysfunction, and upcoming retinitis pigmentosa. MyosinVIIa is an actin-binding motor protein which transports specific cargo to its destination in the cell in an ATP-dependent manner. In mammals, MyosinVIIa is expressed in inner hair cells of the ear, in the retinal pigment epithelium and in some species also in photoreceptors. The zebrafish genome encodes two *myosinVIIa* genes, that we call *myosinVIIa1* and *myosinVIIa2*. Expression of *myosinVIIa1* has been confirmed in hair cells of the inner ear, whereas its localization in the retina remains unclear. The staining pattern of the second paralogue is completely unknown.
In our study, newly raised polyclonal antibodies against both MyosinVIIa paralogues were used to identify the localisation of the two protein variants in adult and larval zebrafish retina.
The MyosinVIIa1 antibody labels an elongated structure along the outer segment of all four cone types present in zebrafish, probably representing the accessory outer segment, whereas no staining pattern in the rod photoreceptors could be detected.

Optic nerve transection and crush lesion increases cell proliferation in the adult rat retina

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In the naïve adult rat retina cell proliferation normally does not occur. In this study, the effect of two different optic nerve (ON) lesions, i.e. ON transection (axotomy) and ON crush lesion, on the mitotic cell activity in the retina of adult female Sprague Dawley rats was evaluated. Bromodeoxyuridine (BrdU) was injected i.p. twice daily up to day 7. BrdU labelled cells were detected and quantified 3, 7, 14 days and 8 weeks following ON axotomy, and 7 days and 8 weeks after ON crush lesion and numbers were compared to naïve retinas. Following ON lesion, the number of BrdU+ cells was increased, with a peak at 7 days after crush. Also, 7 and 14 days after ON axotomy, high numbers of proliferating cells were seen. Co-immunolabeling with nestin, a marker for NPCs, von-Willebrand factor, a marker for endothelial cells, and glutamine synthethase and GFAP, both markers for astrocytes and Müller glia, showed similar cell fractions and cell distribution in all lesion groups. Regardless the type of lesion, approximately 60% of total BrdU+ cells were nestin+. Most of the BrdU+/nestin+ cells were identified as reactive glial cells. Some of the BrdU+/nestin+ cells were associated with endothelial cells. Proliferating cells were mainly found in the inner retinal layers (ganglion cell layer, inner plexiform layer, and inner nuclear layer) but some of them also in the outer plexiform layer. These results suggest a similar cellular activation mechanism with regard to cell division and nestin expression in response to retinal ganglion cell loss independent from the lesion type. Since both, cell division and NPC marker expression are involved in retinogenic processes this could indicate a potential for regeneration after ON lesion.

Impaired Energy Metabolism Leads to Reduced Vision in the Zebrafish *noir* Mutant

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Large-scale mutagenesis screens have been performed in the zebrafish (*Danio rerio)* to identify genes involved in the function of the visual system.
In such a screen the mutant *noir* has been identified due to its dark external appearance and absence of optokinetic behavior. Reduced vision was confirmed by electroretinography which suggested a block of signal transduction from primary to secondary neurons starting at 5 days postfertilization (dpf).
Genetic mapping of the mutation revealed that a defect in a subunit of the Pyruvate Dehydrogenase complex underlies the *noir* mutant phenotype. Absence of enzyme activity leads to the ablation of the ERG b-wave at 5 dpf and to a complete lack of both, a- and b-wave at 7 dpf. At 6 dpfdefects in the inner nuclear layer are observed in histological sections of the retina. This defect extends to the outer retina at 7dpf.
Interestingly, cells of the inner nuclear layer, rather than photoreceptors – the most energy-consuming cells of the retina are initially affected by the mutation suggesting a more complex pathogenesis than simple energy deprivation.
Strikingly, a ketogenic diet consisting of long-chain fatty acids could rescue the *noir* mutant phenotype. Mutant larvae survived longer and the ERG and histological phenotype were significantly improved. The long-chain fatty acids provide an alternative energy source to restore diminished acetyl-CoA levels caused by the block linking glycolysis and Krebs cycle.
We are currently testing two alternative hypotheses. One hypothesizes that the defect in the inner retina is due to aberrant in –growth of blood vessels, while the other proposes a selective damage to cholinergic amacrine cells.
In conclusion, a mutation in a metabolic key enzyme leads to reduced vision and retinal dystrophy in the zebrafish *noir* mutant. The defect initiates in the inner nuclear layer suggesting a complex pathogenesis.

Delayed ribbon precursor sphere formation during photoreceptor synaptogenesis in the absence of Bassoon

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The retinal photoreceptor ribbon synapse is structurally and functionally specialized for the tonic release of neurotransmitter. It is characterized by the presynaptic ribbon, an electron-dense organelle at the active zone, which is covered by hundreds of synaptic vesicles. Recently we showed that the photoreceptor ribbon complex is built from non-membranous, spherical densities – the precursor spheres – during the first two postnatal weeks (1). The precursor spheres are transport units of ribbon and ribbon associated cytomatrix proteins, i.e. RIBEYE, Piccolo, RIM1, and Bassoon. In the developing photoreceptors the precursor spheres are transported to the terminals where they loosely congregate close to the membrane, rapidly change their shape to a ribbon-like appearance and finally attach to the membrane (1). A component of the precursor spheres and a key player in attaching the ribbon to the active zone is Bassoon (1, 2).

We examined the role of Bassoon during the early stages of photoreceptor ribbon synapse assembly (P0 to P14) using mutant mice lacking a functional Bassoon protein.

Antibody labeling for RIBEYE, the major protein component of the ribbon and for Piccolo, a ribbon-associated protein, shows fewer and smaller precursor spheres in the developing Bassoon deficient photoreceptors compared to wildtype controls. Western blot analyses of homogenates from P0 to P14 Bassoon deficient retinae showed lower protein levels compared to controls confirming the immunocytochemical findings. Surprisingly, in addition to reduced protein levels, sphere formation was also significantly delayed in the mutant photoreceptors - in wildtype photoreceptor terminals first spheres were detected around P4, in Bassoon deficient photoreceptor terminals around P10. Upon arrival in the Bassoon deficient terminals, precursor spheres still changed their shape to a ribbon-like appearance but the ribbons never attached to the membrane and rapidly regained a spherical structure.

Our results demonstrate that Bassoon is not only important for ribbon anchoring but also seems to play a role in the early formation and/or stabilization of precursor spheres on their way to the nascent ribbon synapses in the developing photoreceptor terminals.

(1) Regus-Leidig et al. 2008. J Comp Neurol (in press).
(2) Dick et al. 2003. Neuron 37:775-86.
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Hypothyroidism induces changes in adult mouse cone opsin expression and electroretinogram

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Thyroid hormone (TH) plays an important role in the development of the CNS and the retina. Mice with congenital defects in the thyroid hormone receptor ß gene show altered expression of retinal cone opsins, with shortwave (S) opsin in all cones and an absence of middlewave (M) opsin, and a complete lack of light sensitivity in the middle-to-longwave range of the spectrum [Ng et al. 2001]. In the present study we tested whether pharmacologically induced adult-onset hypothyroidism has similar effects on cone opsin expression and retinal function.

C57BL/6J mice were treated from postnatal week (w) 12 with methimazole (MMI) and perchlorate (0.074% and 0.74%, respectively, in the drinking water) to induce hypothyroidism (MMI group). At w24, scotopic and photopic electroretinograms (ERGs) were recorded in the MMI group and in untreated control mice (Control-1 group). In both groups, the expression of S- and M-cone opsins was analyzed by immunohistochemistry. From w24 to w36, mice of the MMI group were returned to normal diet to test whether putative effects are reversible (MMI-Recovery group). The MMI-Recovery group was compared with age-matched controls (Control-2 group).

Measurement of serum TH concentrations confirmed that adult mice treated with MMI for 12 weeks were hypothyroid. MMI mice showed a significant reduction of the scotopic ERG b-wave, without altering the a-wave. A-wave implicit times were increased in the MMI group at medium intensities, but b-wave implicit times were unchanged. Photopic ERGs were performed with UV and green light stimuli, both with and without 523 nm background adapting light. In all cases MMI mice showed smaller responses than Control-1 mice over the whole intensity range tested. Immunohistochemically, MMI mice showed an increase in S-opsin expression in all cones and a decrease in M-opsin expression across the entire retina. Recovery from MMI treatment after w24 restored normal serum TH levels. The pattern of cone opsin expression returned to normal and there was a complete reversal of the effects seen in the ERG. In the scotopic ERG, implicit times as well as a- and b-wave amplitudes in the MMI-Recovery group were identical to those in the Control-1 group and in the case of the a- and b-waves, amplitudes in the MMI-Recovery group were larger than in the Control-2 group. A similar reversal was obtained in the photopic ERG.

In conclusion, MMI/perchlorate-induced adult-onset hypothyroidism leads to a decreased ERG b-wave, both in the scotopic and the photopic illumination range. This effect is reversible. The comparison with the age-matched control even suggests some overcompensation, leading to larger responses in the scotopic ERG. Because the spectral ERG responses do not directly reflect the shifts in cone opsin expression, we assume that additional changes affect ERG responses in systemic hypothyroidism.

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Role of Metabotropic Glutamate Receptors in the Zebrafish Retina

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Background: Metabotropic glutamate receptors (mGluRs) have been identified at all synapses of the vertebrate retina, where they likely regulate neurotransmitter release. The best studied member is mGluR6, which is expressed on ON bipolar cell dendrites and is thought to mediate the ON response in the mammalian retina. This is the only example of an mGluR functioning in direct synaptic transmission. As a first step to elucidate the functional role of mGluRs in the teleost retina, we set out to clone all members of this family and determine their respective expression pattern in the developing and adult zebrafish retina.

Results and Conclusion: The phylogeny of the grm (coding for mGluR proteins) family is comparable to humans and mice with its division into three subgroups. We found two paralogues for each grm ortholog in mammals for most members (grm1, 2, 5, 6, and 8). Those likely arose in a teleost specific whole genome duplication event. RNA in situ hybridization experiments revealed a unique expression pattern for all grms in 5 day old zebrafish, again, supporting the distinct roles of the different mGluRs in the retina. Different expression patterns between two paralogues, as found for example for grm1a and grm1b, suggest a distinct subfunctionalisation of both gene paralogues. We focused on the two mGluR6 paralogues. In contrast to mammals, where mGluR6 is expressed in bipolar cells, we located both genes predominantly in retinal ganglion cells. This surprising result was supported by coexpression with gnao (coding for Goa proteins), the G-protein which is thought to conduct the mGluR6 signal pathway. Since gnao is also expressed in inner retinal cells, we deem likely that mGluR6 is expressed below detection level in bipolar cells. We are in the process of generating antibodies to identify the synaptic localisation of the mGluR6 paralogues. In future, knockdown experiments will determine a function for these genes.

Characterization of Shepherd´s Crook neurons in the chicken optic tectum

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The midbrain is involved in the processing of visual stimuli in vertebrates. It is related to spatial orientation by integrating all available sensory modalities and by generating appropriate premotor signals.
Prominent structures in the midbrain are the optic tectum and the nuclei isthmi. The latter consist of the nucleus isthmi pars parvocellularis (IPC), the nucleus isthmi magnocellularis (IMC) and the nucleus isthmi semilunaris (SLU). These nuclei are reciprocally connected with the optic tectum. In detail, visual information is conveyed retinotopically via retinal ganglion cells (RGC) into the upper layers of the optic tectum [1]. Here, retinal afferents contact a prominent neuron type – the Shepherd's Crook Neurons (SCN). These neurons project further to the isthmic nuclei still maintaining retinotopic organization in SLU and IPC. Neuronal signals are then projected back to the source area in the optic tectum. Projections from the IPC terminate mainly in the upper layers, while projections from the SLU mainly terminate in deeper layers [2]. Both release Acetylcholine into a cartridge-shaped region of the optic tectum. The organization of the IMC is not retinotopic. Neurons in the IMP project to the tectum, to the IPC and the SLU. Their GABAergic terminals are widely arborised. The inhibition from the IMC seems to spare the stimulus site in the optic tectum and also its retinotopic counterparts in SLU and IPC [1].
Possible functions of this feedback loop include the implementation of a winner-takes-all circuit, which might disambiguate stimulus representations, and the focusing of attention onto a (moving) target by depressing activity in all areas of the optic tectum without incoming stimulus. However, many details of these projections are not fully understood.
We are interested in the exact timing and wiring in this circuit. Anatomical data imply that the SCN might play a crucial role. Their characteristic anatomy allows for input from upper as well as from deeper layers of the tectum while relaying spatially organized input to the isthmic nuclei. So far it remains unknown, whether SNCs comprise one or several subtypes regarding their detailed projection pattern or their physiological properties.
Our immunohistochemical stainings were targeted against the transcription factors Brn3A/Pax7 and the Ca^{2+}/calmodulin dependent protein kinase 2 (CamK2). So far, SCNs were immunopositive for Brn3A and immunonegative for Pax7 and CamK2. This indicates that SCNs might consist of only one type. Further neuroanatomical and electrophysiological studies are underway to support these findings by evaluation of projection patterns and physiological data.

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2. Wang, Y., et al., *Columnar projections from the cholinergic nucleus isthmi to the optic tectum in chicks (Gallus gallus): a possible substrate for synchronizing tectal channels.* J Comp Neurol, 2006. **494**(1): p. 7-35.

Subcompartmental distribution of Cx45 on bipolar cells in the mouse retina

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Gap junctions are specialized cell-cell contacts that provide direct communication between cells. Each gap junction channel is formed by two connexons. One connexon consists of six membrane-spanning protein subunits called connexins. In the mouse retina, connexins are expressed by all neuronal cell classes. Previous studies showed that connexin45 (Cx45) is expressed in bipolar cells (BPCs), amacrine cells and bistratified ganglion cells in the mouse retina (Maxeiner et al., 2005; Schubert et al., 2005). Maxeiner et al. (2005) showed that Cx45 is expressed in all OFF BPCs and in ON BPCs of types 5 - 7. Cx45 is located on axon terminals of ON BPC types 5 and 6 (Dedek et al., 2006). However, the location of Cx45 on OFF BPCs is not yet known. Here, we aimed to analyze the subcompartmental distribution of Cx45 on OFF and ON BPCs in the mouse retina.

For this, we used a transgenic mouse line which expresses, in addition to the native Cx45, an EGFP-Cx45 fusion protein which produces an EGFP signal at gap junctions containing Cx45. The distribution of the EGFP signals showed no significant variation from Cx45 immunoreactivity (IR) in wild-type mice. Cx45 IR in the EGFP-Cx45 retina was similar to that in the wild-type retina. This indicates that the expression of the fusion protein corresponded well with the expression of the native Cx45. In addition, glycine IR in Cx45-EGFP mice did not differ from glycine IR in wild-type mice, indicating that neurotransmitter coupling between AII amacrine cells and ON BPCs was functional. Thus, the mouse line expressing a Cx45-EGFP fusion protein provides a useful tool for studying the subcellular distribution of Cx45 in retinal neurons.

To analyze the distribution of Cx45 in the retinas of transgenic EGFP-Cx45 mice, we combined immunohistochemistry with intracellular dye injections. Vertical and horizontal retina sections were stained for EGFP and several BPC markers. Intracellular dye injections combined with a calretinin/EGFP double staining were used to reveal the subcellular distribution of Cx45 in individual OFF BPC types. We found that Cx45 was present on the axon terminals of all OFF BPC types. Occasionally, Cx45 was also expressed on the dendrites of OFF BPCs. Consistent with Dedek et al. (2006), we found EGFP-Cx45 IR both in the axon terminals and in the dendrites of type 5 and 6 ON BPCs. EGFP-Cx45 IR was also present on axon terminals and dendrites of ON BPC types 7 and 8. Further experiments are needed to resolve the distribution of Cx45-EGFP IR in ON BPC type 9.

In the present study we show that Cx45 is located on axon terminals and dendrites of all OFF BPC types and in ON BPCs of types 5 through 8. Whether Cx45 forms homotypic gap junctions or heterotypic gap junctions with Cx36 remains to be seen.

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Electrical image of the nerve fibre layer of the rabbit retina

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The mammalian retina processes and transmits visual information through more than a dozen channels embodied in different types of ganglion cells [1]. Recent studies established that coincident activity of ganglion cells facilitates the transmission from the retina to higher brain areas and facilitates the encoding of visual stimulus information [2]. An implicit assumption for the coincident arrival of afferent input signals at the postsynaptic target cell constitutes the preservation of the temporal activity structure. However, it is unknown if the temporal relation between individual neuronal signals is preserved from the site of initiation along the unmyelinated axons in the retinal nerve fibre layer.

Here we electrically image how action potentials are transmitted across the rabbit retina until they leave the eye. Action potentials were elicited by visual stimuli in the whole mount retina and detected by a multi-transistor array comprising 16000 extracellular sensor sites. We followed the signal propagation of individual action potentials at very high spatial (7.8 µm) and temporal (0.1 msec) resolution [3]. This high spatial sampling was necessary to functionally image axonal populations that curve and cross each other.

The temporal action potential patterns in one axon are unchanged during the transmission in individual axons and bundles thereof. We also recorded misrouted axons with signals propagating away from the optic nerve. These signals were unaffected as well by parallel axons.

Our measurements on more than hundred axons at different retinal positions indicate that the mean conduction velocity (1.2 m/sec) is uniform throughout the rabbit retina. This means that intraretinal unmyelinated axons act as delay lines.

The intraretinal conduction velocity varied among cell types. It was highest (1.5 m/sec) for transient cells and smallest for sluggish cells (< 1 m/sec). Axons from the same cell type conduct at equal velocities thus preserving the multineuronal spike pattern of one given type. However, the temporal relation in multineuronal spike patterns comprising different cell types does change from the site of action potential initiation to the postsynaptic target.

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Retinal cone opsin expression differs between wildtype and albino deer mice

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Failure of melanin synthesis is genetically heterogeneous but results in a common pattern of retinal problems. These include underdevelopment of the central retina, abnormal chiasmatic routing of retinal axons, and a reduction in rod photoreceptor numbers of approximately 30%. The present study sought to establish whether albinism affects the number and the mosaic distribution of spectral cone photoreceptors.

Wildtype (*c*+/*c*+) and albino (*c*/*c*) deer mice (*Peromyscus maniculatus*) were used as models for different ocular pigmentation. Retinas were dissected and prepared as flatmounts. Rod photoreceptors were counted using differential interference contrast microscopy. Cones were identified by opsin immunostaining and their numbers, spectral types, and distribution across the retina determined using epifluorescence microscopy.

Pigmented *P. maniculatus* have a rod-dominated retina (rods: 450.000/mm^2; cones: 3000 - 6500/mm^2). Two cone opsins, shortwave sensitive (S) and middlewave sensitive (M), are present and expressed in distinct cone types (S cones: 5-15%, M cones: 85-95% of the cones). Different from house mouse, S and M cone patterns do not form dorsoventral gradients and coexpression of cone opsins in single cones is exceptional (0 - 2% of the cones). In albino *P. maniculatus*, rod numbers are reduced by approximately 40% (270.000/mm^2). Total cone numbers, the mosaic distribution of generic cones, and the numbers of cones exclusively expressing S opsin are not significantly different from pigmented *P. maniculatus*. However, in contrast to pigmented *P. maniculatus*, in albino retinas S opsin is coexpressed with M opsin in 60-90% of the cones and therefore the population of cones expressing only M opsin is significantly reduced (5-25%).

In conclusion, albinism in *P. maniculatus* results in similar reductions in rod numbers as in laboratory mice. Cones appear not to be affected in their number or general distribution. The pattern of S cone opsin expression, however, is dramatically different in albino *P. maniculatus*. Because mutations at the albino (*c*) locus result in deficits in tyrosinase enzyme function, we hypothesize that tyrosinase activity directly or indirectly affects the regulation of S opsin expression.

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Morphological and functional consequences of inducible ablation of retinal horizontal cells in living mice

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Horizontal cells are laterally-oriented interneurons that form a single layer in the distal inner nuclear layer and unique triad synapses with bipolar cells and photoreceptors in the outer plexiform layer. At these synapses, horizontal cells receive signals from the photoreceptors, and provide negative feedback to photoreceptors and feedforward signals to bipolar cells. Because of extensive electrical coupling, the receptive field of each horizontal cell is much larger than its dendritic field, and each horizontal cell indirectly receives a mixture of rod and cone inputs over an extended area of the retina. Thus, the feedback and feedforward signals from the horizontal cells to the photoreceptors and bipolar cells reflect the average illumination over a large area of the retina.

Recent studies have shown that mouse horizontal cells are coupled by connexin57, a gap junction protein expressed exclusively in these neurons. In order to study the functional role of horizontal cells in more detail, we generated mice expressing the primate diphtheria toxin receptor (DTR) under the horizontal cell-specific promoter of connexin57. Treatment of adult animals (DTR/+, 3 months) with different doses of diphtheria toxin led to complete, selective ablation of horizontal cells in living transgenic animals. Although the gross morphology of the DTR/+ retinas appeared unaffected two weeks after treatment, immunolabeling of retinal sections revealed complete loss of the horizontal cell marker proteins calbindin, neurofilament, Cx57 and GluR2/3. In comparison, bipolar cells stained for PKC and $G_{o\alpha}$ showed only minor morphological changes at this time point: their dendritic trees appeared shorter. At the ultrastructural level, the characteristic synaptic contacts of horizontal cell and bipolar cell dendrites at the synapse triads of rod spherules and cone pedicles were no longer present. Instead, the photoreceptor terminals appeared swollen, with vacuoles partially filled with electron-dense material (debris), and pedicles and spherules contained large mitochondria. These findings indicate that horizontal cells provide structural elements essential for the maintenance of the unique synapse triads in the OPL of the vertebrate retina, and imply that DTR/+ mice treated with diphtheria toxin will become blind. We tested this latter hypothesis using the electroretinogram, and found a clear reduction in the a-wave and a complete loss of the b-wave of treated DTR/+ mice, demonstrating that these animals are indeed blind. Further long-term studies indicate that these animals survive, that their photoreceptors start to degenerate and that bipolar cells begin searching for new partners and grow large dendrites projecting high up into the outer nuclear layer, where they may form ectopic synapses with photoreceptors.

In summary, our data provide evidence that horizontal cells are key elements essential for the maintenance of the unique synaptic contacts at the first synapse in the visual pathway, and for functional visual processing between photoreceptors and bipolar cells within the adult retina.

Light evoked current responses of bipolar cells in a retina with uncoupled horizontal cells

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In the retina, horizontal cells form an electrically coupled network. They sum information from a wide spatial area and can thus influence the photoreceptor and bipolar cell responses from a broad region of the retina. Dedek et al. (2008) used a connexin57-deficient mouse line (Cx57 KO), in which horizontal cells were uncoupled, to study the impact of horizontal cell coupling on ganglion cell light responses. The authors found no differences in the spatial tuning of ganglion cells and its switch from low to high spatial frequencies in response to changes in light intensity. This indicates that horizontal cell coupling does not play a major role in the center-surround antagonism of ganglion cell receptive fields. Like ganglion cells, bipolar cells also show, at least in cold-blooded vertebrates, antagonistic receptive fields. These surround effects might be evoked by amacrine cell feedback, but could also come from horizontal cell feedforward inhibition. In Cx57 KO mice, horizontal cells have smaller receptive fields, a lower input resistance and more depolarized dark resting potentials than in wild-type mice (Shelley et al., 2006). Thus the inhibitory contribution from horizontal cells to bipolar cells could be altered in these mice.

To investigate this, we performed whole-cell voltage clamp recordings from bipolar cells in retinal slices of dark-adapted wild-type and Cx57 KO mice. We measured the light-evoked excitatory cation current ($DeltaI_C$) by clamping the cells at the chloride equilibrium potential (E_{Cl}) and the inhibitory chloride current ($DeltaI_{Cl}$) at the cation equilibrium potential (E_C). Full field light flashes (green light of 520 nm) of various light intensities were focused on the retinal slice and currents were recorded. To identify its type, the measured bipolar cell was filled with Lucifer Yellow and stained with antibodies against calretinin to visualize the stratification level of the axon terminal in the inner plexiform layer.

We characterized light responses of rod bipolar cells and two types of ON cone bipolar cells (types 5 and 7). In wild-type mice, both types of ON cone bipolar cells had their dynamic ranges in the area of 10^2 to $10^{3.5}$ photons μm^{-2} s^{-1}. This was not significantly different in ON cone bipolar cells of the same types in Cx57 KO mice. Also, the decrease of latency with increasing light intensities showed the same dependency in wild-type and Cx57 KO mice. In both genotypes, the sensitivity of the rod bipolar cell was higher than for the tested cone bipolar cells and was not affected by disruption of horizontal cell coupling.

In our investigation, a general loss of horizontal cell coupling seems to have no effect on the light responses of the cone bipolar cell types 5 and 7. Amacrine cells most likely play the major role in evoking inhibitory currents in these bipolar cell types. The question of horizontal cell influence on other cone bipolar cell types is still in progress. However, it is possible that in the mouse retina, horizontal cell function is restricted to the negative feedback onto photoreceptors. This feedback is still functional in Cx57 KO mice (Shelley et al., 2006).

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Effects of temperature on retinal ganglion cell responses

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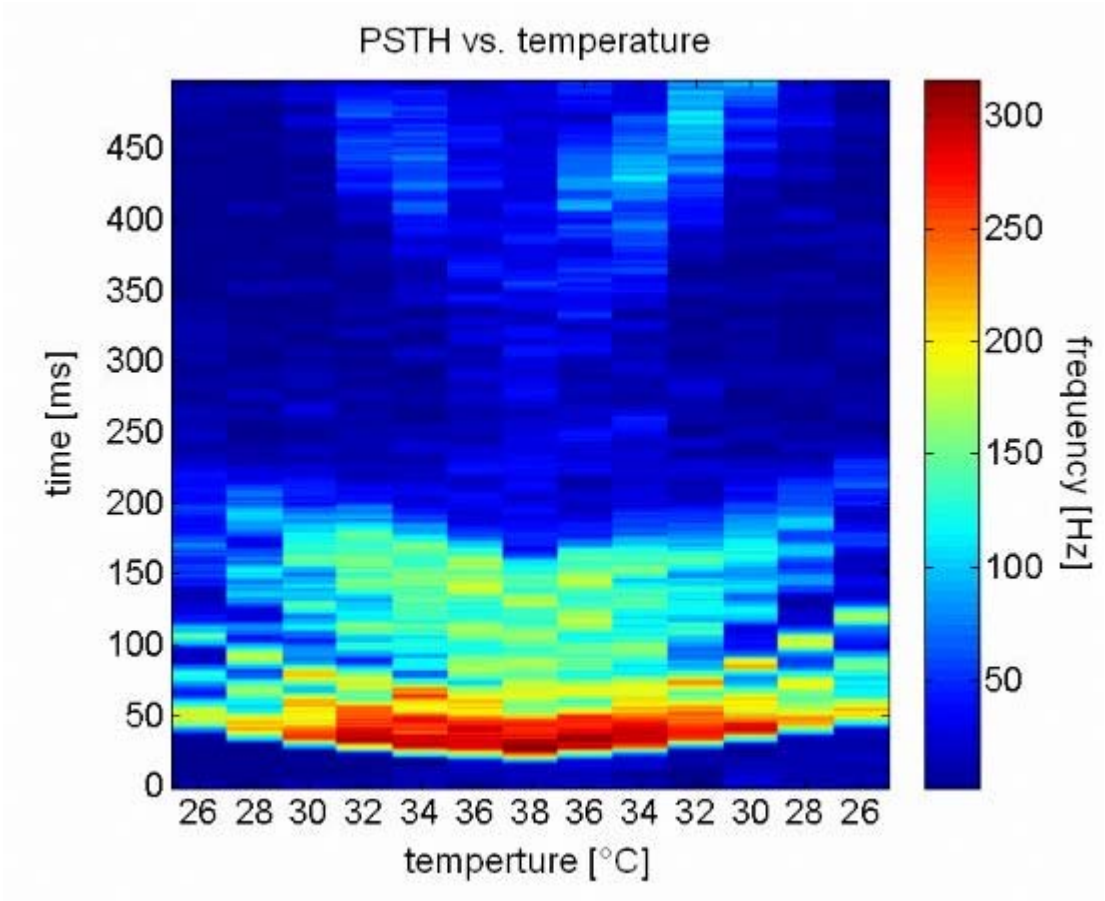
Nervous systems of cold-blooded animals are exposed to significant temperature variations. Their body temperature follows daily, seasonal or otherwise caused thermal changes of their habitats. In the case of warm-blooded animals at least their body surface temperature is affected environmentally. In addition they experience body temperature variations due to their circadian rhythm. Consequently their nervous system is affected by temperature changes, too.

We studied the effects of temperature variations on ganglion cell light responses of isolated retinae. Light responses were recorded with multi electrode arrays from isolated mouse (warm-blooded) and carp (cold-blooded) retinae. A temperature control system was developed that allowed precise temperature variations in the range of 8 °C – 45 °C.

Figure 1 depicts temperature effects on the peri-stimulus time histogram (PSTH) of a mouse retinal ganglion cell. The x-axis displays temperature conditions, the y-axis time after stimulus onset. Spike frequency is represented in grayscale. The retina was stimulated with white, full-field light flashes of 100 ms duration at 0.2 Hz. Light intensity was 39.9 lx. Several light response parameters vary with temperature. For example a temperature increase leads to an increase of spike rate and a decrease of response latency. In addition more complex response properties like burst patterns undergo significant changes with temperature. All observed effects are reversible, indicating that they are caused specifically by temperature variations.

Two important conclusions can be drawn from these results: 1: if retinal temperature during an experiment is not kept reliably at a defined value, the observed neural activity is neither comparable to other experiments nor to the physiological state of the animal. 2: Varying both, temporal contrast of the light stimulus and retinal temperature, leads to ambiguities in stimulus estimations based on classical coding hypotheses like rate and latency: If a decoder in the animals brain has to estimate the temporal contrast of a stimulus by rate or latency alone under different temperature conditions, different stimulus estimations will result. If not compensated, this would impair behavioral performance.


PSTH vs. temperature
time [ms]
0
50
100
150
200
250
300
350
400
450
26 28 30 32 34 36 38 36 34 32 30 28 26
temperture [°C]
frequency [Hz]
50
100
150
200
250
300

Direction-selective retinal ganglion cells in pigmented and albinotic rats

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In all mammals so far investigated albinism has a strong influence on the anatomy and the physiological functions of the visual system. Earlier in vivo analyses in albinotic ferrets and rats revealed a complete loss of optokinetic reactions. Electrophysiological investigations showed that slip neurons in the nucleus of the optic tract and the associated dorsal terminal nucleus of the accessory optic system (NOT-DTN) lack direction-selectivity and seem to have abnormal electrophysiological properties. Until now it is not clear if this dysfunction is localized within the NOT-DTN itself or is posssibly the consequence of a reduced or defective retinal input.
In the recent study we investigated ON-type direction-selective ganglion cells in pigmented Long Evans rats using extra- and intracellular in vitro patch clamp techniques. These cells are known to project to the accessory optic system, which consists of three distinct nuclei: the medial terminal nucleus (MTN), the lateral terminal nucleus (LTN) and the NOT-DTN. The probability of finding direction-selective cells was increased by retrograde prelabelling of retinal ganglion cells with the infrared sensitive dye indocyanine green (ICG), injected into the MTN. In a second set of experiments ICG prelabelled retinal ganglion cells in albinotic Wistar rats were investigated and tested for direction-selectivity. First results showed that in pigmented, as well as in albino rats direction-selective ganglion cells are functional. However, the amount of direction-selective cells in the retinae from albinotic rats seems to be reduced. In pigmented rats 14%, in albino rats 2% of all measured cells were found to be ON-type direction-selective ganglion cells. These data indicate a decreased retinal input to the nuclei of the AOS and can explain the loss of optokinetic function.

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Monkey retinal ganglion cells retain the potential to switch into a strong regenerative state and regenerate axons *in vitro*.<

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Mature retinal ganglion cells (RGCs) of mammals at juvenile or adult stages are normally unable to regenerate axons through the injured optic nerve. This failure has been attributed to inhibitory signals in the extracellular environment and to an irreversible decline in RGCs' intrinsic growth potential. However, axons of RGCs of rats, mice and monkeys are able to regenerate axons in culture. This appears to reflect a change in RGCs' intrinsic growth state: when explanted into culture. In rodents, but not in monkeys intact RGCs exhibit no spontaneous outgrowth and RGCs subjected to axotomy alone show relatively little growth. In contrast, axotomized RGCs exposed to lens injury or to crystallins or to inflammatory factors exhibit a rapid extension of numerous GAP43-positive processes after already 1 day in vitro. To identify the molecular changes that underlie this regenerative ability, we performed immunohistochemistry, western blotting and RT-PCR. We have shown that RGCs exert a growth program which becomes fully developed with respect to growth associated molecules and axon specific markers. In particular, receptors to extracellular matrix and specifically for laminin were expressed together with neurofilaments. When the rate of growth was determined with time-lapse videography, it was similar with that reported for embryonic axons, indicating that regenerating axons recapitulate the principal molecular mechanisms of embryonic development. Proteomic profiling of the regenerating retinas in culture showed different patterns from native retinas at matching ages and from retinas without the opportunity to regenerate axons. In particular, proteins involved in Calcium homeostasis such as calmodulin were regulated and stress proteins such as heat shock proteins were regulated. The molecular changes associated with axon regeneration in mature RGCs are strikingly similar to those reported during rat ganglion cell axon regeneration or peripheral nerve regeneration (e.g. GAP-43). The data show that even after maturation, the molecular mechanisms for axonal growth still exist and can be reactivated to result in stump extension and growth cone formation.
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Spatio-temporal characteristics of identified wide-field amacrine cells (WFAs) in the mouse retina: global contrast-detecting interneurons?

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Using immunohistochemistry and patch-clamp recordings, we investigated the morphological and physiological characteristics of identified wide-field amacrine cells. In the mammalian retina, 20-30 different amacrine cell classes are thought to contribute to the propagation and early processing of visual signals. Amacrine cells are usually associated with complex processing. Aside from a few well studied cell types, such as AII and starburst amacrine cells, the function of most amacrine cell types remains elusive. In particular, cell types with rather large dendritic arbors, called *wide-field amacrine cells* (WFAs), are difficult to investigate on the single-cell level because of their low abundance. In recent years, initial evidence that these cells play an important role in context-dependent signal processing has been presented (Roska and Werblin, 2003).

In this study, we used a transgenic mouse line which expresses the enhanced green fluorescent protein (EGFP) under the control of the choline acetyltransferase promoter (Engelhardt et al., 2007). Immunohistochemical staining for GFP and neurotransmitters revealed that the labeled WFAs are GABAergic neurons. Injection of fluorescent dyes showed two subpopulations, one with cell somata in the inner nuclear layer (INL), and one in the ganglion cell layer (GCL). Both populations have a monostratifying dendritic arbor between sublaminae 2 and 3 of the inner plexiform layer (IPL). Similar amacrine cell types with cell bodies exclusively in the INL have recently been reported from studies in rabbit (Bloomfield and Völgyi, 2007). Using neurobiotin as a neuronal tracer, we show that both subpopulations of WFAs contribute to the dense dendritic plexus in S2/S3 and are interconnected via gap junctions. Additionally, some non-GFP-expressing amacrine cell populations participate in the gap junctional network.

Cells from the GCL were identified by their GFP fluorescence using a two-photon IR laser (>800 nm) in whole-mounted retinae. We performed patch-clamp recordings while stimulating the retina with light from a computer-controlled CRT monitor. The basic light response was found to be a long-lasting ON/OFF depolarization ($\Delta E(m)$ = +20 to +30 mV), matching the cells' stratification level in the middle of the IPL. The amplitude and duration of the depolarization was mainly defined by the short-term change in illumination, i.e. temporal contrast, presented to the retina. The single-cell receptive field was at least 1000 µm in width. Various spatial stimulation patterns proved that the receptive field was not subdivided. Instead, its integrative properties were remarkably uniform over wide ranges.

In further experiments, we tested light responses at different adaptational states. Though already very sensitive under scotopic conditions (1 Rh* / rod), the spatio-temporal response characteristics were almost unchanged in mesopic to photopic light levels (10^5 Rh* / rod). Therefore, we characterize the investigated WFA as a "global contrast detector".
Future studies, including anatomical and pharmacological experiments will give further hints at possible pre- and postsynaptic partners of this cell type.

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Hue, Saturation, and Brightness Values Derived from the Model of Neuronal Color Coding and Elementary Color Sensations in Man

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Color vision in man is adequately described by the model of neuronal color coding (CC) and elementary color (EC) sensations. The CC/EC model precisely predicts the amounts m_i of the six ECs red (R), green (G), blue (B), yellow (Y), black (Bk), and white (W), with $\sum_i m_i = 1$, i= 1-6, from the spectral light intensity distribution. Now, the biological color system (BCS) has been derived from the model, in terms of hue (Ton: T), saturation (Sättigung: S), and brightness (Helligkeit: H): **1)** The hue T of a color sensation is derived as the tuple of the amounts (m) of two of the four chromatic ECs R, G, B, and Y:

$T = (m_i / (\sum_c m_c)$ "EC_i", $m_j / (\sum_c m_c)$ "EC_j"), $i \neq j$; i, j e {1, ..., 4}, and c = 1 - 4,

e. g. T = (0.8 G, 0.2 B), i. e. bluish-green. Two ECs are sufficient to describe a hue, because the members of the two pairs of "opponent" ECs, R/G and B/Y, do not occur simultaneously in an elemental color spot. This is because these pairs are steered by the excitations of two antagonistic coding neurons R/G and B/Y, respectively, with only one value each per instant. **2)** The color saturation S is related to the amounts of the achromatic ECs, Bk and W, which constitute the different shades of gray:

$S = 1 - (m_{Bk} + m_W)$.

3) Besides its constant color, every EC possesses, in addition, a constant specific brightness h_i. The total brightness H of a color sensation is obtained as:

$H = \sum_i h_i m_i$, i=1-6.

4) The subjective difference (dissimilarity) d_{12} of two lights, as measured in usual light-discrimination experiments, is related to the (unconscious) excitations A_i of the three color coding neuron types, via the Euclidean metric:

$d_{12} = (\sum_i (A_{i1} - A_{i2})^2)^{1/2}$, i = 1-6.

5) The set-relational color-difference (dissimilarity) measure $d_{EC,12}$ of two (conscious) color sensations $EC_{1, 2}$ is derived via the city-block metric from the amounts m_i of the ECs that the two color sensations have in common:

$d_{EC,12} = \sum_i I m_{i1} - m_{i2} I$, i = 1-6.

The light-difference measure d_{12} and the color-difference measure $d_{EC, 12}$ respectively describe the results of different judgment types. Because of the nonlinear relationships between A_i and m_i, and because of the different types of metric, both measures are structurally different from each other and thus may not be interchanged.

In conjunction with these measures, the CC/EC model allows now even the simulation of psychophysical experiments with mixed types of judgment, e. g. wavelength-discrimination experiments with lights adjusted for equal bright color sensations, as well as different types of color analytical experiments. Best fits to the results of different experiment types finally allowed for the unique determination of the parameters of the model. Besides the description of most common color vision systems, a color vision system could also be fitted and identified that obviously possesses an unusual blue/yellow CC neuron type, which differs only by swapped appearing signs of an excitatory and an inhibitory synapse. This shows that the CC/EC model enables even the identification and localization of individual differences in color vision systems that are commonly classified to be just normal trichromatic.

Retina-Chip Contact Probed by Thermal Noise

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Multielectrode recordings of ganglion cells, the output neurons of the retina, have successfully been applied in studies of computational neuroscience [1]. The retinal ganglion cells furthermore represent an ideal target to bypass damaged photoreceptors and restore visual function by electrical stimulation [2]. A prerequisite that is important for both issues is a tight contact between the retina and an electrode array with a high electrical resistance that allows efficient recording or stimulation.
Here we present a new method to estimate the resistance of a retina-chip assembly in epiretinal configuration. It relies on the evaluation of the intrinsic Johnson noise of the system that is measured with an array of sensor transistors of a CMOS chip [3]. The method is an extension of an approach used to probe the resistance of cell-chip junctions [4].
The retina is attached to the sensor array with poly-lysine. We record the fluctuating extracellular voltage beneath ganglion cell for time periods without firing. The power spectral density (PSD) of the voltage noise is estimated up to a frequency of 3 MHz. The contribution of the retina-chip contact is obtained by subtracting the noise spectrum of the bare sensors. The net noise spectrum does not show frequency dependence up to about 2 kHz. Using a Nyquist-type relation for a volume conductor, we evaluate a resistivity of 2-5 kOhm cm of the retina. That value agrees with measurements by microelectrodes [5]. Circuit modelling of a volume conductor and of the retina-chip interface indicates that the low-frequency PSD is dominated by the retina resistance and not by the larger resistance of the retina-chip contact.

In summary, the Johnson-noise method is a simple way to estimate the quality of the retina-chip contact and for measuring specific tissue resistance. The method may be applied also with metal electrodes with respect to check the quality of neurophysiological recordings and of neuroprostethic contacts.

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Expression of pannexin2 in the mouse retina

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Pannexins are known as hemichannel-forming proteins with high homology to the invertebrate gap junction proteins, the innexins. Their membrane topology is similar to the vertebrate gap junction-forming connexins, with four transmembrane domains, two extracellular loops, a cytoplasmic loop and intracellular N- and C- termini. But, the sequences of pannexins and connexins do not show a high degree of homology. Thus it is thought that pannexins, innexins and connexins represent independent families of channel-forming proteins. Since pannexin expression has been observed in the mouse retina, there is an ongoing discussion whether pannexin and connexin hemichannels may interact to form functional gap junction channels. Our group is interested in whether this potential type of heterotypic channel is involved in mediating the coupling between cones and rods in the mouse retina. Connexin36 (Cx36) is expressed in cones and mediates rod-cone coupling. However, the corresponding connexin expressed in rods has not yet been identified, even in an extensive study using single-cell RT-PCR on isolated rods with 13 connexin-specific primer pairs.

We studied the expression pattern of pannexin2 (Panx2) in the mouse retina. Using single-cell RT-PCR, we first showed that Panx2 is expressed in rod photoreceptors. We then examined Panx2 expression in retinal cryosections using fluorescence in situ hybridisation (FISH) and immunohistochemistry.

FISH with a Panx2-specific antisense riboprobe revealed moderate Panx2 mRNA expression in the inner segments of photoreceptors and in several cells in the proximal inner nuclear layer (most likely amacrine cells), and prominent expression in certain cell somata of the ganglion cell layer. To determine whether the Panx2 mRNA expression observed in the inner segments really arose from Panx2 mRNA expression in rods, we performed double-FISH with the Panx2-specific riboprobe and a mouse rhodopsin-specific riboprobe. These experiments revealed clear co-localization of rhodopsin and Panx2 mRNA in rod inner segments.

Further immunofluorescence studies with newly-generated antibodies against mouse Panx2 revealed prominent punctate Panx2 immunoreactivity in the outer plexiform layer, predominantly in the distal part, where rod spherules are located. In agreement with the Panx2 mRNA expression observed in the proximal inner nuclear layer and ganglion cell layer, very fine moderate punctate immunolabeling was found in the inner plexiform layer. However, double labeling for Panx2 and Cx36 showed neither co-localization nor even close association of the two proteins in the outer plexiform layer.

In summary, these findings show that Panx2 is expressed in rod photoreceptors and, in particular, the protein is found in the distal outer plexiform layer, where rod spherules are located; there it is more likely to form hemichannels than heterotypic gap junction channels with Cx36 expressed in cones.

A criterion for visual performance: Measuring head movement during the optokinetic reflex

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Most animals compensate a global movement of their visual environment by moving their eyes and head to yield a steady retinal image of the world.

Such compensation movement can be triggered by presenting a moving regular stripe-pattern. The resulting involuntary head- and eye-movement has been observed in several animals and is known as optokinetic reflex (OKR).

In this study, we describe an experimental setup for presenting visual 360° stimuli, which induce OKR in animals while simultaneously tracking their head movements.

For this purpose an IR-reflective marker is attached to the animal's head, which can be video taped under infrared light. In a second step the video tape is analyzed by various image processing steps to extract the spatial orientation of the head.

We show that the velocity of the moving visual stimulus can be derived from the recorded head-movement, making the recording of the head-movement a suitable method to determine whether the reflex was induced or not.

The OKR can be seen as a general criterion for the animal's visual performance and the described experimental method could therefore be used to determine the different visual performances of animals. This is especially useful for knockout animals, which are commonly used in visual science but rarely characterized in their behavior.

Chromatic and Achromatic Temporal Resolution

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Introduction:
Temporal coding properties at all levels of visual processing are an essential element of numerous visual abilities such a motion detection or temporal resolution. In recent behavioral and electrophysiological studies it has been demonstrated that “achromatic” temporal resolution (ATR) in goldfish depends critically upon retinal temporal coding properties. However, the question arises what the temporal resolution ability is under different ambient illumination conditions, stimulus intensities or light of different wavelengths and intensities?
Material and Methods:
Behavioral experiments were performed with a two choice forced procedure (Behrend et al., 2007). First animals were tested with achromatic “white” light, i.e. a steady light (training stimulus, TS) and a flickering light (test stimulus (TeS), 100 % contrast) of 5-50 Hz flicker frequency. Light intensities were varied between 6-13000 cd/m^2, the ambient illumination conditions set to 20 or 60 cd/m^2.
In a second set of experiments animals were presented with light of 404, 450, 500, 550, 600, 640 or 680 nm for both stimuli. Light intensities were set to maximal light intensity of the light source plus monochromatic filter and the FFF determined. The light intensities were reduced in semi-log steps until animals could no longer discriminate TS and TeS.
Results:
ATR. For median light intensities of 50 cd and higher, animals achieved a flicker fusion frequency (FFF) of 40 Hz and higher. For median light intensities decreasing below 50 cd, the FFF decreased in a nearly linear fashion with the log intensity, from about 40 Hz at 50 cd to about 20 Hz at about 3 cd.
Chromatic temporal resolution. Animals were able to discriminate training and test stimulus to varying extent, depending upon the wavelength of light and intensity. Generally, temporal resolution decreased with the quantal flow. If the FFF taken at an quantal flow of 1e12 Q/(m2*s) and plotted as a function of wavelength, discrimination falls off sharply from maximally 24-25 Hz at 450 and 650 nm to 1-2 for light of 404 and 683 nm. In the 450-650 nm wavelength range slips to about 12 Hz. The action spectrum of the chromatic temporal resolution exhibits a peak at about 680 nm, a fall off towards 600 nm and a 2 log increase of sensitivity for 20 Hz flicker. For a 25 Hz flicker signal the peak sensitivity remains at 640 nm, sensitivity for light of longer and small wavelengths decreases by about 1 log unit.

Discussion:
Temporal resolution under photopic illumination conditions under which color vision in goldfish is tetra-chromatic is very good for high light intensities and is reduced by about 50 % if the stimulus light intensity drops by 50 %. However, there does not appear to be an optimal light intensity at which temporal resolution is best.
Chromatic temporal resolution is about half as good as the achromatic temporal resolution. This indicates some kind to interaction or pooling of information provided by the different cone types. It appears as if at least for flicker frequencies higher 20 Hz the highest sensitivity is close to the maximal absorption range of the L-cone. This might indicate a major role of a mainly L-cone driven mechanism for temporal coding in the retina.

Pre-saccadic remapping of the motion after-effect

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Continuously performed eye movements create an ever changing image of the world on the retina. Especially the high frequency of saccadic eye movements (2 - 5 saccades per second) calls for a compensative mechanism to transform the changing visual information into a stable percept. To this end, the brain makes use of internal copies of motor commands, also known as efference copy or corollary discharge. Electrophysiological recordings of visual neurons in the primate lateral intraparietal cortex [1] have shown that the receptive fields of some neurons change their position before the onset of a visually guided saccade. Shortly before the execution of a saccade, the receptive fields jump towards their post-saccadic positions. It was argued that this process is crucially dependent on corollary discharge [2]. These shifts of receptive fields may be responsible for the pre-saccadic transfer of the tilt after-effect (TAE) observed in the perception of human subjects [3]. Since static form adaptation shows pre-saccadic remapping, it is consistent to ask whether a similar remapping can also be shown for motion adaptation. The famous phenomenon of the motion after-effect (MAE), also known as waterfall illusion, occurs after prolonged viewing of a unidirectionally moving stimulus. It becomes manifest in an apparent movement to the opposite direction. We modified the experimental design described by Melcher [3] to test moving adapter displays (random dot kinematograms) as shown in Figure 1. We found a transfer of motion adaptation from pre-saccadic to post-saccadic position when human subjects prepared visually guided saccades. The shift of the point of subjective stationarity, signalling the effect size of the MAE, was in the order of 24 minutes of arc/s. In comparison, this shift was almost absent in the control condition, in which the test stimulus was centred on the initial fixation target. Since the proposed sites for motion adaptation are most likely areas V1 and MT, our results suggest that pre-saccadic remapping extends to these low-level visual areas.

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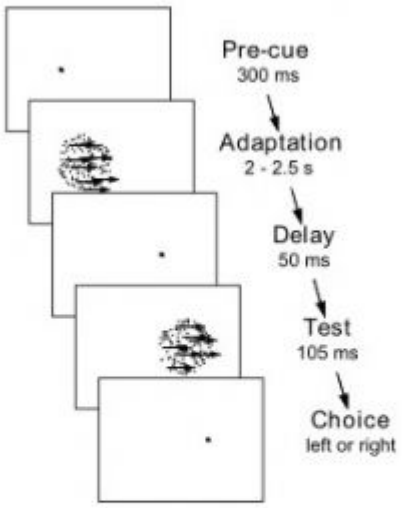

Biber & Ilg
Pre-saccadic remapping
of the motion after-effect
Figure 1

Reorganisation plasticity in the visual cortex of the adult rat after optic nerve crush - a single cell resolution metabolic mapping study

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Incomplete experimental crush of the adult rat optic nerve (ONC) has been suggested to serve as a model of diffuse mechanical axon injury to study the mechanisms involved in recovery of function after degeneration (Genarelli et al., 1989; Kreutz et al., 1998). After incomplete ONC neuronal activity in the contralateral visual cortex is initially decreased, but then recovers during a period of several weeks. The details of how the patterns of neuronal activity in individual cortical columns, layers or cell types change during this recovery process have remained largely elusive. We here investigated the patterns of neuronal activity in visual cortex after ONC in adult rats using thallium autometallography (TlAMG). Thallium autometallography is a novel method for high-resolution mapping of neuronal activity (Goldschmidt et al., 2004). It is based on the fact that in neurons the uptake rates of K^+ and K^+-analogs like the heavy metal ion Tl^+ increase with increasing activity (Keynes, 1965; Landowne 1975). Compared to the 14C2-deoxyglucose method (Sokoloff et al., 1977), which is similar in rationale, thallium autometallography offers the advantage that the tracer can be detected non-radioactively with cellular and subcellular resolution by means of a modified histochemical technique, the Timm-technique or autometallographic method. With this method we find that, after ONC, thallium uptake in the contralateral primary visual cortex is reduced in layers IV and V as compared to the ipsilateral side. During recovery the number of stained layer V pyramidal cells steadily increases, and contra- to ipsilateral differences vanish at around six weeks after the lesion.

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Transsynaptic retrograde labelling in the oculomotor system in rodent using tetanus toxin fragment C and Pseudorabies virus: opportunities and limitations

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Unlabelled non-toxic tetanus toxin fragment C is a useful retrograde transsynaptic tracer that travels into the cell bodies of premotor neurons in the oculomotor system of rodents after an injection into extraocular muscles (1). The traced motor and premotor neurons were detected immunocytochemically with an antibody against fragment C (2). However, this tracer is no longer available.
The present study was undertaken to investigate to which extent two other tracers could be utilized for retrograde transsynaptic labelling in the oculomotor system of rodents: The non-toxic fragment C of tetanus toxin genetically fused to green fluorescent protein (TTC-GFP) (3, 4) and the attenuated Pseudorabies virus strain Bartha carrying DNA for the monomeric red fluorescent protein1 (PrV-614) (5, 6).

After an injection of TTC-GFP (8.5µg/µl) or PrV-614 ($8x10^8$ pfu/ml) into the medial or inferior rectus muscle of mice or rats the corresponding motoneurons in the ipsilateral oculomotor nucleus were intensely labelled. In the PRV-614-injection cases 100h after injection additional virus-infected neurons were found in several areas including the pretectum, the vestibular nuclei and the interstitial nucleus of Cajal. These presumed premotor neurons exhibited the same fluorescence signal intensity as the motoneurons. In the TTC-GFP-injection cases 16h after the injection few weaker labelled neurons were found in the interstitial nucleus of Cajal, not seen at shorter survival times and therefore considered as premotor neurons.
The comparison of both methods revealed that tracing with PrV-614 resulted in strongly labelled premotor neurons, which could easily be detected, whereas premotor neuron labelling was very sparse with TTC-GFP. PrV-614 replicates after reaching the somata of motoneurons thereby functioning as self-amplifying tracer labelling the premotor neurons at undiminished intensity, whereas TTC-GFP tracing is attenuated by a dilution effect and may escape detection. One disadvantage of using PrV-614 is that not every virus injection results in an infection probably due to the individual strength of the immune system. In that respect TTC-GFP is a more reliable tracer with no toxic side-effects, but the quality of retrograde transsynaptic tracing must be improved considerably by using higher concentrations or more sensitive detections methods.

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Diminished plasticity of visual function and sensory maps after cortical stroke in mice

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Stroke is a major cause of death and disability in the industrialized countries. It is an encouraging observation that clinically most patients who do suffer from stroke recover to some degree from the deficits occur. The most straightforward assumption is usually that this is due to plasticity. Many *in vitro* studies indicated that there is an increased plasticity in the perilesional zone of cortical infarcts. In the present study we investigated *in vivo* the impact of a photothrombotically induced cortical stroke on the plasticity of the neighboring visual cortex after short periods of monocular deprivation (MD). Visual function was analyzed behaviorally with a virtual optomotor system. In addition, visual cortical maps were recorded using intrinsic signal optical imaging. After 7 days of MD, control animals showed a significant enhancement of visual acuity of about 18% (0.07 cyc/deg) in the non-deprived eye, from 0.39 cyc/deg at day 1 to 0.46 cyc/deg after 7 days of MD ($p<0.001$, Bonferroni-corrected t-test) and a significant ocular dominance shift towards the open eye in the optical imaging experiments from an ODI of 0.28 (without MD) to 0.03 after 7 days of MD ($p<0.001$, Bonferroni-corrected t-test). In contrast, in animals with a cortical stroke, there was neither a significant enhancement of visual acuity (0.36 cyc/deg at day 1; 0.38 cyc/deg after 7 days of MD; $p>0.05$, Bonferroni-corrected t-test) nor a significant ocular dominance shift (ODI of 0.20 to 0.17; $p>0.05$, Bonferroni-corrected t-test). Thus in contrast to previous in vitro studies, our data rather indicate that plasticity is diminished in the surround of a cortical infarct.

'SPARSIFICATION' OF NEURONAL ACTIVITY IN THE VISUAL CORTEX AT EYE OPENING

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Ongoing spontaneous cortical activity plays a critical role for circuit formation at early developmental stages. In adults, spontaneous activity modulates the integration of stimulus-evoked sensory information. However, the distinct properties of this activity at different developmental stages remain unclear. In the present study, we set out to investigate how eye-opening affects the ongoing spontaneous activity in the mouse visual cortex. We used multi-cell bolus loading technique and video-rate two-photon calcium imaging of neural networks *in vivo*, an approach that allows the detection of action potential firing with a high fidelity and sensitivity.

Here we demonstrate that after eye-opening the spontaneous activity in the *in vivo* mouse visual cortex undergoes a pronounced switch from a dense to a sparse mode of neuronal recruitment. A calcium wave activity emerges in layer 2/3 neurons of the visual cortex at the beginning of the second postnatal week with a low frequency and develops over the next three weeks by a gradual increase in frequency and a decrease in amplitude. Before eye-opening, this ongoing activity is organized in a wave-like activation of the majority of layer 2/3 neurons (75%) that is driven by Up state-evoked neuronal firing. This activity is highly sensitive to antagonists of NMDA receptors. Around eye-opening the fraction of active neurons per wave decreases markedly to 19%, reaching 15% in adults. Importantly, our results suggest that a developmental shift in the excitatory/inhibitory balance underlies the switch from dense to sparse neuronal activity before and after eye-opening, respectively. This sparsification process does not require visual experience since adult mice reared in the dark display similar sparse activity.

Thus, at the onset of vision an immature, dense mode of neuronal recruitment is transformed into a sparse spontaneous activity that may contribute to the feature-selective activation of individual neurons in the mature cortex.

How high is the spatial resolution of the human EEG?

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Mainly for technical reasons, commonly used EEG systems use 32 to 256 recording channels with inter-electrode distances between 1.3 and 4 cm. Thereby it is generally assumed that human EEG has high temporal resolution on the order of milliseconds, but only poor spatial resolution on the order of several centimetres. However, there are only few experimental and theoretical studies quantifying the spatial resolution of the EEG by using well defined criteria. In the present work we use spatial spectral analysis of evoked potential components to quantify the spatial resolution of the EEG. Spatial resolution is thereby defined by the spatial Nyquist frequency, which is twice the maximum spatial component frequency of the signal derived from the spatial spectrum. From the spatial Nyquist frequency the critical inter-electrode distance can be derived, which is required to sample the evoked potential distribution on the head without aliasing. Previous studies using a similar approach to analyse the spatial resolution of evoked potentials conclude that an inter-electrode of about 3 cm is sufficient to reconstruct the evoked surface potential distribution. However, most of these studies are based on recordings with standard EEG-electrodes with diameters and inter-electrode distances on the order of 1 cm, and thus might not have captured spatial fine-structures of the evoked potential on the head, properly. We therefore have designed a flexible, linear band of 64 gold-pin electrodes with a spacing of 3 mm to provide sufficient over-sampling. The electrode-band was positioned between the standard Position O1 and Cz, i.e along the occipital retinotopic gradient proceding from the posterior to the anterior part of the left, dorsal pole of visual cortex. We tried to evoke spatially periodic, retinotopic activity in this cortical region by presenting concentric ring segments of high contrast in the right lower quadrant of the visual field (0.52 s stimulus duration, 1.52 to 2.2 s inter-stimulus-interval). Ring segments were varied in number and size. Spatial spectra of early visual evoked potential components (latencies < 150 ms) displayed an upper cut-off point at about 0.5 cycles/cm. Also, we found that these spatial spectra carried retinotopic information about the applied stimuli in a spatial frequency band between 0.2 and 0.5 cycles/cm. These findings suggest a Nyquist frequency of about 1 cycle/cm. Thus, an inter-electrode distance of about 1 cm would be required to reconstruct the observed retinonotopic spatial fine-structure of the visually evoked potential components without aliasing. Our findings indicate that the spatial resolution of the human EEG might be higher then previously assumed.

The main sequence of human Optokinetic Nystagmus

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Optokinetic nystagmus (OKN) is a reflexive eye movement evoked by visual motion, which serves to stabilize the retinal image e.g. during head movements. It consists of two alternating phases: a slow phase in direction of the stimulus motion and a fast phase in the opposite direction. Two kinds of OKN can be distinguished. The so-called stare-nystagmus is observed when subjects view the stimulus attentively without intentionally foveating any element. It is characterized by small fast-phase amplitudes and a high fast-phase frequency. If subjects intentionally follow single elements of the stimulus they perform a so-called look-nystagmus which is characterized by a low frequency but large amplitude of the fast-phases. While stare-nystagmus is a reflexive eye movement, the slow-/fast-phases of look-nystagmus have often been associated with voluntary pursuit/saccades. Studies comparing the gain of pursuit and slow-phases of stare- and look-nystagmus reported similar values for look-nystagmus and voluntary pursuit. To our best knowledge, however, fast-phases of look- and stare-nystagmus have not yet been compared. The so-called main sequence which describes the tight relationship between amplitude, duration and peak-velocity is widely used for analysing fast eye movements. In this study we aimed at disentangling the relative influence of task and stimulus characteristics on such fast eye movements. To this end we compared the main sequences of fast phases elicited during stare- and, look-nystagmus, as well as those of spontaneous and visually guided saccades.

Eye movements were recorded at 500 Hz with an infrared eye tracker (Eye Link II, SR Research). The subjects' heads were stabilized by a chin rest. Stimuli either were presented on a 22'' (40° × 30° visual angle) monitor or were projected onto a tangent screen (70° × 55°) via a CRT projector. Optokinetic eye movements were elicited by a random dot pattern (RDP, black dots on a gray background) moving horizontally to the left or to the right at 10°/s. Subjects were either instructed to i) stare at the screen without following individual dots (stare-nystagmus condition) or ii) to track individual dots (look-nystagmus condition). Spontaneous saccades were recorded while subjects looked at a homogeneous gray screen without any instructions concerning their eye movements. Finally we recorded visually guided saccades to stationary targets and targets moving at 10 °/s either in the same or the opposite direction as the saccade. A moving RDP with identical properties as in the OKN experiment served as background in this condition.

Across subjects, fast-phases during stare-nystagmus had longer durations and lower peak-velocities than the fast-phases during look-nystagmus. Similarly, spontaneous saccades lasted longer and had lower peak-velocities than visually guided saccades. This indicates that fast eye movements towards visual targets are faster than those without a visual target. Direct comparison of the main-sequence of fast-phases during look-nystagmus with saccadic main-sequences revealed largest similarities to visually guided as compared to spontaneous saccades. Therefore our data support the notion of a close functional relationship between look-nystagmus and voluntary eye movements.

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In vivo dendritic calcium signaling in layer 2/3 neurons of mouse visual cortex

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SPONTANEOUS AND VISUALLY-EVOKED DENDRITIC CALCIUM SIGNALS IN LAYER 2/3 NEURONS OF MOUSE VISUAL CORTEX IN VIVO

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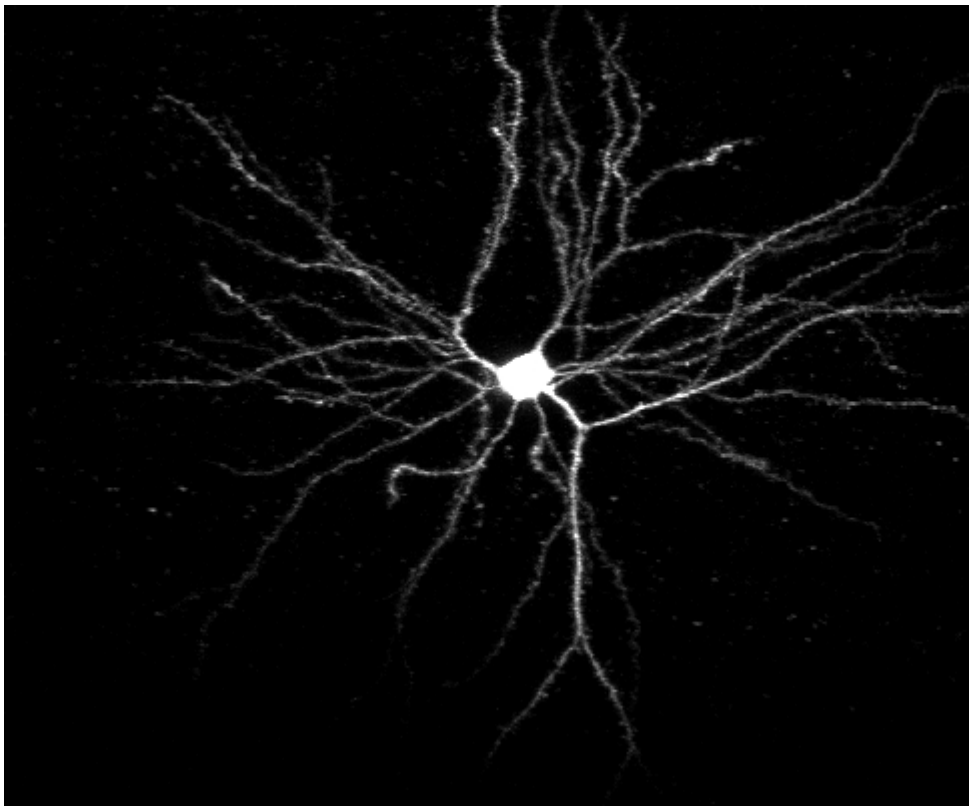
Dendrites are neuronal compartments that are essential for cortical information processing. Up to now, the distribution and integration of visual information in the dendritic tree in vivo remain largely unexplored. In this study, we investigated the spatial profiles of spontaneous and visually-evoked calcium transients in basal and oblique dendrites of layer 2/3 neurons in mouse visual cortex in vivo. By using fast scanning two-photon imaging (based on the use of a resonant galvanometric scanner) and single-cell electroporation with calcium indicators, we recorded calcium signals at focal planes where multiple dendrites of the same cell were visible. We recorded spontaneous activity in darkness as well as activity evoked by oriented moving gratings or white squares.
Our results demonstrate widespread calcium signaling in dendrites during both spontaneous and evoked activity, induced by back-propagated action potentials. We observed that visual stimuli reliably evoke calcium transients in the dendrites of layer II/III neurons of the mouse visual cortex. Dendritic calcium signals were larger and faster compared to those recorded in the somata. The amplitude of the dendritic transients was similar at proximal locations, less than 100 µm away from the soma, and then declined with increasing distance from the soma.
Together, our method and results indicate that back-propagation of action potentials is a robust and reliable property of basal dendrites of layer II/III visual neurons in vivo. These data suggest an active role of basal dendrite signaling for visual information processing in the primary visual cortex.

Figure: Electroporation of layer II/III neurons in mouse visual cortex *in vivo*. A glass pipette filled with 10 mM Oregon Green BAPTA-1 hexapotassium-salt in intracellular Ringer solution, was inserted into layer II/III (~200 µm below the pia). Dye was first injected into the extracellular space in order to visualize with two-photon microscopy individual layer II/III neurons as dark “shadows” within a bright background. The tip of the pipette was then targeted to a selected cell body under visual control. Z-projection of 3D stack of a labelled pyramidal neuron. In all experiments, the location of the imaged neurons in the visual cortex was confirmed *in vivo* by their responses to light flashes and by post-mortem imaging of the stained brain area.



Two-photon imaging of orientation and direction selective neurons in the developing mouse visual cortex

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Although an increasing number of studies are using transgenic mice to identify the molecular mechanisms of plasticity in the visual system, very little is known about the functional properties of neurons in the visual cortex of both developing and adult mice. In this study, we investigated the development of orientation and direction selectivity in the mouse primary visual cortex by using *in vivo* fast scanning two-photon imaging and the multi-cell bolus loading (MCBL) technique with fluorescent calcium indicator Oregon Green BAPTA-1. We imaged the activity of layer 2/3 neuronal populations with single-cell resolution at three developmental stages: at eye opening (P13-P15), during the critical period at 4 postnatal weeks (P26-P30) and after the end of the critical period in adults (P60-P100). In order to investigate the functional architecture of orientation and direction selectivity, we recorded the responses of layer 2/3 neurons to oriented moving gratings.

The proportion of cells responding to moving gratings increased during development from around 15% at eye opening to 48% in the adult mouse visual cortex. At eye opening, the majority of these responding cells were direction selective, whereas the proportion of orientation selective cells was small. Among the responding cells, the proportion of direction selective cells remained stable, while the proportion of orientation selective cells increased by a factor of five during development. Experiments performed in one-month and two-month-old dark-reared animals provided similar results. Taken together, our findings indicate that the development of direction and orientation selectivity involves intrinsic factors and does not require visual experience.

Ocular dominance plasticity in adult visual cortex is limited by the immune receptor PirB

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To explore the function of PirB, a neuronal MHC Class I receptor, optical imaging of intrinsic signals was performed in visual cortex of adult PirB-/- mice. Measurements of the response strength in the binocular zone of visual cortex reveal that the response to contralateral eye stimulation is significantly increased in PirB -/- mice as compared to WT (PirB-/-: 0.65 +/- 0.04; WT: 0.53 +/- 0.03; p=0.03, t-test; n=8). The stimulation of ipsilateral eye resulted in identical responses between the two groups (PirB-/-: 0.23 +/- 0.02; WT: 0.23 +/- 0.03). Consequently, the contra/ipsi ratio, a measure of the contralateral ocular dominance (OD), in adult PirB-/- mice is significantly larger than in WT mice (PirB-/- : 3.2 +/- 0.2; WT: 2.3 +/- 0.2; p=0.009, U Test ; n=8).

Monocular deprivation (MD) of the contralateral eye for 3 days was used to induce an OD shift in PirB-/- or WT mice. Both the mechanism and the extent of the shift differed for the two genotypes. In PirB-/- mice, the OD shift occurred primarily by an unusually rapid and large strengthening of ipsi eye response observed after only 3 days of MD (PirB-/-: 0.50 +/- 0.04; WT: 0.28 +/- 0.02; p=0.004, t-test; n=7). In contrast, OD shift in WT mice resulted from both weakening of the deprived eye and very slow strengthening of the nondeprived eye response. Even though the contra/ipsi ratio between the 2 groups of mice did not differ after 3 days of MD (~1.3), the stronger initial contralateral bias of PirB-/- mice resulted in a larger OD shift. Thus OD plasticity in adult PirB-/- mice is significantly greater than in WT mice.

As shown before, a brief period of MD in WT mice at the peak of the critical period had a “priming” effect on OD plasticity in adults: a second 3 day MD in adult WT mice resulted in larger changes of the contra/ipsi ratio than in adult visual cortex exposed to 3 day MD only once (WT 3dMD: 1.55 +/- 0.23; WT 2 x 3dMD: 1.15+/- 0.05; n=5). However, this conditioning effect is absent in PirB mutant mice: no further OD shift is observed in adult PirB cortex after the second MD (KO 3dMD: 1.32 +/- 0.07; KO 2 x 3dMD: 1.44+/- 0.04; n=7).

These results indicate that PirB normally functions as a “molecular brake” on ocular dominance plasticity, possibly by shifting the threshold for synaptic strengthening vs. weakening. The occlusion of additional OD plasticity by earlier conditioning seen in adult PirB mice suggests that PirB may normally function to prevent excessive recruitment of synapses by any one experience.

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Characterizing the visual system of ageing mice

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Eyesight generally deteriorates in elderly persons (Ferrer-Blasco et al. 2008). This loss of visual capabilities constricts the quality of live, effects ranging from depressed feelings to suicidal tendencies (Mojon-Azzi et al. 2008). Research on the reasons of visual decline in old age, as well as investigations into possible remedies, are thus urgently needed. It is desirable to use the mouse as an experimental animal for such studies, since it allows for a large variety of genetical, pharmacological and surgical manipulations, and a lot of current research on plasticity in the visual system is being conducted in the mouse (Hensch 2005). However, the impact that old age has on the visual system of mice has as yet not been thoroughly characterized. We therefore investigated the visual abilities of ageing mice from 3 months to two years of age using a virtual-reality optomotor system (Prusky et al., 2004). In addition, we visualized cortical maps by intrinsic signal optical imaging in adult (PD109-165), mature (PD208-244) and old (PD 638-757) mice (Cang et al., 2005a; Lehmann and Löwel, 2008).

Visual acuity was 0.39 cycles/degree (cyc/deg) until one year of age and then declined steadily to 0.35 cyc/deg in old mice ($p<0.001$, ANOVA) Interocular plasticity, i.e. the improvement of vision of the open eye after monocular deprivation (Prusky et al. 2006), also significantly declined with age: in adult and mature animals, visual acuity increased by about 22% from 0.39 cyc/deg to 0.51 cyc/deg, compared to an increase of only 12% from 0.35 cyc/deg to 0.37 cyc/deg in old animals ($p<0.001$, ANOVA). Interestingly, visual cortical activity in old mice was also reduced by 33% compared to mature animals (from 2.7 to $1.8x10^{-4}$) and the quality of retinotopic maps (quantified as map scatter according to Cang et al. 2005b) was reduced by 34% (map scatter increased from 1.1 to 1.6).

These results demonstrate that ageing in mice is accompanied by a loss of visual function and a reduction of both interocular plasticity and cortical responses. Our data provide the basis for future research on possible therapeutic interventions to preserve or restore vision in old age.

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Changing functional organization during initial development of orientation maps in ferret visual cortex

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In the visual cortex of higher mammals, preferred stimulus orientation is mapped systematically across the cortical surface. The development of this functional organization is only partially understood. In the ferret, optical imaging of intrinsic signals revealed stably emerging orientation domains around the time of eye opening. However, this method reports only population activity and cannot resolve individual neurons' responses. Electrical recordings identified orientation selective neurons more than a week before eye opening, but their spatial organization was not reported.

Here we use two-photon calcium imaging to map orientation preference at cellular resolution in the developing ferret visual cortex. Around postnatal day 19, neurons exhibit large, spontaneous calcium transients, but visual stimulation evokes only unreliable responses, if any. Within days, and as early as ten days before eye opening, we find a high proportion of visually responsive neurons. Surprisingly, all such neurons at this early time point respond almost exclusively to horizontal stimuli and are distributed uniformly across the cortical surface. Electrical recordings confirm this unusual regime of functional organization. Subsequently, around the time of eye opening, all orientation preferences are present. While most neurons exhibit broad orientation tuning, they are clustered spatially according to preferred orientation, and we never observe spatial mixing of cells preferring different orientations. Adult-like maps emerge as orientation tuning sharpens over the following days.

Our results suggest the following sequence of events in the development of orientation preference. Initially, all visually responsive neurons strongly prefer horizontal stimuli. This early bias could be caused by neuronal activity, or reflect activity-independent mechanisms such as anisotropic connectivity during early axon ingrowth and/or the remodeling of dendritic arbors. Orientation tuning then broadens for all cells as the horizontal bias is lost. Most neurons attain a new preferred orientation, indicating that a fast and dramatic change in orientation preference is a key feature of early orientation map development. Since neurons' new preferred orientations develop in a clustered fashion, we conclude that the development of a neuron's orientation preference is intimately linked to the formation of the orientation map.

Organization of the human MT+ complex as revealed by functional MRI and intraoperative electrical stimulation

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The middle temporal (MT) and medial superior temporal (MST) areas, identified in the superior temporal sulcus of the monkey brain, are considered to be two of the core elements underlying the cortical processing of visual motion. Lesioning of these areas in monkey studies has been demonstrated to result in profound motion perception deficits, i.e. akinetopsia. The human homologs of areas MT and MST (hMT and hMST, respectively) have originally been referred to and treated as one compound, i.e. the human MT+ complex, located in the dorsal/posterior limb of the inferior temporal sulcus. Only recently have areas hMT and hMST been disentangled using functional imaging by demonstrating robust responses to ipsilateral visual stimuli in area hMST not present in hMT. In the present patient study we combined functional MRI with intraoperative electrical stimulation techniques in order to further characterize the organization of the human MT+ complex and its role in visual motion perception. The patient is a 43-year-old female who had been diagnosed with a left temporal tumor after a first episode of word-finding failures. Surgery combined with intraoperative electrical stimulation was intended in the awake patient in order to prevent damage to eloquent brain tissue. Since the posterior parts of the tumor were located in immediate proximity to the posterior limb of the inferior temporal sulcus, we had the opportunity to test the influence of electrical stimulation of hMT+ on motion perception. Prior to surgery, functional MRI was performed in order to separate area hMT from area hMST by resorting to techniques described recently (Becker et al., Eur J Neurosci, 28(8):1674-1685, 2008). In both hemispheres, area hMST could be identified by blood oxygen level-dependent (BOLD) responses to visual motion presented in the ipsilateral visual field. Area hMT was defined by BOLD responses selectively induced by visual motion in the contralateral field. Corroborating earlier findings, area hMST was located immediately anterior to area hMT. Both areas were spared from tumor infiltration. During surgery, the perception of visual motion was assessed by random dot kinematograms presented for 300 ms in the left or the right visual hemifield. All stimuli involved coherent visual motion (100% coherence) in either of the four cardinal directions (up, down, right, or left). Motion direction discrimination was tested in this four-alternative forced-choice paradigm in the absence and presence of the intraoperative electrical stimulation delivered at amplitudes of 2 to 6 mA. Akinetopsia was induced by electrical stimulation of a small patch of cortex (approximately 2 cm x 3 cm) in accordance with the location of hMT+ as determined by fMRI. While deficits in the contralateral visual field were observed after stimulation of the posterior part of this focus, stimulation of its anterior part induced (also) ipsilateral motion blindness. On the basis of these observations, we conclude the following. (i) The human MT+ complex reveals an anterior-posterior organization with area hMST located anterior to hMT. (ii) Lesioning of area hMT as well as lesioning of area hMST results in akinetopsia in humans. (iii) Ipsilateral motion blindness observed after stimulation of hMST but not hMT most likely reflects influences on the contralateral hMT+ complex mediated by monosynaptic projections.

Spike Sorting Errors: Statistical Differences of Cortical 'Single Unit' and 'Single Neuron' Activity.

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Extracellular recording techniques are the preferred experimental means to monitor neuronal spiking activity in the nervous system of animals. Extracting the so-called 'single-unit' activity (SUA) from the extracellular signal involves two steps. First, 'spikes' that are assumed to reflect action potentials (APs) of nearby neurons are detected, e.g. by thresholding. Second, the spike sorting procedure assigns each individual spike to a particular 'unit'. All spikes of one unit are supposed to correspond to the APs generated by one single neuron. However, detection and sorting are error-prone. Spikes may be falsely assigned to a particular unit (false positives, FPs), and some spikes are missed and not assigned to the respective unit (false negatives, FNs). Indeed, several publications have estimated the amount of errors obtained in the process of spike sorting and FP/FN rates on the order of 10-15% (e.g. Pouzat et al., 2004; Joshua et al., 2007) seem plausible.

We investigated the effect of spike sorting errors on statistical properties of cortical spike trains. In particular we focused on 3 statistical features of cortical spike trains, namely (1) the negative serial correlation of neighboring intervals (Lebedev & Nelson, 1996, Nawrot et al., 2007, Engel et al., 2008), (2) the interval variability as measured by the coefficient of variation (CV), and (3) the spike count variability quantified by the Fano factor. To test the effect of FPs and FNs on these statistical measures, we used two methods of generating surrogate data sets with FPs and FNs. Firstly, we used a point process model with a realistic interval distribution and serial interval correlations (Farkhooi et al., 2008) and randomly inserted or deleted spikes from numeric realizations. Secondly, we used in vivo intracellularly recorded spike trains from cortical neurons (Nawrot et al., 2007) and mixed spikes of different independent recordings.

Our results demonstrate that (1) serial correlation is lost and becomes insignificant for a FP rate of about 10-15%, (2) the coefficient of variation monotonically increases with increasing FP or FN rate, and (3) the Fano factor increases even more strongly than the CV as negative serial correlation is lost which reduces the spike count variance in single neurons (Nawrot et al., 2007; Farkhooi et al., 2008). Thus, we conclude that a realistic rate of spike sorting errors can severely alter the statistics of the true single neuron spike train. Our results suggest that spike sorting may lead to a general over-estimation of single neuron variability, and that it conceals serial spike train statistics that are observed in true single neuron spike trains.

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Effects of feature-directed attention on the representation of speed and color changes of superimposed objects

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Previous studies have shown that directing attention to a particular feature of a target object is associated with the co-selection of other, non-relevant features of that object, thus confirming the key prediction of object-based attention theory. In contrast to these findings we recently showed that co-selection of non-attended features is not mandatory. Instead, directing attention to a target feature may result in suppression of the representation of non-attended features at both attended and unattended objects (Wegener et al, Vision Res., 2008). However, these results have been obtained by using objects separated in space, while former results supporting object-based selection used overlapping stimuli. Hence, the question arises whether the feature-based selection mechanisms we have shown may be overwritten by mechanisms of object-based selection when the target object itself is subject to ambiguity, as for overlapping objects. If whole objects are the entities of attentional selection, extracting the correct target object in this case requires binding of its constituent features, which presumably will result in enhanced processing of non-relevant features of the target object. In contrast, if attention might be directed to features alone non-relevant features will not be co-selected but may instead be subject to noise reduction mechanisms in order to enhance the signal-to-noise ratio for the attended feature.

In order to test for this assumption we re-examined our previous findings by using a Posner paradigm and spatially overlapping random dot patterns at the centre of gaze. Subjects were required to attend to one of two target features, either speed or colour, on one of the two objects, and to give speeded responses whenever they detect a change of one of the two features. Assuming co-selection of non-relevant object features one should have expected a processing benefit for the non-attended feature of the attended object. However, our results do not support this prediction. Instead, and in accordance with our former study, the data support a feature-based selection account that is associated with delayed reaction times for non-attended features. These data are in contradiction with strict object-based selection accounts. They favour a view centring task demands as the decisive determinants for neuronal selection mechanisms.

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A multi-electrode array for chronic recordings in monkey area V1 allowing for bidirectional movement of electrodes and fast electrode exchange.

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Neurophysiological studies on brain function often imply the requirement to obtain data from many neurons at the same time. However, when using awake animals the number of electrodes that can acutely be inserted is restricted. According to this, several techniques have been developed that allow for chronic implantation of multi-electrodes arrays. Still, for the work with awake monkeys, a disadvantage of most of these techniques is that they i) have been designed for electrophysiological measurements in the cortex of small animals (rodents and birds), or ii) only allow for controlled movement of electrodes in one direction, or iii) do not allow fast and easy replacement of electrodes. We here present a new technical approach that overcomes these limitations and allows for recordings of single cell activity and local field potentials over prolonged periods of time. The system fulfills the specific requirements for multi-electrode recordings in awake behaving primates. It allows moving the electrode in forward and backward direction in steps of less than 80µm and for a distance within the tissue of up to 15mm, exchanging the set of electrodes in very short time and without the need of an additional surgical procedure or anesthetic intervention, and sterile closure of the trepanation. The system can be easily build and at very low cost.
We present data of the first prototype of this multi-electrode array that was used to record neuronal signals from brain area V1 over a period of 11 weeks. We introduce a new and automated mapping procedure for V1 local field potentials and demonstrate that the new system enables stable recording of population receptive fields in different depths of the visual cortex over many recording sessions. Moreover, stabilizing a neuron by directed forward and backward motion of the electrode facilitated recording of spikes from single cells over a complete recording session.
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Simvastatin improves spatial vision in mice following acute retinal ischemia /reperfusion

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Statins are 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors broadly used to treat hypercholesterolemia. Recent data suggest that statin therapy has pleiotropic effects, including protection against acute and chronic neurodegeneration following ischemic stroke, Alzheimer's disease and inflammatory central nervous system pathologies. In the retina, statins increase levels of stress proteins after an optic nerve lesion and enhance survival of retinal ganglion cells (RGCs). The aim of this study was to evaluate the effect of statin delivery on visual capabilities and cortical maps in mice following acute retinal ischemia/reperfusion.

We used a model of global retinal ischemia by transient elevation of intraocular pressure above systolic blood pressure for 30 or 60 minutes. The statin Simvastatin (20mg/kg) or vehicle was delivered intraperitoneally 24, 48, 72 and 96 hours after retinal ischemia. Mouse vision was quantified by means of a virtual-reality optomotor system (Prusky et al., 2004) on days 1, 3, 6 and9 after ischemia. Visual cortical maps were recorded by optical imaging of intrinsic signals (Cang et al., 2005; Lehmann & Löwel, 2008). Finally, the number of RGCs surviving the insult were determined after specific labelling with an antibody against b-III-tubulin in flat-mounted retinae 9 days after the injury.

Sixty minutes of retinal ischemia caused a complete loss of visual function and a significant reduction in the thickness of retinal layers (~ 15%). After 30 minutes of ischemia, spatial vision was reduced by ~ 0,24 cyc/deg on day 1 after the lesion and a significant loss of RGCs was observed (control: 106.000 cells/retina; ischemia: 90.000 cells/retina). While we observed an improvement of visual acuity in all groups (statin and sham treatment) until day 9 after the lesion simvastatin treatment starting 24 h after the lesion significantly improved both RGCs survival (statin: 103.000 cells/retina) and spatial vision: from 0,17 cyc/deg on day 1 to 0,33 cyc/deg on day 9 in statin-treated animals and from 0,16 cyc/deg on day 1 to 0,27 cyc/deg on day 9 in sham-treated animals (t-Test, $P<0,05$). Optical imaging revealed that the activity of visual cortical maps differed between statin-treated and control animals (t-Test, $P < 0,001$) and between statin- and sham-treated animals (t-Test, $P < 0,05$).

Administration of statins may constitute a suitable approach to treat neurodegeneration associated with retinal ischemia or other ocular diseases such as diabetic retinopathy and glaucoma.

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Proprioception in the extraocular eye muscles of different species

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The aim of this study is to review the variety of sensory terminals in eye muscles and investigate their function using neuroanatomical methods.

In vertebrates extraocular muscles differ in many ways from skeletal muscles. Although there is no stretch reflex in extraocular muscles, proprioceptive signals do reach the brain [1] and may be important for eye alignment during fixation and slow eye movements. Three different types of proprioceptors are found in extraocular muscles: muscle spindles, Golgi tendon organs and lastly, palisade endings which are unique to eye muscles [2]. There is a considerable variation among different species with regard to the presence of proprioceptive organs. Whereas spindles and Golgi tendon organs are well developed in sheep and pig, neither are found in cat, and only poorly developed muscle spindles are present in human. Palisade endings are cuffs of nerve terminals located at the myotendinous junction [4], and have been found in all vertebrates. While the proprioceptive function of muscle spindles and Golgi tendon organs is well established, the function of palisade endings (PEs) is not clear. Furthermore the, location of their cell bodies is also unknown. PEs give rise to terminals with both motor or sensory characteristics. Whereas a motor function of PEs is suggested by their expression of cholinergic markers the vast majority of neurotendinous junctions support a sensory function [3].

In an attempt to localize the cell bodies of the palisade endings neuronal tract-tracers (cholera toxin subunit B; WGA-HRP) were injected into the myotendinous junction of extraocular muscles in monkeys. Sections of the trigeminal ganglia were treated with antibodies against the tracer and choline acetyltransferase (ChAT).

The analysis revealed that a consistent population of small and medium-sized neurons is tracer-labelled within the ophthalmic part of the trigeminal ganglion. A small fraction of these neurons exhibits ChAT-immunoreactivity, which is an unexpected property of sensory neurons. Mainly based on their cholinergic properties palisade endings have been considered as motor nerve endings. Although more experiments are necessary to find out the functional role of palisade endings, the present work provides new data to support the hypothesis that palisade endings could arise from an unusual population of cholinergic sensory neurons in the trigeminal ganglion.

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Neuronal correlates of luminance change in the pigeon brain – a population code of the visual Wulst<

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The avian visual wulst is widely proposed to be a functional analogue to the mammalian primary visual cortex (V1) based largely upon its physiological properties and input connectivity. However, we have recently shown that the spatiotemporal dynamics of its response to oriented gratings differs in many respects to V1, casting further question upon its actual functional role in the avian visual system.
Applying in vivo voltage-sensitive dye imaging to the anesthetized and paralyzed pigeon, we show that the visual wulst contains two spatially distinct regions that each code for a different direction of luminance change. The visual stimulus is a uniform change in luminance across a large screen covering ~120º x 80º of the visual field. Lowering luminance locally activates an anterior part of the wulst, followed by gradual spread of activity; whereas the opposite (luminance increase) initiates activation at a posterior locus.
We also demonstrate that the strength of luminance modulation is represented by the number of recruited neurons and by changes in the amplitude of the peak response.
A similar mapping of luminance change is known to exist within thin stripes of the secondary visual cortex (V2) in primates. Our data provides further evidence that the visual wulst is not a simple analogue of V1 but incorporates V2 functions as well.

Measuring optokinetic response in adult zebrafish and medaka

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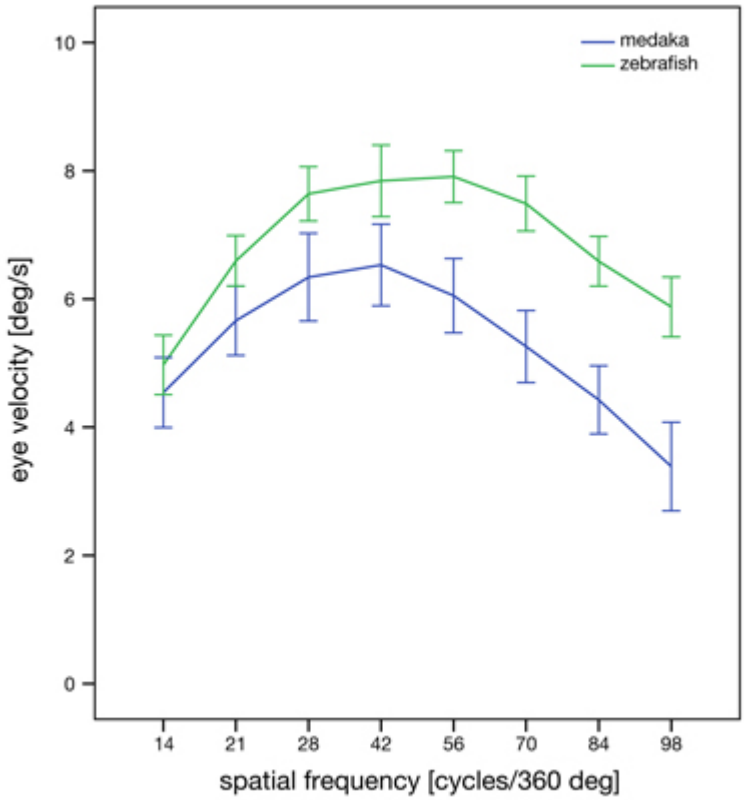
The optokinetic response (OKR) is a robust visually evoked behavior that does not need prior training. Therefore, it is an ideal tool to screen for visual defects in vertebrates. This assay is well established in larval zebrafish. Larval zebrafish can be immobilized in methylcellulose, and because of their transparent body, the dark pigmented eyes can easily be recognized and tracked by computer programs.

Applying the OKR to adult zebrafish is met by two technical hurdles: First, the fish have to be restrained in movements without using any anesthetics or neuromuscular-blocking drugs, since the fish need to be conscious and muscles need to be fully functional. Using methylcellulose would not work for adult fish, since a constant water flow has to irrigate the gills to keep the fish alive and to minimize the amplitude of ventilatory movements. Second, the eyes cannot be tracked as easily as in larval zebrafish, since the body of adult zebrafish is not transparent anymore and the contours of the eyes in transmitted infrared light is not visible as clearly as in larvae. In addition, eye movements in adult zebrafish are much faster and more jittery than in larval fish, making it necessary to record eye movements at higher framerates and to modify evaluation methods to filter saccadic eye movements and to extract reliable eye velocities of OKR slow phase.

We could successfully restrain fish by gently clamping the body between two pieces of sponge fitted in a flow-through chamber, continuously supplying the fish with oxygenated water. By shading body pigmentation through overlay of a virtual white triangle image, the eyes could reliably be tracked.

We applied this technique to show that eye velocity is higher and more constant in the temporal-to-nasal than in the nasal-to-temporal direction. A comparison of the OKR of adult zebrafish and medaka revealed only minute differences in contrast sensitivity and temporal resolution. Only for the spatial resolution we found significant differences: spatial resolution seems to be lower in medaka fish. In addition, saccade frequency is higher in medaka than in zebrafish.


medaka
zebrafish
eye velocity [deg/s]
0
2
4
6
8
10
14
21
28
42
56
70
84
98
spatial frequency [cycles/360 deg]

Analysis of temporal flash patterns in the visual system of the European starling (*Sturnus vulgaris*)

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The faster an animal is propagating through the natural environment the better should be its visual temporal resolution. Accordingly, the critical flicker-fusion frequency (CFF) is higher in birds than in humans (e.g., Nuboer, et al. 1992, *Brit. Poultry Sci.* 33: 123). The CFF describes the response properties for steady-state visual signal processing. For the processing of transient visual signals, however, the doublepulse resolution (DPR) provides a better description of the processing performance. This measure reports the threshold for detecting a temporal gap between two light flashes in relation to the size of the gap. In humans, the DPR can reach about 10 ms (e.g., Treutwein und Rentschler 1992, *Clin. Vision Sci.* 7: 421).
The DPR of four European starlings (*Sturnus vulgaris*) was measured in an operant Go/NoGo procedure. Four humans were tested as well for comparison. The individuals had to detect double-flash stimuli (DFS) in a background of repeated single-flash stimuli (SFS). The duration of each flash in the DFS was 5 ms, and the gaps were varied between 1 and 60 ms for starlings and 25 and 60 ms for humans. SFS varied in duration from 11 to 70 ms. The luminance of the stimuli was also varied. Resulting psychometric functions were analyzed via signal detection theory. For a d' of 1.8, starling thresholds ranged from 18.2 to 29.9 ms, and human thresholds obtained with the same stimuli ranged from 30.7 to 37.8 ms ($p=0.005$, t test). This finding suggests that the starling's visual system has a higher temporal resolution for transient tasks than the human visual system.
The comparison of responses to SFS and DFS has also been used to investigate audio-visual integration. In humans, single light flashes accompanied by multiple auditory beeps can lead to the illusionary perception of multiple flashes (Shams, et al. 2000, *Nature* 408: 788). Similarly, single auditory beeps accompanying multiple flashes can lead to the illusionary perception of fewer flashes (Andersen, et al. 2004, *Brain Res. Cogn. Brain Res.* 21: 301). Here we tested whether the European starling would be a suitable model organism for the investigation of such a multisensory interaction.
The influence of auditory beeps onto the visual perception of flashes was investigated in four starlings via an operant Go/NoGo procedure. Like in the DFS threshold determination, the starlings were trained to detect double-flashes in a repeated single-flash background. Beeps that were not synchronized to the background SFS were presented with a probability of 20 %. All possible combinations of 0 to 2 flashes accompanied by 0 to 2 beeps synchronized to the flashes (except for the 0 flashes 0 beeps condition) were used as test stimuli. The gap between flashes in the DFS was fixed to a duration for which response rates of 30 % were expected. Beeps were presented at 85 or 95 dB SPL. The starlings' probability of response to the one flash one beep condition did not differ from the one flash two beeps condition in both series of experiments ($p>0.05$, least significant difference t test). Also, the response probability in the two flashes two beeps condition did not differ from that of the two flashes one beep condition ($p>0.05$, least significant difference t test). These results suggest that the starling lacks the visual fission or fusion illusion that can easily be elicited in humans.

Stronger activation of the medial superior temporal area in migraineurs with aura compared to patients without aura

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Differences between people with and without migraine on various measures of visual perception have been attributed to abnormal cortical processing due to the disease. However, the issue concerning the degree of involvement of later visual cortical areas in the interictal phase of this disease and the direction of the related cortical excitability change in migraine patients has been heavily discussed. The aim of the present study was to explore the dynamics of the basic interictal state with regard to the extrastriate, motion-responsive middle temporal area (MT) and medial superior temporal area (MST) using blood oxygenation level dependent (BOLD) functional magnetic resonance imaging (fMRI). fMRI at 3 Tesla was performed on 18 migraine patients (9 with aura (MwA) and 9 without aura (MwoA)) and 9 healthy subjects, with different coherent and incoherent moving dot stimuli. To improve group analysis, the individual cortical folding pattern was accounted for by using a cortical matching approach. All motion stimuli activated a distributed cortical network, including striate and extrastriate areas. Compared with healthy controls and MwoA patients, MwA patients showed significantly higher signal changes in response to visual stimulation in bilateral MST, but not in MT. This implies an enhanced responsiveness of MST of MwA patients in the interictal period and strengthens the hypothesis that hyperexcitability/enhanced responsiveness of the visual cortex in these patients goes beyond primary visual areas. The present study demonstrates for the first time the significant differences in the BOLD response of visual area MST in patients with aura compared to control subjects and patients without aura.

Receptive field shifts in macaque primary visual cortex induced by saccade adaptation

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The saccadic system is capable of rapidly adapting to changes in oculo-motor conditions (e.g. changes in the mechanics of the eyeball) that otherwise would lead to movement inaccuracy and poor vision - an effect usually referred to as saccade adaptation. This adaptation challenges the neuronal mechanisms that guarantee visual perceptual stability across eye-movements. The neural basis of this perceptual stability is currently unknown. In our current study we therefore have mapped receptive fields (RFs) in primary visual cortex while the monkey performed a saccade adaptation task.

RFs were mapped by presenting Gaussian luminance patches at random positions on a CRT monitor under three different conditions: during fixation, in a classical saccade task and during saccade adaptation. Saccade adaptation was elicited by a perisaccadic displacement of the saccade target by an amount of 15% of saccade amplitude against the direction of the eye movement. To obtain spatiotemporal RFs we used a stimulus response correlation technique.

Response latencies did not differ between the three conditions. However, RF positions were different during saccade adaptation trials as compared to fixation or saccade trials. RF locations calculated from stimuli presented during an early postsaccadic period (0...50 ms after saccade offset) in adaptation trials were shifted in the direction of the saccade (i.e. opposite to the direction of the target displacement) compared to non-adapted saccade and fixation trials. These shifts in RF position compensated for about 20 percent of the gain change of the saccade amplitude. RFs calculated from stimuli presented later than 100 ms after saccade offset, did not exhibit this effect any more.

We conclude that eye movement signals do have an influence on spatial information processing in V1 - a mechanism probably involved in the maintenance of perceptual stability across saccadic eye movements.

Human Ocular Following Response to Sampled Motion

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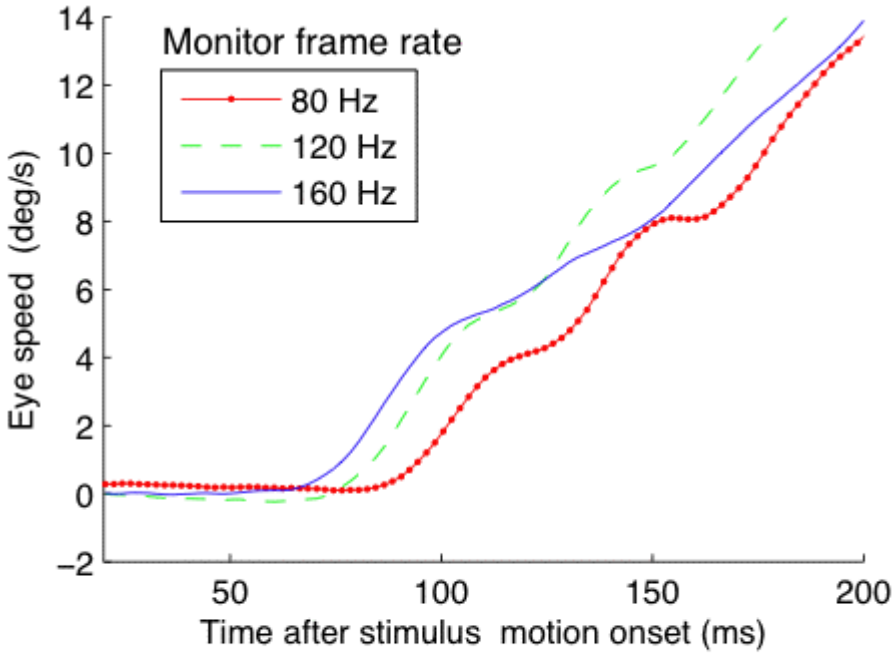
It is nowadays common practice in visual experiments to use computer-generated stimuli presented on a monitor. We find that the short-latency ocular following response (OFR) to a moving random dot pattern considerably depends on the frame rate of the presented stimulus in the range of 80 to 160 Hz, which is far above the human flicker fusion limit. More specifically, the latency of the response decreases and the acceleration increases, so that the overall response becomes stronger for higher monitor frame rates. Also, we find an increase of OFR strength on double-frame presentations, that is, with every frame being displayed twice. Concluding, the temporal discreteness and mode of presentation of digital stimuli might be more important to oculomotor and motion perception studies than commonly assumed. We show that all effects can be explained on the basis of spatiotemporally oriented receptive fields early in the visual pathway.



Mean ocular following response (141 trials) of subject LB to repetitive stimulation with random dot patttern moving at 30 deg/s, displayed at 3 different monitor frame rates.

Pigeons being spoilt for choice: a study on hemispheric dominance

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The avian visual system is asymmetrically organized. While the left hemisphere is mainly concerned with detailed analyses of visual features, the right hemisphere is mainly in charge of configurational processes and recognition of individuals in a social context. This functional lateralization is accompanied by various anatomical asymmetries. Even though much research has been devoted to lateralization we still do not know the exact neuronal mechanisms leading to functional asymmetries. Work on functional asymmetries commonly focuses on comparing performance measures between the hemispheres, hemispheric dominance, however, is rarely investigated in animal models.

The aim of the present study was to investigate if the left hemisphere, which performs better in visual discrimination tasks, also dominates the right hemisphere during visual discrimination. Pigeons were trained monocularly on a colour discrimination task. In this forced choice task they learned two stimulus pairs consisting of an S+ and an S- each. One stimulus-pair was trained with the right eye occluded, the other pair with the left eye occluded (Figure 1A+B). After reaching learning criterion the animals were tested binocularly with compound stimuli. They had to choose either the S+ of the right eye combined with the S- of the left eye or the S+ of the left eye combined with the S- of the right eye (Figure 1C).

In these tests the pigeons chose significantly more often the S+ they had learned with the right eye. Since the optic nerve of avians is almost completely crossed, we conclude that the left hemisphere does not only show better performance in visual discrimination, it also dominates the right hemisphere during visual discrimination.

To investigate the underling mechanisms of this dominance the role of the visual wulst was examined in a second part of the experiment. Test trials were conducted with Tetrodotoxin or Saline injections into the left or right visual wulst. While blocking of the right visual wulst had no influence on performance, blocking of the left visual wulst resulted in an increased pecking on the S+ the animals had previous learned with the left eye. In other words, blocking the left visual wulst led to a loss of dominance and in some animals even to a reversal of dominance. The visual wulst therefore plays a key role in shifting the dominance to the left hemisphere.

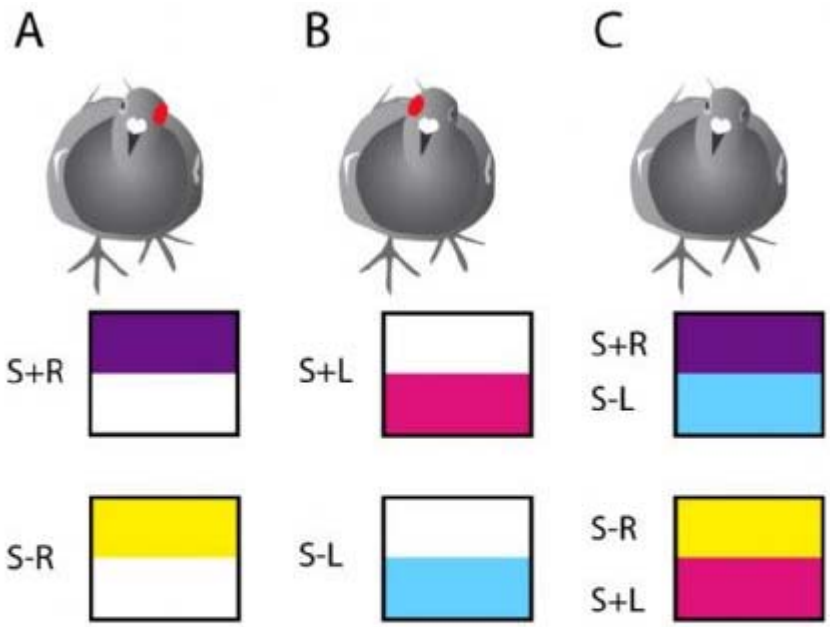

Figure 1: Colour discrimination in a forced choice task. Pigeons learned one stimulus pair with the right eye (A) and anotherone with the left eye (B). Afterwards they were tested with combined stimuli (C).

The onset of cortical activity after visual stimulation can be identified with multifocal visual evoked potentials (mfVEPs)

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Purpose: Multifocal visual evoked potentials (mfVEP) are usually applied to simultaneously record the evoked cortical activity from many parts of the visual field. We here present a different application of the mfVEP method to identify the temporal onset of cortical activity after visual stimulation. The aim of this study was to compare the interindividual variability of this new latency measure with the variability of the P100 peak implicit time in standard visual evoked potential (VEP) recordings.
Methods: 30 visually normal subjects participated in the experiment. Binocular mfVEPs were recorded as voltage difference between two electrodes placed 4 cm above and below the inion. Dartboard patterns with 60 fields were presented within a circular stimulus field with a diameter of 41°. Within the dartboard fields checkerboard patterns with a mean luminance of 86,1 cd/m2 and a contrast of 99.8% were reversed in contrast following m-sequence stimulation. Second order mfVEP traces were squared and averaged across all 60 fields resulting in one multifocal power function (MPF) for each subject. Due to the squaring procedure noise fluctuations do not cancel out but generate a MPF pedestal. Cortical activity after visual stimulation leads to an increase of the MPF regardless of the mfVEP waveforms. Latency was defined by the onset of a sudden MPF rise above the noise pedestal. For comparison VEPs to reversing checkerboard patterns with a check size of 0.4°, a mean luminance of 45 cd/m2, and a contrast of 99.7% were recorded for each subject using a Oz-FPz derivation. The implicit time of the major positive peak near 100ms (P100) was used for analysis.
Results: (1) The averaged MPF across all subjects showed a steep rise starting 43.8 ms after visual stimulation and leading to a MPF increase by a factor of 6.6 during the following 10 ms. (2) Each of the 30 subjects showed a sudden steep MPF rise between 40 and 51 ms. The MPF waveform differed strongly between subjects for later time intervals. (3) Average results (mean ± SD, n=30) were 45.0 ± 2.8 ms for MPF latency and 101.9 ± 5.4 ms for P100 implicit time.
Conclusions: MPF analysis of mfVEP recordings clearly indicates the onset of cortical activity after visual stimulation. The low interindividual variability of the MPF latency suggests that this measure of signal transmission time is less modulated by the individual cortical anatomy than the implicit time of the P100 component. The MPF latency data demonstrate that about half of the P100 implicit time is required for signal transmission to the visual cortex while the other half reflects intracortical processing. Thus MPF may help to distinguish between pre-cortical and intracortical involvement of neural dysfunction, e. g., in cases of demyelinating diseases of the visual system.

Vision and visual cortical maps in mice with a photoreceptor synaptopathy: Bassoon mutant mice have reduced but robust visual capabilities

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How little neurotransmission in the visual system is sufficient to promote decent visual capabilities? This question is of key importance for therapeutic approaches to restore vision in patients who suffer from degenerative retinal diseases. In the retinae of mice, mutant for the presynaptic scaffolding protein Bassoon (Bsn), signal transfer at photoreceptor ribbon synapses is severely disturbed due to impaired ribbon attachment to the active zone. Here we have investigated Bsn-/- mice as a model system to study the central processing and visual capabilities of animals with severely disturbed retinal processing by using two different behavioural tasks and optical imaging of intrinsic signals. Both visual acuity and contrast sensitivity were significantly reduced in mutants compared to littermate controls: Visual acuity of Bsn-/- mice was reduced by about 0.2 cycles/degree (cyc/deg) compared to Bsn+/+ littermates in both the optomotor and the visual water task (Bsn-/-: 0.22 resp. 0.37 cyc/deg; Bsn+/+: 0.39 resp. 0.56 cyc/deg) and contrast sensitivity was reduced by a factor of 2 to 6, depending on the tested spatial frequency. Daily testing from eye opening showed that Bsn-/- mice reached adult values of both visual acuity and contrast sensitivity already at four weeks of age. Interestingly, optical imaging of visual cortical activity revealed essentially no differences between adult Bsn-/- and Bsn+/+ mice in both amplitude of the cortical signals and quality of retinotopic maps and cortical maps were also adult-like at four weeks of age. These results show that i) while Bassoon-dependent fast exocytosis is essential for normal vision surprisingly good visual performance – at least at higher image contrasts – can be achieved in spite of a severely impaired signal transfer at photoreceptor ribbon synapses, ii) both the development and maintenance of visual cortical maps does not depend on the presence of photoreceptor synaptic ribbons and iii) visual development in Bsn-/- mice is completed at four weeks of age indicating that the later developing ectopic synapses do not seem to improve vision or affect visual cortical maps. Thus, the central visual system can make use of slow and weak retinal signals to subserve surprisingly robust vision.

Impact of oxytocin on cells of the primary visual cortex in albino and pigmented rats

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Albinism is a genetic defect that causes a number of distinctive phenotypic features and in addition a number of visual and visuomotoric dysfunctions. One visuomotoric deficit is the loss of the optokinetic nystagmus (OKN). In combination with the loss of the OKN, a change in the intracellular chloride concentration and in the chloride reversal potential of pyramidal cells of the primary visual cortex (VC) in albino rats was found.
In this context we presume changes in the chloride Cotransporters KCC2 and NKCC1, which are responsible for the extrusion (KCC2) respectively the uptake (NKCC1) of chloride.
Tyzio *et al*. (2006) observed a significant reduction of the intracellular chloride concentration in the fetal and neonatal rat hippocampus on day of delivery. This is due to the peptide-hormon Oxytocin, which is assumed to down-regulate the activity of the cotransporter NKCC1 and to shift the excitatory action of GABA to inhibitory. This mechanism should protect fetal neurons during parturition. These findings raise the question if the treatment of albino rats with oxytocin can shift the chloride reversal potential of cells of the visual cortex to values found in cells of pigmented rats. To directly investigate the effect of oxytocin, we injected oxytocin from the postnatal day 7 or only one-time and compared the physiological properties of visual cortical pyramidal cells in pigmented and albino rats.
Significant differences were found between animals which were treated only once with the peptide hormone and such treated with NaCl for control. The chloride reversal potential is shifted towards more negative values (-73 mV) in cells of albino rats treated once with oxytocin in comparison to neurons of albino rats treated with NaCl for control (-44 mV). The neurons of the NaCl-treated animals show the same reversal potential of chloride such as the cells of untreated animals. The treatment with oxytocin for only one-time shifts the reversal potential to values observed in neurons of untreated pigmented rats.
Application of Carbachol was performed to elicit seizures in slices of the visual cortex both of pigmented and albino rats. Comparing the seizures of the both strains we want to examine if cells of the visual cortex of albino rats are more exciting than cells from pigmented one.

Gaze allocation during natural behavior in the real world

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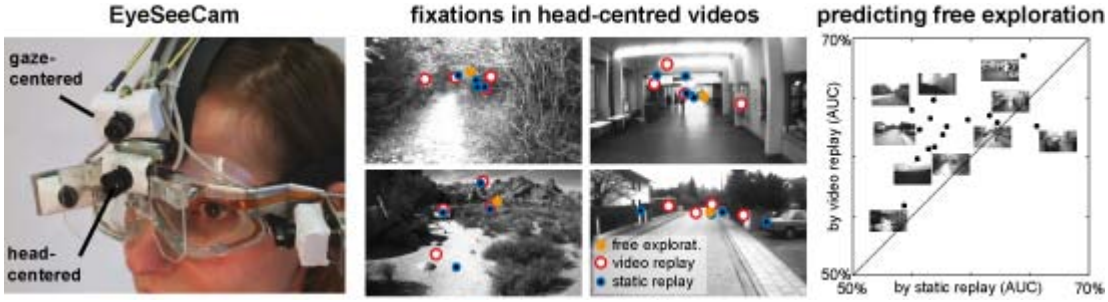
"Natural" gaze allocation is typically measured by tracking observers' eye position while they view photographs of complex scenes. Despite the obvious advantages of such well-controllable experimental conditions, typical laboratory experiments do not adequately capture the rich dynamics of natural input. In part these are generated by head-in-world movements, which provide a coarse centering on potentially interesting ("salient") regions of the visual field, subsequently refined by eye-in-head movements. Furthermore, stimuli and display properties have to be carefully chosen to avoid biases by the artificial setup. These constraints raise the question as to what extent laboratory experiments are informative for gaze allocation under truly real-world conditions? Using a novel mobile eye-tracking setup ("EyeSeeCam"), we measure gaze position during free exploration of various in- and outdoor environments, while simultaneously recording head-centered and gaze-centered videos. Head-centered videos are then replayed in a standard laboratory setup, either preserving their temporal continuity ("video replay") or as sequences of temporally unrelated 1-second frames ("static replay"). We find that the statistics of natural gaze allocation and thus natural retina-centered input exhibit a rich dynamics, which are not adequately modeled by a series of static images interleaved by saccades. Laboratory conditions show a pronounced bias of eye position to the center of the stimulus, which is stronger for static than for dynamic presentations. Consequently, inter-observer consistency is higher in the static replay condition, though not fully explained by spatial bias alone. This leaves room for image-specific bottom-up models to predict gaze beyond generic biases. Indeed, the "saliency map" model predicts eye position above chance in all 3 conditions, but best for continuous replay. Real world gaze is better predicted by the video replay condition than by the static replay condition in 14/15 environments ($p<0.001$, sign-test). However, since prediction of real-world gaze allocation is far from optimal in either laboratory condition (maximally 69% area under ROC curve), real-world recordings are inevitable to probe the validity and restrictions of laboratory results for the real world. These results show that experiments and models benefit from preserving the spatial statistics and temporal continuity of natural stimuli to improve their validity for real-world gaze behavior.



Stimulus evoked neuronal synchrony is reduced under saccadic viewing conditions

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A growing number of studies investigates influences of natural viewing conditions on stimulus coding in the visual system. We recently investigated neuronal coding properties of multi-unit activity (MUA) and local field potentials (LFP) recorded in primary visual cortex of macaque monkey under passive and saccadic viewing conditions [1], focusing on mean responses.
A different feature of neuronal activity that has been proposed to support object perception is synchrony of cell ensembles representing a stimulus [2].

We analysed multi-channel recordings from primary visual cortex (V1) of one and V4 of two macaque monkeys with respect to differences in signal coupling across different viewing conditions. Correlations in LFP and MUA were significantly reduced when an object was brought into the RF by a saccade, as compared to when it was switched on in the RF during fixation. LFP coherence in the gamma range was about 40% lower in the saccadic viewing condition than in the fixation condition.

The abrupt onset of a stimulus in the RF, as used in many studies investigating coding properties in the visual system, evokes transient stimulus locked oscillations of neuronal activity. Similar results were recently reported for saccades in darkness [3]. However, the underlying processes do not seem to interact constructively, at least not under the conditions of our experiments, where the presented objects had no behavioral relevance.

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[2] von der Malsburg (1981)
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Internal Report 81-2

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Transient Cortical Excitation at the Onset of Visual Fixation
Cerebral Cortex 18(1): 200-209

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Spatio-temporal topography of saccadic suppression

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Visual perception is modulated during saccadic eye movements. Contrast thresholds for the detection of luminant visual stimuli are significantly increased briefly before, during and after saccades. Previous studies measured the temporal evolution of this suppression but did not investigate any position dependency. Accordingly, in our current study we explored the contrast threshold for visual stimuli presented during saccades at different positions in the visual field.
Eye movements were recorded in human subjects with an infrared eye tracker (EyeLink 2, SR-Research) running at 500 Hz. Visual stimuli were presented on a CRT monitor or on a large tangent screen in front of the subjects who initially fixated a target left from the center on the horizontal meridian. 500 to 1000 ms after trial onset, the fixation target was switched off and a saccade target appeared right from the vertical meridian. Visual stimuli were presented perisaccadically for 10ms at different positions in the visual field.

The detection rate of stimuli was reduced during saccades as compared to steady fixation. However, this reduction was not constant across space but increased with retinal eccentricity. Also the time of peak saccadic suppression turned out to be a function of stimulus position within the visual field. Maximum suppression occurred earlier at and near the saccade target as compared to regions at and around the initial fixation target.
We developed a numerical model to get a better insight into the underlying neural processes. In essence, our model combines previously described psychophysical data on retinal contrast sensitivity and perisaccadic shifts of attention. Based on our model data we conclude that visual perception is perisaccadically suppressed at a global scale. The observed spatio-temporal topography of saccadic suppression most likely results from the eye movement dependent stimulus eccentricity and additional attentional effects.

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Single cell responses to instantaneous speed-changes of various positive and negative amplitudes in macaque area MT

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Motion perception has been extensively studied in psychophysics and physiology, and neuronal activity of macaque area MT has been directly related to the subjective perception of both speed and speed-changes. However, most of the present understanding of differential motion detection is limited to psychophysical studies and little is known about the underlying neuronal mechanisms. The small number of physiological experiments addressed this issue mainly with gradually accelerating stimuli. We here present first data from macaque area MT estimating responses of single- and small multi-units to instantaneous speed changes of different amplitude. Neurons were stimulated with a Gabor patch of preferred motion direction and spatial frequency while the animals were engaged in a dimming task at fixation. Gabors moved at one of two basic speeds (2.15 and 3.6°/s) for 750ms and were then rapidly accelerated or decelerated by 5, 10, 15, 20, 25, 50, 100, or 200%, and moved at this speed for another 500ms. Prior to this, all neurons were characterized for direction and frequency tuning, and an extensive characterization of their constant speed responses to all of the 32 speeds used in the study was carried out.

Preliminary analysis of a set of about 75 neurons revealed real speed tuning for about 20% of the cells, whereas 80% show an independent tuning for spatial and temporal frequency, being in agreement with previous findings on speed tuning properties of MT. As a first step to analyse neuronal sensitivity to speed changes of positive and negative amplitude, we separately computed the average firing rate of the transient and the sustained response component after the speed change. It might be assumed that transient and sustained responses differ in such a way, that the transient may generally reflect a change in stimulus speed, whereas the sustained response represents the amplitude of the change. Yet a first comparison of the acceleration index that can be computed by comparing the cell's activity before and after the speed change does not show systematic differences between transient and sustained response components. Instead, for accelerating as well as for decelerating events even the transient component closely follows the cell's speed tuning. This seems to be at least true for smaller changes in the stimulus' speed, whereas for larger changes responses to positive and negative speed changes are more likely to deviate from the cell's speed tuning curve, frequently being associated with overestimation of small speeds. Ongoing analysis will aim to make a closer connection between the cell's speed tuning and its ability to represent speed changes of different amplitudes by using support vector machines for analyzing the specific information content of transient and sustained responses.

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FUNCTIONAL ARCHITECTURE OF SUPERFICIAL HORIZONTAL CONNECTIONS.

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This study sheds light on the role of long range horizontal connections that originate from pyramidal neurons in layer 2/3 of the neocortex. Adjacent pyramidal cells extend their axons laterally to converge in patches of synaptic boutons. Viewed from the cortical surface these axonal patches appear like the etals of a flower, the so-called "Daisy Architecture" (DA). We injected intracellularly 30 superficial pyramidal neurons (Layer 2 and 3) in the cat primary visual cortex. Simultaneously we used Optical Imaging to acquire functional maps of the same cortical area. The individual neurons were reconstructed in 3D and aligned with the functional maps. With this method we are able to look at the morphology of physiologically characterized pyramidal cells in relation to the activity of the whole population. Despite the fact that all the cells belonged to the class of layer 2 and 3 pyramidal neurons and had the typical patchy organisation of their boutons in common their axonal arborizations were highly inhomogeneous. The number of patches per neuron ranged between 2 and 8. These findings are consistent with the findings from Binzegger et. al. 2007. Single axonal arms branching close from the cell body can form 1 or 4 distinct patches along their way through the superficial layers. Neurons sitting very close to each other, up to 20 um, can show a similar or dissimilar distribution of their patches and contribute to the same or different patches. The relation of the morphology and the functional maps showed that neurons located in the center of an orientation domain send their axons to their neighbouring orientation domains but do not project to all of their neighbouring domains. We conclude that single superficial pyramidal neurons provide a very selective and individual patchy output to their same layer target neurons. A neuron channels the common input from the center of the daisy individually to different petals of the DA thus contributing only partially to the whole DA. One daisy needs a whole set of these architecturally different neurons to be complete.

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How do prior expectations shape contour integration?

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Contour integration is a process which links oriented edge elements that are aligned colinearily into a coherent percept. This process is thought to rely on feedforward or recurrent integration mechanisms, making its percepts very salient and independent of top-down or on-going processes ("pop-out"). However, recent studies have shown that attention can strongly modulate contour integration, suggesting the confluence of bottom-up (sensory input) and top-down (prior expectations) processes might even be a necessary prerequisite for contour integration. To uncover neural substrates and mechanisms underlying the influence of prior expectations and attention on contour integration, we combine psychophysical with electrophysiological investigations: Participants had to carry out two experiments with identical visual stimuli but different behavioural tasks: a detection task (A) and a discrimination task (B). Stimuli consisted of vertical and horizontal ellipses formed by colinearily aligned Gabor elements, which were embedded in a field of Gabors with random orientations and positions. Each hemifield could contain either one vertical, one horizontal, or no ellipse. All combinations of these three basic configurations were possible, totalling to nine stimulus categories for the two hemifields. In experiment A participants had to give a yes response whenever one stimulus contained at least one ellipse, in experiment B observers had to give a yes response only when a target was present (this target could be either a horizontal or a vertical ellipse, in any hemifield of the stimulus).

In the detection task, reaction times (RT) for horizontal ellipses are ~70 ms shorter than for vertical ellipses. In the discrimination task, RTs for targets are consistently shorter than for distractors, even if the bias for horizontal ellipses is taken into consideration. The presence of redundant targets (e.g. two horizontal ellipses instead of only one horizontal ellipse) also shortens RTs. Thus, the psychophysical data clearly demonstrate a pronounced influence of higher cognitive processes on contour integration.

In our EEG recordings, we find pronounced differences in event-related potentials (ERPs) between stimulations with and without the presence of contours. These differences appear at about 110-160 ms after stimulus onset in the occipital regions of the cortex. We also discovered strong modulations of the evoked potentials with the number of contours present (about 140 ms after stimulus onset in the same regions). Neural correlates related to top-down processes are weaker and need the collection of more data, or the application of more advanced methods in data analysis, for obtaining consistent results.

Further acquisition of behavioral and electrophysiological data is carried out to support the psychophysical finding that top-down processes influence contour integration. We also aim at uncovering the mechanisms of contour integration by focusing on the temporal dynamics of this process being revealed by the shapes of the ERPs.

This work has been supported by the BMBF as a part of the National Bernstein Network for Computational Neuroscience (Bernstein Group Bremen).

Attentional alteration of direction tuning of neurons in macaque area MT to two spatially separated motions

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Attending to a spatial location or to non-spatial features of visual stimuli modulates neuronal responses in the visual cortex of primates. Previous extra-cellular recording studies have shown that switching attention from outside the receptive field (RF) to a single stimulus inside the RF of neurons in the extrastriate visual cortex causes a multiplicative modulation of the neuron's tuning curve. It is unknown whether attention acts in a similar manner for tuning curves created by a systematic variation of more complex stimuli. We investigated this issue by recording single neurons from the middle temporal visual area (MT) of two rhesus monkeys using two random dot patterns (RDPs) moving within two spatially separated stationary apertures, sized and positioned to fit within the classical RF.
The monkeys were trained to attend to one of the two patterns (the target) while maintaining its gaze on a fixation spot and ignoring the other pattern. The target was specified by a cue that preceded every trial. The monkey was required to detect either a luminance change in the fixation spot (*attend-fix* condition) or a transient change of direction or speed in the RDP either inside the RF (*attend-in* condition) or far outside the RF (*attend-out* condition). In the latter two conditions the cue appeared at the same location and moved in the same direction as the target pattern. The two RDPs inside the RF always moved with a relative angle of 120 deg. Tuning curves were determined in the *attend-fix* and *attend-in* conditions by systematically varying the RDPs' directions. In the *attend-out* condition a pattern with either preferred or null direction was attended while motion in the preferred direction was present in the RF.
The tuning curves showed two peaks corresponding to the two stimulus configurations in which one of the patterns inside the RF moved in the neuron's preferred direction. Comparing the tuning curves in the *attend-in* and the *attend-fix* conditions, we found a strong attentional enhancement for the peak corresponding to the target pattern. Furthermore, this peak was significantly broader in the *attend-in* condition. One of the two monkeys showed a significant attentional suppression of the second peak. Changes in neuronal responses to the preferred direction caused by spatial and feature-based attention were in agreement with earlier findings (see [Treue & Martinez Trujillo 1999]).

Thus, our findings indicate highly non-multiplicative changes to the direction-tuning of MT neurons to bi-vectorial motion when attention is switched from outside to inside the RF. This can be accounted for, at least partially, by a combination of modulatory influences of spatial- and feature-based attention. We have also collected data on attentional modulation of MT neuronal responses to bidirectional, but spatially overlapping RDPs (see contribution by A. Lochte et al.). That study has shown an even higher enhancement (without broadening) of the peak corresponding to the attended pattern and no evidence for the second peak suppression. A possible explanation for the latter might be the fact that our design, unlike the transparent case, allows selective modulation of the two stimuli by spatial selection and by changes of the RF profile.

Visual evoked activity in V1 of anesthetized rats: from gratings to natural images

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In the primary visual cortex, preliminary studies showed that spiking activity dynamic is strongly influenced by the level of complexity of visual stimuli. In particular, it has been shown that spiking activity becomes sparser and more reliable under natural like condition, compared to the classical use of simplified artificial stimuli (such as moving gratings). Since in-vivo-experiments often rely on anesthetized animals, stimuli that were used to mimic natural conditions have been movies or static images animated according to a realistic eye-scan. Note, however, that this differs from real natural conditions, in which the occulomotor feedback is present.

In the present study, we want to investigate the dynamics of visual evoked activity in the primary visual cortex of anesthetized rats. For that purpose, we used extracellular recording techniques to monitor both spiking activity and local field potential (LFP) of a cortical volume using different types of visual stimulations. We have chosen to study V1 of rat because recent studies showed that the response properties of these neurons are surprisingly well tuned, with cells responding to a highly specific set of stimulus parameters and having receptive field organization characterized by complex interactions between center and surround components. Such findings indicate that the responses of these rodent V1 neurons are as specialized, in many ways, as those of highly visual animals (cat, primate). Technically, we recorded network activity with a 3 x 4 array of extracellular electrodes arranged in a plane perpendicular to the cortical surface. The visual stimulation paradigms we used are characterized by different levels of complexity, ranging from simple full-field bright flashes and drifting gratings to moving natural scenes. For the natural like condition, we animated a static picture by a saccadic eye movement model. The model was build according to the features of the saccadic behaviour of the rat described in the literature and includes fixations, saccades, micro-saccades and drifts. It is a major aim of this study to reveal how stimulus complexity is reflected in the spatio-temporal patterns of sensory input-evoked activity, and how dynamic properties of the network change according to it.

Consistent with previous studies, preliminary results showed that V1 neurons are indeed tuned to the features of artificial stimuli, such as orientation, spatial and temporal frequency of moving gratings. Under natural like conditions, we observed that activity dynamics changed and became sparser and more reliable as previously described in cats and monkeys. In particular, we observed that the major increase in reliability occurs within transient epochs of strong firing rate increase. In contrast to previous studies, however, these transient episodes of high activity seem not to be related to fixation onsets but rather to the saccades themselves.

Stimulus and task-related gamma oscillations in monkey V1 induced by local and global contours

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Gamma activity has been linked to mechanisms relevant for visual contour integration and selective attention. Recently we have shown that gamma activity can also be modulated by expectancy.Here we further investigate this effect by means of arrays of Gabor patches containing embedded Gestalt figures.

Our previous findings have shown that gamma oscillation strength increases steadily in the course of the trial for responses to drifting gratings. This suggests that the anticipation of a behaviorally relevant event is associated with increased neuronal synchronization. In the present study, we observe a similar effect using complex stimuli, both for central and peripheral (calcarine sulcus) receptive fields, indicating a spatially widespread effect. Expectancy could modulate gamma synchronization at the main frequency component induced by complex stimuli demanding contour integration (mean frequency, 53 Hz). Overall, these results support the existence of a gain mechanism capable of spatially non-selective modulation of gamma activity in the primary visual cortex. These findings are in accordance with the general notion that widespread coordination of oscillatory activity is required for bridging sensory inputs and internally-generated ongoing signals into a unified cognitive act.

Task-dependent viewing behavior in school children

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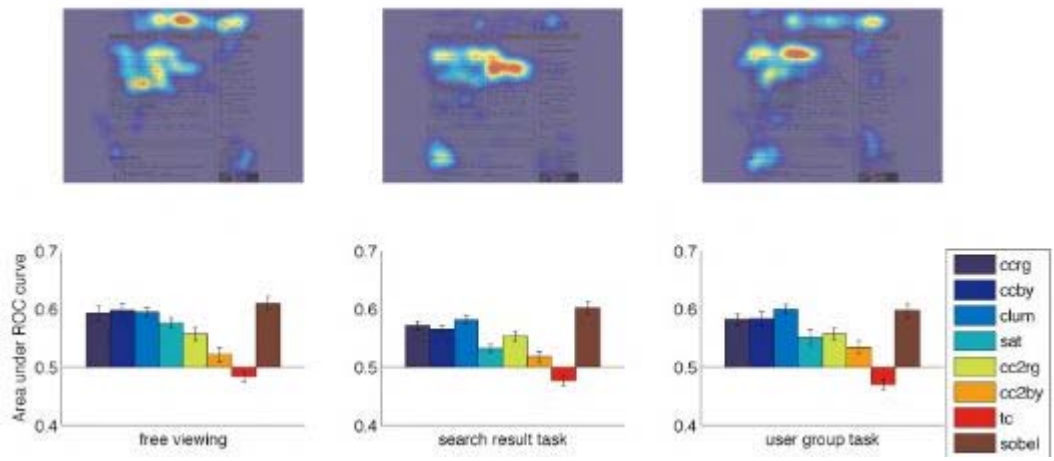
Task-dependent viewing behavior was investigated by tracking eye-movements in school children. Furthermore, we analyzed whether such behavior, if present, is accompanied by changes in the correlation between low-level image features, assumed to be part of the bottom-up hierarchy of visual processing, and fixation points.

In the study 24 pupils, aged 11 to 12 years, viewed screen-shots of 60 different web pages. The experiment was divided into three task blocks: subjects were instructed (1) to look at the pages carefully (free viewing); (2) to rate the relevance of a page in relation to a previously presented search term (search result task); (3) to pick the prototypical user that best matched the page from a selection of five possible users, presented after the stimulus had been seen (user group task).

Our analysis revealed that the fixation patterns differed significantly for all pairwise comparisons of tasks ($p<0.01$, established with a bootstrapping technique). The different fixation patterns on a sample stimulus can be seen in the upper part of the figure. To assess the correlation between fixations and different image features, we then calculated the area under the ROC curve (AUC) for separating fixations from control locations for eight different image features (red/green contrast, blue/yellow contrast, luminance contrast, saturation, 2nd order red/green contrast, 2nd order blue/yellow contrast, texture contrast and edges; see figure bottom part). An ANOVA with feature and task as factors and AUC as the dependent variable showed significant main effects ($p<0.01$) with no interaction ($p>0.5$). This demonstrates that, although the total magnitude of the feature-fixation correlations varied with tasks, the relative weighting of different features in the bottom-up hierarchy was not changed.

Our results show that in the used paradigm, task-dependent viewing behavior is present in school children, and that it is not accompanied by changes within the bottom-up hierarchy. In a related study investigating young adults in a similar setting, largely similar results were reported (Betz et al., submitted). We conclude that the effects found by both studies cannot be explained by a modulation of the influence the features investigated have on fixation selection. They are likely to be genuine top-down influences that are independent of bottom-up image processing (strong top-down). In that case, independent top-down processing would either be an inherent feature of visual attention or learned at a very early stage.



Gamma oscillations in the visual Wulst of the owl: a comparative study

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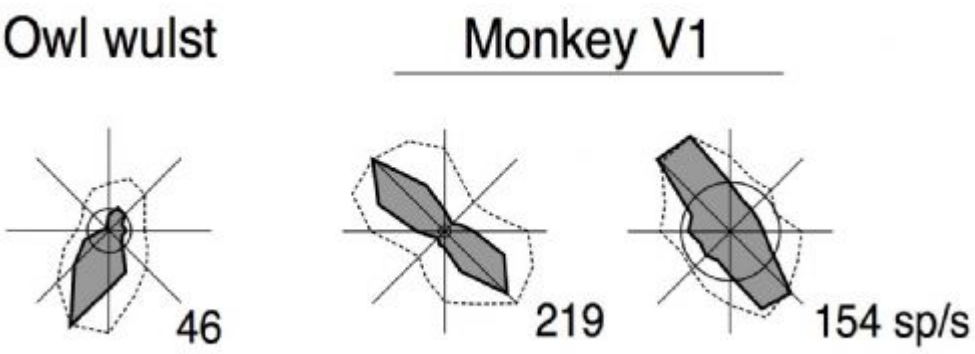
Gamma oscillations have been associated with various integrative processes in the visual system. Numerous studies in the mammalian cortex show an increase in power for the gamma frequency band (30 to 60 Hz) in response to visual stimuli, such as drifting gratings and plaids. Here we extend this observations to a non-cortical structure, the visual wulst of the owl.

We used drifting gratings to determine the dependence of gamma-oscillations on the direction of movement. Simultaneous recordings were obtained from the foveal and parafoveal representation of the visual wulst in seven awake owls. We compared direction tuning of LFP signals with the tuning of multiunit and single-unit activity recorded simultaneously from the same electrode. In many instances, we found a close correspondence in tuning between LFP within the gamma band and spiking activity. Our results are interesting in two respects: 1) they suggest that, as proposed to be the case in the mammalian visual cortex, gamma-band oscillations may play an important role in visual processing in the owl forebrain; 2) they are consistent with the notion that a columnar organization of direction selectivity exists in the visual wulst.

Figure1. Comparison of tuning properties for responses to moving gratings in the wulst and in V1. Solid lines, tuning curves for single cell responses. Dashed lines, curves for gamma power of the LFP recorded from the same electrode. Notice that in the wulst, similar to V1, the tuning characteristics of the LFP matches the properties of single neurons.



Toral lateral line units of goldfish, *Carassius auratus,* are sensitive to the position and direction of sphere vibration

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According to theoretical studies (Curcic-Blake and van Netten 2006, J. Exp. Biol. 209, 1548-1559; Goulet et al. 2008, J. Comp. Phys. A 194, 1-17) the spatial pattern of water motions caused by a vibrating sphere contains information about sphere position and sphere vibration direction. To investigate if midbrain (Torus semicircularis) lateral line units code for sphere position and/or the direction of sphere vibration, we stimulated goldfish, *Carassius auratus*, with a vibrating sphere (diameter 10 mm, frequency 50 Hz, stimulus duration 500 ms). Sphere vibration directions were 0° (parallel to the long axis of the fish), 45°, 90° (perpendicular to the surface of the fish) and 135°. The sphere was positioned in 5 mm steps along the long axis of the fish, the distance between fish and sphere was varied between 5 and 20 mm. Recordings from the Torus semicircularis revealed that lateral line units had single or multipeaked receptive fields. While none of the units (n = 15) responded exclusively to a certain sphere position, in some units a small change in sphere position led to a phase shift of 180°. In addition, the response amplitudes and/or the response phase of some midbrain lateral line units changed dramatically, if the direction of sphere vibration was altered. Although an increase in the distance between fish and sphere caused a decrease in evoked activity, the shape of the receptive fields always remained unaltered. With respect to phase and shape the receptive fields of some midbrain lateral line units showed similarities with the receptive fields of medullary lateral line units and primary lateral line afferents of goldfish.

Neural Encoding of Bulk Water Flow in the Midbrain of the Goldfish (*Carassius auratus*)

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Fish use their lateral line to detect weak water motions and pressure fluctuations that occur in naturally flowing bulk water. How the brain processes bulk flow information is not known, however. Here we report initial investigations of the responses of midbrain lateral line units in the torus semicircularis of the goldfish (*Carassius auratus*) to controlled unidirectional water flows.

The water velocity in a circular 35 l tank was increased from 0 to 14 cm s^{-1} in either a head-to-tail or tail-to-head direction (see figure, upper part of the figure and lower respectively). The mean spike frequency (bin width 1s) of the recorded units was calculated (black traces) and correlated to water current velocity. A hotwire anemometer (grey traces) and hydrophone were used to visualize the experimental water flow and pressure (acoustic) stimulus components, respectively. The recordings were performed with 1MO tungsten electrodes placed in the midbrain contralateral to the side of stimulus application.

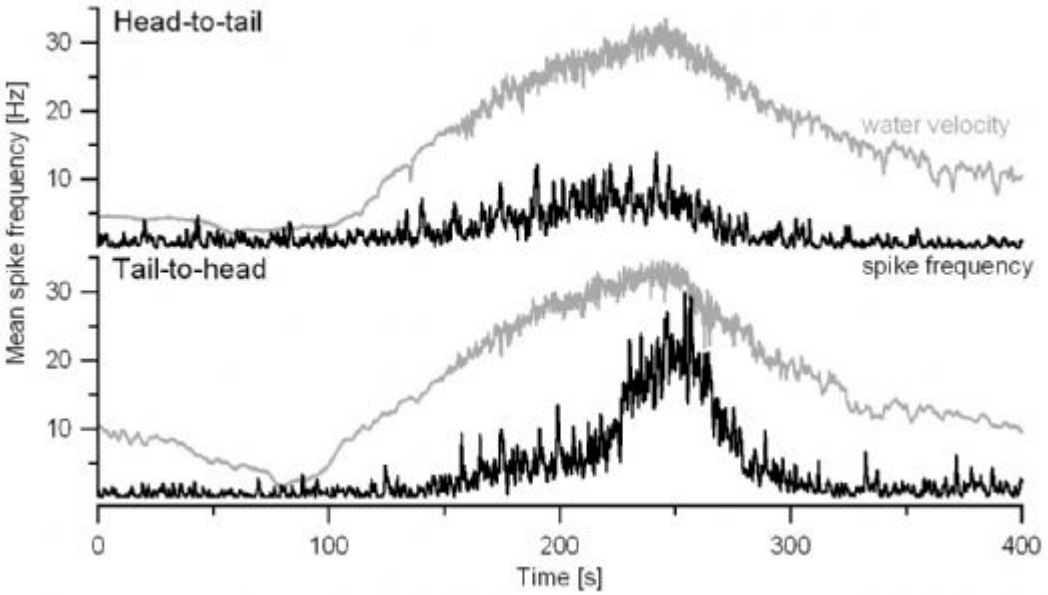
Most units increased their spike frequency when increasing the water velocity, but the background activity, response shape and response variance differed between units. The largest spike frequency could often be correlated with the highest water velocity, but in some cases the response decreased before the highest water velocity was reached, suggesting tuning to a particular flow velocity.

The anemometer recordings revealed that, for our experimental set-up, the amount of high-frequency energy in the water flow increased rapidly above a critical velocity (see grey traces after 150s). This is a result of micro turbulences superimposed in the flow. Nearly all units responded only after the appearance of this high frequency turbulence in the flow stream. The responsiveness to high frequency water turbulences is in principal consistent with the theory that these turbulences are cross correlated to determine the water velocity with a neuronal delay line (Chagnaud et al., J. Neurosience 28(17), 2008).

In contrast to peripheral lateral line nerve fibres, which do not code bulk water flow direction (Chagnaud et al., Zoology 111, 2008), we found units which showed huge asymmetry in response between head-to-tail and tail-to-head flow (see figure), or responded almost only in one direction.

Thus midbrain neurons in the goldfish are responsive to the velocity, direction and fine structure of bulk water flow and can correlate these parameters with input from other sensory modalities.

Supperted by DARPA and the EU


Head-to-tail
Tail-to-head
water velocity
spike frequency
Mean spike frequency [Hz]
30
20
10
0
100
200
300
400
Time [s]

Object localization using sensor equipped artificial lateral line canals<

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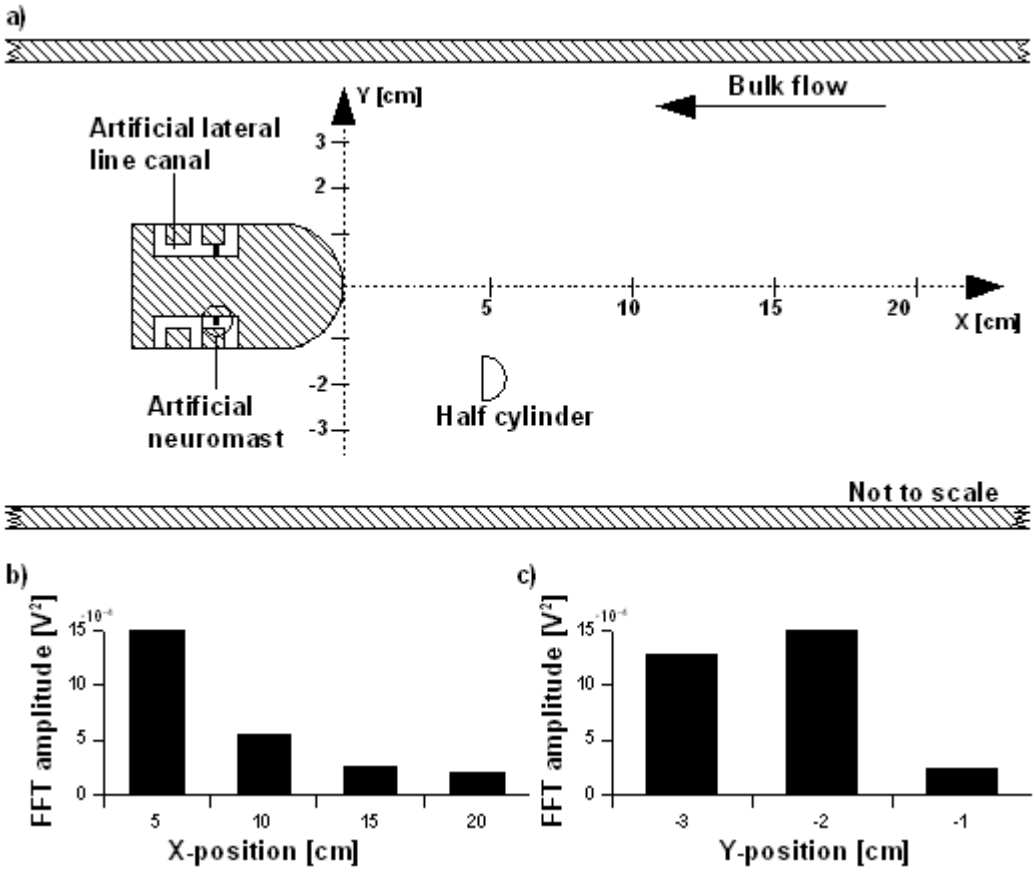
Fishes use the mechanosensory lateral line to detect weak water motions. The lateral line of fishes consists of up to several hundred superficial neuromasts and of neuromasts embedded in canals. We equipped two artificial lateral line canals (ALLCs) with artificial neuromasts and exposed them to a vortex street that was generated with a cylinder (or half cylinder) placed in running water. Using the voltage output of the artificial neuromasts we found that vortex streets cause oscillations in the ALLCs whose temporal and spatial distribution depend on the position and size of the cylinder. Since the vortex shedding frequency is a function of both, cylinder diameter and bulk flow velocity, the Fourier patterns of the water motions not only can be used to detect and localize an object (cylinder) but also to calculate its size, provided bulk flow velocity is known.

Figure 1a shows the setup. A half cylinder (1.5 cm) was placed at 28 positions upstream to the ALLCs. Bulk flow velocity was 7.9 cm/s. The frequency amplitude of the voltage output of the artificial neuromasts (one of which is marked with the circle in Fig. 1a) decreased with increasing distance between the half cylinder and the ALLCs (Fig. 1b, data for Y = -2 cm and X = 5, 10, 15, and 20 cm). Oscillations were only present if the cylinder was placed ipsilateral to the canal that housed the neuromast (Fig. 1c, X = 5 cm, Y = -3, -2 and -1 cm).

We propose that simple ALLCs can be used to measure hydrodynamic landmarks and thus for navigation.

Supported by DARPA


a)
Bulk flow
Y [cm]
Artificial lateral line canal
3
2
-2
-3
5
10
15
20
X [cm]
Artificial neuromast
Half cylinder
Not to scale
b)
FFT amplitude [V²]
15
10
5
0
5
10
15
20
X-position [cm]
c)
FFT amplitude [V²]
15
10
5
0
-3
-2
-1
Y-position [cm]

Discrimination of complex hydrodynamic stimuli in rheophilic fish

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Vortex streets are common in aquatic habitats. They are generated, for instance, by the undulatory body movements of a swimming fish or by submerged objects situated in running water. Predatory fish use fish generated vortex streets to detect and track down prey fish. If exposed to a vortex street, rheophilic fish may alter their body kinematics such that they save locomotive energy. Despite the ubiquitous presence of vortex streets in the aquatic habitat and their biological importance we do not know whether and to which precision fish can detect and discriminate vortex streets. We trained rheophilic fish (ides *Leuciscus idus*) to swim to a food dispenser if exposed to a vortex street generated with an object placed in running water. Our behavioural experiments clearly show that fish can recognize vortex streets. We currently investigate whether and to which precision fish can discriminate vortex streets that differ with respect to the vortex shedding frequency and/or the direction of vortex rotation.

Dipole detection and discrimination by the oscar, *Astronotus ocellatus*

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Fish detect hydrodynamic stimuli with the mechanosensory lateral line and the inner ear. We wanted to know how well oscars, *Astronotus ocellatus* can detect and discriminate sinusoidal water motions of different frequencies and which type of sensory information they use for these tasks. In addition, we studied whether discrimination performance is affected by background noise in the form of turbulent water motions.

Five Oscars were trained by food reward to approach a stationary sphere (radius 4mm) that was vibrating at 100Hz (S^+). Correct responses were scored if fish turned towards and approached the sphere during stimulus presentation (3 seconds). Blank trials (no sphere vibration) were interspersed between stimulus trials to determine spontaneous approach rates. In still water, Oscars showed between 71% and 85% correct responses to S^+. Across animals, the percentage of false alarms during blank trials was smaller than 20%. Detection thresholds for the 100Hz stimulus ranged between 0.003µm and 0.01µm (mean 0.006µm) peak-to-peak water displacement on the surface of the fish (values calculated according to Harris und van Bergeijk 1962, JASA 34: 1831-1841). To study frequency discrimination, fish were trained to ignore the sphere when it was vibrating at a different frequency (S^-). Four fish learned to ignore 70Hz, two fish learned to ignore 80Hz, and three fish learned to ignore 150Hz, i.e., they discriminated these frequencies from 100Hz. Fish did not learn to discriminate 90Hz or 130Hz from 100Hz. In turbulent water fish showed a slightly inferior response to S^+ (between 61% and 73 % correct responses), but they were still capable to discriminate 70Hz from 100Hz. After treatment with streptomycin, an antibiotic that blocks the lateral line without affecting the inner ear, fish showed substantially decreased response rates indicating that stimulus detection and discrimination were impaired.

The results show that Oscars learned to discriminate between sinusoidal wave stimuli based on sensory information received by the lateral line. Performance in turbulent water was not different from that in still water indicating that background noise was effectively filtered by the lateral line.

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Goldfish neuromasts are sensitive to low-frequency electrical stimulation

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With the lateral line system fish detect pressure differences in their environment caused by motions of prey, predators and conspecifics. The receptive units of the lateral line are neuromasts that occur freestanding on the surface of the skin or within lateral line canals. Each neuromast is comprised of a patch of sensory hair cells covered by a gelatinous cupula. The responses of the fish lateral line to various types of mechanical stimuli have been well studied. However, one of the questions that has hardly been investigated is to which degree the filter properties of the lateral line depend on the peripheral morphology and/or on peripheral and central integration mechanisms, respectively. For instance, it is not known to which degree amplitude response and frequency range of a neuromast are determined by the peripheral biomechanical filter properties and/or by the physiological properties of the hair cells, the synaptic transmission between the hair cells and the afferent fibers, and the frequency of spike generation at the spike initiation site.
To address these questions we stimulated individual lateral line neuromasts in goldfish, *Carassius auratus*, with local electric fields while recording neuronal activity of individual primary afferent fibres in the posterior lateral line nerve. First, the location of the innervated neuromast on the fish surface was determined by moving a mechanical dipole (sphere with 8 mm diameter, vibration frequency 100 Hz) alongside the fish and determining the location from which the dipole elicited the strongest response. Then, electric stimuli (sine and square waves) were delivered to the neuromast through a pair of carbon electrodes placed at a distance of 2 mm above the neuromast.
Afferent fibers showed a clear phase-coupled response to electrical stimulation with an increase in spike rate in response to the rising slope of a sine stimulus and a suppression of neuronal activity in response to the falling slope. With increasing frequency (0.2-10 Hz) the number of spikes per stimulus cycle decreased whereas phase-coupled response persisted. In order to determine the relative contributions of biomechanics and physiology to the filter properties of the peripheral lateral line, are presently studying responses to higher frequencies in the range of 20-100 Hz that have been used in the past to study lateral line responses to mechanical stimuli.
Supported by the EU (project CILIA, no. 016039)

Two different modes of gain control in a single auditory interneuron (AN2) in crickets

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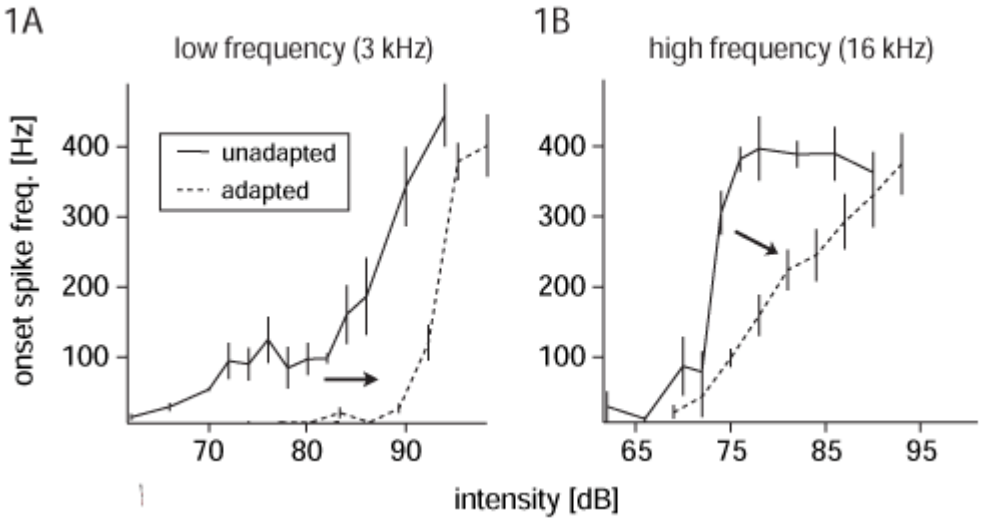
Spike frequency adaptation (SFA) changes the coding properties of sensory neurons in response to persistent stimulation. Possible functions of SFA are the maximization of information transfer, the generation of intensity invariance or background suppression. Signal transmission properties as modified by adaptation can be observed as alterations of the input-output (IO) function of a specific neuron. A shift of the IO curve produces a change a threshold, while a change of the slope and thus the gain of the IO-function alters the dynamic range of the respective cell. Here, we studied SFA by extra- and intracellular recordings from an identified auditory interneuron (AN2) of crickets (*Teleogryllus leo*) that is sensitive to two different ranges of sound carrier frequencies: low frequencies for mate recognition and high frequencies for predator detection. In order to examine how adaptation modifies the IO curves in these different frequency ranges, we performed three sets of experiments: (1) adaptor and test stimuli were of the same frequency, (2) the neuron was adapted to one frequency and tested at the respective other frequency, (3) the neuron was adapted intrinsically by current injection and then tested acoustically at both frequencies. Additionally, we presented constant stimuli of 3s duration to investigate the time course of adaptation in both frequency ranges.

Adaptation in the low frequency channel shifted the IO-function to higher intensities, resulting in large changes of the threshold, but a very small change in gain (Fig.1A). At higher sound frequencies, the slope of the IO-function was substantially decreased by SFA and thus the dynamic range increased, but the threshold remained unchanged (Fig.1B). Neither adaptation to the respective other frequency nor to intracellularly applied current stimuli affected the IO functions. We conclude that most of the carrier-frequency-specific adaptation is located in the pathways peripheral of the auditory interneurons under study.

We also observed a significant difference in the time course of adaptation: at lower frequencies, the time course was best described by a single exponential fit with a time constant between 50 and 80ms. At higher frequencies, a double exponential fit yielded best results: one time constant was between 120ms and 160ms and the other between 600 and 1200ms.

In summary, we observed large differences in the adaptation of signal transmission properties for two classes of stimuli in single auditory interneurons. This likely reflects the different requirements for processing of the two signal classes with respect to behavioural context and is probably implemented by placement of the main site of adaptation peripherally to the respective interneuron.


1A
low frequency (3 kHz)
1B
high frequency (16 kHz)
onset spike freq. [Hz]
400
300
200
100
unadapted
adapted
70
80
90
65
75
85
95
intensity [dB]

Effects of contralateral noise stimulation and low frequency biasing on DPOAE – Changing the operating state of cochlear amplification?

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The mammalian efferent medial olivo-cochlear (MOC) system is capable to modulate the active amplification of low-level sounds in the cochlea. Changes of the cochlear amplifier can be monitored by measuring distortion product otoacoustic emissions (DPOAE). The quadratic distortion product f2-f1 is sensitive to changes in the operating point of the transfer function of the cochlear amplifier. For a better understanding of the impact of the MOC efferent system on the cochlear amplifier we investigated the effect of contralateral broadband noise stimulation, known to elicit efferent activity, on DPOAEs in the mongolian gerbil. In a second approach, we additionally biased the position of the cochlear partition and hence the operating point of the cochlear amplifier periodically by a low frequency biasing tone (5 Hz).

During contralateral broadband noise stimulation, a significant increase of the amplitude of f2-f1 was found, which already occurred at low contralateral stimulus levels (20 dB SPL). Maximum level increase of f2-f1 was about 5 dB, while 2f1-f2 was not affected. This observation can be interpreted as the result of a change of the operating point of the cochlear amplifier due to efferent activity. Biasing the cochlear partition by the low frequency tone resulted in a phase related amplitude modulation of f2-f1, dependent on the level of the bias tone. This modulation pattern was changed pronouncedly during contralateral noise stimulation, in dependence on the noise level.

The current results suggest that efferent effects on DPOAE might be produced by changes in the operating point of the cochlear amplifier transfer function and were in good agreement with a simple model based on a Boltzman function to simulate the consequences of shifts of the operating point on the distortions.

This work was supported by the DFG and Jürgen-Manchot-Stiftung.

Generation of a tamoxifen inducible hair cell specific TRß1 knock-out mouse model

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Thyroid hormone receptor ß 1 (TRß1) dysfunction leads to deafness in humans and mice (Refetoff et al. 1993; Forrest et al. 1996). Deafness in TRß1 mutant mice has been suggested to result from TRß1-mediated control of fast-activating BK currents in inner hair cells (Rüsch et al. 1998). New results however, suggest that deafness is not a result of delayed BK currents, but may have an origin outside the hair cells (Winter et al. under revision). For further investigations to verify this presumption, we aimed to delete the TRß1 receptor restrictively in hair cells, within the critical time window prior to the onset of hearing. To obtain a hair cell-specific deletion of TRß1, we use a well established CreLoxP system and a transgenic mouse model (Math1CreER), in which the expression of the Cre-recombinase is under control of the Math1 promotor and its activation is inducible by application of Tamoxifen (Chow et al., 2006). In the cochlea, Math1 is only expressed in hair cells, from E13 to P7, therefore Cre expression and gene deletion is presumed to be active during that time. This makes it possible to generate mice with a hair cell specific deletion of TRß1 prior to the onset of hearing. By crossing the Math1CreER^{TM} mouse with a floxed TRß1 mouse, the obtained mouse model is ready for functional and cellular phenotyping. First data will be presented that describe the hearing function of these inducible hair cell specific TRß1-knock-out animals as well as the phenotype of hair cells.

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Interaction partners of otoferlin

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One of the genes underlying hearing impairment in mice and humans is the sequence coding for otoferlin. Mutations within *OTOF* lead to a recessive disorder called DFNB9. Several studies have indicated otoferlin's association with ribbon synapses of cochlear sensory hair cells, as well as data showing the protein's presence in neurons, nerve fibers and hair cells, suggesting a more ubiquitous function. Otoferlin's co-localization not only with ribbon synaptic proteins, but also with additional endosomal (EEA1) or Golgi proteins (GM130) were motivation for a search for further binding partners of otoferlin by a yeast two-hybrid screen in a rodent cochlear cDNA library (P3-P15). This screen identified some novel interacting partners, substantiated by transient co-expression and co-localization in HEK 293 cells and co-immunoprecipitation of the complex using tagged proteins *in vitro* and native proteins from cochlea. This finding implies that otoferlin could be a part of components contributing to trans-Golgi trafficking.

This work has been supported by Deutsche Forschungsgemeinschaft DFG-Kn-316/4-1 and Landesgraduiertenförderung Baden-Württemberg.

Feature Selectivity of Grasshopper Auditory Interneurons Determined by Spike-Triggered Covariance Analysis

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A focus of sexual selection in acridid grasshoppers lies on acoustic cues: species and gender recognition, as well as the localization of potential sexual partners, depend on the amplitude modulations of acoustic signals produced during courtship. The metathoracic network is a three-layer feed-forward network, consisting of the receptors as the input layer, an intermediate layer of local interneurons and ascending interneurons as the output layer to the brain. It forms an information bottleneck about acoustic stimuli for the decision centres in the brain. Recent evidence suggests that the network of metathoracic interneurons processing sound in the periphery is conserved across species of the taxon (Neuhofer et. al. 2008). Thus, the representation of stimuli by neurons within this conserved network constrains the features on which sexual selection can rely.

Past studies examining the processing properties of auditory interneurons have relied mostly on simple stimuli, consisting of single sinusoidal amplitude modulations or of patterns known to elicit behavioural responses in certain species. However, to fully understand the array of features being represented in the network, a more general approach is adopted here: Using white-noise random amplitude modulated stimuli, we determined the feature selectivity of auditory interneurons in the metathoracic ganglion by means of spike-triggered analysis techniques. This allows us to fit a linear-nonlinear cascade model: firstly, the stimulus is fed through a set of filters, and secondly, the filters' outputs are transformed by a non-linearity to the neuron's firing rate. The filters can be determined easily from the moments of the spike-triggered stimulus ensemble. The output nonlinearity is estimated by finding the function between filter-output and measured firing rate. The traditional spike-triggered average assumes that a neuron is sensitive to one single feature only. However, we expect auditory interneurons - especially at the output layer of ascending neurons - to be sensitive to multiple features. Spike-triggered covariance analysis allows extracting all features that contribute to a neuron's spiking behaviour. Furthermore, it enables us to determine how these multiple features interact to produce the output of a neuron.

Insights into the feature selectivity of the meta-thoracic network should further the understanding of the processes underlying song recognition as well as of the evolution of the signals used.

Neuhofer, D., Wohlgemuth, S., Stumpner, A., and Ronacher, B. (2008). Evolutionarily conserved coding properties of auditory neurons across grasshopper species. *Proceedings. Biological sciences / The Royal Society*, 275(1646):1965-1974.

The Role of L-VDCC for activity-dependent BDNF transcription: a cell culture model

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Brain-derived neurotrophic factor (BDNF) plays a crucial role for activity-dependent plasticity, alteration of synaptic efficacy and balance of inhibition and excitation (Korte et al., 1995). The transcription of BDNF itself was shown to be regulated in a calcium-dependent manner whereby Ca^{2+} influx through L-VDCCs plays a crucial role (Tao et al., 2002). We could show in various studies that BDNF expression in spiral ganglion neurons is altered in aging animals (Rüttiger et al., 2007), after tinnitus inducing acoustic trauma (AT) (Tan et al., 2007) and after salicylate application (Panford-Walsh et al, 2008). Altered BDNF levels in the periphery of the cochlea therefore may be the trigger for pathological imbalances of neuronal activity in the central auditory system. To further test this hypothesis we are particularly interested whether AT- or salicylate- induced changes in BDNF expression are L-VDCCs dependent as described previously (Tao et al. 2002; Tippens et al., 2008). In the cochlea up to now, only BDNF exon IV/IX and VI/IX transcripts are shown to be expressed (Tan et al., 2007).
We use a cell culture system in which we transfected YFP- and CFP- tagged BDNF transcripts (exon IV and exon VI), including their appropriate promoter regions. Here first experimental approaches will be presented, that examine the usefulness of the *in vitro* culture system for elucidating presumptive trauma- and Ca^{2+} dependent BDNF signalling pathways.

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The role of the $Ca_V1.2$ channel in the cochlea

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Voltage-gated L-type Ca2+ channels (LTCCs) play an important role in synaptic transmission, secretion and gene expression. In the cochlea the expression of Cav1.2 and Cav1.3 has been shown using RT-PCR (Green et al., 1996).

Within the auditory system the function of Cav1.3 is already known. Constitutive Cav1.3 KO mice are deaf due to the complete absence of L-type currents in the cochlear inner hair cells and degeneration of outer and inner hair cells (Platzer et al., 2000).

In situ hybridisation shows Cav1.2 mRNA expression in spiral ganglios neurons of adult rat.

Cav1.2 protein is located under the hair cells (Waka et al., 2003) and in spiral ganglion neurons in adult mice shown by immunohistochemistry using a a1C calcium channel subunit specific antibody.

Until now the function of Cav1.2 for hearing has not been elucidated. Mouse models, in which Cav1.2 is deleted, are available but lethal with birth. Therefore, a conditional KO mouse model is required. To generate this model a mouse line with Cre expression under the promoter of spiral ganglion neurons specific genes would be essential. We considered the promoter of two genes as possible candidates in driving Cre expression: (1) TASK-5, (2) Pax-2. In addition to a distinct expression profile in auditory nuclei of TASK-5 described by Karschin et al. (2001), we detected TASK-5 in spiral ganglion neurons.

Pax-2 is one of the earliest markers of the otic placode, the inner ear's progenitor (Pfeffer et al., 1998). Pax-2/Cre/R26R mice show most cells in the cochlea duct and vestibular sensory epithelia (derived from the otocyst) reporter-positive at postnatal day 0 (Ohyama et al., 2004). A conditional Cav1.2 KO mouse model could help to understand functions and role of this channel in physiological conditions and auditory pathologies as acoustic trauma and tinnitus.

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Responses of brainstem lateral line neurons in goldfish, *Carassius auratus*, to different water flow directions and velocities

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Recent studies show that the activity of primary afferents in the lateral line nerve encodes the fluctuations within a water flow. This information can be used by brainstem neurons to determine flow direction and flow velocity by comparing inputs from fibers that innervate two or more peripheral receptors organized in series. If so, these neurons should respond preferentially to particular flow velocities and flow directions.

Here, we recorded activity from brainstem neurons in goldfish, *Carassius auratus*, in a water flow and determined velocity thresholds, temporal activity patterns, and directional sensitivity. A propeller generated water flow in a flow tank containing upstream and downstream collimators. Flow was from anterior to posterior (AP) or from posterior to anterior (PA). Neuronal activity was monitored under two stimulus conditions: pulsed flow (discrete velocity steps to 0.7, 2.6, 4.4, 6.2, 8.8, 10.0 and 12.0 $cm*s^{-1}$) and ramped flow (continuous increase in velocity from 0 to 10 $cm*s^{-1}$ and/or 0 to 12.0 $cm*s^{-1}$).

Single unit recordings were made from 34 brainstem neurons in 14 fish. Thus far we gathered data from 19 units that were stimulated with pulsed flow and from 13 units that were stimulated with ramped flow. Fifteen of the 19 units stimulated with pulsed flow changed discharge rate in response to at least one of the applied velocities. With increasing flow velocity, discharge rate increased in seven units and decreased in four units to both AP and PA flow direction. Three units showed no systematic change in discharge rate to increasing flow velocity and, two units increased discharge rate to AP flow but decreased their rate to PA flow.

When stimulated with ramped flow, four units increased and one unit decreased discharge rate during the entire duration or at least over a few seconds of the ramp in response to both flow directions. In eight units activity depended on flow direction. Two units showed a rate decrease to AP flow and a rate increase to PA flow, two other units decreased rate to AP flow but did not respond to PA flow and, four units did not respond to AP flow but to PA flow (in one case with a rate increase and in three cases with a rate decrease).

The data indicate that brainstem lateral line neurons exhibit a wide range of response behaviors to a water flow. In some neurons responses to water flow appears to be independent of flow direction, whereas in other neurons responses are clearly different to AP and PA water flow indicating that these neurons encode flow direction. Presently there is no evidence for a tuning of brainstem neurons to a particular flow velocity or to a range of velocities.

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Microtubule-associated proteins shape mechanoreceptors in Drosophila

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Drosophila mechanoreceptors such as those found in campaniform sensilla in the wings and halteres and in the Johnston's organ in the fly's ear have a prominent microtubule-based cytoskeleton. Both receptor types are ciliated and possess characteristic ciliary modifications: the microtubule-rich tubular body in campaniform receptors and the ciliary dilation in the Johnston's organ. These modified cilia most likely serve as the site of transduction in both mechanoreceptors. We have identified two microtubule associated proteins (DCX-EMAP, katanin-like) that are specifically expressed in these mechanoreceptors, that show a characteristic subciliary localization and that, when disrupted, lead to sensory defects and cause specific ultrastructural alterations within the modified cilia. These studies show that the microtubule cytoskeleton plays a key functional role in mechanotransduction in these classes of sensory cells.

Auditory Pattern recognition by brain neurons of the grasshopper *Chorthippus biguttulus*

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Chorthippus biguttulus (Acrididae) is an acoustically communicating grasshopper in which pair formation is achieved by duetting between the sexes. Females use male song patterns as a cue for species recognition and mate choice (v.Helversen, v.Helversen 1994, Fortschr. d. Zool. 39: 253-284). The females' decision to answer to a conspecific male depends on a number of song features, such as the syllable-pause structure and the syllable onsets and offsets (Balakrishnan et al. 2001, J Comp Physiol A 187: 255-264). The males react to a conspecific female song by turning towards the sound source, running or jumping a short distance and singing themselves (v.Helversen, v. Helversen 1997, J Comp Physiol 180: 373-386).

A major problem for a male is to simultaneously localize the sound source and analyze the amplitude modulation pattern for signal recognition. Behavioural experiments have shown that both tasks are processed in parallel neural pathways: the information reaching the two tympanal organs is summed up in the pattern recognition pathway thereby eliminating directional information. In contrast, in the sound localization pathway binaural intensity differences are amplified through contralateral inhibition. Important steps of this computation take place in the metathoracic ganglion, which harbours an important module for processing of auditory information. However, the final decision about attractiveness and direction of the sound source occurs in the brain (Ronacher et al. 1986, J Comp Physiol A, 158 363-374).

We therefore focus on the processing properties of brain neurons. Special emphasis is laid on how directional and pattern info is processed and whether their two pathways remain separated in the brain.

According to the sex and species specific parameters, patterns of different attractiveness have been constructed and were tested in behavioural experiments. Afterwards the same stimuli were used for intracellular recordings in the OSG of the animals tested before. Local interneurons with a potential contribution to either pathway were characterized, and their possible role for the evaluation of song pattern attractiveness - as revealed in the behavioural experiments – has been studied.

Novel Findings in Inner Hair Cells of Hypothyroid Rodents Showing Exocytosis in the Absence of Otoferlin

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Thyroid hormone (TH) is essential for the development of hearing. A lack of TH in a critical developmental period from embryonic day 17 till the onset of hearing at around postnatal day 12 (P12) leads to morphological and functional deficits in the organ of Corti and the auditory system in rats and mice (Deol, 1973; Uziel, 1986; O'Malley et al., 1995; Knipper et al., 2000, 2001). In control animals the onset of hearing is accompanied by up- and downregulation of Ca^{2+} currents leading to a more efficient exocytosis such that less Ca^{2+} ions are needed for neurotransmitter release (Johnson, Marcotti and Kros 2005, Sendin et al. 2007, Brandt et al., 2007). Spontanous Ca^{2+} action potentials present in the first postnatal week cease enabling the inner hair cell (IHC) to generate graded receptor potentials from P12 onwards (Johnson, Marcotti and Kros 2005).

Recent studies show that expression of BK channels in IHCs is severely delayed or missing in hypothyroid animals and in athyroid Pax8 knockout mice (Brandt et al. 2007, Sendin et al. 2007). Cav1.3 channels are present but Ca^{2+} currents remain immature-like, thereby generating Ca^{2+} action potentials in these animals even until postnatal day nine. Interestingly, otoferlin, a candidate for the Ca^{2+}-sensor of IHC exocytosis (Roux et al. 2006), is not expressed in IHCs of hypothyroid animals but is present in Pax8 knockout mice (Brandt et al. 2007). To assess why IHC under hypothyroid conditions show robust exocytosis, despite the absence of otoferlin, we performed molecular studies to identify possible candidates subsituting for the supposed Ca^{2+}-sensor function of otoferlin.

Contralateral sound alters the f2-f1 distortion product otoacoustic emission – Impact of primary tone level and frequency specificity

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Outer hair cells (OHC) in the organ of corti of the mammalian cochlea are responsible for the amplification of low-level sounds. They are strongly innervated by the medial olivocochlear (MOC) efferents which are capable of modulating the electromotile OHC response characteristics. To investigate this efferent modulation of cochlear amplification, the efferents can be activated by contralateral sound stimulation. Measuring distortion product otoacoustic emissions (DPOAEs) evoked by two-tone stimulation is a reliable and non-invasive method reflecting the activity of the cochlea amplifier.
DPOAEs were recorded in the Mongolian gerbil under light anesthesia while activating the contralateral ear with different sound stimuli. Our focus was the quadratic difference tone, f2-f1, which is known to be sensitive to efferent effects. DPOAE-recordings lasted 2.6 seconds including a period of contralateral acoustic stimulation (duration 800 ms). Several primary tone frequencies from 2.5 to 15 kHz and various contralateral sound stimuli (noise bursts or pure tones, 30-70 dB SPL) were tested.
DPOAE growth functions were measured that describe the dependency of DPOAE level on the primary tone level. The f2-f1 functions often included a notch as it is known from the 2f1-f2 DPOAE. Typically, contralateral stimulation with broadband noise caused a distinct change (mainly increase) of the f2-f1 level. The change in f2-f1-level depended on the primary tone levels with strongest effects at low to moderate tone levels, especially around the notch. Only small changes were obvious for the 2f1-f2 DPOAE. To determine a frequency-specific effect of the MOC efferents, the contralateral ear was stimulated with pure tones (0.5-38 kHz) and frequency-specific shifts of the f2-f1 level were detected. The typical pattern included small level changes over a broad frequency range and pronounced effects at contralateral frequencies slightly below the primary tones.
The results show that the f2-f1 difference tone is well suited to detect efferent effects on cochlear amplification in the mammalian ear. The frequency-specificity can be related to the anatomical pattern of MOC projections to the cochlea.

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Vibration sensitivity of leg scolopidial organs in Mantophasmatodea

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In Mantophasmatodea, males and females communicate via substrate vibrations that are produced by tapping the abdomen on the substrate surface. Males have a pronounced drumming organ on the subgenital plate, while females tap in the absence of any specialised organ. Vibrational signals are species- and gender-specific, and are of great importance for mate recognition and location. This communication system shows several intriguing similarities to that of other animal groups using acoustic or vibrational communication cues.

The mechanoreceptors responsible for the transduction and reception of the vibrational signals are as yet unknown, but are probably scolopidial organs within the legs. We therefore investigated the responses of the leg nerves to substrate vibrations applied to the tarsus, and we tried to identify responsive mechanoreceptors by ablation experiments. Males and females of the species *Karoophasma biedouwense* and a yet undescribed, sympatric species which belongs to the same family (Austrophasmatidae) were used in this study. Intensity and frequency characteristics were determined, and compared to the intensity and frequency ranges of the communication signals.

Best frequencies were between 40 and 640 Hz; the total frequency range where responses were recorded in the leg nerve was between 5 Hz and 2560 Hz. These values agree well with the frequencies observed in the communication signals. Threshold intensities at the best frequencies were close to 0,005 m/s^2, or 0,5 nm displacement (peak-to-peak values) at 640 Hz. The responses of the leg nerves of different legs (fore-, mid-, and hindlegs) did not differ much.

Ablation experiments indicate that both, subgenual organ and femoral chordotonal organ respond to vibration stimuli, whereas the tarso-praetarsal chordotonal organ is not involved in vibration reception.

Factors improving and impairing song pattern recognition in a grasshopper

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Acoustic communication is a prerequisite for mate recognition and species isolation in the acridid grasshopper *Chortippus biguttulus*. In this bidirectional communication system males produce species- and sex-specific calling songs that will trigger response songs in a female, if she is receptive and likes the detected signal. Subsequently, songs are emitted alternately by both sexes, enabling the male to phonotactically approach the female. Decisive for song recognition is the characteristic temporal pattern of amplitude modulations, the signal's envelope. Earlier studies with model songs have shown that a certain relation between the durations of syllables and intermittent pauses is crucial for *C. biguttulus* (von Helversen 1997 J Comp Physiol A 180:373). In the biotope however, the temporal structure of signals is frequently masked and distorted on the way between the sender to the receiver. Degrading factors amongst others are atmospheric turbulences (wind, moving ground vegetation) that account for amplitude fluctuations at low frequencies. We hypothesized that amplitude fluctuations in the frequency range of the signal's envelope would be more derogatory to song recognition than higher frequencies.

In behavioural tests song models were gradually degraded in 3 dB steps, using narrow noise bands (100 Hz or 200 Hz bandwidth), scanning ranges from 0-800 Hz. Indeed female responses indicate that noise frequencies between 0- 200 Hz were most detrimental for song detection. Compared to noise bands containing 200 to 800 Hz, noise band < 200 Hz led to a 3 to 5 dB reduced noise tolerance.

Furthermore we investigated whether certain signal traits exist that may improve song recognition in a noisy environment. We compared female reactions to song models showing syllables with onset accentuation versus no such onset accentuation, while songs where gradually degraded. The presence of an onset accentuation in the syllables improved noise tolerance by ~4 dB compared to syllables without accentuation. Hence, the necessity of a reliable song recognition in the presence of noise may have been a driving force that led to the strongly amplitude modulated structure of acoustic signals observed in many grasshopper species.

As a next step we then restricted the degradation to specific time windows of the signal, in order to detect those parts of the signal where recognition is particularly susceptible to noise.

The behavioural results will then be related to neurophysiologic data, using the same stimuli and degradation protocols.

Characteristics of the receptor current in locust auditory receptor cells

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The functional properties of auditory receptor neurons of locusts are well understood in terms of a signalling cascade composed of a sequence of linear filters and static nonlinearities. The filter elements were measured indirectly from the spike response recorded from the auditory nerve, thus leaving the ear intact [1,2]. However, only little is known about the biophysical mechanisms of the underlying auditory transduction process. In this study we investigate properties of the receptor current that can be indirectly inferred from the interspike interval statistics of the spike train response.

Intracellular recordings were performed from auditory nerve fibres of *Locusta migratoria* during simultaneous acoustic stimulation with pure tones of various intensities. The obtained interspike intervals (ISIs) showed high variability with CVs up to 0.8. The ISI histograms were successfully fitted with the probability density function of ISIs (p(T) in fig. A) that was derived for the perfect integrate-and-fire (PIF) neuron driven by white noise [3], suggesting that the receptor current drives the spike generator in the superthreshold limit-cycle regime. In addition, the PIF theory allows to infer indirectly the noise strength of the receptor current in the intact ear. This noise strength as a function of sound intensity (see fig. B) shows a pronounced peak approximately in the middle of the dynamic range of the receptor neurons. This is in accordance with the binomially distributed number of open channels for a population of two-state (open/closed) ion channels that also shows the maximum variance for an open probability of ½ (see e.g. [4]). Thus, this finding supports our hypothesis that the receptor current is indeed carried by stochastic opening channels in the receptor cells' membrane and that their open probability changes with increasing sound intensity. By means of simulations (see fig. C) of single-compartment conductance-based models we test the validity of this indirect approach and the possibility to make further inferences on receptor channel number or single channel conductivity. Future voltage-clamp studies directly at the somata of auditory receptor neurons within Müller's organ will allow us to quantify the receptor current directly and to measure its reversal potential, maximum conductance or possible adaptation. The results from the indirect methods are important to ensure that direct measurements do not severly damage the auditory transduction machinery.

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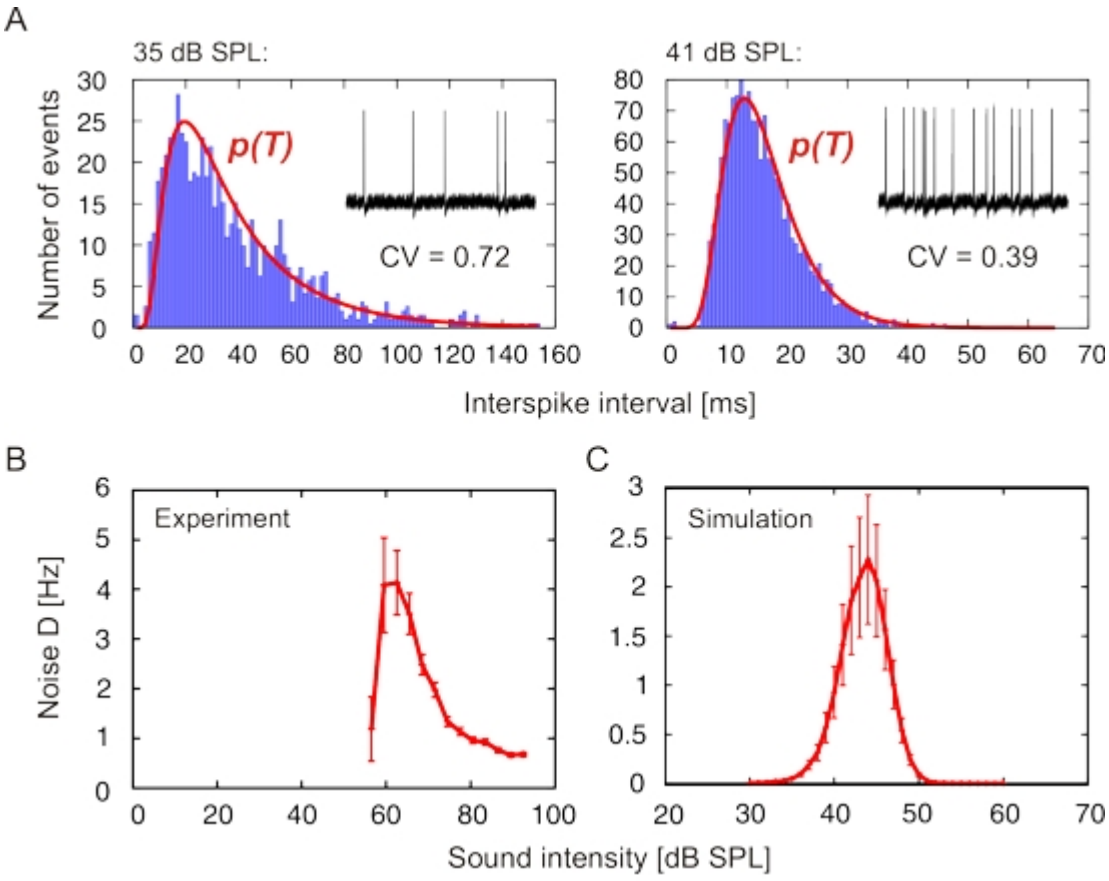
A
35 dB SPL:
41 dB SPL:
Number of events
p(T)
CV = 0.72
CV = 0.39
Interspike interval [ms]
B
C
Experiment
Simulation
Noise D [Hz]
Sound intensity [dB SPL]

Mechanical feedback amplification in Drosophila hearing requires a specific subset of NompC-expressing, sound-sensitive mechanosensory cells

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Gravity sensing and hearing in *Drosophila melanogaster* rely on Johnston´s Organ (JO), a chordotonal stretch-receptor organ consisting of ca. 500 mechanosensory neurons residing in the 2nd antennal segment. These JO neurons transduce stimulus-induced antennal displacements and, in addition, generate motions to boost and amplify the minute displacements which had elicited the response in the first place (Göpfert et al., PNAS 2005). Previous studies have shown that this latter mechanical feedback requires the transient receptor potential (TRP) channel NompC (=TRPN1) (Göpfert et al., Nat Neurosci. 2006), which is apparently expressed in sound-, but not gravity-sensing JO neurons (Kamikouchi et al., in press). Mechanical measurements now confirm that the NompC-expressing, sound-sensitive neurons account for mechanical feedback amplification by JO. The gain of the feedback remained largely unaltered if gravity-sensitive JO neurons were ablated, ablating sound-sensitive neurons disrupted the feedback exerted by JO. This latter effect associated with a reduced sensitivity of sound-evoked nerve responses, which was also found to result from the disruption of NompC. Linking mechanical feedback amplification by the fly´s JO to a specific population of JO neurons, our analysis provides functional support for the restricted expression of the NompC channel in JO neurons and shows that NompC-expressing neurons are required for sensitive sound-detection by JO, whereas the gravity-sensing neurons are of minimal to none importance for the feedback amplification.

The virtual ear: Deducing transducer function in the *Drosophila* ear

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Mechano-electrical transduction, i.e. the transformation of mechanical stimuli into electrical signals, constitutes the heart of the hearing process. Recent studies of vertebrate and invertebrate hearing systems have shed light on the functional workings of the transduction machinery, yet how auditory relevant genes contribute to the process of hearing largely remains unclear. The antennal hearing organ of *Drosophila melanogaster* is an emerging model system to study hearing mechanisms *in vivo*: recently, a physical/mathematical framework that describes the fly's auditory performance has been proposed, which represents the *drosophila* ear as two opposed populations of transduction modules that couple to harmonic oscillator that corresponds to the fly's antennal sound receiver. The use of functional (as opposed to generic) components in this model opens the way to understand the functional role of specific gene products in the fly's hearing organ. Here, we present the virtual ear, an *in silico* implementation of the fly's ear, that aims at being a high-throughput tool to analyze experimental data of wildtype and mutant fly ears, as well as a framework for the *in silico* exploration of possible phenotypes that result from molecular parameter changes. Using the virtual ear, we can link alterations of the macroscopic mechanics of the fly's hearing organ due to specific genetic mutations to parameter value alterations of the virtual ear, thereby linking genes and their function in the transduction machinery of a hearing organ *in vivo*. The virtual ear will be made freely accessible and can be used as a ready-to-use research and teaching tool to explore fundamental mechanisms in hearing.

Presynaptic Processing in Auditory Afferents of Bushcrickets

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Bushcrickets (*Mecopoda elongata*) produce complex acoustic signals for intraspecific communication. The sound signals are used for mate attraction and spacing of individuals in the biotope and are modulated in amplitude and frequency. The ears are located in the front legs and a set of 55 afferents project into the prothoracic auditory neuropil. A tonotopic representation of best frequencies in the peripheral arrangement of the afferents in the *crista acoustica* of the ear and of their central axonal projections in the prothoracic gangion has been demonstrated by intracellular recordings in other species of bushcrickets (Römer 1983, Oldfield 1988, Stölting and Stumpner, 1998).

We analysed the central mechanism of frequency processing in the auditory afferents using intracellular recordings and optical imaging of Ca^{2+} sensitive dyes (Oregon Green), which were iontophoretically injected into the axons. Our main findings are:

1. Imaging Ca^{2+} responses during acoustic stimulation in the presynaptic terminals reveals the frequency tuning of the afferents and matches the frequency tuning as determined by intracellular recordings. Staining afferents consecutively demonstrates the tonotopic representation of sound frequencies in the prothoracic auditory neuropil.

2. Upon acoustic stimulation, intracellular recordings close to the presynaptic terminals reveal graded membrane depolarisations of 2-3 mV which occur besides the spike activity. These depolarisations are derived from both ipsilateral and contralateral sources and are probably driven by GABAergic inputs to the presynaptic terminals.

3. Imaging and intracellular recordings demonstrate that the tuning of the graded potentials in the terminals may be different to the afferent's spike tuning. Furthermore they may act to different degrees on different terminals of the same afferent. They may therefore contribute to shape the frequency processing in the bushcricket auditory pathway.

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Spatial acuity of active electrolocation of objects in complex scenes by the weakly electric fish, Gnathonemus petersii

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The weakly electric fish *Gnathonemus petersii* generates electrical signals with an electric organ in its tail. During each electric organ discharge an electric field builds up around the fish. The animal perceives its own electric emissions with epidermal electroreceptor organs. Objects are detected and distinguished by analysing the electric images, which are projected onto the skin surface of the fish – a process called active electrolocation.

In the first part of this project we determined up to what distance *G. petersii* can discriminate between objects of different shapes (metal pyramid versus metal cube) and of different sizes (small (2x2x2cm) versus large (3x3x3cm) metal cubes) during active electrolocation. In addition, we measured whether the presence of different types of backgrounds (plastic-, metal- and water plant - backgrounds) can interfere with object discrimination. In the second part of the project, we investigated whether *G. petersii* can detect a gap between two objects (metal or plexiglas cubes). Our goal was to determine the minimal detectable separation distance between two objects during active electrolocation.

In order to test these features, the animals were trained in food-rewarded two-alternative forced-choice procedure. An aquarium was divided into two compartments by a plastic partition, which contained two gates. The fishes were trained to discriminate between two objects of different shapes or sizes, which were presented on a platform 5 mm behind each gate. Fish had to swim through one of the gates in order to make a decision and receive a food reward.

The thresholds for the maximal distance at which objects could be distinguished from one another were obtained by presenting the objects at distances of 1 cm to 8 cm from the gates. Our results show that *G. petersii* can discriminate between the shapes or sizes of metal objects up to a distance of 3 - 4 cm.

Interestingly, the performance was not impaired when discrimination had to be performed in the presence of a non-conducting plastic background which was positioned 0.5 cm behind the objects. Currently we are measuring the effects of other backgrounds, such as large metal plates (conducting background) or water plants (natural background), on the discrimination performance of the fish.

The results of the second part of the project showed that *G. petersii* can detect gaps between objects and discriminate them for one solid object of equal length. When two metal or two plexiglas cubes (both 2x2x2cm) with a gap of 3 cm between them were presented to the fish, they could discriminate them from a continuous metal or plexiglas object of the same overall length. This performance was maintained when the size of the gap between the objects was reduced, and we are currently determining the threshold for this gap detection ability.

In summary, our data show that *G. petersii* is able to discriminate between objects of different shapes even in the presence of a uniform background and fish are able to detect gaps between objects. These results indicates that the spatial acuity of the fishes during active electrolocation is better than was previously expected based on the physical superposition effects of the electric images when several objects are present.

Using *Drosophila* to trace candidate deafness genes

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Johnston's organ (JO), the hearing organ in the second segment of the *Drosophila* antenna, seems a valid system to trace genes for hearing as JO neurons and vertebrate inner-ear hair cells are developmentally specified by homologous atonal-family genes. To identify candidate genes for hearing, we compared gene expression in the second antennal segments of *Drosophila* atonal mutants and controls using whole-genome microarrays. 275 genes were identified that are preferentially expressed in JO. These genes include 5 known JO genes, 8 known ciliary compartment genes, and genes that are implicated in intracellular signalling (32 genes) , protein modification (26 genes), nucleic acid regulation (23 genes), cell metabolism (9 genes), transport (12 genes), and sensory perception (18 genes). 14 genes encode cytoskeletal components, 19 encode ion channels, and 13 molecular motors. Every 7th gene identified by our screen seems related to a candidate human deafness gene. Ongoing functional analyses show that disrupting genes identified by our screen affects fly hearing.

Relating filter properties of auditory transduction chains to biophysical mechanism.

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Hearing is enabled by specialized receptor organs, that convert sound into neural signals, i.e. action potentials. Using an indirect iso-response (IR) approach, Gollisch et al. (2005) obtained a functional description of the auditory signal transduction chain from the intact ear of locusts. The signaling cascade is composed of two linear filters and two static nonlinearities. The linear filters reflect (i) the mechanical properties of the tympanum of the locust ear and, supposedly, (ii) the electrical integration of the transducer current in the receptor neuron.

Whereas the tympanic filter is completely revealed by the IR method, only the tail of the second filter component is captured by the method. First, the rising part of the filter kernel that reflects the shortest time constants in the system cannot be measured. Second, the initial part of the filter is distorted by the first filter component. Thus, only the slowest time constant can be estimated from the tail of the second filter as obtained by the IR method, provided the second filter kernel is substantially longer than the first filter. Since this longest time constant is, however, quite short (ca. 0.5 ms), we conclude that the transduction channels and the spike-generator must be in close proximity to each other.

Using dynamic spike-generator models we demonstrate that all integral components of the spike-generator are fully absorbed into the second linear filter. Therefore, the description of the electrical integration and the spike generator as separate elements in the signaling cascade, i.e. as a linear filter and a static nonlinearity does not reflect two separate biophysical mechanisms. Due to the nonlinearity of spike-generators the second filter is only an approximation of the full dynamics of electrical integration in the receptor neurons.

In Drosophila, much more is known about the biophysical properties of the auditory transduction chain than in locusts. In particular, a gating-spring model successfully describes various aspects of the transduction machinery (Nadrowski and Goepfert, 2008). A functional description of this system with a cascade model differs from that obtained from locust in three aspects: (i) there is no second linear filter for electrical integration since this integration seems much faster than the first filter and thus cannot be resolved experimentally. (ii) The first filter not only captures the mechanical properties of the sound receiver, but also includes the feedback-dynamics arising from the transduction modules. (iii) Due to the gating-springs the system contains nonlinearities hampering descriptions by linear filters.

The two examples demonstrate that a functional description of auditory signaling cascades as a sequence of linear filters and static nonlinearities in general cannot be directly related to biophysical mechanisms. Either, as in Drosophila, many different mechanisms are captured in a single filter element, or, as in locust, a single mechanism might be described by more than one filter element. Notwithstanding these limitations, the functional description of transduction cascades is useful for assessing their computational properties.

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Nadrowski B, Alberg JT, Goepfert MC (2008). Curr. Biol. 18: 1365-1372

Insertion -Force and -Depth of Laser Fibers into a Cochlea Model

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Objective: Optical stimulation of the cochlea for hearing restoration is a potential alternative to the present cochlear implant (CI). It has been hypothesized, and demonstrated in preliminary animal studies, that laser stimulation within the cochlea can achieve more frequency-specific activation, thus potentially improve hearing performance, compared to conventional electrical stimulation. While there are various insertion studies investigating CI arrays into the cochlea, to our knowledge, there is no existing insertion study with laser fibers. Therefore, we evaluated the insertion depth and force of different laser fibers into the cochlea and correlated those measurements to the core and cladding diameter as well as the design of the fibers.
Methods: The fibers had a core diameter ranging from 20 to 105 µm with varying cladding diameters. Single or bundle of fibers with or without silicon coating were inserted into a scala tympani model made of Teflon (Cochlear Ltd.) and measurements were made using an Instron 5543 force device.
Results: Our results showed that insertion of a bare fiber requires more force than silicon coated fibers. Furthermore bare fibers are less resistent to bending stress and exert greater forces along the outer scalar wall. Silicon coated fibers (e.g., bundle of 5 fibers each with a 50 µm core and 5 µm cladding diameter) enabled insertion into the first turn with forces and force profiles comparable to what has been achieved with conventional CI electrode arrays.
Conclusion: These findings are encouraging as to the safety of fiber implantation within the cochlea and the ability to reduce insertion forces through different types of coatings. Further studies are needed to identify the optimal type of fiber and coating to achieve the desired flexibility, insertion forces and biocompatibility for a non traumatic implantation of an optical cochlear implant.

Sound induced vibration pattern on the tympanal membranes of the bushcricket *Mecopoda elongata*

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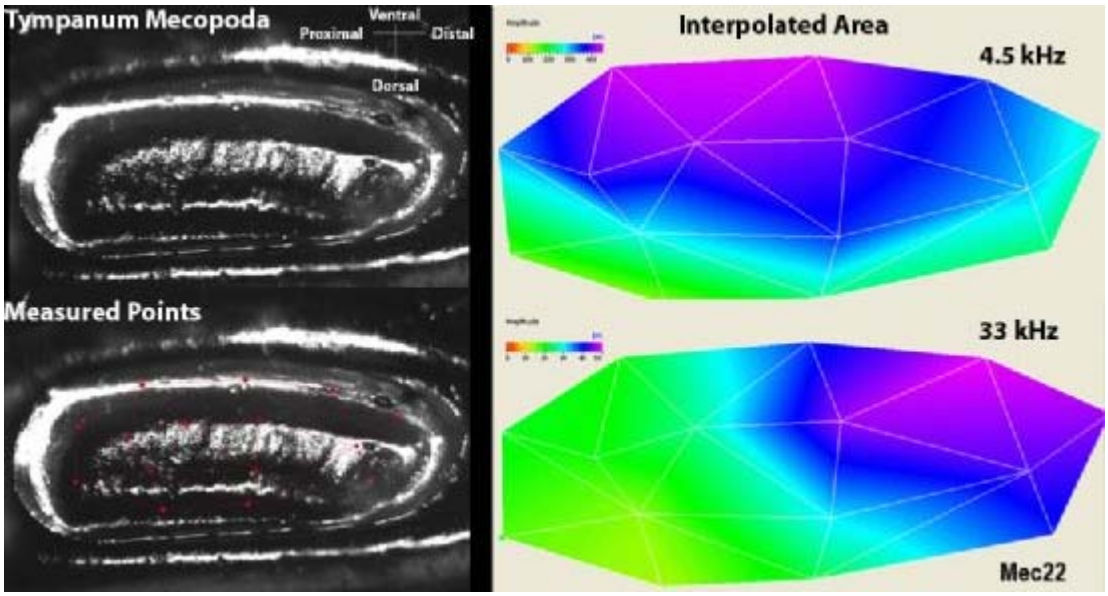
Bushcrickets possess complex tibial receptor organs with similar arrangements of receptor and accessory cells in all three pairs of legs. But only the foreleg organ acts as a detector of sound, as its receptors are associated with structures suitable for the transmission of airborne sound, two tympanal membranes and the acoustic tracheal system [Bangert et al. 1998 Hear Res 115: 27-38]. The combination of a linear arrangement of the receptors within the organ, their tonotopic organization - proximal: low frequencies, distal: high frequencies - and the ultrasonic hearing range make these animals a promising model organism to study the mechanisms of high frequency hearing.

To determine the role of the tympanal membranes in the hearing process, we investigated their vibration pattern in the bushcricket *Mecopoda elongata* (Tettigoniidae) using a Laser-Doppler Scanning Vibrometer (MSA 500, Polytec). Preliminary data of sound induced tympanum vibration measurements show a frequency depended distribution of the amplitude maxima (see figure). Stimulation with sound frequencies below 20 kHz induces in-phase vibrations of the whole tympanal membrane. When stimulated with higher sound frequencies, the vibration shows a complex amplitude pattern with a significant phase shift along the proximal-distal axis of the tympanum. The maximum amplitudes of these vibrations occurred towards the distal end of the tympanal membrane. Planed measurements of the receptor organ itself (*Crista acustica*) will help to clarify whether these findings provide first evidence that the tympanal membranes contribute to the realization of tonotopy in the hearing organ of bushcrickets.

We thank Polytec (Waldborn Germany) for providing the laser vibrometry equipment. Our research was supported by stipends from the Evangelisches Studienwerk (to D.M.) and the Jürgen Manchot Stiftung (to M.W.).

Figure: ***Left site****: anterior Tympanum of Mecopoda elongata and measured points.* ***Right side****: interpolation of the measured vibration magnitude at different stimulus amplitudes.*



Sound Localization in Lizards: How a Pressure-Gradient Receiver may Function

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Due to their small interaural distance, lizards have developed a special hearing mechanism, the "pressure-gradient receiver". The lizard auditory peripheral system differs from the mammalian one by a coupling of the ear drums through the internal mouth cavity.
We present a three-dimensional analytical model of the pressure-gradient receiver. The central aspect of the coupling of the membranes with the internal mouth cavity is hereby realized by the boundary conditions.
Additionally, the lizard's middle ear, a simple lever construction called columella, is asymmetrically attached to the tympanic membrane. That leads to the question of how the middle ear influences the spatial amplitude profile and the frequency distribution of the tympanic membrane vibration.
Finally, we show results from numerical calculations of the eigenfrequencies of the internal mouth cavity. To this end, we have constructed the complex geometry from a cast imprint of the mouth cavity with the help of three-dimensional scans.

How crickets determine the direction of a flow field.

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Crickets and other Orthoptera such as locusts and cockroaches possess two sensor organs, called cerci, on the back of their body. They are covered with a variety of different sensor types, viz., acceleration sensors, chemoreceptors, touch sensors and, what we focus on here, flow sensors. The flow senors, called filiform hairs, consist of a long thin hair, coupling viscously to the motion of the surrounding air, and a mechanosensory hair cell, translating the deflection of the hair into spikes in the ascending nerve fibers. The ascending axons project into the terminal abdominal ganglion and form a map, representing the direction of the air flow around the cerci. Four of the interneurons (R10-2a, R10-3a, L10-2a and L10-3a) arising from the cricket's terminal abdominal ganglion seem to encode the direction of the flow field by voting through maximal firing rates for one of four directions approximately given by 45°, 135°, 225° and 315° w.r.t. the long axis of the cricket in its plane of motion (G.A. Jacobs, J.P. Miller and Z. Aldworth, 2008). Behavioral studies (e.g. E. Tauber and J.M. Camhi, 1995) indicate that determining the stimulus direction is an essential feature of an appropriate response.
We propose that the four interneurons implement a population vector code and show that it is capable of estimating the direction properly. To test this hypothesis, we have computed a realistic flow field around the cerci caused by an attacking predator so as to determine the stimulus to the sensor system during attack. A good description of the motion of a filiform hair is that of a harmonic oscillator with the hair oscillating in the plane of preferred direction. By applying pop vector code to randomly distributed hairs of different lengths and directions all over the cercus, we obtain a good neuronal reconstruction of the direction of an attacking predator.

Coding of stimulus amplitude by electrosensitive neurons in a weakly electric fish

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The weakly electric fish *Gnathonemus petersii* uses a self-generated electric field to scan its environment. This phenomenon is called active electrolocation. During active electrolocation, the fish elicits brief electrical pulses (each ~ 400 µs in duration) with an electric organ situated in the tail. Objects within the electric field and with different impedance than the surrounding water have an effect on the amplitude and waveform of the local electric organ discharge (EOD). Hence, an "electric image" is projected onto the fish's skin. The local EOD modulations are detected by specialized cutaneous electroreceptors (mormyromasts) and conveyed to the first central stage of ascending electrosensory pathway, the electrosensory lateral line lobe (ELL). The mormyromasts contain two different sensory cell types: A-cells and B-cells. A-cells respond in proportion to amplitude modulations of the EOD, whereas B-cells additionally sense distortions of the local EOD waveform. The processing of sensory input allows the fish to obtain detailed information about its surroundings, even in complete darkness. Former investigations by Engelmann et al. (2008) showed that primary afferents of the sensory A-cells responded to a local increase of the electric field amplitude with a decrease of spike latency, while the rate of the responses usually remained constant. This gives evidence that the afferent fibres from the A-cells code the EOD amplitude within the range of amplitude modulations that occur due to small objects in form of a latency code. That means that primary afferents use first spike latency to encode the objects resistive properties. Is this coding strategy also used by central processing units?

In this study we investigated the coding strategies of electrosensory units in the medial zone of the ELL. This region receives input from the amplitude encoding A-cells of the mormyromasts. Thus we recorded extracellularly with NaCl-filled (3 mol/L) glass microelectrodes (1-3 MO) from excitable (E) and inhibitable (I) units. Curarized fish were stimulated with an artificial whole-body stimulus, resembling the fish's own EOD in amplitude. For testing, we moved small cubes (4x4x4 mm^3) made either of plastic or metal through the centre of the receptive field (RF) of a neuron. To analyse the coding strategy, we determined spike rate and first spike latency. While spike rate was determined as the number of spikes within a stimulus interval, first spike latency represents the time delay between a stimulus and the occurrence of the first spike following the stimulus. Simultaneously, we recorded the peak-to-peak (p-p) amplitude of the local EOD in the centre of the RF of the unit. This allows analysing the neuronal responses with respect to the actual modulation of the electric field due to the presence of the objects. For good conductors, the local p-p-amplitude increases while it decreases for non-conductors. To conclude, we can say that the local field geometry caused by the material properties of an object has a very strong influence to the responses of electrosensitive neurons. In contrast to the primary afferents, which use mainly the latency for encoding object properties, we found a mixed coding in the ELL of *Gnathonemus petersii*. Some units only showed a change in rate *or* latency, but most of them used both analysed coding strategies, rate *and* latency, to encode the stimulus amplitude.

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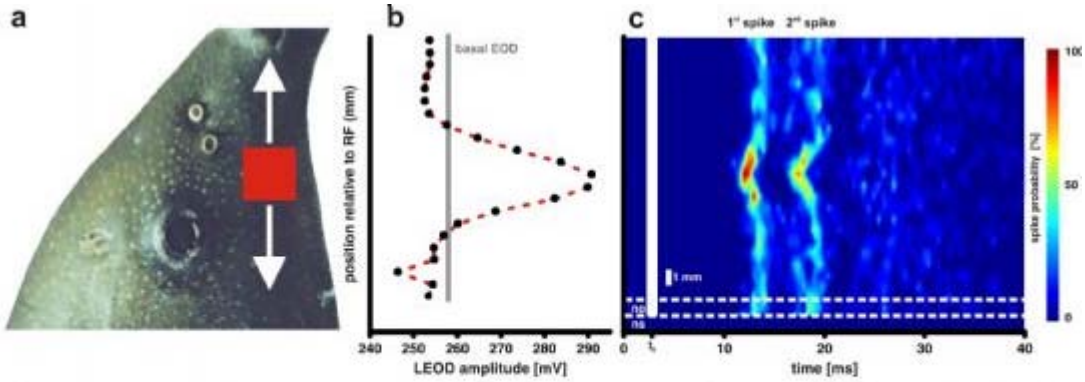

Electric image coded by a sensory E-unit. **a** Side view of the head of the fish with the location of the centre of the receptive field of the E-unit shown by the *red cube*. The *white arrows* indicate the relative movement of the metal cube. **b** Amplitude of the local EOD measured at the centre of the receptive field while moving the object laterally through the receptive field. The *vertical line* represents the basal EOD amplitude in the absence of the object, measured at the centre of the receptive field. **c** Colour coded PSTH showing the spike probability measured at each position and the timing of spikes per EOD (*ns: no stimulus; no: no object; vertical line: timing of stimulus*).

Physiological and anatomical evidence for sensory integration in the electrosensory lateral line lobe of *Gnathonemus petersii*

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Mormyrid fish use reafferent input (EOD) for active electrolocation. The sensory organs involved (Mormyromast) contain differently tuned A- and B-receptor cells. While A-cells are mainly tuned to amplitude changes in the reafferent input, B-cells respond to both amplitude- and phase-changes. Changes of the EOD-phase, i.e., alterations of its waveform, are typical for the presence of prey-like items in the environment, whereas pure amplitude changes are indicative of innate objects in the environment. Since B-cells respond to both parameters (phase & amplitude), the animal must integrate the input of A- and B-cells centrally in order to unequivocally determine the nature of a stimulus. This ability has been shown in behavioural studies and is probably based on a subtractive mechanism: subtracting A-cell information form B-cell input and comparing the result to the activity of A-cells should allow the animal to distinguish amplitude and phase information.

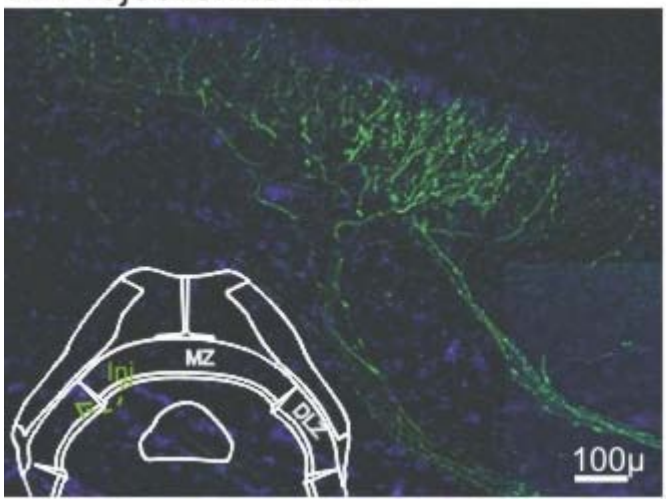
Here we investigate if an initial integration takes place in the ELL (see fig A). Within the ELL, both receptor cells convey their input to separate regions: the medial (MZ) zone receives exclusive A-cell input, whereas the dorsolateral zone (DLZ) is the sole target for B-cell input.

While recording cells in either the MZ or the DLZ, regular and phase-shifted EODs (+10°, -10°) were presented in alternation. Based on the known projection of A- and B-cells, we expected that pure phase-shifts would not influence neurones of the MZ, but should strongly influence neurones of the DLZ. However, both major classes of neurones of the MZ and DLZ - neurones that are inhibited (I) or excited (E) by the EOD - were responsive to such shifts. I-cells of the MZ consistently decreased their rate in presence of positive phase-shifts (6 of 20 cells), and increased their rate in response to negative shifts (11/20). E-cells of the MZ (3/9) responded oppositely to I-cells. Cells of the DLZ responded in opposition to those of the MZ. Et-cells generally responded to negative shifts by increasing their rate and vice versa (4/5), while the opposite was seen for I-cells (2/4).

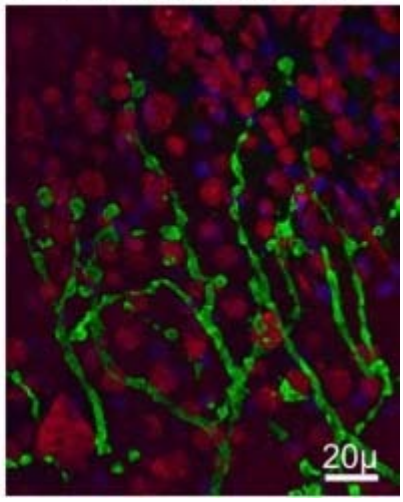
Since A-cells of the mormyromsts are not responsive to these phase shifts, interzonal projections, which we show to be coming from two cell-types, are responsible for the influence of phase on the firing of MZ-cells (figure A). The most prominent interzonal projection is due to LMI cells that interconnect between DLZ and MZ (figure B) in a somatotopic manner. A second class of neurones, so called interzonal cells, was labelled and also mediated interzonal connections. However, these cells were only labelled following Neurobiotin injections, suggesting electrotonic coupling to LMI cells.

In summary we show that phase-information is processed in both the DLZ and the MZ and leads to opposite response in both zones. This is the first evidence for the merging of information needed to explain the behavioural capability to discriminate electrical impedances. The opposite response in both zones to similar phase-shifts probably results in an enhanced contrast between the amplitude- and phase-information at later sensory processing stages.

A. Projection to DLZ



B. Terminals in DLZ



Can Optoacoustic Stimulation replace OHC Amplification in the Cochlea?

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The success of conventional hearing aids and electrical cochlear implants have generally been limited to hearing in quiet situations, in part due to a lack of localized (i.e., frequency specificity) sensorineural activation and subsequent impaired speech discrimination in noise. A new type of stimulation mode using laser stimulation may improve overall activation properties. Laser stimulation (813 nm) of the cochlea has shown to induce localized basilar membrane motion and cochlear microphonic potentials (Fridberger et al. 2006). Compound action potentials have also been elicited using 2.12 µm laser pulses through activation of auditory nerve fibers (Izzo et al. 2006). We sought to assess if optoacoustic stimulation of the cochlea, especially remaining hair cells would be possible.

Frequency-specific and click evoked auditory brainstem responses (ABRs) were recorded preoperatively in ketamine-anesthetized guinea pigs to confirm normal hearing. The bulla and then the cochlea have been exposed. Optically evoked ABRs (OABR) were then recorded in response to laser stimulation with a 50-µm optical fiber (532 nm, 10 ns pulses, 500 repetitions, 10 pulses/s; Nd:YAG laser, Quantel Brilliant B, France) of the tympanic membrane (TM), soft tissue surrounding the bulla (ST), otic capsule (OC), round window directed towards the basilar membrane (RW), modiolus underneath the RW (MD).

OABRs similar in morphology to acoustically evoked ABRs were obtained for stimulation of the TM, OC, RW, MD with energy levels between 3-30 µJ/pulse. The OABRs were similar to those elicited with acoustic stimulation except for shorter latencies. They increased with increasing energy level reaching a saturation level around 23 µJ/pulse and remained consistent across stimulation over time, including stimulation at 30 µJ/pulse for over 10 minutes, indicating minimal or no damage within the cochlea to laser stimulation.

Overall we have demonstrated that laser light stimulation with 532 nm has potential for a new type of auditory prosthesis that can activate the cochlea at the hair cell level without apparent damage to the cochlea. Further studies are needed to determine the optimal laser parameters and fiber placement locations for localized and tonotopic activation.

Neurophylogeny of the Ensifera Auditory Systems

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In Ensifera, the homology of hearing organs is disputed for either common or convergent origin. Furthermore, it is not established whether atympanate Ensifera represent the ancestral or a secondarily reduced state. The auditory organs of Ensifera are located in a tibial sensory organ complex, comprising both subgenual organ and tympanal organ (Grylloidea: Gryllidae + Gryllotalpidae) or subgenual organ, intermediate organ and crista acustica (Tettigonioidea: Tettigoniidae, Haglidae, Anostostomatidae). For atympanate taxa, only Rhaphidophoridae have been investigated neuroanatomically; their tibial organ consists of subgenual and intermediate organ.

Here, comparative data on the tibial sensory complex from representatives of three major atympanate taxa of Ensifera are presented (Gryllacrididae, Stenopelmatidae, Schizodactylidae). In all three taxa, structures homologous to the Tettigonioid crista acustica are present. Using summed recording from the tibial organs, the entire organ complex is shown to be receptive for vibrational stimuli.

This neuroanatomy is in accordance with morphology-based phylogenies of Ensifera (Ander, 1939; Desutter-Grandcolas, 2003), and provides evidence for the phylogenetic affinity of Schizodactylidae to Tettigonioidae. The organisation of the tibial organ in Ensifera separates the Grylloidea and Tettigonioidea. With respect to origin of audition, neuroanatomy argues for the basal position and pre-auditory situation of Rhaphidophoridae (also Stritih and Stumpner, in press). This is in accordance with phylogeny based on both morphology and recently molecular data (Fenn et al., in press).

Thus, in Tettigonioidea, tympana and auditory function would have evolved after a sophisticated tibial organ. The investigated atympanate taxa would possess a precursor organ for auditory receptors. This hypothesis invites further investigation of auditory organ structure and central projection, as well as evolution of communication modes.

Differential dynamic processing of semicircular canal signals in separate subpopulations of frog extraocular motoneurons

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Self-generated body motion provokes retinal image displacements with a resultant degradation of visual information-processing. To maintain visual acuity during locomotion, compensatory eye movements are activated by the transformation of semicircular canal and otolith signals into extraocular motor commands. These vestibular signals cover a wide dynamic range from tonic changes of head position to high acceleration profiles during rapid body movements. This requires that the sensory-motor transformation occurs in parallel, frequency-specific pathways. The dual organization of phasic and tonic central vestibular neurons suggests that a frequency-specific signaling might also occur at the level of the extraocular motoneurons. Antidromically identified abducens and oculomotor motoneurons were recorded in an isolated frog brain *in vitro*. Intrinsic membrane properties were determined by intracellular current injections and synaptic inputs were evoked after bilateral electrical stimulation of individual semicircular canal nerves. The waveform of subthreshold synaptic compound responses and spike discharge patterns evoked by frequency-modulated electrical pulse trains to semicircular canal nerves, in combination with intrinsic membrane properties were used to define different functional subtypes of motoneurons. Multiunit recordings of extraocular motor nerves *in vitro* using the same synaptic stimulus paradigm allowed the analysis of the firing pattern of individual axons by an advanced spike sorting algorithm. Based on the responses evoked by intracellular current injections and the profile of synaptically elicited compound responses after semicircular canal nerve stimulation, three subpopulations of extraocular motoneurons could be distinguished. The first population consisted of rather tonic type motoneurons with low-pass filter properties and synaptic response profiles that appeared to be exclusively mediated by presynaptic tonic-type vestibular neurons. A second population had highly phasic membrane properties and received inputs only from phasic-type vestibular neurons, based on the evoked compound response waveform and the spike pattern. These two subtypes were supplemented by a third population with intermediate intrinsic properties and semicircular canal nerve-evoked responses that suggested a convergence of presynaptic inputs from both types of vestibular neurons. Identified single units in extraocular nerve recordings confirmed the presence of separate subpopulations based on their synaptically evoked discharge patterns. These data comply with the activity of extraocular motoneurons during natural head movements. Accordingly, distinct motoneuronal populations exist that either code eye position, eye velocity or a combination of both parameters. Although some convergence occurs at the level of the motoneurons, a separation of phasic and tonic vestibulo-ocular signal components is maintained in particular neuronal populations and thus corroborates the idea of a signal processing in parallel frequency-tuned pathways.

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Modeling the origin of functional heterogeneity among auditory nerve fibers

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It has been known for more than 30 years that the sensitivity of auditory nerve fibers in mammals is correlated to their spontaneous firing rate and that the variability of these two properties is linked to the auditory system's capability to reliably encode sounds with intensities spanning about 4 orders of magnitude. However, neither the origin nor the coupling of these properties are yet fully understood. The key players at this early level of the auditory system are the presynaptic active zone of inner hair cells, the postsynaptic spiral ganglion neurons and efferent synapses onto spiral ganglion neurons.
We use modeling to investigate how each of these components could contribute to modify the spontaneous rate and sensitivity.

On the presynaptic side, the effect of varying Ca^{2+}-channel number, density and their distance to vesicles has been explored. On the postsynaptic side, we test the effects of firing threshold and adaptivity of the afferent fiber, as well as the strength of a tonically active or intensity-driven feedback inhibition by the efferent fiber.
Parameters for the modeling of the hair cell ribbon synapse are restrained by measurements from in-vivo and in-vitro experiments including fast confocal imaging of synaptic calcium signals, current fluctuation analysis derived calcium channel counts, STED-microscopy based calcium channel cluster size estimates, as well as auditory nerve properties from single unit recordings.
Although not frequently considered in the literature, presynaptic parameters are found to strongly influence spontaneous rate and sensitivity.

Intensity Invariance Emerging in a Feed Forward Network

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Many sensory systems need to encode a wide range of stimulus intensities in their spike frequency while being sensitive to small intensity differences. Both must be achieved under biophysical constraints on the neuron's maximum spike frequency. On the single neuron level a steep spike-frequency-Intensity-curve (f-I-curve) is optimal to distinguish small intensity differences but the intensity range that can be encoded is necessarily small. A shallow f-I-curve allows for encoding of a broad intensity range but reduces the resolution. This conflict can be resolved by spike-frequency adaptation that shifts the dynamic range of a steep f-I-curve to the mean stimulus intensity. This way, fast changes of the stimulus are encoded while information about the mean stimulus intensity is lost. Although sensitivity adjustment by adaptation has been revealed in many sensory systems, it usually remains unclear how it is achieved by different adaptation mechanisms on different levels of the sensory pathway. This study investigates the emergence of intensity invariant coding in a feed forward network composed of receptor neurons with steep f-I-curves and different sensitivities that converge to a single interneuron. This kind of network matches early stages of the auditory system of crickets and locusts. In respective experimental studies local interneurons displayed f-I-curves with slopes comparable to that of individual receptors, but, unlike the receptors, shifted their dynamic range over a considerable intensity range according to the mean intensity of the presented stimulus. We use both a firing rate model and a network of integrate and fire neurons to investigate under which constraints to the network elements such steep and strongly shifting f-I-curves are generated. In particular we are interested to specify which network elements may or need to adapt. The model parameters were chosen to reproduce experimentally observed onset and steady state f-I-curves of auditory receptor neurons. Our results show that adaptation on the receptor level alone is insufficient to achieve both, steep f-I-curves and a shift of the dynamic range to the mean stimulus intensity. By simple summation of receptor responses the resulting f-I-curve is shifted but its slope is considerably smaller than that of individual receptors. Ongoing studies investigate how adaptation in the interneuron itself or synaptic dynamics may contribute to generate the desired properties of the interneuron. Hypotheses derived from this study will be used to design further experiments in the cricket auditory system.

Neurophysiology and neuroanatomy of the lateral line system in *Xenopus laevis*

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The African clawed frog *Xenopus laevis* uses a mechano-sensory lateral line (LL) system for localising wave sources (e.g. insect prey) on the water surface. About 180 water-velocity sensitive organs are systematically distributed on the frog's body to pick up wave signals, and generate wave source representations in the CNS. To approach the latter, the effects of the wave frequency, curvature, and stimulation angle on LL responses in the CNS were studied, and recording sites reconstructed.

Wave sources were placed relative to the midpoint of the frog's rostro-caudal axis at distances from 4 to 10 cm, and at different angles from 30° to 180°. Above the submerged frog's centre the wave amplitude and spectral composition of incoming wave stimuli were stabilised.

Of 221 extracellularly recorded LL-responses to monofrequent surface waves, 169 showed a significant change in their response rate for different wave frequencies from 10–40 Hz ($p<0.05$; Wilcoxon-test). 34.9% of the units responded best to frequencies =25 Hz while 65.1% showed a best frequency (BF) >25 Hz. The BF distribution was non-uniform ($p=0.001$; chi-square-test). Randomised stimulation of the frog with surface waves from various distances (resulting in variable wave curvatures) elicited curvature-dependent response rates in 112 central LL-units ($p<0.05$; Wilcoxon-test). 85.7% of these units displayed a response-rate change =50%. When using six different wave source angles, 43 direction-sensitive LL-units (of N=45) showed significant response rate changes ($p<0.05$; Wilcoxon-test). Best responses were not uniformly distributed across source angles ($p=0.001$; chi-square-test), and occurred for a stimulation angle of 90° in 34.9% of the units.

After recordings, neurobiotin was iontophoretically applied to pinpoint recording sites and their likely inputs. Brain sections were only analysed if the injection site did not extend beyond the borders of a single nucleus. Histological data was collected for tracer injections into the optic tectum (OT; N=8) and the principal nucleus of torus semicircularis (TP; N=5) where 96 of the 221 LL-recordings were obtained. In all animals and for both injection sites, substantial retrograde labelling of cell bodies was observed ipsilaterally in three tegmental subdivisions (i.e. dorsal and ventral tegmentum, nucleus isthmi; $N_{\mathbf{OT\text{-}inject.}}=23$; $N_{\mathbf{TP\text{-}inject.}}=32$; mean total number of cells; also below). In the laminar nucleus of the torus semicircularis, ipsilateral retrograde transport was stronger after injection in the TP compared to the OT ($N_{\mathbf{OT\text{-}inject.}}=16$; $N_{\mathbf{TP\text{-}inject.}}=39$). In stark contrast to OT injections, only TP injections demonstrate a significant ascending input from decussating fibres that originate in the lateral line nucleus of the medulla ($N_{\mathbf{TP\text{-}inject.}}=16$). Both the OT and the TP receive descending fibres from diencephalic nuclei ($N_{\mathbf{OT\text{-}inject.}}=13$; $N_{\mathbf{TP\text{-}inject.}}=6$).

The results confirm that both distance- and direction-dependent wave parameters that could serve to compute source positions are reflected by central LL-responses. Among recording sites, the TP has been known as a main projection area of medullary LL-neurones, however, also the tegmentum and the laminar torus semicircularis potentially influence LL-processing. Descending diencephalic projections are likely to convey visual information, but might also serve efferent LL-control mechanisms.

Coding at high precision in the velocity-regime: Application of information theory to lateral-line detection

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The mechanosensory lateral line system of fishes, especially the peripheral organization and physiology, has been studied intensively. This sensory system, unique to aquatic animals, is characterized by the presence of two classes of sensory units, the so-called superficial neuromasts and the canal neuromasts. The anatomical differences between both sub-systems are associated with physiological differences; most importantly, canals filter the responsiveness to low-frequency perturbations of a flow field as long as the scale of the latter is smaller than the inter-pore distance.

Recent advances in research on the biophysics of the stimuli to the lateral line have lead to the hypothesis that the stimulus to both systems can be analyzed in a similar manner, and that boundary layer effects can be neglected. One prediction of this analysis is that the most efficient stimulus to superficial neuromasts should be the water velocity field on the skin of a fish. Physiological evidences for this idea have been found.

Through band-pass filtered noise as a stimulus we now elucidate which stimulus features superficial neuromasts are responsive to. In order to do so, we follow the reverse-correlation approach using either simple linear reverse correlation or a covariance analysis that is not restricted to a single filter. The advantage of the latter is that it does not a priori limit the number of stimuli that a neuron might be responsive to.

All investigated afferents responded with high reproducibility to noise stimuli in a frequency band between 10 to 150 Hz. The correlation between the stimuli and their reconstructions were high, reaching up to 70%, and depended on the stimulus intensity. Covariance analysis consistently resulted in two eigenvector that were of similar frequency but shifted by Pi/2. The first eigenvector is similar to the first Wiener kernel (STA) and firing of the neurons depends on the mean frequency and phase of the stimulus, in accordance with the directional sensitivity of the lateral-line periphery. The second eigenvector contributes only weakly to the spiking. We conclude that functionally the STA is likely to represent a smooth version of the velocity of the stimulus and is neither related to the acceleration nor to a fractional derivative of the stimulus velocity. Surface neuromasts are therefore more likely to be pure velocity detectors. As a side-remark we note that we can confirm our conclusion regarding the physiology by means of a theoretical model based on spiking neurons.

Dimensional Changes of tectorial membrane due to mutations in TRß and the tectorins.

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Alpha-tectorin is a noncollagenous component of the tectorial membrane which plays an essential role in auditory transduction. In several DFNA12 families mutations in TECTA, the gene encoding alpha-tectorin, were shown to cause hearing impairment (HI) with different phenotypes depending on the location of the mutation. As well there are developmental changes in the tectorial membrane caused by lack of specific hormones like the Thyroid-Hormone or Receptors like TRß. Here we will show two different ways of affecting the tectorial membrane formation.

A Deletion of thyroid hormone receptor ß (TRß) causes deafness in mice and men. Delayed maturation of inner hair cells (IHCs), due to delayed expression of the fast-activating BK current, has been discussed to contribute to hearing loss. In Contrast an increasing thickness of tectorial membrane and enhanced tectorin level caused by deletion of TRß leads to reduced DPOAEs and cochlear microphonics. This suggests that disturbance of mechanical performance is the primary cause of deafness following TRß deficiency. Here different transgenic mice strains were generated using the PCre/Lox system amongst others. For the observations of tectorial membrane we used Trß-/- mice. As well as epoxin embedded slides for ultrastructure analysis, OCT embedded slides for immunolabeling were used. It could be presented that we have a frayed tectorial membrane that shows an increased immunostaining against a- and ß-tectorin. Further Western blotting and densitometric analysis were performed using dissected cochlear tissue to confirm the assumption that not the frayed structure is reasonable for a stronger immunolabeling but rather more protein is translated. The intensities of both a- and ß-tectorin were normalized to the level of the house keeping protein ezrin. So it was possible to show enhanced tectorin levels.

A Turkish family displaying autosomal dominant inherited HI. Linkage analysis revealed significant cosegregation (LOD score: 4.6) of the disease to markers on chromosome 11q23.3- q24. This region contains the TECTA gene which was subsequently sequenced. A nucleotide change in exon 13, 4526T>G, was detected leading to a substitution from cysteine to glycine at codon 1509 of the TECTA protein. This cysteine is located in vWFD4 domain, a protein domain which is supposed to be involved in disulfide bonds and protein-protein interactions. Conclusions: It is conspicuous that the phenotype in this family correlates with other families, also displaying mutations involving conserved cysteines. In all three families these mutations result in progressive HI involving high frequencies. In contrast, mutations which are not affecting the vWFD domains seem to provoke mid-frequency sensorineural HI. Furthermore, evaluation of clinical data in our family revealed a gender effect for the severity of hearing impairment. Males were significantly more affected than females. The identification of the third family displaying a missense mutation in the vWFD domain of alpha- tectorin underlines the phenotype-genotype correlation based on different mutations in TECTA. (Pfister et al., 2004)

Two modes of information processing in the electrosensory system of the paddlefish, *Polyodon spathula*

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Paddlefish (*Polyodon spathula*) use their passive electrosensory system for the detection of their prey, small water fleas. With anatomical and electrophysiological techniques, we investigated how electrosensory information is processed in the brain. Primary afferent fibers innervate a single area in the hindbrain, the dorsal octavolateral nucleus (DON). Secondary neurons in the DON project then to the mesencephalic tectum and to two areas in the dorsal tegmentum, the torus semicircularis, and the lateral mesencephalic nucleus. We found here that the projections to the tectum and dorsal tegmentum arise from two different populations of neurons in the DON. Cells in the anterior DON project to the contralateral tectum whereas cells in the posterior DON project to the tegmentum, bilaterally. These cells are one order of magnitude more sensitive than the cells projecting to the tectum and respond better to lower frequencies. This makes them better suited to detect prey at a distance when signals are weaker and have lower frequency components. In contrast, DON cells projecting to the tectum can work in a proximity mode only, when the prey is close to the receptors in the skin. In this mode, spatial resolution is high and electrosensory information can directly converge with visual information in the tectum to guide prey capture behavior. This is the first report of two modes of information processing in the octavolateral system and may help to understand the functional role of the torus semicircularis as opposed to the tectum.

Delay-sensitive neurons of the auditory cortex in the Phyllostomid bat, *Carollia perspicillata*

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In insectivorous bats that use frequency-modulated (FM) or a combination of constant frequency and FM signals (CF-FM) sonar signals, cortical delay tuning for target range estimation is well studied. The aim of the present study was to determine the mechanism and properties of delay-sensitive neurons in the auditory cortex (AC) of the fruit-eating FM-bat *Carollia perspicillata*. In this species, combination-sensitive (delay-tuned) neurons are embedded in clusters within the high-frequency field (HF) field of the AC. These FM-FM neurons are responsive to the same FM component in the echolocation pulse and echo (e.g. FM_1+FM_1, FM_2+FM_2).

In 27 anesthetized bats, a total of 221 frequency-tuned neurons of the AC in *Carollia perspicillata* were recorded. Delay-tuned neurons were stimulated by using behaviorally relevant stimuli. *Carollia perspicillata* produce multiharmonic, downward FM signals of broad bandwidth, short duration, high frequency and low intensity for navigation (Thies, 1998). The call consists of 4 harmonics. In 140 units combinations of all harmonics were tested (FM_1+FM_1, FM_2+FM_2, FM_3+FM_3, FM_4+FM_4). The majority (36.43 %) responded to the combination of the 2nd (FM_2+FM_2) or the 3rd harmonic (FM_3+FM_3). In 38 out of 140 units (27.14 %), the cortical neurons were responsive exclusively to stimuli mimicking the pulse and echo of the 2nd harmonic. These results correlate with the natural echolocation calls of *Carollia* where the main energy is at the 2nd and 3rd harmonic.

The delay-tuned neurons were also tested with ‘unnatural’ stimuli (FM↑up). In 48 out of 58 cases (82.76 %) the delay-sensitive neurons responded both to FM↓down and FM↑up stimuli that mimicked certain echolocation pulse-echo delays. The conclusion is that FM-FM neurons in *Carollia perspicillata* are responsive to the broad frequency content of the FM signal, whereas the direction of the modulation seems not to be significant.

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Distribution of Na^+/Ca^{2+} exchangers NCX and NCKX in the developing rat auditory brainstem

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The survival and development of auditory neurons is crucially dependent on the intracellular Ca^{2+} concentration. By extruding Ca^{2+}, Na^+/Ca^{2+} exchangers contribute to Ca^{2+} homeostasis. Two families of Na^+/Ca^{2+} exchangers can be distinguished within the superfamily of cation/Ca^{2+} exchangers: the NCX family (NCX1-3) of Na^+/Ca^{2+}exchangers and the NCKX family (NCKX1-6) of $Na^+/Ca^{2+}/K^+$ exchangers. The additional thermodynamic driving force imparted by K^+ allows NCKX to extrude Ca^{2+} even if the Na^+ gradient is diminished. All NCX isoforms as well as NCKX2-4 and NCKX6 are differentially expressed in the brain, suggesting different functional roles. NCKX5 mRNA could also be detected in brain but localization or protein expression has not been analyzed so far. Here, we have investigated the distribution of NCX and NCKX isoforms in auditory brainstem structures of the rat, namely the cochlear nucleus (CN), the superior olivary complex (SOC), and the inferior colliculus (IC), each comprising several subregions. To account for ontogenetic aspects of NCX/NCKX distribution, rats of two ages were analyzed [P4, i.e. before hearing onset (at P12), and P60].

Immunohistochemically, expression of NCX1-3 and NCKX2 protein was detected in CN, SOC and IC neurons of P4 and P60 rats. Within the CN, immunoreactive (IR) signals were detected in all subnuclei. In P60 CN, NCKX2 labeling was mainly observed in the neuropil, whereas NCX1-3 showed a mainly somatic labeling pattern. At P4, NCX1-3 as well as NCKX2 showed mainly neuropilar labeling. Within the SOC, all subnuclei showed IR signals for all investigated isoforms at both ages. The IC of P4 and P60 rats showed IR signals mainly in neurons of the dorsal and external cortex. Additionally and to include NCKX3-6, against which no antibodies are presently available, analysis was performed at the mRNA level. The mRNA of all investigated isoforms was detected in CN, SOC, and IC tissue of both ages, corresponding to the observed expression of NCX1-3 and NCKX2 proteins. To quantify expression levels, real-time PCR was performed. In all investigated tissues, NCX1 and NCX2 transcription was upregulated during development. NCX3 mRNA was upregulated in the CN, downregulated in the SOC and showed no significant regulation in the IC. NCKX2 and NCKX6 were downregulated during development in all tissues while NCKX4 showed downregulation in the CN and SOC, but no changes in the IC. NCKX5 mRNA was upregulated during development in the CN and IC and downregulated in the SOC. In summary, real-time PCR experiments showed in the majority of cases an age-dependent upregulation of NCX isoforms, yet a downregulation of NCKX isoforms. Transcription was also found to be regulated between the different tissues within one age group. Since none of the investigated nuclei shows a unique expression pattern, all investigated isoforms of Na^+/Ca^{2+} exchangers appear to act together in Ca^{2+} regulation in the auditory brainstem.

Distribution and kinetics of synaptic AMPA receptors in adult MSO neurons

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In mammals the location of low frequency sounds in the horizontal plane is initially processed in the medial superior olive (MSO) by a neuronal coincidence mechanism. These coincidence detector neurons are highly sensitive to the timing of binaural excitatory and inhibitory inputs. However, the kinetics and distribution of the underlying excitatory receptor and synaptic currents are unclear. Furthermore, it is not known if dendritic filtering affects the temporal profile of the synaptic currents and/or potentials due to their location on the dendritic tree.

We investigated the distribution and kinetics of AMPA receptor currents of MSO neurons in matured, 30 day old Mongolian gerbils. The AMPA receptor distribution was determined using UV-laser uncaging of 1 mM circulating MNI-glutamate combined with voltage-clamp whole-cell recordings. Calibration experiments revealed an effective uncaging spot size of ~7 µm full width at half maximum. Synaptic evoked AMPA receptor currents were elicited with a stimulation glass electrode. Recordings regarding receptor distribution were carried out at room temperature; other recordings were obtained at ~35°C.

We analysed the peak currents and rise times of pharmacologically isolated AMPA currents optically evoked along the dendrites of MSO neurons. We found an average 2-fold decrease in peak current along the dendrite up to a distance of 135 µm from the soma, however this was accompanied by 2-fold increase in the 20-80 % rise time of these currents, indicating the presence of dendritic filtering.

Synaptically activated AMPA-receptor currents displayed extremely fast kinetics. We found large excitatory postsynaptic currents (EPSCs) ranging between 1 and 6 nA. The decay time constants of these currents ranged from 200-400 µs. This decay time course is expected to be an overestimation due to the inevitable attenuation of the signal by the series resistance of the whole-cell voltage-clamp condition. Therefore, offline series resistance compensation is needed to determine the actual speed of these fast and large EPSCs. These results provide first insights into the conversion of EPSCs to EPSPs in the MSO.

Virtual space technique as a measure to investigate the role of the barn owl's facial ruff

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Barn owls (*Tyto alba*) have an excellent auditory system, allowing them to localize sound targets with an accuracy of up to 3° in both the horizontal and vertical plane, respectively. The hearing range of barn owls covers frequencies from 200-12000 Hz. This is possible because of two morphological specializations: the barn owl's characteristic facial ruff, and asymetrically arranged ears. The left ear and flap are positioned higher at the head than the right ear and flap, and are more sensible to sound coming from the lower hemisphere, and vice versa for the right ear. Incoming sound is specifically filtered in a direction dependent manner. Sound arrives with an interaural time delay (ITD), which systematically varies with the spatial angle of the sound source. Additionally, especially high frequency components are stronger attenuated at the ear which is opposed to the sound source. The resulting interaural level difference (ILD) likewise depends on the sound source position.

Although ITD and ILD occur for any binaural hearing animal, the asymmetric ears and the facial ruff of the barn owl change the spatial distribution pattern in a characteristic way. If sound is played from specific spatial positions, the direction dependent transfer functions can be recorded at the owl's eardrum. These so-called head-related transfer functions (HRTFs) can be used for filtering of arbitrary sounds in order to create a virtual space. This technique allows to manipulate certain characteristics of the facial ruff. In the presented study, we removed the facial ruff of an anesthesized barn owl. We recorded a set of HRTFs for representative spatial positions (varying azimuth and elevation). We then stimulated owls via headphones with broadband noise stimuli filtered with these HRTFs, either with or without facial ruff, respectively. Thereby, the influence of the ruff and the asymmetric ear arrangement could be investigated separately.

Adaptation in the auditory midbrain of the barn owl (*Tyto alba*) as demonstrated by a two-stimulus paradigm

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Neurons typically respond with a higher rate to the onset of a stimulus than during ongoing stimulation. This decline in their initially high response rate to a steady-state at later phases of the stimulus presentation is termed spike-frequency adaptation. The underlying mechanism is different from adaptation-of-excitation, where the capacity of a neuron to respond to subsequent stimuli is reduced, when the first stimulus was excitatory itself. Adaptation has been observed at almost all levels of the auditory system and is linked with effects like the “dynamic-range problem” or novelty detection.

In this study we were interested whether adaptation occurs in monaural responses of the narrowband neurons in the central nucleus of the inferior colliculus (ICC). Working with anaesthetized owls and using standard extracellular recording techniques, we first determined the best frequency (BF) of a neuron. Stimuli were tones of varying frequency with 100 ms duration and 5 ms cosine-squared on and off ramps. Secondly, a monaural rate level function (RLF) was recorded at the BF. We then tested adaptation at BF by presenting two consecutive stimuli. The first stimulus was presented at a given level (10%, 30%, 50%, 70%, 90% of the saturating responses); the second stimulus was presented either at varying inter-stimulus intervals (ISI, ISI tuning, ISI varying from 25 ms to 1600 ms, equal levels) or at varying levels of the second stimulus (level tuning, ISI = 0 ms, level varying in 5 dB steps from 0 to 25 dB louder). The change of the response was measured as the response ratio, i.e. the quotient of the response to the second stimulus divided by the response to the first stimulus multiplied by 100.

Preliminary results from 17 neurons demonstrate a decrease in response in level tuning to about 50% for the second stimulus for both ipsi- and contralateral stimulation when the level of the second stimulus was equal to the level of the first stimulus. An increase of 10.85 dB (for 10% of the ipsilateral RLF) to 15 dB (for 50% of the contralateral RLF) of the level of the second stimulus was necessary to reach a response level as observed to the first stimulus. After a mean ISI of 1025 ms, the response of the second stimulus was equal to the response to the first stimulus. ICC neurons are, therefore, highly adaptable to code for subsequent stimuli varying in either level or temporal occurrence.

Estrous cycle-dependent plasticity of auditory cortical activation in mice

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Emotions, sensations, cognitive performances, and even the expression of mental deseases show rhythmic changes during the menstrual cycle of women (e.g. Parlee MB, Psychol Bull 1983;93:539-48; Derntl B et al, Horm Behav 2008;53:90-5). Female mice show estrous cycle dependent differences in the perception of the biological significance of wriggling-call models in behavioural tests (Schmid C, Diploma-Thesis, University of Ulm, 2007; Ehret G, Schmid C, submitted). They discriminate the quality of call models only in diestrus and proestrus (not in estrus and metestrus). Here, we demonstrate that the behavioural variations of emotional auditory perception are represented in auditory cortical activation during the estrous cycle.
Mouse pups emit series of harmonically structured low-frequency "wriggling calls" when an adult animal is in a nursing/warming position on the litter. By changing acoustical parameters, wriggling calls can be modelled to be efficient (biologically significant) or inefficient (biologically insignificant) releasers of maternal care in mothers (Ehret G, Riecke S, PNAS 2002;99:479-82; Geissler DB, Ehret G, PNAS 2002;99:9021-25) For our acoustic stimulation we used an efficient and an inefficient call model as releaser of maternal care, as has been found in mothers. Neuronal activation was imaged and quantified via c-Fos immunocytochemistry by counting c-Fos positive cells in frontal sections of the auditory cortex of both hemispheres (Geissler DB, Ehret G, EJN 2004;19:1027-40). Among our results are the following: (1) There is a left-hemisphere advantage in the number of Fos-positive cells in the second auditory cortical field (AII) both for efficient and inefficient call models. (2) The lowest numbers of Fos-positive cells occur in the left hemisphere in diestrus when the animals perceive the efficient call model (significant difference to stimulation with inefficient model). (3) There is a left-hemisphere advantage in the number of Fos-positive cells in the dorsoposterior field (DP) when the animals perceive the efficient call model (significant in estrus and diestrus). (4) In DP there are no estrous dependent differences in labeling neither for the efficient nor the inefficient call model. These data show that the activation of AII reflects specifically in diestrus the discrimination ability of the quality of wriggling call models for the release of maternal behaviour. Furthermore our results support the suggestion that AII plays an important role in the identification ("what") of communication sounds and that this processing seems to be left-side specific. In DP the left-hemisphere advantage of processing efficient call models is obvious as has been shown already for mothers (Geissler DB, Ehret G, EJN 2004;19:1027-40).

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Auditory motion perception following unilateral cortectomy

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The perception of motion is an essential prerequisite to responding adequately to the dynamic aspects of sensory information in the environment. The neural substrates of auditory motion processing are, at present, still a matter of debate. It has been hypothesized that motion information is, as in the visual system, processed separately from other aspects of auditory information, such as stationary location. Here we report data on auditory perception of stationary and motion stimuli from a subject with right-sided resection of the anterior temporal-lobe region including medial aspects of Heschl's gyrus, and from three subjects with unilateral (right-sided or left-sided) hemispherectomy. All these subjects had undergone cortectomy decades earlier. The subjects with hemispherectomy were completely unable to perceive auditory motion, but showed slight to moderate deficits in judging stationary location. The subject with temporal lobectomy exhibited quite similar stationary auditory deficits as found in the subjects with hemispherectomy, but was completely normal in judging auditory motion. Thus, there was a clear dissociation of the effects of unilateral temporal lobectomy and hemispherectomy on auditory motion perception. Collectively, these findings suggest that the unilateral anterior temporal-lobe region plays a significant role in the analysis of stationary, but not moving, sound. One may assume that the cortical "motion network" is distinct from the "stationary network", and is located either in the most posterior aspects of temporal lobe, or in non-temporal, most likely parietal, areas.

Potassium channel expression in the avian auditory brainstem – comparison of in vivo and in vitro development

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In the auditory brainstem, physiological adaptation to computational tasks can be associated with biophysical specialisations of neurons. Two potassium channel types, Kv1.1, which mediates a low-threshold K-current (LTK), and Kv3.1b, which mediates a high-threshold K-current (HTK), are highly expressed in the auditory system of birds. The interaction of these potassium channel types with basic membrane properties and voltage-activated sodium inward currents produce specialised biophysical properties of the neurons. Many studies demonstrate the importance of Kv1.1/Kv3.1b in the auditory brainstem. However, little is known about the development of these channels.

We thus compared protein expression of Kv1.1 and Kv3.1b in brainstem samples from different embryonic stages to samples from developing primary cultures of auditory brainstem at corresponding timepoints. Functional synaptogenesis and random spiking took place in the auditory brainstem culture, but no rythmic or recurring activity, as is known from recordings in the embryonic brainstem, could be found. Thus our culture system approach allowed us to differentiate between the signalling roles of the onset of synaptogenesis (ie transsynaptic activity) and rythmic bursting activity.

We found, that between E10 and E14, at the time of functional synaptogenesis in vivo, Kv3.1b protein levels increase markedly. Surprisingly, this increase was also found after corresponding periods of cultivation, also during a phase of synaptogenesis. The increase in Kv3.1b was reflected in an increase of HTK in the in vitro neurons. Also, voltage-activated inward currents strongly increased during cultivation. We thus conclude, that spiking elicited by beginning transsynaptic activity is a sufficient trigger for Kv3.1b expression.

Kv1.1 protein expression, on the other hand, did not increase in vitro. Correspondingly, LTK was very low. Therefore it can be concluded, that Kv1.1 needs a more intricate developmental signal not found in the in vitro situation.

With computer models of the in vitro neurons we explored the impact of the gradually changing conductances during development. This model accounted for the diversity of firing patterns found in developing auditory brainstem and in vitro. Additionally, the in vitro neurons were compared to model neurons of nucleus laminaris in simulated physiological tasks like high-frequency firing and coincidence detection.

In conclusion we suggest that an activity dependent upregulation of Kv1.1, which is missing in the in vitro neurons, is necessary for the development of auditory function.

Time course gene expression profiling identifies candidate genes for maturation and function of the rat superior olivary complex

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Auditory structures are promising model systems to study the development of neurosensory circuits. Hearing onset occurs at postnatal day (P) 12 in rats and mice, and maturation processes continue up to P25-P30. The superior olivary complex (SOC) represents a prominent structure within the auditory brainstem. It is the first station in the auditory pathway where information from both cochleae converges. This binaural information is used for sound localization by processing interaural intensity differences and interaural time differences. Here, we report on a time course gene expression profiling experiment in the SOC in order to identify candidate genes for maturation and function. To this end, its genome wide expression pattern was characterized at P4 (pre-hearing), P16 (shortly after hearing) and P25 (mature system) using a microarray platform with more than 40,000 different probes. For comparison, the transcriptome of the entire brain was also investigated at P4 and P25. This enabled the identification of SOC specific expression patterns.

More than 2,600 probes were up-regulated or down-regulated between P4 versus P16 or P25. This large change in gene expression correlates with the switch from spontaneous to sensory input driven activity in the SOC. In contrast, only ~ 90 probes were differentially expressed between P16 and P25.

Hierarchical cluster analysis of significantly regulated probes revealed 5 different expression profiles during SOC development. The majority of probes showed a strong up-regulation from P4 versus P16 and a gradual increase from P16 to P25; or exactly the opposite, a drastic decline from P4 to P16 and a gradual decrease from P16 versus P25. Another set of probes displayed a constant, nearly linear up-regulation or down-regulation over the 3 time points. Interestingly, several probes showed peak expression at P16.

Similar to the SOC, about 3,000 probes showed differential expression in the entire brain between P4 and P25. However, at both stages, we noted considerable differences in gene expression between the SOC and the entire brain. At P4, ~1,500 probes were differentially expressed between the SOC and the brain. This number increased 1.6 fold (~2,600 probes) at P25 and indicates development-dependent specification of the SOC. Both in the SOC and in the brain, ~ 1,300 probes were regulated in the same direction during development, reflecting common aspects during maturation of neuronal networks. However, 1,800 probes demonstrated SOC-specific developmental changes. In summary, our study identifies both general and SOC-specific candidate genes underlying maturation and function of neuronal circuits.

Electrophysiological recordings from the primary auditory cortex of the unanesthetized and anesthetized house mouse (*Mus musculus*)

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The majority of studies that concentrate on questions concerning the neuronal processing of acoustic stimuli in the central auditory system, are conducted on anesthetized animals. It is broadly known, however, that many response properties of neurons can be influenced or even dramatically changed by anesthetic agents (e.g. Zurita et al. 1994, Schulze and Langner 1997, Gaese and Ostwald 2001).
In this present study we examined the influence of a ketamine/xylazine anesthesia on the neuronal responses of single- and multi-units from the cortical layers III – IV (200- 400 µm depth) in the tonotopically organized primary auditory fields AI and AAF of the house mouse (*Mus musculus*). We presented pure tones (PT) and 100% sinusoidally amplitude modulated (AM) tones to eight weeks old female mice. In line with previous studies on different species, we observed that neurons in anesthetized animals compared to unanesthetized ones showed longer response latencies to both PT and AM tone stimulation. In addition, neurons in anesthetized animals had a considerably lower spontaneous activity and a reduced evoked rate at their best frequency. The effect of the anesthesia was most obvious when comparing the capability of the neurons to synchronize their response to the time structure of the envelope of the AM tones. The neurons of anesthetized animals mostly showed no phase-locked discharge patterns at all or were only able to phase-lock up to a modulation frequency of 2 – 6 Hz. Responses to AM tone stimulation recorded from unanesthetized animals showed phase-locking up to modulation frequencies of about 100 Hz. The primary auditory fields AI and AAF did not indicate significant differences with regard to the effects of anesthesia on neuronal responses to AM tones. This study provides further evidence that the anesthetic state changes the neuronal responses in the primary auditory cortex in a significant way. Thus neuronal data from anesthetized animals may not provide a relevant basis for studying cortical mechanisms of auditory perception and recognition.

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General properties and synaptic input-output functions of neurons in the dorsal lateral lemniscus of Mongolian gerbil

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Neurons in the dorsal nucleus of the lateral lemniscus (DNLL) receive strong excitation from nuclei of the superior olivary complex (SOC). It appears that *in vivo* the precision of spike timing of DNLL neurons recembles largely that of SOC neurons during auditory stimulation. Thus, DNLL neurons are capable of temporal precise high frequency firing. It is suggested that this characteristic is supported by the neurons robust action potential (AP) initiation properties and possibly by its long integration constants. However, quantitative discribtion of the AP properties and its inititation as well as the EPSC to EPSP convertion are lacking in these neurons. Therefore, we investigated the biophysical properties and the synaptic input-output relation of DNLL neurons.

Whole-cell voltage- and current-clamp recordings were used to measure excitatory postsynaptic currents (EPSC) and potentials (EPSP) respectively from visually identified DNLL neurons from Mongolian gerbils of postnatal day 14 to 17. Pharmacologically isolated EPSCs and EPSPs were evoked by stimulating afferent fibres with a glass electrode. Recordings were obtained out at ~35°C bath temperature.

First we measured the APs characteristics and the basal membrane parameters of DNLL neurons. In general DNLL neurons appear to by highly variably with an input resistance ranging from 60 to300 MOhm, a membrane time constants between 5 and 30 ms and a resting potential between -49 and -68 mV. However all neurons displayed large APs and sustained firing behaviour during 300 ms depolarising current injections.

Synaptically activated AMPA-receptor EPSCs decayed with an average of 1.6 ms. The relation between EPSC decays, rise times and peak amplitudes indicated that excitatory inputs are subject to dendritic filtering. Besides the AMPA mediated EPSCs a substantial, synaptic NMDA component is present. Investigation the EPSP generation revealed that the NMDA component contributed to the prolongation of the EPSPs during spatial and temporal summation. In addition, pharmacologically isolated excitatory fibre stimulation usually evoked postsynaptic APs. The amount of APs elicited by a single fibre stimulation pulse depended nearly linearly on the elicited charge of the AMPA EPSC.

Together this shows that DNLL neurons integrate slowly but are capable of temporal precise high frequency firing as they adept firing rates to the input strength. Thus the physiology of these neurons is suitable for integrating precise temporal information.

Parallel electrophysiological and behavioral analysis of layer-specific electrical microstimulation in primary auditory cortex - implications for the subcortical-loop hypothesis

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For the development of sensory cortical neuroprostheses it is important to determine the generators of electrically evoked cortical responses and their neuronal dynamics. One obstacle for a detailed understanding of how intracortical microstimulation (ICMS) interfaces with cortical processing is that the roles and forms of intracortically (IC) and thalamocortically (TC) evoked neuronal activities, as well as the role of corticothalamic (CT) backpropagating feedback loops in particular, are not well known [1]. Behavioral studies revealed that infragranular stimulation has the lowest threshold [2] that could in principle be due to activation of subcortical loops.

The objective of this study was a parallel electrophysiological and behavioral analysis of layer-specific ICMS in primary auditory cortex AI of the Mongolian gerbil. We used current source density (CSD) analysis to compare acoustical stimulation and ICMS. Electrically evoked and acoustically evoked CSD profiles were similar in terms of spatiotemporal arrangement of current sources and sinks suggesting the recruitment of similar neuronal elements. Then, intracortical sources of activity were suppressed by topical application of $GABA_A$-agonist Muscimol to disentangle TC and IC contributions to the CSD profiles [3]. During the pharmacological blockade a layer IV-sink, corresponding to the lemniscal TC input, was still visible with acoustical stimulation. But even for strong infragranular electrical stimulation we still found remaining sinks and sources when the stimulation site was less than 600 µm afar from the recording axis. This however indicates the principal relevance of intracortical connections for the broad cortical spread after ICMS, but points to an additional activation of precise feedback-connections of the thalamocortical system.

In order to exploit the neuronal dynamics evoked by ICMS for neuroprosthetic applications [4] we determined the laminar threshold variation in a behavioral ICMS detection task using a shuttlebox paradigm. Analysis of psychometric functions revealed that ICMS in the granular and infragranular layers (IV-VI) yielded lower behavioral thresholds compared to ICMS in supragranular layers. We found biphasic bipolar stimulation to be more reliable and efficient than biphasic monopolar stimulation.

Further implications of the combined electrophysiological and behavioral results for the role of subcortical loops in ICMS-evoked perception will be discussed.

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TRAUMA-INDUCED ALTERATION OF BDNF AND ARG3.1/ARC EXPRESSION IN THE AUDITORY SYSTEM

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Altered neuronal activity in the central auditory nuclei has been shown, in humans and mice, to be a physiological correlate of tinnitus (Eggermont and Roberts, 2004; Kaltenbach et al., 2000; Mahlke and Wallhäuser-Franke, 2004). Our objective was to investigate changes in activity dependent gene expression in the cochlea (BDNF) and in the auditory cortex (Arg3.1/Arc) of animals that perceive tinnitus. Perception of tinnitus was studied in an animal behavioural model (Rüttiger et al., Knipper, 2003). Animals were trained to show tinnitus by altering their behaviour. Animals were exposed to acoustic trauma of different intensities (80dB, 100 dB, 120 dB) for 1 or 2 hours at 10 kHz. Hearing measurements are performed and tinnitus induction was analysed at different time periods post trauma (h, d, weeks). Cochleae and brains were embedded for sectioning or collected for RT-PCR. Microtome sections of the brains enabled us to detect mRNA and protein on the same section, while cochleae were analysed for mRNA and protein on neighbouring sections. In this study we show the correlation of BDNF expression in the cochlea and Arg3.1/arc expression in the auditory cortex, dependent on noise intensity and generation of tinnitus.

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Effect of spoken and sung syllables on brain activity

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An early form of music by humans is singing. Singing has characteristics of both music and speech. Speech is thought to be processed predominantly in the left hemisphere and music more dominantly in the right hemisphere [1]. In accordance with this hypothesis, passive listening to sung lyrics compared to spoken lyrics led to stronger activity in the right temporal lobe. Nevertheless, differential activity was also found in regions of both hemispheres [2]. The present fMRI-study investigated which brain regions are involved in the perception of sung and spoken syllables in a discrimination task instead of passive listening.
Eight subjects had to differentiate between sung and spoken syllables. Each subject participated in two sessions, in which syllables sung or spoken by a man or a woman were presented.
Sung and spoken syllables activated the superior temporal gyrus and sulcus, insula, inferior frontal gyrus, middle frontal gyrus and other regions in both hemispheres. Stronger activity to spoken compared to sung syllables were mainly seen in the right hemisphere, i.e. in the inferior frontal gyrus, anterior insula, and posterior medial frontal gyrus. Stronger activity to sung compared to spoken syllables were seen in several regions in both hemispheres, e.g. in left and right precentral gyrus, left and right posterior insula, left anterior superior temporal sulcus, and right inferior occipital gyrus.
In contrast to a study with spoken and sung lyrics [2] we found a clear right hemispheric bias for spoken syllables in contrast to sung syllables. One reason for this discrepancy might be the presentation of meaningless syllables in our study in contrast to lyrics. Perception of sung syllables in contrast to spoken syllables seems to recruit several additional areas in both hemispheres with a bias to the right hemisphere, which is assumed to preferentially process music [1].

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Processing of intonation in spoken language

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Intonation is important because it emphasises the intention of the speaker and helps to resolve semantic ambiguities. In the current fMRI study we investigated the neuronal activities in the human brain during the processing of distinct intonations in sentences. These sentences have been recorded during communication of subjects with a machine in a tower of Hanoi experiment. In the fMRI study 12 subjects listened to these short sentences, which can be subdivided into three categories:
1. the object of the sentence is emphasized ("the triangle"),
2. the direction of the movement is emphasized ("to the right"),
3. no distinguishable difference in intonation of the object or the direction.
Every 16 sec one sentence was presented, all together 30 sentences of each condition. The subjects had to sort them into the correct category by pressing one of three possible buttons.
Sentences without distinguishable differences in intonation elicited greater activation in a number of areas. We found a significantly greater activation ($t = 2.8$) in the right posterior insula (BA 13), right thalamus, bilateral posterior cingulate gyrus, right middle temporal gyrus (MTG) and superior temporal gyrus (STG) during sentences without distinguishable differences in intonation compared to the other categories. Additionally, sentences in which the object was emphasized produced greater brain responses in the right insula, right STG and MTG compared to sentences of the second category. Emphasis of the direction of the movement elicited greater activation in the left putamen compared to sentences of the first category.
The results support the view that speech prosody perception involves widely distributed regions in both hemispheres (Tong et al., 2005). Furthermore, we found a number of brain areas differentially activated depending on which information of a sentence is emphasized.
We presume that activation in the left putamen is correlated to the processing of information related to the direction of the object whereas activation in the right MTG and STG as well as the right posterior insula are more associated with the object itself.

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Binaural Interactions in Congenital Deafness

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The functional development of the auditory cortex is severely affected by total absence of auditory input. It has been shown that diversity of responses to monoaural peripheral stimulation is dependent on auditory experience. The aim of the present study is focused on the classification of binaural interaction in the naïve primary auditory cortex.

The present study was undertaken in four congenitally deaf cats (CDC) and four hearing controls, all in adult age (> 6 months). Control animals were acutely deafened by intracochlear application of neomycin. Cortical responses were evoked by pulse trains (500Hz, 3 pulses) presented uni- and bilateral at intensities 0-12 dB above response thresholds. Intracortical activation was recorded in the primary auditory field AI by means of 16 channel microelectrode arrays (Michigan probes).

Binaural interactions were classified according to the responses to monoaural stimulation. In hearing controls, three types of interactions were observed: EE – both monoaural stimulations evoke responses, EO – contralateral stimulation evokes response only, and OE – ipsilateral stimulation evokes response only. Cortical positions, which respond to binaural stimulation only, were classified as preferentially binaural (PB). Binaural interactions (except of PB class) were further classified as facilitation (evoked firing rate to binaural stimulation of more than 120% of the sum of monaural contra- and ipsilateral responses), neutral interaction (less than 120% of the summation, but more than 80% of the smaller monoaural response) and inhibition (less than 80% of the smaller monoaural response). In CDCs, all types of binaural interaction were repeatedly observed despite of complete absence of hearing experience. Significant difference between the frequencies of contralateral (EO) and ipsilateral preference (OE) and between the frequencies of facilitation and inhibition in binaural interaction was found in hearing controls, but not in congenitally deaf animals.

The present results demonstrate the preservation of basic binaural interactions in naïve primary auditory system and thus to some degree a preserved subcortical binaural processing. Cortical responses were, however, found in significantly smaller proportion of recording positions. The contralateral preference and facilitation typically found in control animals was reduced in CDCs. These two latter findings demonstrate compromised binaural interaction processing in the deprived auditory cortex.

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Analysis and Simulation of the Neurophonic Potential in the Laminar Nucleus of the Barn Owl

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It is a challenge to understand how the brain represents temporal events. One of the most intriguing questions is how sub-millisecond representations can be achieved despite the large temporal variations at all levels of processing. For example, the neurophonic potential, a frequency-following potential occurring in the network formed by nucleus magnocellularis and nucleus laminaris in the brainstem of the bird, has a temporal precision below 100 microseconds.
Here we address the question of how the neurophonic potential is generated and how its remarkable temporal precision is achieved, using a theoretical model. The neurophonic potential consists of at least three spectral components, and our studies aim at revealing their origin. Our hypothesis is that magnocellular axons are the origin of high-frequency component of the neurophonic, whereas action potentials in the laminar neurons are the origin of the 1-2 kHz component. We present an advanced analysis of in-vivo data, numerical simulations of the neurophonic, and analytical results to test this hypothesis. The analysis of the signal-to-noise ratio of the high frequency component of the neurophonic potential lets us estimate the number of independent sources to be at least 250, further implicating the magnocellular axons and indicating that the laminaris neurons alone can not be the source of neurophonic potential.
This work was supported by the BMBF (Bernstein Collaboration in Computational Neuroscience: Temporal Precision, 01GQ07102) and the DFG (Emmy Noether, Ke 788/1-4).

Chloride Homeostasis in the Avian Auditory Brainstem

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GABA is the major inhibitory transmitter in the brain. In most brain regions of adult vertebrates the inhibitory effect is based on hyperpolarisation. Chloride ions enter the neurons via GABA-A receptors following the electrochemical gradient. The potassium-chloride-cotransporter 2 (KCC2) is presumably responsible for the low intracellular chloride level. Early in development, the electrochemical gradient for chloride is reversed in cortical and sub-cortical neurons. Chloride ions leave the neurons via GABA-A receptors and induce a depolarisation. Many authors hold the sodium-potassium-chloride-cotransporter 1 (NKCC1) responsible for the high intracellular chloride level.

In the auditory brainstem of birds (chicken), the depolarising effect of GABA persists into adulthood. Nucleus laminaris and N. magnocellularis receive GABAergic input from the Nucleus olivaris superior. This input is depolarising but inhibitory due to a shunting inhibition. The intracellular chloride level seems to be increased. The molecular basis for this increase is still unknown.

We report here the expression patterns of two chloride transporters in the chicken auditory brainstem on protein level. We find expression of NKCC1 protein in the auditory nuclei of chicken at different developmental stages (E7, E10, E14, E16, E18 tested). Unexpected, we also find KCC2 expression at the same time points.

Further, we performed two-photon fluorescence lifetime imaging with the Cl-sensitive dye MQAE to compare the relative amount of chloride ions in Nucleus laminaris and N. magnocellularis with neighbouring non-auditory neurons.

Complexins are required for auditory synaptic transmission beyond the hair cell

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Complexins (CPX) are small cytosolic proteins that regulate the presynaptic SNARE complex. We explored the role of CPXs in afferent auditory neurotransmission. Inner hair cells, which form ribbon synapses with spiral ganglion neurons, were negative for CPX I-IV mRNA and protein.We observed CPX I protein by immunohistochemistry in the spiral ganglion and in the cochlear nucleus. Auditory brainstem responses were normal in CPX II, III and IV single knockout mice as well as in CPX III/IV double knock-out mice. However, we observed a neural hearing impairment across all frequencies in CPX I knock-out mice. While hair cell transmitter release and subsequent sound encoding were normal, we found an impaired afferent synaptic transmission from SGN to cochlear nucleus neurons. *In vivo* extracellular recordings from single neurons of the ventral cochlear nucleus reported a decreased onset rate as well as increased first spike latency and jitter. We attribute these changes to the reduced release probability of the CPX I-deficient presynaptic terminals, which we observed in acute slices of the ventral cochlear nucleus. We conclude that CPX I is required at central auditory synapses and normal hearing, but dispensable at the hair cell synapse.

Acoustic startle response in the wild-type and domesticated Mongolian gerbil

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The acoustic startle response in rodents is a fast reaction to a sudden loud noise. Startle and especially its modulations have been studied in rodents in great detail and serve as animal models in several respects. Almost only domesticated rats and mice participated in these studies. The Mongolian gerbil (*Meriones unguiculatus*), one of the standard subjects in auditory research because of its auditory threshold comparable to humans, has been used to investigate the influence of domestication on auditory-related behavior, which we extend to acoustic startle behavior.

We characterized the acoustic startle response in gerbils and determined the influence of domestication by directly comparing animals from a domesticated with a wild-type strain (ancestors recently caught in the wild). Mongolian gerbils showed a strong and reliable acoustic startle response to noise burst above a threshold of 77-80 dB SPL. They showed short-term habituation to repetitive stimulation by between 4% (wild-types) and 34% (domesticated). The startle response can be modulated by a variety of factors in spite of its reflex-like appearance. Inhibition of the acoustic startle response by noise burst or gap-in-noise prepulses in gerbils was strong, maximum prepulse inhibition induced by noise bursts was between 65% (wild-types) and 87% (domesticated). Differences between domesticated and wild-type gerbils were even more pronounced for gap-prepulse inhibition. Percent inhibition in domesticated gerbils (80%) at specific stimulus conditions was almost double the inhibition in wild-types.

The obvious differences between domesticated and wild-type gerbils lead to the question how this fits into the pattern of behavioral changes by domestication. As found in the data on short-term habituation, there was a tendency for stronger startle responses in domesticated gerbils compared to wild-types, while startle threshold was not different between the two strains. This is in contrast to the more common pattern of domestication which includes reduced aggression and reduced defensive behavior.

The described strong prepulse inhibition in gerbils can be very useful as a tool to determine auditory sensitivity in a very fast and efficient way. Such reflex modification audiometry, based on differences in the inhibitory effect, was used by others to determine auditory temporal resolution in rats. Variants of this behavior as a test paradigm can help to investigate disorders of auditory processing. In a recent application of this approach perceptual changes related to tinnitus were determined. This provides insight into defective auditory processing which is otherwise very difficult to quantify. The presented data encourage combining reflex audiometry with physiological approaches on audition in gerbils.

Missing cochlea activity leads to anatomical changes and delayed development of NMDA receptor-mediated transmission in the superior olivary complex

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L-type Ca_v 1.3 channels are the major source for transmitter-releasing calcium influx in the inner hair cells of the cochlea. Due to this reason, Ca_v 1.3 knock-out mice (KO) are deaf (Platzer et al., 2000, Cell 102, 89-97) and believed to virtually completely lack spontaneous activity in the auditory nerve [before the onset of hearing at postnatal day (P) 12]. We therefore employ these mice to assess the influences of missing cochlea-driven activity on the development of the superior olivary complex (SOC).

Nissl staining at P12 revealed a reduced volume of the superior periolivary nucleus (SPN) by 25%, the medial nucleus of the trapezoid body (MNTB) by 43% and the lateral superior olive (LSO) by 58% in KO compared to wild-types (WT). Moreover, the number of the cells was lower in the KO SPN (20%) and the KO LSO (33%). In addition, the typical U shape of the LSO was not observed in the KO and the cell density was higher.

For that reason, we investigated the physiological properties of the excitatory, glutamatergic projections from the cochlear nucleus (CN) to the LSO, as the CN is the first station of the central auditory pathway and may be affected to a high extent by the missing activity. Whole cell patch clamp recordings of LSO neurons were performed with holding potentials of -70 mV and +40 mV. Electrical stimulation of the afferents from the CN to the LSO at -70 mV activated only the nonNMDA component. The peak amplitude of the nonNMDA component increased from P3 to P12 in WT and KO (P3 WT: 57 ± 8 pA; P3 KO: 49 ± 10 pA; P12 WT: 155 ± 21 pA; P12 KO: 132 ± 23 pA). No further changes were observed from P12 to P19 (WT: 95 ± 16 pA; KO: 119 ± 20 pA). Decay time constant and rise time did not differ between age groups or WT and KO. These results show that the development of the nonNMDA component does neither depend on cochlea-driven spontaneous activity nor on sound-induced activity.

The NMDA component was recorded at +40 mV and isolated digitally. In WT mice, its peak amplitude showed a steady decrease with age (P3: 56 ± 12 pA; P12: 23 ± 5 pA; P19: 5 ± 1 pA). The decay time constant also decreased (P3: 111 ± 9 ms; P12: 35 ± 4 ms; P19: 19 ± 3 ms), consistent with the idea that NMDA receptors are needed in the early stage of development and undergo a change from receptors rich in NR2B subunits to those rich in NR2A subunits. This development was delayed in KO, as there was no decrease of the peak amplitude from P3 (39 ± 10 pA) to P12 (64 ± 19 pA) and a significantly higher decay time constant at P12 (55 ± 5 ms). This suggests that the developmental down regulation of the NMDA component, before hearing onset, depends on cochlea-driven spontaneous activity. However, no differences between WT and KO could be observed at P19 (KO: 8 ± 2 pA; 19 ± 4 ms), demonstrating that this development only partly depends on activity and, like the nonNMDA component, appears to be driven by other factors. In addition, VGlut staining of the SOC showed no differences between WT and KO, again implying a normal development of excitatory transmission.

Together, these results reveal a functional maturation of the CN-LSO projection which is largely unaffected by the missing cochlea-driven activity, although major anatomical differences are observed.

Functional implications of the cochlear nucleus in dolphins

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Despite the outstanding auditory capabilities of dolphins, only a few papers concerning the cytological and fibre architecture of the medullary auditory nuclei in these animals have been published so far. Moreover, a comprehensive characterization and classification of the various neuron populations along the auditory pathway including their homologization with corresponding neuron populations in other orders of mammals is still lacking.

To resolve this lack of information we investigated the cochlear nuclei (CN) of five brains of common (*Delphinus delphis*) and La Plata (*Pontoporia blainvillei*) dolphins using routine microslide series of the three main anatomical planes.

In general, the CN in dolphins comprises the same set of subnuclei as in other mammals. However, the volume ratio of the dorsal cochlear nucleus (DCN) in comparison to the ventral cochlear nucleus (VCN) of dolphins represents a minimum among the mammals examined so far. Since in cats the DCN is necessary for reflexive orientation of the head and pinnae towards a sound source, the massive restrictions in head moveability in dolphins and the absence of their outer ears may be correlated with the reduction of the DCN.

Moreover, the same set of main neuron types were found in the dolphin CN as in other mammals, including octopus and multipolar cells. Since the latter neuron populations are thought to be involved in the recognition of complex sounds, including speech, we speculate that, in dolphins, they may be involved in the processing of their communication signals.

Comparison of our two delphinid species revealed that large spherical cells (LSC) are present in the La Plata dolphin but absent in the common dolphin. These neurons are known to be engaged in the processing of low-frequency sounds in terrestrial mammals. Accordingly, in the common dolphin, the lack of LSC seems to be correlated with a shift of its audiogram to the high-frequency range. However, the existence of LSC in the VCN of the La Plata dolphin suggests that this species uses lower frequencies.

Effects of endogenous shifting of auditory attention in rats

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Attention can be directed to a target either cue-directed through external stimuli or through an internal, voluntary decision. The former is referred to as exogenous, the latter as endogenous shifting of attention. While the effect of exogenous attention shifting on performance in visual and auditory tasks has been extensively investigated, the effect of endogenous attention shifting has so far only been examined in the visual domain. Since a performance-enhancing effect through endogenous attention shifting was clearly demonstrated in visual target detection tasks involving rats, we tried to extend this principle to the auditory domain using a covert orienting paradigm.

Nine rats were trained in an experimental setup containing three positions: the starting position in the middle flanked by two target positions (left, right) where animals were also rewarded. Voluntary shifting of attention was induced in an auditory detection task, where the target location was "cued" by its spatial probability as a function of time. With increasing waiting time the probability for target appearance shifted from right to left. Rats were trained to remain in the central starting position until a target noise burst was played within a random waiting period (200 – 1000 ms). After short waiting periods (200 – 600 ms), right targets were more probable but, with increasing waiting periods (600 – 1000 ms), left targets were increasingly more likely to emerge.

After intensive training, rats were able to perform well in this paradigm with hit rates above 85%. The difference in response latency between left and right depended strongly on target probability, thereby indicating the internal shifting of attention. The advantage in response latency for high probability targets was up to 50 ms at short waiting periods which decreased systematically for increasing waiting periods and switched to the opposite side above 600 ms (difference 20 ms at a waiting period of 1000 ms). This advantage became even more apparent as we increased task difficulty by lowering target intensity from 60 dB SPL to 30 dB SPL in 10 dB increments. Differences in response latency for short waiting periods increased further to 80 ms (at 50 dB SPL) and even 140 ms (30 dB SPL). Small but significant differences in error rates depended also on side and waiting period. Short response latency correlated with reduced error rate.

Based on these findings we deduce that our rats had learned the temporal-spatial pattern of target occurrence and consequently shifted their attention as indicated by response latency and error rate. They consequentially reacted faster and with more precision if the target occurred on its high probability side. This was most obvious when animals had little preparatory time and while task difficulty was increased.. In summary, this demonstrates the rats' ability to shift their attention endogenously based solely on experience and free of external cueing. Endogenous attention shifting can not only affect auditory tasks but also improve performance in such tasks in an extremely tangible way.

Detection of auditory evoked potentials and mismatch negativity-like responses in the awake and unrestrained rat

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Recording of auditory evoked potentials (AEPs) can be combined with presentation of deviant sounds embedded in a homogeneous series of standard sounds (oddball stimulation paradigm). Low probability deviant sounds evoke a mismatch negativity (MMN), a late component of the AEP, which can be studied as a fundamental aspect of auditory perception. Impaired MMN is found in Alzheimer's and Parkinson's disease, dyslexia and schizophrenia, and underlying mechanisms are of great interest.
MMN has been recorded in various species including primates, cats, guinea pigs, rabbits and rats. In rats, the existence of a component comparable to that of human MMN remains controversial (Ruusurvita et al., 1998; Lazar and Metherate, 2003; Tikhonravov et al., 2008). MMN studies are normally performed in anesthetized rats even though it is known that narcotics influence central sensory processing. In the present study we aimed to demonstrate auditory evoked MMN in non-anesthetized rats.
In order to detect an MMN-like potential in awake, unrestrained rats we set-up a telemetric recording system (TSE Sytems) using chronically implanted epidural electrodes above the auditory cortex. We recorded AEPs after stimulation with bandpass-filtered noises with carrier frequencies from 5 to 18 kHz. In the oddball stimulation paradigm, we used fixed standard and deviant stimuli, which were presented pseudo-randomly with a deviant probability of 0.05-0.2. Subsequently, stimuli for standard and deviant were interchanged and the oddball experiment was repeated. MMN was calculated as the difference between the average of the two standard recordings and the average of the two deviant recordings.
A first series of experiments with 5 Lister hooded rats showed that stimuli evoked AEPs of variable shape, depending on the frequency bands used. Repetitive recordings at subsequent days revealed that AEP amplitudes elicited with the same stimulus increased with time after electrode implantation, and reached their maximum level after 14 days at the earliest. In contrast to the variability of AEP amplitudes, peak latencies were stable over time. The first positive peak (P1) arose 23 ± 3 ms, the following negative peak (N1) 37 ± 4 ms after stimulus onset. These two components were the most stable peaks recorded.
MMN-like responses were recorded in all 5 rats in the latency range of the N1, especially near the falling edge of the N1-wave. The shape of the potential shift in the MMN-like response, however, varied among animals and even intraindividually.
We conclude that (1) MMN-like auditory evoked potentials can be telemetrically recorded in non-anesthetized rats and that (2) the potential alteration induced by the change from a standard to a deviant stimulus seems to be inhomogeneous under non-anesthetized conditions.

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Signal detection in modulated maskers with different envelope shapes: a study of masking release in the mouse

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For improving the detection of signals in background noise, the auditory system can make use of correlated amplitude fluctuations (i.e., comodulation) in different frequency bands of the background noise. When the bandwidth of a comodulated background noise masker exceeds the auditory filter bandwidth, the signal detection threshold for a tone centred in the masker is decreased compared to the threshold in maskers lacking the correlation. This effect is referred to as comodulation masking release (CMR). CMR has already been shown to occur in the house mouse (Weik et al. 2005, 30th Neurobiology conference Göttingen, Klink and Klump, unpublished data), but it appears to be limited largely to processing within auditory filters.

Spectral and temporal characteristics affect the amount of CMR. Here, the effects of the envelope modulation frequency of the masker and the shape of the modulator of the masker were evaluated in the NMRI mouse. Five mice were trained in a go-nogo-procedure with food rewards under constant-stimuli conditions to report the detection of a 10-kHz pure tone of varying level in a comodulated or unmodulated random-noise masker. To produce comodulated noise maskers, band-pass noise centred at 10 kHz and with a bandwidth of 400, 3000 or 10,000 Hz was multiplied with one of three different types of modulators: square wave, sinusoidal and trapezoid. Modulation frequencies of 10 and 100 Hz (except for the trapezoid) were used at a modulation depth of 100%. The spectrum level of the masker in all conditions was 40dB SPL/Hz. The modulated-unmodulated difference (MUD) is determined by subtracting the threshold in the modulated masker from that in the unmodulated masker.

Here we report preliminary data from an ongoing study. The masked threshold obtained with unmodulated maskers was as can be expected from the large auditory filter bandwidth (i.e., a median threshold of 72 dB was observed). At all modulation frequencies, thresholds were lower in the modulated masker compared to the unmodulated masker (i.e., a positive MUD was observed). In general, a smaller MUD was observed for maskers modulated at a rate of 100 Hz than for maskers modulated at a rate of 10 Hz.For maskers modulated by a 10-Hz square wave or trapezoid, a much larger MUD was observed than for sinusoidal modulators. The MUD for the 10-Hz square wave or trapezoid indicated that the mice were able to reach absolute thresholds during the brief period (50 ms or 38 ms for square wave or trapezoid maskers, respectively) in which the masker was switched off. The data of the present study suggest that temporal masker structure is an important factor in determining the amount of masking.

Intracranial Local Field Potentials can be estimated from auditory brainstem function by Artificial Neural Network simulations

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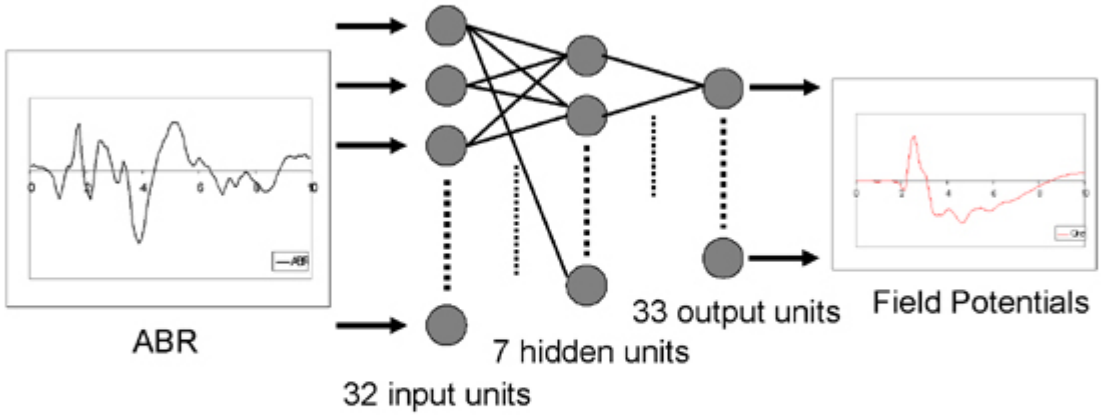
In humans and animals hearing function can objectively be determined by measuring the electrical response of the brainstem to auditory stimuli (auditory brainstem responses, ABRs). The ABR represents a 'brainwave function' containing characteristic positive and negative peaks attributed to the electrical activity in distinct areas of the afferent auditory pathway. Until now however the accurate assignment is still controversially discussed.

To unravel the single components contributing to the waves within the ABR function, a micro-electrode was stereotactically positioned in the cochlear nucleus complex, the superior olivary complex, the nuclei of the lateral lemniscus, the inferior colliculus and the medial geniculate body and field potentials were recorded synchronously to the extra cranial ABR recordings. The auditory stimulus used was a broad-band click stimulus with sound pressure levels between 0 and 100 dB SPL.

Using a three layer feed-forward artificial neural network, the extra cranial ABRs can be correlated with the intra cranial field potentials, unknowing the actual detailed interaction of the auditory nuclei. Using the ABR-data as input to the trained network, a precise prediction of latency and amplitude of the local field potentials in the measured nuclei could be made. These results may be of clinical importance for a better interpretation of ABR-data. Further experimental data will verify the reliability and robustness of the model and the neural network will be tested for generalization limits, portability and individuality.

Supported by DFG-Kni 316-4-1


ABR
32 input units
7 hidden units
33 output units
Field Potentials

Cricket brain neurons – song pattern recognition and control of walking

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During auditory communication in crickets, silent females walk towards singing males. Two fundamental processes underlie this behaviour: the female must recognize the song of conspecifics and localize its source in space. Cricket calling song is made up of chirps, repeated at 500 ms intervals. Each chirp comprises 3-6 syllables of 21 ms length, which are themselves repeated at ca. 42 ms intervals. It was previously assumed that recognition takes place before localization, but the findings by Hedwig and Poulet (Nature 430, 2004; JEB 208, 2005) have revealed that the females start steering with a latency of 55-60 ms after the onset of the first chirp, which is well before any pattern recognition could have taken place in the brain recognizers. Further behavioural investigations revealed that pattern recognition gates unselective rapid steering (Poulet and Hedwig, PNAS 102, 2005).

The auditory afferents transmit information from the ears to auditory interneurons (AN1, AN2) in prothoracic auditory neuropils. The auditory information is carried to the brain where it is relayed to local and descending neurons, which may form a pattern recognition network. Work on local auditory brain neurons by Schildberger (1984) showed that higher order local neurons in the brain lose the syllable coding properties of ascending auditory cells, but they still respond more strongly to the songs with syllable repetition intervals (SRIs) that are similar to the natural song. However, these findings do not explain the rapid steering movements, which suggest the preservation of single syllable coding from the sensory system all the way through to the motor output.

We have developed an apparatus that enables us to record the activity of single brain neurons in a behaving cricket. We identified several auditory local neurons that connect left and right sides of the brain and branch in the lateral accessory lobes. Their response latency (ca. 34 ms) suggests these neurons might be involved in rapid steering. The magnitude of the response (in APs/chirp) showed strong selectivity for the SRI of cricket song; moreover, they all copied the chirp syllable structure. Another local neuron of similar morphology copied single syllables with an even greater accuracy, but with a significantly longer response latency (60 ms), suggesting it might be a part of the modulatory circuitry that gates unselective steering. To compare the copying of syllabic structure and selectivity for cricket song SRIs between the lower and higher order neurons, we recorded from an ascending auditory interneuron, AN1. The copying of chirp syllable structure was similar to that of some local neurons, but the magnitude of response (APs/chirp) was not selective for the SRI of the cricket song.

We have also described several descending auditory neurons that control walking. Their common feature is a dendritic arborization in the lateral accessory lobes. Injection of depolarizing current into these descending cells was sufficient to elicit walking in crickets. Their response latency to cricket calling song was ca. 44 ms. Based on the auditory response latencies of the investigated cells (24 ms for AN1, 34 ms for local neurons connecting lateral halves of the brain and 44 ms for descending cells), we propose that these findings suggest a possible auditory pathway underlying phonotactic behaviour. As the output regions of AN1 are in the ventral protocerebrum and the dendritic areas of the local and descending cells are situated in the lateral accessory lobes, we expected to find auditory neurons that connect these two areas. We have indeed identified two such cells, both with the dendritic area overlapping the AN1 output region and the axonal beaded output area located in the lateral accessory lobes.

The BAEP audiogram of the lesser spear-nosed bat *Phyllostomus discolor*

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The neotropical bat *Phyllostomus discolor* has widely been used as an animal model in auditory and communication research (review: Esser, K.-H. (2003) Speech Communication 41, 179-188). A couple of studies investigated the dependency of auditory thresholds on sound frequency. These studies used different methods to determine the species' audiogram, ranging from behavioural tests to single-cell recordings and the measurement of otoacoustic emissions. A comparison of the hitherto established threshold curves indicates a large variation in both the spectral and threshold domain. To clarify these inconsistencies, it was necessary to re-determine hearing thresholds in *P. discolor*.

Here, we used extracranially recorded **brainstem auditory evoked potentials (BAEP)** as the method of choice for the following reasons. (i) Unlike the results of behavioural tests, BAEPs are independent of the animal's evaluation of an auditory stimulus. (ii) BAEP thresholds are based on the functionality of a number of nuclei in the auditory pathway and not of a single relay station like in most cellular-level recordings. (iii) Further, thresholds determined by state-of-the-art BAEP recordings are largely unaffected by arbitrary criteria or the experimenter. Up to now, the BAEP-method has not been used to study hearing in *P. discolor*.

Studies in humans, mice, rats and chinchillas have already shown that thresholds determined by BAEPs are similar to the results of behavioural hearing tests. However, BAEP thresholds published for bats, generally appear too high presumably due to an insufficient signal-to-noise ratio in the measurements. BAEP-threshold determination is based on the detection of the disappearance of evoked potentials. Therefore, the signal-to-noise ratio must allow that even evoked responses to acoustic stimuli near the absolute threshold are still detectable. Due to the optimization of recording conditions (for details see poster) and an increase in the number of averaged measurements, we succeeded in reaching a signal-to-noise ratio that fulfils these demands.

A comparison of the *P. discolor* audiogram determined here to previously established ones and to those of other microchiropteran species led to the conclusion that the BAEP-threshold curve (present study) is in accordance with the hearing abilities of *P. discolor*. In contrast to most of the previous studies on *P. discolor*, we considered the species' entire hearing range. At 60 dB SPL reliable auditory responses were found in the range of 3 – 130 kHz. Furthermore, we can provide conclusive evidence for the origin of a prominent notch in the threshold curve that occurs around 50 kHz. By comparing our BAEP results with previously audiograms, we found hints indicating that this notch is generated in the outer ear of the bat. The established audiogram provided here can be used as a standard for future studies of hearing in this species.

Cells and Kinases: How to protect the Ear from Noise

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Exposure to high sound pressure levels (noise) damages the cochlear sensory outer (OHC) and inner (IHC) hair cells, resulting in permanent hearing loss, hyperacusis and tinnitus. Cochlear hair cells are most susceptible to noise damage, and regeneration has not yet been described in mammals. Effective protection mechanisms are needed to guard the auditory system from noise damage and permanent hearing loss.

Cochlear hair cells have prominent efferent innervation from the medial and lateral olivary complex (MOC and LOC) through the crossed and uncrossed olivo-cochlear bundle. The efferents terminate on the somata of the outer hair cells (MOC-bundle) and make axo-dendritic terminals on the afferent Type-I fibers below the inner hair cells. Olivo-cochlear efferents are known to modulate the membrane resistance by opening channels for K+ and Cl- ions and play a crucial role in cochlear filtering, pattern shaping and improving the detection of signals in noise. However, their role in noise protection is still obscure.

Our aim was to pharmacologically and functionally modulate the olivo-cochlear efferent system and unravel the importance of the LOC and MOC efferents for protection from noise trauma in a rat animal model. In a complementary approach, the role of cyclic GMP (cGMP) dependent protein kinase type I (cGKI) in cochlear tissue was analyzed in noise. cGKI is a central player in nitric oxide (NO) activated second messenger pathways. The NO-cGMP signaling pathway is proposed to play a crucial role for normal cellular function and for response to traumatic situations. We detected cGKI in IHCs and OHCs and in spiral ganglia neurons. In the cochlea, excessive NO production could be observed in inner ear disorder. In cGKI knockout mice (cGKI-/-), exposure to traumatic noise led to a substantial hearing loss following acoustic trauma, indicating that the cGMP-signaling pathway, suggested in hair cells and spiral ganglia neurons, may determine the trauma response via the activation of cGKI.

The possible interaction of efferent innervation and cell protective signaling pathways are discussed.

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Temporal response properties in the receptive fields of mouse auditory midbrain neurons

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The central nucleus of the auditory midbrain inferior colliculus (ICC) is a center of high convergence of ascending and descending pathways of the mammalian auditory system. The neuronal response properties both in the spectral and temporal domain have been shown to be divers (e.g. Ehret, 1997, In: The Central Auditory System, Oxford University Press, New York, pp 259-316). Possible relationships between temporal and spectral responsiveness of neurons in the ICC have rarely been analyzed. To provide such an analysis for the mouse (Mus musculus) ICC is the central topic of our study.

Neuronal activity in response to tone bursts of various frequencies were studied by extracellular single-unit recordings in ketamine/xylazine anesthetized animals. Response latencies and temporal patterns of responses were studied together with excitatory and inhibitory receptive fields (see Egorova et al., 2001, Exp. Brain Res. 140, 145-161) from response threshold up to 85 dB SPL in 138 neurons. Among our results are the following. (1) Response latency was constant for tone levels higher than 30 dB above threshold in 77% of the neurons. (2) Phasic-tonic or tonic responses changed to pauser or phasic responses at tone levels higher than about 30 dB above threshold in nearly 33% of the neurons. (3) Neurons with primary-like (class I) and inhibition-dominated (class II, see Egorova et al., 2001) frequency tuning curves had significantly more tonic, phasic-tonic, pauser, and long-latency responses (84% of class I and 68% of class II) than phasic responses, while broadly tuned neurons (class III, see Egorova et al., 2001) had significantly more often (72%) phasic responses. (4) The shape of the frequency tuning curve did not correlate with tone response latency.

These data show that temporal response properties in about 1/3 of the neurons depend on the sound level which implies that a level independent coding of tones in these neurons may be realized only by their onset response. The relationships between the shape of the frequency receptive field and the temporal response patterns in many neurons suggest that both neuronal properties are influenced by the same mechanisms, which include excitatory and inhibitory interactions and the local convergence of response patterns already determined at the level of the lower brainstem nuclei.

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Collision-like interaction of acoustic and electric stimulation in gerbil (*Meriones unguiculatus*) auditory cortex A1

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To investigate possible processing modes of sensory cortical neuroprostheses we investigated the interaction of acoustic and direct electric stimulation ("acoustic-electric collision") in gerbil primary auditory cortex A1. We applied both acoustic and electric stimuli in anaesthetized animals with parametrized stimulus onset asynchronies (SOAs), in which the acoustic stimulation (200 ms pure tone) was shifted against the electric stimulus (biphasic, chargebalanced single pulse).
Two stimulation electrodes (1 mm interelectrode distance) were implanted tangentially in cortical layer IV, in a region of high frequency representation and low frequency representation, respectively. For the recording of local field potential (LFP) depth-profiles, a shaft-multielectrode (23 channels) was implanted radially in the vicinity of one of the stimulation electrodes; usually in the low frequency range (0.5 - 2 kHz) near the caudal border of A1.
Interaction was then examined by comparing averaged field potentials and current source density (CSD) plots of a “well-seperated” condition (SOA = 250ms, putativley no or only little interaction between the two stimuli) with the other conditions (SOAs: +- 0, 2.5, 5, 10, 25, 50, 100 ms). Viability of different methods of data analysis will be shown and discussed.

Effects of bilateral lesioning of the medial nucleus of the trapezoid body on behavioural sensitivity to interaural time differences

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Differences in the arrival time of sounds at the two ears (Interaural Time Differences, ITDs) are the main cue to localize low-frequency sound sources in azimuthal space. Neural sensitivity for ITDs is first established in the medial superior olive (MSO), where neurons perform a coincidence detection of inputs from left and right ear. Besides binaural excitatory innervation, MSO neurons additionally receive prominent inhibitory inputs. One major source of these inhibitory projections is the medial nucleus of the trapezoid body (MNTB), which shows pronounced specializations for high-fidelity temporal transmission. Recent electrophysiological studies in the Mongolian gerbil (*Meriones unguiculatus*) have revealed the importance of this precisely timed inhibition for the processing of ITDs: Blocking the inhibitory inputs shifted the response distribution of MSO cells and degraded the neuronal ITD sensitivity.
In this study we investigate the significance of glycinergic inhibition mediated by the MNTB for the behavioural ITD sensitivity. To do so, we assessed the effects of bilateral lesions of the MNTB on the behavioural localization ability in gerbils. Specifically, we quantified the precision of azimuthal sound localization in a two-alternative-forced-choice (2AFC) paradigm before and after lesioning of the MNTB by injection of kainic acid. The animals were trained to classify a low-pass filtered noise, presented over loudspeakers located at various positions (ranging from 7° and 52.5°) in the frontal hemisphere, as either left or right. Additionally, we compared the behaviourally collected data with electrophysiological in-vivo recordings from ITD-sensitive cells in gerbils with a unilateral MNTB lesion. Hence, this study probes the significance of the MNTB mediated inhibition for behavioural sound source localization.
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Amplitude modulation coding in the mammalian auditory midbrain in the presence and in the absence of noise

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The ability to extract a target sound from a complex mixture of sounds is crucial to acoustically communication. Temporal modulations of acoustic signal amplitudes convey important information for recognition and categorization of sounds. Processing of sound envelopes is therefore of crucial importance to the discrimination of communication signals from competing background noise. Although neural processing of sound envelopes has been extensively studied, the neural coding principle for sound envelopes at the level of the auditory midbrain remains obscure. According to a prevalent hypothesis, amplitude modulations are encoded in temporal activity patterns at the level of the auditory brainstem and this code is then transformed into a rate based periodicity code along the ascending auditory pathway. This assumption, however, neglects the possibility of a temporal code for amplitude modulations at the level of the auditory midbrain. A temporal code however might be beneficial in complex acoustic situations.
We recorded in vivo from single units in the auditory midbrain of Mongolian gerbils that were acoustically stimulated with two sets of synthetic temporally modulated stimuli (sequences of noise bursts or sinusoidal amplitude modulated noise) and two sets of natural stimuli (falcon calls or speech). Every stimulus set was presented in the presence and in the absence of a spectrally matched unmodulated masker. Neuronal response patterns were then analysed using a spike distance metric. The distance metric employed in this study allows for an estimate of the temporal acuity at which the signal can best be classified. Since temporal resolution of the spike distance metric is a free parameter, the contribution of spike rate and spike timing to stimulus discrimination can be analyzed separately.
We investigate neurometric performance within each class of stimuli based on the temporal pattern of the response and on spike rate of the responses respectively. We provide evidence that discrimination of responses to both synthetic and natural stimuli is best at a resolution of approximately ten milliseconds. Temporal acuity remains beneficial for discrimination performance when the target signal is presented under masking conditions and consequently, discrimination depends strongly on timing of spikes. Taken together, at the level of the IC, knowledge of the temporal pattern of neural responses is advantageous for discrimination of amplitude modulations in quiet and in the presence of a masker.
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Representation of complex communication sounds in secondary fields of the mouse auditory cortex

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Secondary fields of the left auditory cortex in mammals are considered to play a major role in processing and recognition of species-specific vocalizations. There are only few electrophysiological studies about sound representation in these fields so far. Here we examined the representation of models of mouse pup wriggling calls in the left secondary auditory (AII) and dorsoposterior (DP) field of the house mouse (*Mus musculus*) auditory cortex. Wriggling calls release pup-caring behaviour in adult mice. Single- and multi-unit recordings from cortical layers III and IV were carried out in anesthetized (ketamine/xylazine) mothers and naïve females. We synthesized five different call models. They contained either one, two or three main harmonics of the calls (3.8, 7.6, 11.4 kHz) or three harmonics with 1 kHz amplitude modulation. In addition, the duration of the inter-call intervals in a series of four calls with all three harmonics was varied between 50 and 700 ms. All call models have been tested before for their ability to release maternal behaviour (Ehret and Riecke, PNAS, 99: 479-482, 2001; Gaub and Ehret, J Comp Physiol A, 191: 1131-1135, 2005). Thus, call models of high, medium and low biological significance were presented and the auditory cortical responses were analysed.

Among our results are the following: (1) The neural response rate evoked by either of the stimuli is significantly higher in DP than in AII and higher in mothers than in naïve females, while the spontaneous rate is also higher in DP than in AII but did not differ between animal groups. (2) The response rate of the neurons was determined by the position of the frequency components of the call models in the neuron's receptive field and by facilitatory and inhibitory interactions of the frequency components. (3) There were various degrees of influence of the response to one call model in a series on the response to the next. In general, the strongest effect (lower response rate) was seen for the shortest inter-call interval from the response to the first on the response to the second call in a series. Thus, the present results show complex spectral and temporal integration in high-order fields of the mouse auditory cortex.

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Investigation of neural circuits in the auditory brainstem via light-sensitive ion-channels

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A significant part of the processing of auditory information is performed by subcortical auditory centres located in the brainstem and midbrain. These nuclei interact with each other through a complex network of excitatory and inhibitory projections. To understand the computations performed by these circuits, it would be desirable to precisely control distinct nuclei and study their effect on other centres of the network.
A promising strategy is the use of light-sensitive ion channels such as Channelrhodopsin 2 (ChR2) from the green alga *Chlamydomonas reinhardtii,* or light-sensitive chloride pumps such as Halorhodopsin from the archaeon *Natronomonas pharaonis.* Both proteins show sufficiently fast kinetics to simulate at least some patterns of activity, and the activation of small groups of neurons or even single cells is possible. The proteins were introduced into the brainstem of Mongolian gerbils (*Meriones unguiculatus*) by using viral vectors. The viruses were administered by stereotactic injection into the Medial Nucleus of the Trapezoid Body (MNTB) and the Inferior Colliculus (IC).
Stable *in vivo* expression of both proteins was obtained. Lentiviral vectors equipped with a synapsin promotor provoked cell type specific expression. Alternatively, injection of a Semliki-Forest Virus (ChR2 only) led to less specific, but much faster expression at a high level. Functionality of the channels was tested *in vitro* using the patch-clamp technique. Hence, this results give way to a broad range of options in the investigation of neural circuits in the auditory brainstem.

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Developmental changes of $GABA_B$ receptor function in the medial superior olive

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Gamma-aminobutyric acid (GABA) is an important inhibitory transmitter during the early postnatal development of the auditory brainstem. Besides its direct role in inhibitory synaptic transmission GABA also modulates the release probability of inhibitory and excitatory neurotransmitter on presynaptic terminals via $GABA_B$ receptor activation. The main mechanism proposed for this effect is the inhibition of calcium channels located on the presynapse resulting in decreased vesicle release. Antibody staining against $GABA_B$ receptors shows profound labeling of neurons in the medial superior olive (MSO), a nucleus in the auditory brainstem involved in the processing of interaural time differences (ITDs). MSO neurons analyze ITDs by integrating two glutamatergic excitatory inputs from the ipsi- and contralateral cochlear nucleus and two glycinergic inhibitory inputs from the medial and the lateral nucleus of the trapezoid body (MNTB and LNTB).

Using whole cell patch-clamp recordings in acute brainstem slices of P8 - P19 gerbils we show that each of these inputs can be modulated by $GABA_B$ receptor activation. Application of baclofen, a $GABA_B$ receptor agonist (0.03µM - 10µM), significantly reduced the amplitude of evoked IPSCs and EPSCs depending on the animal's age. Before hearing onset (P12), the sensitivity of the excitatory inputs to baclofen was significantly higher compared to the inhibitory inputs and decreased with age. Inhibitory inputs underwent the opposite developmental change. Since baclofen application increased the paired pulse ratio and decreased the sIPSC and sEPSC frequency this effect is most likely due to presynaptic $GABA_B$ receptor activation.

$GABA_B$ receptor activation also directly affected the glycine receptors (GlyRs) at the postsynaptic site. Bath-application of baclofen (100µM) increased the amplitude of glycinergic mIPSCs significantly. To test whether G-proteins are involved in this potentiation mechanism GTP-γ-S was intracellularly applied to activate the ßγ-subunits of G-proteins. This doubled the amplitude of the glycinergic input compared to control conditions as previously shown in spinal cord cell cultures (Yevenes *et al*. Nat. Neurosci. 2003).

Dependent on the GABA concentration released into the synaptic cleft presynaptic depression and postsynaptic potentiation could interact to change the balance of excitatory and inhibitory inputs.

The MNTB-">This suggests a modulatory role of $GABA_B$ receptors for ITD processing in the MSO during development and possibly also in mature animals.

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Distribution of int-2/FGF3 mRNA expression in distinct neuronal populations of the adult mouse brain.

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Int-2/FGF3 is a cellular oncogene originally identified as one of two genomic integration sites, designated int-1 and int-2 (Nusse and Varmus, 1982), for the mouse mammary tumor virus (MMTV). Northern blot analysis of mRNA from mouse embryos detected int-2 transcripts in 7.5-day egg cylinders, which were no longer detectable in early somite stage embryos 24h later, thus indicating a role for the int-2 gene product restricted to early mouse development. This suggestion was confirmed by several other studies demonstrating int-2 expression during embryogensis in the mouse with special emphasis on int-2 being necessary for the normal development of the inner ear, cerebellum, retina and teeth. The present study, however, reveals expression of int-2 mRNA in non-neoplastic adult brain tissue. The anatomical localization of int-2/FGF-3 mRNA- expressing neurons in the brain of adult, male C57/Bl6J mice was studied with in situ hybridization histochemistry using a radio-labeled synthetic oligodeoxy-nucleotide probe complementary to the mRNA of mouse int-2. The highest levels of int-2 mRNA were found in pyramidal cells of the 5th layer in several areas of the neocortex, i.e. in the lateral orbital, frontal, parietal, occipital and temporal regions, and in the granular insular cortex, as well as in the most superficial cell body-containing layers throughout the piriform and entorhinal cortices. A somewhat less intense labeling was found in several structures of the thalamus, e.g. the anterior ventral, rhomboid, paraventricular, paratenial, medial, mediodorsal, submedius, ventromedial, angular, posterior, paracentral and lateral posterior nuclei, whereas neurons of the medial and subgeniculate parts of the medial geniculate nucleus exhibited labeling almost as strong as that found in the neocortex. Taken together, in addition to earlier studies demonstrating int-2 expression mainly during very early developmental stages, as well as in close relationship with the development of peripheral structures of the auditory pathway, we now report a strong labeling of central structures involved in, among others, processing of auditory information, such as the medial geniculate body and auditory neocortical areas.

Tone lateralization is affected by both linguistic roles and physical properties

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Whether tones in tonal languages are processed in the left hemisphere is a question under debate. It touches upon the key issue of how our brains are functionally organized, namely, does the functional organization of the brain depend on the nature of the tasks, such as language or music, or the physical properties of the input signals, such as slow-changing spectral cues or fast-changing temporal cues.

Tone is used to distinguish lexical meanings by pitch variations. According to the "*task-dependent*" view, tone perception should be left lateralized, because it bears language function and the left hemisphere is the language dominant side. However, the "*cue-dependent*" view predicts that tone perception should be right lateralized, since the right hemisphere has an advantage in processing pitch changes, which are the physical properties of tones. There is supporting evidence for both of these views. For example, two behavioral studies [1,2] using the **dichotic listening** paradigm have shown a right ear advantage (REA, left hemisphere advantage) on tone perception, whereas a recent **ERP** (Event-Related Potential) study [3] using the MMN (Mismatch-Negativity) paradigm has shown a right hemisphere advantage on tone perception around 200 ms after the stimulus onset under the preattentive condition.

Here we conduct an **ERP** experiment, which has a high temporal resolution, to examine tone lateralization following the **dichotic listening** paradigm. We examine the effects of both the ***semantics*** and the ***physical property***. We adopt a 2×2 design, with real and pseudo-syllables as two levels in the factor of the semantics and tone and stop-consonant dichotic trials as two levels in the factor of the physical property.

Thirty-two native Mandarin subjects with normal hearing have participated in the experiment. In the experiment, subjects first hear two different syllables in their two ears simultaneously. After seeing the indication of "left" or "right" on the screen, subjects are asked to identify both the initial consonant and the tone of the syllable that they hear in their left or right ear.

The results support that both the ***semantics*** and the ***physical property*** affect tone lateralization. By measuring the differences of the P200 (a positive peak around 200 ms after the stimulus onset) peak amplitude between the homologue left and right electrodes, C3 and C4, a two-way repeated-measures ANOVA shows a significant main effect of the semantics ($F(1,31) = 8.437$, $p < .007$) and a significant main effect of the physical property ($F(1,31) = 5.560$, $p < .025$) (see Fig. 1).

Given the evidence that the linguistic role and the physical property affect tone lateralization, we argue that the functional organization in the brain is an adaptive system affected by both tasks and input signals. The "*task-dependent*" and the "*cue-dependent*" views complement each other to form a more complete picture of speech perception.

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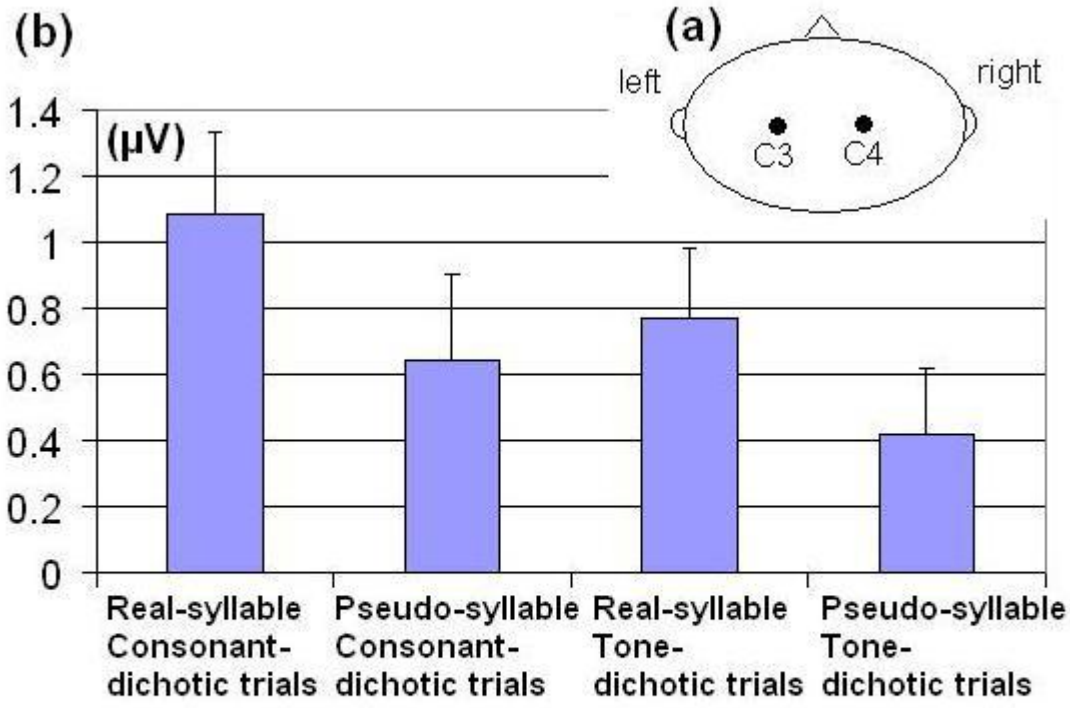

Fig. 1. (a) An illustration of the positions of electrode C3 and C4.
(b) The mean value of the differences of the P200 peak amplitude between C3 and C4 under each condition.
(Each error bar denotes one standard error.)

P300 and reaction time as measure of hearing effort of cochlear implant users and normal hearing listeners during sound discrimination in noise

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In auditory discrimination tasks with background noise, cochlear implant (CI) patients as well as normal hearing (NH) subjects show an increase of the latency of the P300 event related potential with increasing noise level already at signal-to-noise ratios (SNR) where subjective discrimination is still unambiguous (Igelmund et al. 2008, FENS Abstr. 4: 188.12). The prolongation of the P300 latency is interpreted as reflecting an increase of the discrimination effort with decreasing SNR, suggesting that the P300 may be a useful objective measure of the hearing effort. To check this interpretation, the P300 latency was compared to the reaction time, a parameter which is accepted as an indicator of hearing effort.

The study was conducted with adult CI and NH listeners. Speech sounds (ada-ama) were presented at a convenient level and masked by white noise at various SNRs. In an auditory discrimination task (oddball paradigm, 80% standard, 20 % deviant stimuli), the subjects had to respond to the deviant stimuli by mouse click.

For NH listeners, the subjective discrimination of the speech sounds was nearly 100% correct down to SNR = -15 dB and steeply declined at lower SNR. In contrast, masking noise consistently induced an increase of the latency of the P300 at far higher SNRs. At SNR = - 6dB and -18 dB, the P300 latency of NH subjects was prolonged by 53±5 ms (mean ± SEM) and 225 ± 24 ms, respectively, as compared to presentation in quiet. Surprisingly, the simultaneously measured reaction time was not prolonged by masking noise down to SNR=- 6 dB. Further decrease of the SNR induced increase of both the P300 latency and the reaction time. For CI patients, the results were comparable although shifted to higher SNRs, with higher interindividual variation and with lower correlation between the reaction time and the P300 latency.

The results suggest that for normal hearing listeners as well as for CI patients, the latency of the P300 is a reliable measure for hearing effort during sound discrimination in noise, with higher sensitivity at moderate SNRs and lower variability as compared to the reaction time. Thus, it may be a valuable objective tool for the optimization of cochlear implants, e.g. in the evaluation of the benefit of new speech processing strategies.

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Call frequency control by neurons in the vocal motor nucleus of Greater Horseshoe Bats

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In a previous study, it could be shown that frequency, duration, and interpulse interval of bat echolocation pulses are affected by changes in the synaptic activity within the vocal motor nucleus, the nucleus ambiguus (NA), of horseshoe bats: injections of various excitatory and inhibitory transmitter agonists and antagonists into NA affected different sets of laryngeal motoneurons yielding specific changes in these call parameters. The results suggest a specific activity pattern in cricothyroid motoneurons, which control call frequency: lower frequencies are not caused by a reduction in excitatory input to these motoneurons. Instead, they possess an intrinsic spontaneous activity, which is independent of glutamatergic synaptic input and only modulated by $GABA_A$. When not calling, this spontaneous activity is constantly suppressed by GABAergic input from premotor structures. During call emission, premotor input releases this inhibition, and the amount of release from inhibition is indirectly proportional to call frequency.

In the presented study, we tested this model by recording single unit activity from the NA in horseshoe bats while they were spontaneously echolocating and adjusting call parameters in response to electronically altered auditory feedback signals of their calls. We found one type of NA neurons, which exhibited pre-vocal excitatory activity that was directly proportional to call frequency. This pre-vocal activity was suppressed by muscimol and slightly elevated by bicuculline, whereas glutamatergic drugs had no effect. Another type of neurons was inhibited during call emission and exhibited an inverse correlation between spike count and call frequency. The activity of these neurons was increased by application of glutamate (and AMPA) and reduced by GABA (and muscimol). This type of neuron may represent the source of the GABAergic inhibition to those NA motoneurons that control call frequency.

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Modelling transmission at the bushy cell synapse in complexin-deficient mice

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Complexins (CPX I-IV) are small cytosolic proteins that regulate the presynaptic SNARE-complex. An accompanying poster by Strenzke et al. describes that hearing is impaired in CPX I knock-out mice and that the first affected synapse is the endbulb of Held in the cochlear nucleus (CN). *In-vivo* extracellular recordings from auditory nerve fibres (ANF) and CN-cells as well as *in-vitro patch-clamp* recordings from bushy cells document the neuronal activity pre- and postsynaptic to the endbulb of held.
This extensive experimental characterization allowed us to construct a tightly constrained model of synaptic transmission at the endbulb of held. The synapse is driven with random spike trains mimicking ANF activity. The synaptic plasticity (depression in control mice vs facilitation in $CPX^{-/-}$ mice) and the threshold behaviour of the bushy cells are taken into account to predict spike timing in bushy cells. The output of the model is compared to *in-vivo* activity of CN neurons.
The model correctly reproduces the observed sharpening of first-spike latency (FSL) distributions from ANF to bushy cells. Furthermore it describes the observed increase in average FSL in $CPX^{-/-}$ mice. The model predicts a strong influence of the spontaneous firing rate of the ANFs on the fibres ability to drive the postsynaptic bushy cell: high spont fibres are capable of driving the bushy cells with equally short FSL in $CPX^{-/-}$ and control mice while low spont fibres cause more delayed first spikes. This prediction could be scrutinized in those rare recordings, when pre-potentials from the presynaptic ANF and postsynaptic spikes are picked up by the same extracellular electrode.

Multi-Electrode Recordings of Delay Lines and Neuro-phonic Potential in the Auditory Coincidence Detector Circuit of Birds

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To subserve the detection of interaural time difference, which is used to localize the azimuthal position of a sound source, the Jeffress-model [1] has suggested three levels of processing: frequency specificity, delay lines and coincidence detection. This system is supposed to be realized in birds in the third-order nucleus laminaris (NL), the first nucleus where binaural signals are processed and coincidence detection takes place.

We use the auditory system of birds (chicken, barn owl) to study the neurophonic potential (NP), a frequency-following potential with a temporal precision of some 10 μs, occurring in the network formed by nucleus magnocellularis (NM) and NL in the brainstem. We are focused on two aspects. First, we expect to find out more about the origin of the NP. Possible sources of the NP are the NM afferents, their synapses and on the postsynaptic side the action potentials of NL neurons. Second, we want to show directly that the NM projections in the owls brainstem form delay lines, as proposed by the Jeffress model.

Acute coronal slices of the brainstem (300-500 µm thick) were prepared from barn owls (*Tyto alba*) and chickens at different ages (P2-P8). Recordings were carried out on perforated 8x8 MEAs (multi-electrode arrays) from Multichannel Systems (Reutlingen, Germany) while stimulating extracellularly at different loci (contra-/ipsilateral NM or along the projections of NM). The latencies of the averaged response were determined by calculating the time difference between corresponding extrema to show the progression of the signal. DNQX, AP-V and Ca-free medium were used to separate the different sources of the NP, TTX to show the neuronal origin of the response.

Latencies changed within the NL of the barn owl from medial to lateral as well as in the dorso-ventral direction in response to contralateral stimulation. Latencies between two neighbouring electrodes (distance: 200 µm) were about 34 to 250 µs corresponding to propagation velocities between 0.8 – 5.9 m/s at 35 °C. The responses vanished after application of TTX and came back after washing out. In the chicken, we found different conduction velocities for ipsi- and contralateral projections of NM, consistent with other studies [e.g. 2]. First experiments with application of the NMDA-receptor antagonist AP-V showed little impact on NP in contrast to the AMPA/Kainat-receptor antagonist DNQX.

Our data from the owl provide the first direct evidence for delay lines in NL, indicating the realisation of the Jeffress-model. Pharmacological data are currently analysed. Those will provide the basis for elucidating the sources of the NP.

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DIFFERENT FRUIT ODORS PRODUCE WIDELY DIVERGENT DYNAMIC RESPONSES IN DROSOPHILA ANTENNAL OLFACTORY RECEPTOR NEURONS

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Olfactory sensilla are located on the third antennal segment of Drosophila melanogaster. Each sensillum contains 1-4 bipolar sensory neurons, with odorant receptor molecules (ORs) in the distal sensory dendrite. Insect ORs are seven-transmembrane receptors, but are significantly different to other G protein-coupled receptors, with inverted membrane topology. Recent work has indicated that heteromeric combinations of OR molecules produce odorant-activated cation channels, but it is not clear whether they operate as direct ligand-gated ion channels, or whether there is G-protein activation and nucleotide signaling between components.

Our primary interest is in the dynamic properties of chemotransduction in Drosophila basiconic antennal neurons. Detailed knowledge of receptor dynamics should provide important clues about the mechanisms of transduction, about the range of information that is transmitted to the central nervous system, and the contributions of olfaction to behavior. We previously developed a new stimulation system to provide accurate and reproducible control of odorant concentration, allowing characterization of the dynamic properties of odorant receptor neurons.

Here, we recorded single unit responses from Drosophila antennal large basiconic sensilla to a battery of fruit odors, using the tungsten electrode technique. Action potential records from single neurons were separated from multiple action potential amplitude recordings by a template matching algorithm. Random binary on-off sequences drove a gas flow valve that delivered a primary air stream containing propylene as a tracer gas plus odorant chemicals (15 µl at 1-10% in mineral oil) evaporated from filter paper. This primary stream was released into a fan driven secondary air stream that diluted both odorant and tracer, while delivering laminar air flow to the antenna. Propylene tracer concentration was measured by a miniature photoionization detector with its probe located within 2 mm of the antenna, providing a surrogate measure of odorant concentration at the antenna. Fourier transforms were used to convert both odorant concentration and action potential signals to the frequency domain, where they were used to estimate frequency response and coherence functions for odor transduction and encoding by the sensory neurons.

Odorants tested included Ethyl acetate, Phenylethyl acetate, Ethyl butyrate, Methyl salicylate and Hexanol. Each sensory neuron responded to several different odorants, but with distinctly different frequency response functions, and even polarities. Frequency response functions were reliably fitted by combinations of linear filter models that were characteristic for each odorant. Equal mixtures of two odorants with different frequency response characteristics and opposite polarities (Ethyl butyrate and Phenylethyl acetate) gave responses that were closely similar to Phenylethyl acetate alone, rather than a combination of the two responses.

These data suggest that multiple pathways exist between ORs and receptor currents in Drosophila odorant receptor neurons, and that there are strong interactions between these pathways.

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Neuronal correlates of pattern recognition in a social insect

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Ants discriminate colony members (nestmates) from members of a different colony (non-nestmates) by colony-specific patterns of hydrocarbons on the cuticle (label). During nestmate recognition, it is thought that the label is compared to a neuronal template (label-template matching) and a mismatch leads to aggression. It is not known where label-template matching takes place and how a template is realized in the nervous system. Different mechanisms are possible which may even occur in combination with each other. A first possible mechanism is that a sensory filter in the periphery of the nervous system acts as a template, where receptor neurons are adapted to the ever-present nestmate label and only non-nestmate labels are detected. A second possibility is that label-template matching takes place in the central nervous system and the template consists of an internal representation of the nestmate label. Sensory information is compared to this internal representation in olfactory integration centers of the ant brain. A third mechanism possibly involved in label-template matching is that sensory information is specifically modified along the olfactory pathway to allow discrimination, with specific modification acting as a template. In order to study the neuronal representation of the label, we presented dummies treated with extracts of postpharyngeal glands (which contain hydrocarbon patterns similar to those on the cuticle), and measured the responses in the olfactory system of workers of the Florida Carpenter ant *Camponotus floridanus*. Neuronal activity in olfactory receptor neurons was monitored by electroantennography and stimulation with nestmate or non-nestmate label resulted in measurable responses. Responses to nestmate label would not be expected, if adaptation at receptor level played an important role for label-template matching. We investigated the first olfactory neuropile of the ant brain, the antennal lobe, which receives input from receptor neurons, processes this input and relays it to the higher integration centers of the central nervous system. Responses in olfactory antennal lobe output neurons were measured by calcium imaging and again stimulation with both nestmate and non-nestmate label resulted in neuronal activity. A major role of adaptation at receptor neuron level is thus unlikely. Currently, we compare the neuronal representation of nestmate and non-nestmate labels in the antennal lobe in search for specific modifications of sensory information.

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Correlating social organization and neuroanatomical characters in leaf-cutting ants

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Division of labor is fundamental in the complex organization of social insects. In the leaf-cutting ant genera *Atta* and *Acromyrmex*, division of labor in the worker caste is related to size polymorphism. Small workers take care of brood and fungus cultivation, large workers specialize in leaf-cutting and transport. Behavioral experiments show differences in the trail following behavior (Kleineidam et al. 2007) and in the sensitivity to trail pheromone components between large and small workers.

Within the Attine species *Atta vollenweideri*, we compared different characters of the olfactory system, which may relate to differences in olfactory-guided behavior and even to olfactory specialization. As expected, the antennae of large workers have a much higher number of olfactory sensilla and receptor neurons compared to small workers. The functional units of the antennal lobe (AL), the glomeruli, are bigger in larger workers. We also found that the number of glomeruli in the ALs of large workers is by 50 glomeruli higher compared to the ALs of very small workers. These glomeruli were found in the dorsal part of the antennal lobe, but their function is not yet clear. The most prominent difference in the olfactory system of large and small workers is a substantially enlarged glomerulus - the macroglomerulus (MG) - near the antennal-nerve entrance (Kleineidam et al. 2005). In the MG, information about the releaser component of the trail pheromone is processed.

We compared the neuroanatomy of the AL across 25 Attine species in order to investigate the evolutionary origin of the MG. We studied species of all three groups of Attini (lower, higher and leaf-cutting Attini) with differences in social organization and ecology. Only in the polymorph leaf-cutting ants a MG was found, whereas the MG was absent in the monomorph lower and higher attine species with less complex social organization. We found a MG in almost all investigated leaf-cutting Attini, but fewest developed in two *Acromyrmex* species (*A. striatus* and *A. balzani*). Interestingly, these two species have a less elaborated foraging trail system, which further supports the idea that the MG is an important adaptation for processing of information about the trail pheromone.

We conclude that the MG in large workers of leaf-cutting Attini is a derived character, resulting in specialization in polymorph leaf-cutting species. Neuroanatomical differences that underlie specializations in the behavior of workers play an important role in division of labor and social organization.

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Multi-unit recordings in the dual olfactory pathway of the honeybee

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Odors play a crucial role for communication and orientation in most animal species. Because of a dominant role of olfaction in communication, this is especially true for social insects. Odor information is encoded in neuronal signals and transferred by olfactory receptor neurons (ORNs) to the primary olfactory centers and from there by interneurons to secondary olfactory centers in the brain. The first relay station in insects is the antennal lobe (AL), the analogue of the vertebrate olfactory bulb, where the first stage of odor processing occurs in olfactory glomeruli. In the honeybee neuronal information is conveyed from the AL glomeruli via two separate uniglomerular projection-neuron (PN) output tracts, the medial and lateral antennoprotocerebral tracts (m- and l-APT), to higher olfactory integration centers in the mushroom bodies (MBs). This dual PN olfactory pathway to the MBs is a special feature in Hymenoptera (Kirschner et al. J Comp Neurol 2006; Zube et al. J Comp Neurol 2008).The MBs are known to be involved in higher-order sensory processing as well as learning and memory.

The aim of this study is to analyze odor processing, in particular temporal parameters, along the dual olfactory pathway in the honeybee. For multi-unit analyses neuronal activity was recorded extracellularly with multiple thin insulated wire electrodes (method adapted from Okada et al. J Neurosci, 2007). The customized recording setup includes preamplifiers with relays that allow either recording or electrical stimulation (Tucker-Davis Tech., USA) and custom-built amplifiers (NPI electronics, Germany) with free selectable reference channels. Data was collected using a self-written acquisition software (LabVIEW, National Instruments, Germany) and finally processed and analyzed for spike-sorting and clustering with Spike2 (CED, England). We record multi-unit activity within the AL and from PN output tracts to characterize the two major PN tracts by their olfactory response profiles. Furthermore, we use extracellular recording techniques to gain information about the responses of the target cells of the dual olfactory pathway in the MBs - the Kenyon cells (KCs).To analyze the discharge characteristics of KCs in response to variable temporal input we use selective electrical stimulation of both PN tracts.

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Adult neurogenesis in the olfactory system of homing pigeons supports the impact of olfactory cues for navigation

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Pigeons use olfactory cues to navigate over unfamiliar areas[2]. Accordingly, the OBs of homing pigeons are enlarged compared to non-homing breeds representing a functional adaptation to olfactory-guided homing behavior[4]. Apart from the hippocampus, the OB in the mammalian brain is the only region giving lifelong rise to new nerve cells revealing a plastic mechanism contributing to the perceptual and memory functions performed by the bulb[3]. Enhanced perceptual and memory demands associated with olfactory-guided homing behavior suggests neurogenesis also in the avian OB but this has not been verified yet. Therefore, we performed BrdU-injections (3 x 100 mg / 1000g body weight i.p.) to estimate neurogenesis rate in the OB of one year old pigeons and combined BrdU-labeling with an immunohistochemical characterization of bulbar neuron types.

Immunohistochemistry demonstrated a bulbar organization similar to the mammalian one with calbindin-positive cells confined to the granule cell layer while the glomerular layer was characterized by tyrosine hydroxylase-positive cells. Two weeks after BrdU-injection, BrdU-labeled cells could be detected around the ventricular zone and within the granule cells layer. Within the following six weeks, the mean number of BrdU-labeled cells further increased with granule cells representing the major newborn cell population. Remarkably, increase in newborn cells number was more pronounced in the right OB supporting its stronger impact in olfactory-dependent homing behaviour[2]. After four weeks, the first BrdU-positive cells could be identified in the glomerular layer but a substantial number of newborn cells was present only after eight weeks survival time. This pattern suggests a considerably long differentiation time compared to the mammalian OB[2]. The piriform cortex likewise displayed BrdU-labeled cells whereas only a very small number of newborn cells could be detected within the hippocampus. Especially the V-shaped layer, which is assumed to be comparable to the dentate gyrus[4] the only area of the adult mammalian hippocampus giving rise to newborn neurons was lacking BrdU-labeled cell.

Doublecortin (DCX)-immunolabeling verified the generation of new nerve cells in the OB. However, only cells around the ventricular zone and cells migrating into the glomerular layer displayed DXC immunoreactivity. This labeling pattern supports that newborn granule cells differentiate more rapidly than periglomerular interneurons comparable to the developmental pattern observed in the mammalian OB[3]. In sum, the present study verified adult neurogenesis in the avian olfactory bulb. The parallel presence of newborn cells in the piriform cortex indicates that neurogenesis in the olfactory system of pigeons is involved in neuronal processes mediating olfactory-guided navigation presumably in a lateralized manner.

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Confocal life-cell imaging in the microvillous layer of the sensory epithelium using an intact whole-organ preparation of the mouse vomeronasal organ

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In most mammals, olfactory stimuli are detected by at least two different sensory systems, the main olfactory epithelium (MOE) and the vomeronasal organ (VNO). The VNO, a blind-ended tube at the ventral part of the nasal septum is specialized for detection of social cues that convey important information about gender, social, and reproduction status. So far, the majority of physiological studies investigating vomeronasal signal transduction processes used either freshly dissociated vomeronasal sensory neurons (VSNs) or acute coronal sections of the VNO. While these approaches provided a wealth of important insights into the molecular machinery of vomeronasal signaling, such preparations are inherently limited by mechanical perturbation of the natural cellular environment.

Here, we report a novel highly intact mouse VNO whole-organ preparation which is suitable for physiological recordings of pheromone-induced Ca^{2+} signals at the dendritic surface of the vomeronasal sensory epithelium. Using this method, the sensory epithelium is essentially undamaged and axonal projections to the accessory olfactory bulb are in sound condition. Combining life-cell confocal fluorescence microscopy of Ca^{2+}-sensitive reporter dyes with specific pharmacological approaches, we are able to investigate the role of various signaling enzymes and ion channels. Our current findings confirm a critical role of phospholipase C as well as members of the TRPC ion channel family in vomeronasal signal transduction. Employing this novel whole-mount preparation method in future studies, we envisage to obtain significant new insights into social signaling mediated by the accessory olfactory system.

Pectine neuropils of the scorpion - serotonine immunoreactivity and similarities to insect and crustacean olfactory lobes

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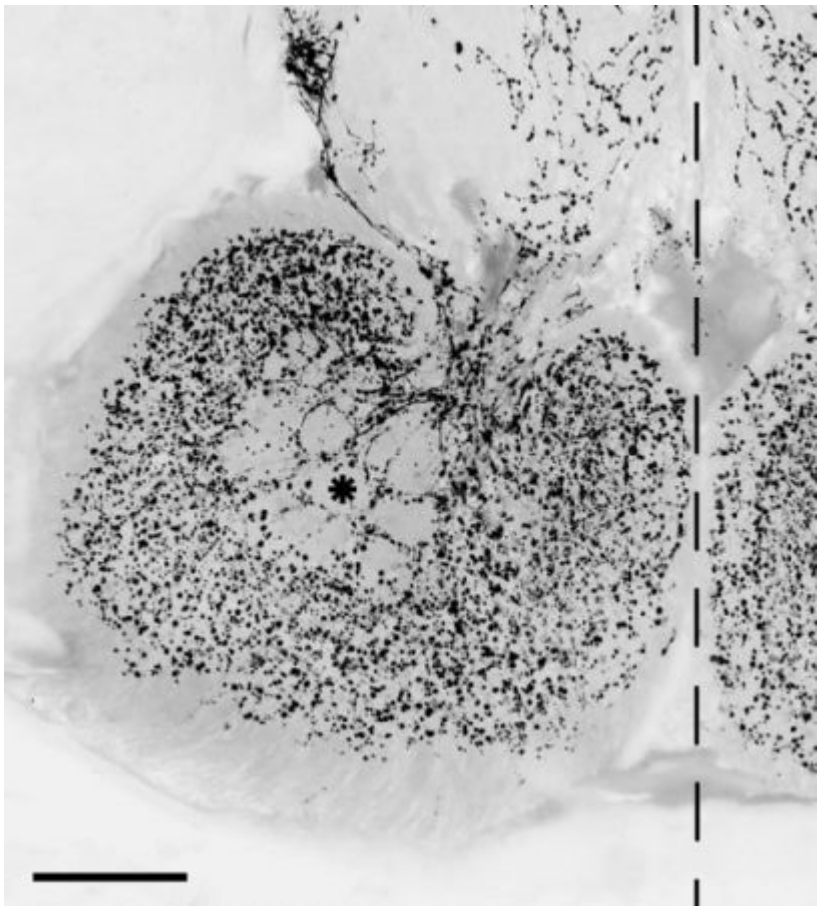
The pectines of scorpions are mechano- and chemosensory appendages located ventrally behind the walking legs, employed in probing the substrate, courtship, and related behaviours. The sensory afferents of the pectines supply conspicuous large neuropils that bear intriguing similarities to insect olfactory lobes and crustacean antennal lobes, but they also possess clear distinctive features. The most notable similarity is a glomerular structure, even though these glomeruli are not actually globular, as in insects, but rather flattened and heterogeneous in shape, "lobular" (Wolf (2008) Arthropod Structure and Development 37, 67-80).

One characteristic property of insect and crustacean olfactory lobes is their supply by a single large serotonergic neuron. Among others, this feature has been proposed to support homology of the olfactory lobes in the two arthropod groups, and it has functional significance in olfactory signal processing (review in Schachtner et al. (2005) Arthropod Structure and Development 34, 257-299). Despite this apparent diagnostic and functional importance, a possible serotonergic innervation has not yet been studied in the scorpion pectine neuropils.

We examined serotonine-immunoreactivty (-ir) in the pectine neuropils of *Androctonus australis* and *Pandinus imperator*, both species yielding similar results. The neuropil cortex and the matrix between the lobuli are supplied by a dense network of serotonine-ir arborisations and varicosities (see figure). According to previous studies (Wolf ibid), the areas exhibiting serotonine-ir contain the axons of the sensory afferents (cortex and matrix) and of output interneurons (matrix). The lobuli themselves are spared from serotonine-ir. The serotonine-ir supply of the pectine neuropils has two origins. The first is a single ipsilateral neuron soma, slightly larger (up to 30 mm) than the surrounding cell bodies and associated with the neuromeres of the genital and pectine segments. This situation is reminiscent of the serotonergic supply of insect and crustacean olfactory and antennal lobes.

The second serotonergic innervation of the pectine neuropils is from a group of some 10-20 ipsilateral neuron somata of slightly smaller size (ca. 15-20 mm), similar to the surrounding cell bodies. These somata are part of a much larger serotonine-ir group comprising 70-90 somata. The whole group is located more anteriorly than the single soma mentioned above, and associated with the neuromere of the last (4^{th}) walking leg.

The figure shows a frontal (cross) section of the right pectine neuropil of Androctonus. *Serotonine-ir arborisations and varicosities are black; note the spared lobuli, a central one indicated by ä. Ganglion midline is indicated by stippled line; scale bar 50mm.*



Characterization of responses to TAAR-specific amines in the olfactory system

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G protein-coupled receptors of the olfactory receptor (OR) family expressed in the ciliary membrane of olfactory receptor neurons (ORNs) in the olfactory epithelium (OE) are responsible for the detection of odorants. Recent studies have shown that a second family of G protein-coupled receptors, the trace amine-associated receptors (TAARs), are also expressed in the OE of various vertebrate species. Trace amine-associated receptors were originally thought to be activated by a group of neuromodulatory compounds, the so-called trace amines. Now it has been shown that, at least in mice, TAARs are expressed in ORNs in a similar way as ORs and that TAARs detect specific amines, including amines present in mouse urine. It has therefore been hypothesized that TAARs may primarily act as chemosensory receptors for amine odorants.

Here we demonstrate that amines, including TAAR-specific amines, are suitable olfactory stimuli in larval *Xenopus laevis*. We then thoroughly characterized the amine-induced responses of individual ORNs using functional Ca^{2+}-imaging and tested the influence of amine odorants on the tadpoles' behaviour. In addition we verified that TAARs are expressed also in the olfactory organ of larval *Xenopus laevis*. The collective evidence of the present study sheds new light on amines, in particular TAAR-specific amines, as olfactory stimuli and on which receptors they are transduced by.

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DoOR - a Database of Odorant Responses in *Drosophila melanogaster*

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Odors are coded by combinatorial activity patterns across olfactory receptor neurons, a coding logic that is maintained into the first processing network, the vertebrate olfactory bulb or the insect antennal lobe. Therefore, in order to understand the physiological representation of an odor in the brain, it is not sufficient to know the odor-response profile of individual receptors, but rather odor-response profiles of all receptors need to be measured. This is a formidable task even in the numerically simple system of the adult fruit fly Drosophila melanogaster, where approx. 45 receptors are expressed.
Nevertheless, many studies report odor-response patterns for individual or groups of receptors. Here, we developed a computational approach that allows pooling odor-response profiles from different studies, even if the measured magnitudes differ (e.g. action potentials vs. calcium influx, or in situ measurements vs. heterologous expression). Applying this approach to all receptors of the fruit fly, and pooling all available published data, as well as additional measurements from our lab, we generated a new open source database in which all odor-response profiles are accessible: DoOR (Database of Odorant Receptors).

We present a consensus odor response matrix across odorants and olfactory receptors. Users can query the database for the information of a particular odorant receptor, yielding a consensus odor-response profile, including error estimates and flagging of inconsistent datasets. Users can also query the database for an odor, yielding complete combinatorial odor-response patterns. DoOR handles the construction of odorant response matrices and visualizes the calculated olfactory responses in the antennal lobe. The reconstructed odorant response matrix allows for the application of linear algebra, and can therefore be combined with other matrices, for example with an odorant distance matrix to estimate hitherto unknown odorant responses.
Both the database and the software will be open source and freely available on the internet. Our framework allows to easily integrate future datasets in order to constantly improve our knowledge about combinatorial odor coding. Due to its flexible design, this DoOR can also be opened to odor responses in other species.

Novel techniques for the exploration of the honeybee antennal lobe.

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The antennal lobe (AL) is the first instance of olfactory information processing in the insect brain. Here, signals from receptor cells converge onto glomeruli, the coding units of the AL: each olfactory stimulus is represented by a combinatorial pattern of glomerulus activity. Going beyond solely representing the collective receptor input, the AL also o ers the hardware that can putatively implement functions such as gain control, odorant mixture e ects or even learning by interfering with glomerular patterns in the temporal or spatial domain. The hardware for odor processing consists of 60.000 receptor (input) neurons, 800 projection (output) neurons, and at least two networks of local neurons interconnecting the glomeruli [1]. Structure and function of these networks that consist of about 4000 neurons, are, however, largely unexplored.

Optical recordings utilising calcium-sensitive dyes have provided a means of making the AL accessible to experimental research, enabling us to monitor changes in combinatorial activity upon presentation of an odorant stimulus. For these recordings, we selectively stain projection neurons (PNs) that relay glomerular output to higher-order brain centers, i.e. we see glomerular activation patterns that are the result of receptor input and subsequent processing in the AL.

Here, we use pharmacological techniques and generate novel image analysis tools that will help us to further understand the function of the AL. We have developed an algorithm that facilitates the identification of glomeruli in recordings from the honyebee AL [2]. By detecting statistically independent signals in the recordings, we construct maps of the part of the AL under the microscope that we then project onto the standard atlas of the honeybee AL. Thus, we are now able to automatically extract glomerular patterns from large collections of datasets, proposing, for the first time, a deterministic glomerulus identification method to replace error-prone manual approaches. Further, the algorithm allows to distinguish signals from spatially overlapping glomeruli that could only be analyzed as a mixture in previous work. Our improvements in image analysis enable us to detect even subtle changes in glomerular patterns that may be the result of the interglomerular networks.
In our pharmacological approach we actively interfere with neurons in the AL in order to elucidate the role of neurontransmitters. Previous works have shown that both GABA [1] and histamine [3] provide inhibitory input to AL neurons. We find that bath application of acetylcholine, one of the main excitatory transmitters in arthropodes, induces a strong and fast increase in intracellular Ca^{2+} concentration and thus is at least one of the transmitters that mediates the activation of PNs. Currently, we are investigating the e ect of octopamine onto AL neurons. Octopamine is released into the AL through the VUM neuron [4] and is known to mediate learning in the AL [5,6].

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Daytime-dependent effects of cAMP and octopamine on the pheromone transduction of the hawkmoth *Manduca sexta*

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The pheromone-dependent mate search in moths is controlled by unknown circadian pacemakers and unknown coupling signals which synchronize the distributed pacemakers. Previous studies suggested that circadian changes in the hemolymph concentration of the biogenic amine octopamine (OA) are important circadian coupling signals which might act via adenylyl cyclase (AC) activation. To investigate the daytime-dependent modulation of pheromone transduction of olfactory receptor neurons (ORNs), the membrane-permeable cyclic nucleotide analogue 8bcAMP, OA and its precursor tyramine (TA) were applied over the recording electrode in long-term tip recordings of trichoid sensilla of *Manduca sexta*. In addition, the OA-receptor antagonist epinastine (EPI) was employed. To search for daytime-dependent differences the recordings were performed either at Zeitgebertime (ZT) 22-1, 1-4, or 8-11 (ZT 0 = lights on). Using a non-adapting pheromone-stimulation protocol in control recordings the responses to bombykal stimuli of 10 or 1 µg remained constant over the recording duration of 180 min. The perfusion of the sensillar lymph with 1 mM OA and TA increased both the sensillar potential (SP) amplitude and the action potential (AP) frequency daytime-dependently. Also daytime-dependently, 8bcAMP increased the SP amplitude continuously, but in contrast had no effect on the initial AP frequency, while EPI decreased the AP frequency without effects on the SP amplitude. The daytime-dependent effects varied greatly for the agents tested. OA and TA increased both the SP amplitude and AP frequency at ZT 8-11. The SP increase by OA was 2.5 fold stronger than the TA-dependent increase. OA, but not TA also increased the SP amplitude at ZT 1-4, the increase was by the factor 9.7 weaker than in recordings at ZT 8-11. 8bcAMP increased the SP amplitude at ZT 1-4 and ZT 8-11. In addition EPI strongly decreased the AP frequency at ZT 8-11 but had only a very weak effect at ZT 22-1 although a 10-fold higher EPI-concentration was used. At ZT 22-1 no effects of OA, TA and 8bcAMP were found. In addition, daytime-dependent differences in the AP distribution in responses were observed: In control recordings at ZT 8-11 a shift from phasic to tonic responses was recognizable over the recording duration and in addition the APs elicited in the first 100 ms of the response were decreased. This decrease in the temporal resolution of the ORNs was antagonized by application of OA and TA at ZT 8-11. In contrast, EPI enhanced the shift to tonic responses at ZT 8-11 and also shifted the responses to a tonic distribution at ZT 22-1. 8bcAMP had no effect on the AP distribution. Furthermore, several effects on the spontaneous AP frequency were observed. OA and 8bcAMP increased the mean spontaneous AP frequency and the percentage of APs occurring in bursts at ZT 8-11. More and slightly longer bursts were generated. EPI decreasedthe mean spontaneous AP frequency at ZT 22-1 and ZT 8-11 and also lowered the percentage of APs in bursts at ZT 22-1. Therefore, we hypothesize that circadian release of OA modulates ORNs daytime-dependently via at least two different OA-receptors. One OA receptor appears to couple to an AC and affects the SP and the generation of spontaneous APs, while the other does not couple to an AC and restores the ability to generate phasic responses to pheromone stimuli. [Supported by DFG grant STE531/13-1,2]

Investigating the contributions of Ga_o and Ga_q to information processing in *Drosophila* olfactory sensory neurons

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Heterotrimeric G proteins signaling downstream of 7-transmembrane receptors are signal transducers of major importance in almost every cell. *Drosophila* olfactory sensory neurons (OSNs) express several Ga-subunits, but their contribution to odor responses in these cells is largely unknown. Focussing on Ga_o and Ga_q, we have therefore selectively blocked their function both individually and in combination and performed behavioral and physiological experiments.

In order to target OSNs, we used the Or83b-GAL4 line. We expressed pertussis toxin to inactivate Ga_o and Ga_q-RNAi to reduce expression of Ga_q. Either one resulted in behavioral deficits in a simple odor-choice paradigm. Blocking both of them led to a further non-additive decrease in performance.

To examine the underlying physiological mechanisms we focussed on OSNs expressing the receptor Or92a, using Or92a-GAL4 to drive the expression the calcium-sensitive reporter GCaMP. We recorded odor-evoked responses at two levels of information processing within these neurons, (1) the somato-dendritic compartment on the antenna which is the location where olfactory transduction takes place, and (2) the axon terminals in the antennal lobe where OSNs are presynaptic to other neurons.

In the somato-dendritic compartment, changes in odor responses after Ga_o and Ga_q downregulation were only visible at high odor concentrations. However, consistently strong effects were found at the level of axon terminals. In male flies, expression of pertussis toxin resulted in an increase in response strength, while knocking down Ga_q led to weaker responses as compared to the control. When both Ga-subunits were silenced together, response strength was back to control levels.

We conclude that primary signal transduction downstream of the olfactory receptor in Or92a-expressing OSNs does not depend on the G proteins examined here. Both Ga-subunits appear to be involved in mechanisms modulating the output of OSNs. Further studies are planned to characterize localization and mode of action of these modulatory mechanisms.

Calmodulin is Important for Pheromone Adaptation in Vomeronasal Sensory Neurons

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The mammalian vomeronasal organ (VNO) plays an essential role in the detection of chemical cues such as pheromones that convey significant information about gender, status, and individuality. The vomeronasal sensory neurons (VSNs) are among the most sensitive chemosensors in mammals and are able to detect stimuli over a broad concentration range. Understanding VNO signaling requires knowledge of the dynamic processes that regulate the sensitivity of pheromone detection in VSNs. Recent findings revealed that pheromone-induced activation of VSNs ultimately leads to an increase in intracellular Ca^{2+}, presumably via G protein-mediated activation of phospholipase C and generation of the second messenger molecules IP_3 and DAG. We recently showed that DAG, in turn, activates a Ca^{2+}-permeable conductance that is critical for membrane depolarization and significantly impaired in mice lacking the TRPC2 ion channel.

Here, we report that pheromone-induced Ca^{2+} entry plays a crucial role as a negative feedback modulator of VSN sensitivity. Using electrophysiological methods (electro-vomeronasogram as well as whole-cell experiments) we show that VNO responses undergo effective sensory adaptation that requires the influx of Ca^{2+} and is mediated by calmodulin (CaM). Removal of the Ca^{2+}-CaM feedback eliminates pheromone adaptation. The activation of diacylglycerol-operated cation channels in inside-out membrane patches excised from the dendritic endings of VSNs is strongly inhibited by Ca^{2+}-CaM revealing a key role of these ion channels in vomeronasal sensitivity regulation. Our analysis provides insight into a previously unrecognized feedback mechanism in the VNO that is essential for adjusting the sensitivity of pheromone detection.

Smells like nurse bee spirit?

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In honeybee colonies the female worker caste expresses a pronounced polyethism. Summer bees live for ~6 six weeks and perform a rich behavioural repertoire ranging from various indoor duties (e.g. feeding, building) to foraging nectar, pollen and water outside the hive. Switching between these behaviours depends on age and goes along with plastic changes in the synaptic architecture of the mushroom-body calyces.
However, division of labour in a honeybee colony is not purely age-dependant and as rigid as described above. In fact, the transition from nurse bees to foragers may additionally be modulated by chemical communication signals enabling the colony to respond in a flexible manner to environmental changes by shifting the work force between indoor and outdoor duties.
One of these chemical cues is ethyloleate (EO) which has earlier been described to delay the onset age of foraging (Leoncini et al., PNAS, 2004). EO is apparently produced in high concentrations in the honey crop of foraging bees. As honeybees continuously feed each other via trophallaxis EO is passed throughout the entire colony, thus making it an easy accessible signal for work force distribution. Therefore the concentration of EO can be regarded as a primer pheromone that accelerates or delays onset of foraging.
Whether EO is received as an olfactory and/or gustatory cue or whether it is internalised still remained unclear. Here we investigate if EO is received as an olfactory cue. Since EO is of low volatility at room temperature, heated air or heated dummy stimulation were used to evoke behavioural or physiological responses. This stimulation was first used in electroantennography (EAG) recordings of whole antennae as a first indication that EO is at least partly received via the olfactory system. Furthermore, Ca^{2+}-Imaging using bath application or selective staining of projection neurons with calcium sensitive dyes is performed to investigate the neuronal representation of EO within antennal-lobe glomeruli. The results provide evidence that EO is at least partly received as an olfactory cue suggesting that it may mediate plastic changes in brain during the transition of nurse bees to foragers via the olfactory pathway.

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Imaging Olfactory Learning in the Mushroom Body Lobes of the Honeybee

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In the honeybee the mushroom bodies (MB) are required for learning and memory retrieval. The lobes are their main output region connecting the MB with different regions of the brain including pre-motor regions or the input side of the MB. We imaged MB extrinsic neurons during differential appetitive conditioning and retrieval of short term odor memory. The behavioral performance of each animal was monitored during imaging and later quantified. The morphology of the imaged structures was investigated and partly reconstructed.

We observe MB extrinsic neurons which show associative plasticity correlated to the behavioral performance of the animal. However other extrinsic neurons do not seem to be subject to this form of plasticity. By investigating the morphology of the neurons we aim on assigning the different experimental outcome to subtypes of extrinsic neurons.

Biological activity and composition of cuticular lipid extracts of the cricket *Gryllus bimaculatus.*

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The cuticular lipids of the cricket *Gryllus bimaculatus* (DE GEER) serve as contact pheromones, that are essential, in combination with tactile, visual and acoustic cues, for aggression and courtship behaviour (Tregenza & Wedell 1997, Anim. Behav. 54:979-984; Adamo & Hoy 1994, Anim. Behav. 47:857-868). To investigate the chemical basis of sex-recognition and sex-specific behaviour more closely, whole body hexane extracts were produced. These were tested for biological activity in a behavioural assay, and analyzed by gas chromatography – mass spectrometry (GC-MS) with regard to qualitative and quantitative composition.

The behavioural assay yielded that courtship could be elicited unequivocally with female- and aggression with male-extracts only. This verifies the behavioural relevance of the cuticular lipids, which is conserved in the hexane solution. The GC-MS analysis of the extracts revealed that males have a higher quantity of cuticular substances than females. In both sexes a large variety of hydrocarbons, with a chain-length ranging from 25C to 31C, was found. The compounds comprise saturated, mono- and diunsaturated hydrocarbons as well as mono- and dimethylalkanes with internal and terminal branching positions. The extracts of males and females differ in both their quantitative and qualitative composition. The differences cannot be pinpointed to a single component or a certain class of substances, which suggests that the pheromones are complex composites. Further experiments will focus on producing, testing and analyzing hydrocarbon fractions, both singularly and in combination.

The stimulatory heterotrimeric G-protein Ga_s is involved in olfactory signal transduction in *Drosophila*

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Olfactory signal transduction starts with the activation of olfactory receptors, which are known to recognize of a wide range of structurally highly variable substances. While vertebrate olfactory receptors are 7 transmembrane proteins which activate heterotrimeric G-proteins after ligand binding, olfactory receptors in *Drosophila* were reported to have an inverse membrane topology compared to classical G-protein coupled receptors, with an intracellular N- and extracellular C-terminus. Moreover, it was show recently that the receptors can function as ligand gated ion channels. Controversial findings were reported concerning the additional involvement of heterotrimeric G-proteins in olfactory receptor signaling. One arising question is therefore, whether these 7 transmembrane receptors also couple to heterotrimeric G-proteins, in addition to the reported novel way of signaling. Our data involving *in vivo* electrophysiological recordings and protein redistribution, as well as investigations using recombinantly expressed olfactory receptors, now demonstrate that olfactory receptor signaling in *Drosophila* indeed involves G-proteins for signal transduction. Moreover, our results provide compelling evidence that the stimulatory Ga_s protein is involved in odorant detection. In conformity with Ga_s signaling we could show that increased cAMP levels lead to excitation of the olfactory sensory neurons.

HF Magnetic Field Disrupts Magnetic Orientation in Zebra Finches

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Zebra finches can be trained to use the geomagnetic field for short distance orientation (Voss et al., 2007). The mechanisms for magnetoreception are as yet unknown but two hypotheses are currently discussed. One focuses on the existence of clustered magnetic particles in the upper beak of birds which are thought to be affected in their orientation by the ambient geomagnetic field. The second hypothesis is based on a light-induced radical pair mechanism within molecules of the retina of birds that can be influenced in its outcome by a magnetic field.
To determine which of the two explanations applies for the geomagnetic orientation in our previous experiments we trained zebra finches to search for food with only the geomagnetic field as an orientational cue. When the birds had learned the task, their performance was first tested in a magnetic field shifted by 90° to affirm that orientation was solely based on the magnetic field. We then investigated whether the orientation performance was affected by superimposing a 1.156 MHz oscillating field over the ambient earth magnetic field. High frequency oscillating currents are said to disturb magnetoreception by inducing resonance effects on radical-pair spin states. The birds were well oriented in the natural geomagnetic field as well as in the 90° horizontally shifted field. If a high frequency oscillating field was superimposed the ability of zebra finches to orient by the geomagnetic field was eliminated. Our results therefore clearly indicate that magnetic field orientation in zebra finches depends on radical pair mechanisms.
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A dual olfactory pathway in honeybee antennal lobes: Innervation pattern of local neurons.

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Dual pathways, that is anatomical separation, and parallel pathways, that is functional separation, often coincide with each other and provide means to process elementary aspects of the same modality. A well known example for this concept is the processing of "what" and "where" in the visual system.
In the honeybee a "dual olfactory pathway" separates the first olfactory neuropil, the antennal lobe into hemilobes (Kirschner et al 2006). Glomeruli in the ventral-rostral hemilobe mainly receive input from sensory axons of the T1 tract and are innervated exclusively by uniglomerular projection neurons of the lateral antennoprotocerbral tract (ACT), while glomeruli in the dorsal-caudal hemilobe are associated with sensory input mainly from the T3 tract and exclusively with uniglomerular projection neurons of the medial ACT. The functional significance of this anatomical separation is unknown.
Local neurons interconnect the about 160 glomeruli of the honeybee antennal lobe. So far, two major classes of local neuron morphologies have been described: homogeneous interneurons branch uniformly within several glomeruli, while heterogeneous interneurons invade a single glomerulus strongly, and several others sparsely.
Here we asked whether the arborisation of local neurons is limited to one hemilobe, or whether local neurons may as well interconnect the hemilobes.

Forager honeybees were anesthetized by cooling and restrained in custom-made mountain brackets. The head capsule was opened and glands as well as tracheae were removed as far as necessary to expose the antennal lobes. Intracellular dye application was achieved using sharp electrodes, filled with 4% Alexa-488 hydrazide in 0.2M KCl. After successful encounter of a single neuron, apparent by a drop in membrane potential and vigorous firing, Alexa-488 was iontophoretically injected by applying depolarizing current pulses of 0.3-3.6 nA at 1Hz.
Brains were dissected and underwent standard morphological treatment of fixation, dehydration and clearing, followed by taking wholemount confocal images. The software AMIRA was used for image processing and reconstruction of the neuron’s morphology.

We found two groups of local neurons with distinct patterns of ramification: one group exclusively innervated glomeruli belonging to one of the hemilobes. The other group branched throughout the antennal lobe and interconnected both hemilobes.

Our findings provide evidence that the dual pathway of the honeybee olfactory system is not an exclusive property of antennal lobe input and output, but extend to a subpopulation of local neurons.
The presence of local neurons restricted to one hemilobe could possibly provide means for the network to separate certain aspects of an odour. The existence of local neurons branching within both hemispheres on the other hand might allow for processing of stimulus identity independently from the transmitting input pathway.

Processing of imperfect odor mixtures in the honeybee antennal lobe

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Most odors in the environment consist of many substances, and still they are perceived as unitary entities (synthetic olfaction). On the other hand, it is often possible to detect and identify individual components in an odor mixture (analytic olfaction). Several studies have shown that odor mixtures lead to non-linear interactions within the olfactory system, including potentiation and suppression of responses in olfactory cells, from the level of receptor cells to central processing neurons. This was taken as the basis for our synthetic capacities. One situation where components of a mixture could be accessible to the olfactory system is the occurrence of "imperfect mixtures". Unlike "perfect mixtures", which arise from a unique source and in which the components are distributed homogeneously with constant concentration ratios in space and time, in the case of "imperfect mixtures" plumes of odors emanate from different sources and mingle incompletely, resulting in an odor stimulus with discontinuous concentration ratios. We hypothesize that these discontinuities in an imperfect mixture as opposed to the perfect mixture contain information that can be used to detect the mixture components. We therefore expect different representations of perfect and imperfect mixtures in early stages of olfactory processing. We investigated the representation of perfect and imperfect mixtures in the first olfactory processing stage in the honeybee brain, the antennal lobe. Projection neurons were retrogradely stained with the calcium indicator Fura2-dextran. Single odor components as well as their binary perfect and imperfect mixtures (generated by shifting the onset of the components) were delivered to the antennae while projection neuron responses were recorded by 1- or 2-photon imaging. We have found first evidence that imperfect and perfect mixtures differ in quality and quantity of inhibitory mixture interactions. We observed instances where the processing of a preceding odor suppressed or even abolished the projection neuron response to a subsequent odor. However, these effects varied between animals and glomeruli. Possibly, previous olfactory experience of the bees might influence the processing of imperfect mixtures. The observed effects could enable the honeybee to distinguish between the two types of mixtures and therefore allow for the discrimination of odors in an imperfect mixture.

Imaging Temporal Odor Representation in the KENYON CELLS of honeybees

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Mushroom bodies are higher order sensory integration centers in insect brains where olfactory information is integrated and processed. In honeybees (*Apis mellifera*), each mushroom body consists of 170,000 Kenyon cells.

Several studies have dealt with the characterization of olfactory input into the honeybee antennal lobe, the first olfactory neuropil, and the output from the antennal lobe to the mushroom bodies via projection neurons. So far, little is known about odor processing in Kenyon cells. Imaging - and electrophysiological studies in honeybees, moth, and locusts revealed a sparsening of Kenyon cell responses to odors in the temporal domain.
We have investigated this temporal sparsening effect and show that Kenyon cells do not code to the length of an applied odor stimulus. We observe a brief phasic response after stimulus onset independent from stimlus length. This sparse response may be a result of general or specific inhibition. Inhibition in the insect brain is usually mediated by the neurotransmitter GABA. Therefore we used GABA-receptor blockers and investigated the effect on Kenyon cell responses.
For a single stimulus we found a stronge effect on the response strength but no influence on the temporal dimension. The application of two or more stimuli revealed odor specific effects.

For our experiments we used the calcium imaging technique with calcium sensitive dye Fura 2 and combined it with odor stimulation in order to analyze the neural activity of Kenyon cells in mushroom body calyces *in vivo*.

Cannabinoids modulate odor sensitivity in the olfactory epithelium

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Olfactory information processing heavily depends on the environmental as well as the internal state of an animal including the appetitive, arousal, and reproductive conditions. The involved neural substrates and chemical signalling systems are poorly understood as yet. In this regard recent data clearly demonstrate that various neuromodulators act already at the peripheral level, the olfactory epithelium.

Also the endocannabinergic system has a profound impact on the responsiveness of individual olfactory receptor neurons in larvae of *Xenopus laevis*. Previous data indicated that the block of CB1 receptor led to a desensitisation of olfactory receptor cells. Here, we clearly demonstrate that the CB1 receptor activation increase the sensitivity of olfactory receptor neurons. Moreover, the activation of the endocannabinergic system is able to enhance the spatiotemporal response pattern of olfactory receptor cells to single odorants.

Taken together our data specifies the role of the endocannabinergic system in the olfactory epithelium. Moreover we demonstrate for the first time that the peripheral endocannabinergic modulation is important for the detection of odors and thus for olfactory coding in general.

Dis- and Reassembling Complex Natural Blends by Linked Gas-Chromatography - Optical Imaging Techniques in *Drosophila melanogaster*

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Natural odors occur in complex blends consisting of many different components. In order to identify the components and to characterize their activity patterns in the olfactory system, we established a new tool of odor coding investigation by combining gas chromatography (GC) and calcium imaging techniques. Using the model organism *Drosophila melanogaster*, we measured physiological responses of receptor neurons in the olfactory organ, the antenna, and the first olfactory neuropil, the antennal lobes. As stimulus we used banana extract which was either presented as a whole or fractured into its single components. To identify those odor components in the banana extract which evoke neuronal activity in the receptors neurons the extract was injected into the GC and from there directed to the fly's antennae via a transfer line. During the stimulations we measured the intracellular calcium concentration changes in olfactory receptor neurons. In a second step we identified the active components of the banana extract by coupled gas-chromatograph/mass spectrography (GC/MS) analysis. By disassembling the banana odor and identifying neuronal activation patterns of its single components we are able to compare an artificially reassembled pattern (simple additive pattern without any signal processing by the olfactory network) with the activation pattern elicited by the whole banana extract (putatively processed by the olfactory network). This puts us in a position to investigate network properties like mixture interactions leading to pattern processing of odor blends. In that way we hope to elucidate the processes behind the perception of complex natural blends and the role of the peripheral olfactory network.

Peripheral and central olfactory processing of sex pheromone in an insect model

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Although much progress has been made recently, many questions are still open in the understanding of olfactory processing in antennal lobes and the transformation they operate on the antennal input into the output to higher brain centres. We are currently investigating these questions by focusing on how sex pheromone signals are processed within the male-specific macroglomerular complex (MGC). Sex pheromone communication is crucial for mate encounter and therefore species survival. In our model species, the noctuid moth *Agrotis ipsilon*, the central olfactory structures are easy to access *in vivo* and the pheromone compounds involved in mate attraction are well known.

By means of extra- and intracellular recordings and stainings of peripheral and central olfactory neurons, we are describing the different neuronal elements of the MGC of sexually mature, virgin males. For recordings, the antenna is stimulated with 200 ms pulses of the pheromone blend or its individual components at different concentrations. Both receptor neuron (RN) and projection neuron (PN) responses are quantitatively analysed to specify how the characteristics of the pheromone signal, *i.e*. concentration and duration of single and repeated pulses, are encoded by these neurons. The glomeruli forming the MGC can be identified by analysing the arborisations of physiologically characterized RNs and PNs with the help of the antennal-lobe atlas available in *A. ipsilon*.

Single sensillum recordings show a long lasting response of the pheromone-sensitive RNs, whereas a large proportion of the intracellularly recorded and stained PNs arborising in the MGC respond with a shorter bi-phasic excitation / inhibition pattern to pheromone stimulation. However, other response types are observed from PNs in the MGC which indicate that PNs in the sex pheromone pathway present a diversity of response patterns. These results suggest that the message transmitted by RNs is temporally shaped by interactions between AL neurons. According to studies in other insects, the inhibition period we observe in most of our PN responses would be due to an inhibitory input from local neurons (LNs). Unfortunately intracellular recordings from LNs have been very rare so far in our preparation and more LN responses to pheromones will have to be recorded before their precise role in the shaping of the incoming information can be understood. Current injections during intracellular recording presently in progress should give access to electrical properties of PNs.

In parallel, extracellular recordings, using two glass microelectrodes located near the MGC, analysed with appropriate algorithms, allowed us to extract the spiking activity of 2 to 5 neurons at the same time. Long lasting recordings revealed interactions of certain neurons in spontaneous activity and a high degree of synchronisation during pheromone stimulation.

These various experimental data are being integrated in quantitative models of individual MGC neurons and of RN/PN/LN interactions within the MGC network. These models are intended to fit the experimental data sets, to help interpreting them and to suggest new experiments.

Neuronal representations of olfactory and visual associative learning in the honeybee (*apis mellifera*)

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The mushroom body (MB) is a central neuropil structure in the protocerebrum of the honeybee brain and plays a crucial role in sensory integration and memory formation. In order to elucidate the role of MB extrinsic neurons in olfactory and visual associative learning and memory formation, we perform extracellular long-term recordings of single neurons in the output region of the MB for up to 4 days, while the bee performs a classical conditioning task on each day. The Protocerebral Tract (PCT) neurons have recurrent connections from the output to the entrance region of the MB. We ask, whether single PCT neurons are responsive to either or both sensory modality, olfactory and/or visual, and wether they change their spiking patterns due to learning.

First, we present a set of odors and colors before training to get the baseline neuronal activity. Then we pretrain the bee differentially with two colors, one rewarded, the other not. Afterwards, the same colors are simultaneously presented with two different odors (compound training). Again, one set of pairs is rewarded, the other is not.
30 minutes after trainng, all odors and colors, seperately and pairwise, are tested. Responses are defined as a rate increase or decrase within the time of stimulation. A rate change is statistically assigned as a response as soon as it deviates more then two standard deviations from spontaneous activity.

We typically find neither responses to odor nor to color before training.
In most neurons, we find no responses during training on day 1, while during the retention test performed 30 minutes after training, the neuron respond clearly to either all odors or all colors or both. Some neurons respond specifically to the rewarded odor or color. On the consecutive days, we apply the same training and test procedure as on day 1. We find responses in the training sessions as well as in the retention tests to the stimuli, the neuron already responded to before.

These results indicate that the recurrent PCT neurons change their spiking pattern after learning. They are rather heterogeneous with respect to their receptive sensory modalities and might thus support sensory integration. The fact that the cells rather respond to all stimuli within one or two certain sensory modality after learning and not only specifically to the rewarded stimuli let us conclude that the PCT`s feedback information about the context in which the reward system is involved.

Experience modulates pheromone sensitivity in moths

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Male moths innately have a high sensitivity to female-produced sex pheromones. In the noctuid moth *Spodoptera littoralis*, however, behavioural responses to the pheromone can be further increased by brief pre-exposure to the sex pheromone. An increase in behavioural responses was shown within two distinct time windows: 15 minutes and 27 hours after pheromone exposure (Anderson *et al*. 2007). In parallel, an increase in sensitivity of neurons within the primary olfactory centre, the antennal lobe, was observed 27 h after pre-exposure with pheromone. To test if the observed effect is a form of general sensitization or rather a phenomenon of selective attention, we tested effects of pre-exposure with different sensory stimuli on the behavioural and central nervous sensitivity to sex pheromones.

In behavioural tests on a locomotion compensator, we were able to reproduce the effects of a pheromone exposure on the behavioural response to sex pheromone. The threshold of responses to sex pheromone was significantly lower 27 h after exposure to a female equivalent of pheromone. In addition, exposure to an artificial bat sound, a strong predator signal, also lowered the threshold for pheromone responses after 27 h. This behavioural effect was correlated with an effect on the sensitivity of antennal lobe neurons. Although neurons in bat sound-exposed male moths did not reach the same sensitivity as in pheromone-exposed males, thresholds were still significantly lower than in naïve males. To test if this cross-modality effect is indeed an indication that the pre-exposure effect might be a form of sensitization, we will now investigate if exposure to other olfactory stimuli, such as host plant odours and to attractive and aversive gustatory stimuli has an effect on pheromone responsiveness on the behavioural and central nervous level.

3D Standard Brain of the Red Flour Beetle *Tribolium castaneum:* A Tool to Study Sex Dimorphism, Adult Plasticity and RNAi

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Tribolium castaneum is emerging as a further standard insect model beside *Drosophila*. Its genome is fully sequenced and it is susceptible for genetic manipulations like RNA interference (RNAi). Tribolium provides an ideally suited organism to study adult brain development. As a beetle, Tribolium occupies a basal phylogenetical position among the holometabola and thus contrasts the highly advanced Diptera.
Here we created a volumetric standard brain of freshly eclosed adult males (A0) for defined brain areas, thus enabling us to compare brain morphology between sexes, different ages, and physiological states combined with RNAi. To obtain the desired datasets we labeled whole brains with an antiserum against the synaptic vesicle protein synapsin to visualize neuropil areas, analyzed the staining using a confocal laser scanning microscope (Leica TCS SP2), and 3D reconstructed the major brain neuropils (including antennal lobes with selected olfactory glomeruli, mushroom bodies etc.) using AMIRA (Mercury Systems).
In detail, we compared neuropilar volumes between freshly eclosed males and females, found immense post-eclosional neuropilar growth within the first week of adulthood, and present evidence for a successful RNAi knockdown of the tachykinin precursor. Currently we are studying the cause of adult specific neuropilar plasticity by examining the involvement of proliferating neuroblasts and programmed cell death using BrdU and TUNEL labeling.

Homeostatic Plasticity in Basal Vomeronasal Neurons: Activity-Dependent Expression of Ether-à-Go-Go Related Gene Potassium Channels

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Conspecific chemosensory communication controls a broad range of social and sexual behaviors. In most mammals, social chemosignals are predominantly detected by sensory neurons of a specialized olfactory subsystem - the vomeronasal organ (VNO). The behavioral relevance of social chemosignaling puts high demands on the accuracy and dynamic range of the underlying transduction mechanisms. However, the physiological concepts implemented to ensure faithful transmission of social information remain largely unknown. Here, we show that sensory neurons in the basal layer of the mouse VNO dynamically control their input-output relationship by activity-dependent regulation of K^+ channel gene expression. Using large-scale expression profiling, immunochemistry and electrophysiology, we provide molecular and functional evidence for a role of ether-à-go-go related gene (ERG) K^+ channels as key determinants of cellular excitability. Our findings indicate that an increase in ERG channel expression extends the dynamic range of the stimulus-response function in basal vomeronasal sensory neurons. This novel mechanism of homeostatic plasticity in the periphery of the accessory olfactory system is ideally suited to adjust VNO neurons to a target output range in a layer-specific and use-dependent manner.

Modulation of firing activity of olfactory receptor neurons by background odorants

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Pheromone communication in moths serves for mate recognition and mate finding. Female moths release a specific blend of pheromone components in precise and stable ratios. Each component of the blend is detected by olfactory receptor neurons (ORNs) with appropriate receptors. Mate finding and recognition depend on chemical specificity and ability of the olfactory system to follow rapid changes in pheromone concentration. While pheromone components are emitted in minute amounts the natural environment of moths is rich in volatile organic compounds. The main sources of these odorants are plants. It is known that in *Spodoptera littoralis* such non-specific odorants (among them linalool, Z3-hexanol and geraniol) modulate ORN firing activity in response to pheromone components.

S. littoralis is thus a suitable model for analysis of detection of a specific signal in a noisy environment and the interactions of specific and unspecific signals. We have recorded the responses of ORNs housed in long trichoid hairs of the antennae of male *S. littoralis* to the main component (MCP) of pheromone blend, Z9E11-14:Ac, in the presence of linalool. Linalool is a monoterpene released by different plants including maize and cotton, two major host plants of *S. littoralis*. Diffusion of odour over distance from the source creates pulsed stimuli. In natural conditions the probability of two compounds from different sources reaching the sensory structures at the same time is very low. Linalool was therefore presented either simultaneously with MPC, as a single pulse during sustained stimulation with MPC or as the background to a single pulse of MPC. We performed single-sensilla tip recording and monitored changes in ORN firing-rate and time pattern in response to stimuli. Firing rate changes were used for evaluation of excitation and suppression events. They were determined statistically by a homemade program written in R, using the slope of cumulative curve of firing events.

In most cases the maximum firing activity was significantly lower in the presence of linalool. When MPC and linalool were presented simultaneously, there was no significant change in response latency, however the response duration was somewhat shortened compared to the responses to MPC alone. When linalool was presented as the background, we observed one peak just after the beginning of the MPC stimuli and one after the linalool background was turned off. Linalool stimulation did not produce excitatory response and only occasionally the after-peak was observed when the linalool was turned off. When a single puff of linalool was presented during the MPC stimulation, the excitatory response was suppressed. After linalool was turned off, the excitatory response recouped. When MPC was presented in regular short pulses against a background of linalool, the temporal pattern of firing followed the stimuli pattern more closely compared to MPC pulses without linalool background. We conclude that (1) an odorant background affects pheromone detection despite high degree of chemical specificity (2) temporal information is better preserved than intensity coding (3) effects on behaviour will depend upon integration in the CNS but a reduction of response intensity does not necessarily have a negative effect on orientation. The pheromone communication system appears very robust to noise.

Signaling Protein Distribution in Olfactory Sensory Neurons

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The olfactory system is a unique part of the central nervous system since it retains neuronal turnover and regenerative capacities in adulthood. This makes it a interesting system to understand sensory signal transduction as well as neurogenesis. Cilia play important roles in allowing us to sense the environment, to see, hear, and smell. Although olfactory signaling proteins are highly enriched in cilia, little is known regarding the mechanisms of trafficking and subcellular localization in general and after activation. Movement of proteins to and along cilia is not only necessary for the assembly, maintenance, and length control of (sensory) cilia, but is also important for the sensory activity of cilia, implying a direct role for ciliary transport processes in signal transduction. Given the important biological function of transport along cilia, we aim to develop a precise understanding of the role of ciliary transport of signaling molecules for olfactory signal transduction. In a previous study using a proteomics approach we identified candidate proteins from olfactory epithelium. To gain insight into the signal transduction and trafficking we use cell biological, biochemical, and physiological approaches. In our studies we investigate signal transduction interaction partners in general and after activation of olfactory receptors.

Characterization of ion channels involved in olfactory transduction in the antenna of the hawkmoth *Manduca sexta.*

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Females of the hawkmoth *Manduca* sexta moths attract conspecific mates by the release of a species-specific sex-pheromone blend from abdominal glands. The male moths detect the pheromones with specialized pheromone-sensitive olfactory sensilla trichoidea on their antennae. Each trichoid sensillum is innervated by two olfactory receptor neurons (ORNs) one of which always responds to bombykal, the main sex pheromone component. Patch clamp studies on cultured ORNs suggests the following hypothesis of pheromone transduction in *M. sexta*: Bombykal binds to a G-protein-coupled odorant receptor (OR) which activates a phospholipase Cß (PLCß). PLCß hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP2) into inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). Our patch clamp recordings found evidence for directly or indirectly IP_3 - and DAG-dependent ion channels in olfactory receptor neurons with properties of transient receptor potential (TRP) channel. These ion channels cause a transient influx of Ca^{2+} into the sensory neurons. In addition, IP3 appears to bind to IP3 receptors (IP3R) in the endoplasmic reticulum to mediate Ca^{2+}-release from intracellular stores. The transient Ca^{2+} rise then triggers the activation of directly Ca^{2+}-dependent ion channels with properties of TRP channels. Under conditions of short-term adaptation prolonged Ca^{2+} rises together with DAG activates a protein kinase C (PKC), which then opens PKC-dependent nonspecific cation channels. After conditions of long-term adaptation with long, adapting pheromone stimulation it was shown before that cGMP rises in trichoid sensilla in *M. sexta* antennae. The cGMP then activates different sets of cyclic nucleotide-gated (CNG) ion channels in the antenna. Among these are at least two types of CNG-type nonspecific cation channels and one type of hyperpolarization-activated cyclic nucleotide-modulated nonspecific cation channel (HCN). In addition, also potassium selective CNG channels were described in patch clamp recordings.
With RT-PCR we partially identified one HCN and one IP_3 receptor gene in the antenna, as well as two splice variants of a TRPL-like transcript and a CNG channel gene in brains or antennae of *M. sexta*. In addition, as previously reported, we confirmed the presence of the ether a-go-go (eag) channel in the antenna.With in situ hybridization we attempt to localize the partially cloned ion channels in the antenna of *M. sexta* to determine their possible function in pheromone- or odor transduction. [Supported by DFG STE531/13-1]

Neural basis of mating-dependent olfactory plasticity in a male moth

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Most animals, including insects, have developed different strategies and mechanisms to recognize relevant information in a changing environment. External factors, including experience with a sensory stimulus, as well as the physiological state of an animal might directly influence their behaviour. Evidence is accumulating that insects, through neuronal plasticity, have developed a variety of mechanisms that allow them to modulate their behaviour. The insect olfactory system is an attractive model for the study of neuronal wiring and information processing underlying behavioural plasticity, because of well-defined stimuli and a relatively simple and accessible peripheral and central nervous system, which shares nevertheless important features with the vertebrate system. In moths, the sensitivity to sex pheromone may vary according to the time of the day, age, mating status, as well as experience[1]. Male moths of *Agrotis ipsilon* (Lepidoptera: Noctuidae) are no longer attracted to the female sex pheromone following mating[2]. This plasticity is not only seen at the behavioural level, but is accompanied by a change in the sensitivity of central olfactory neurons. The loss of sensitivity after mating is restored during the next day[2]. This transient neuronal plasticity serves as an energy-saving strategy by switching off the olfactory system and therefore preventing males from mating unsuccessfully.

In the present study, we analyzed the response pattern of peripheral and central neurons to sex pheromone in virgin and newly mated *A. ipsilon* males, by extracellular recordings of antennal receptor neurons and intracellular recording and staining of antennal lobe neurons. Detailed analysis of the recorded activity of the different neuron types will allow us to understand how the neural network is remodelled/modulated in order to change the sensitivity of the olfactory system. In addition, we investigated whether mating might also affect the central processing of non-pheromonal odour information (*i.e*. plant volatiles). By analyzing the sensitivity threshold, the instantaneous firing rate, the total number of spikes during a response, as well as the latency and the duration of the response, we could show how mating changes the central processing of pheromonal information, whereas the input at the peripheral level (*i.e*. responses of pheromone receptor neurons) remains constant. Moreover, we show that this mating-dependent neuronal plasticity is restricted to the sex-specific pheromonal system and does not affect the processing of general odours such as plant-volatiles.

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ß-arrestin2 mediated desensitization of mammalian odorant receptors

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Odorant receptors comprise the biggest subfamily of G-protein-coupled receptors. While the endocytic mechanisms of other G-protein-coupled receptors have been characterized extensively, almost nothing is known about the intracellular trafficking of odorant receptors. We investigated the endocytic pathway of mammalian odorant receptors and found that these receptors bind ß-arrestin2 with high affinity and are internalized via a clathrin-dependent mechanism. After prolonged odorant exposure receptors are not targeted to lysosomal degradation but accumulate in recycling endosomes. Moreover, ß-arrestin2 is redistributed into the dendritic knobs of mouse olfactory receptor neurons after treatment with a complex odorant mixture. Prolonged odorant exposure resulted in accumulation of ß-arrestin2 in intracellular vesicles. Adaptation of olfactory receptor neurons to odorants can be abolished by the inhibition of clathrin mediated endocytosis, showing the physiological relevance of the here described mechanism of odorant receptor desensitization. To get further insight in the mechanisms of adaptation and sensitization in the olfactory epithelium we investigate the odorant receptor trafficking and the interactions of odorant receptors with ß-arrestin2 and other trafficking proteins in living cells.

Functional characterization of the scaffolding protein, Multiple PDZ Domain Protein 1, MUPP1, in olfactory signal transduction

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The unique ability of mammals to detect and discriminate between thousands of different odorant molecules is governed by the diverse array of olfactory receptors (ORs) found on the dendrites of olfactory sensory neurons (OSNs), in the nasal epithelium. ORs are 7-transmembrane-domain G-protein coupled receptors and comprise the largest gene superfamily in the mammalian genome. Interestingly, certain OR subtypes possess a classical PDZ domain binding motif in their C-terminal regions. It has previously been shown that PDZ proteins (PSD-95, Discs large, ZO-1) specifically recognize short peptide motifs in the C-terminus of their target proteins. They have been established as sites for protein-protein interaction and play a central role in organizing diverse cell signalling assemblies, thus providing a possible mechanism for receptor modulation of membrane protein function independent of G-protein activation.

We are particularly interested in Multiple PDZ domain protein, MUPP1, which we have shown to be expressed in the dendritic knobs and cilia of OSNs, the sites of olfactory signal transduction. Upon odorant exposure, we found a significant increase in MUPP1 expression in the dendritic knobs, which was exposure-time-dependent. Moreover, on co-expression in Human Embryonic Kidney (HEK293) cells, with various ORs containing putative PDZ motifs in their C-termini, MUPP1 exhibits a membranous expression pattern, compared to its usual diffuse cytosolic expression. In a GST fusion protein interaction assay, a specific interaction was found between the C-terminus of olfactory receptor HS_OR and PDZ 1+2 of MUPP1. The physiological function of this interaction will be further investigated via RNAi of MUPP1 in HEK293 cells, followed by transfection of ORs and ratiometric calcium imaging. Furthermore, by transfecting OSNs i*n vitro,* with a dominant-negative mutant of the interacting PDZ domains, we hope to elucidate the physiological function of this interaction in the olfactory signal transduction cascade.

Brain structure of *Scutigera coleoptrata* (Myriapoda: Chilopoda): New insights into the evolution of mandibulate olfactory centers

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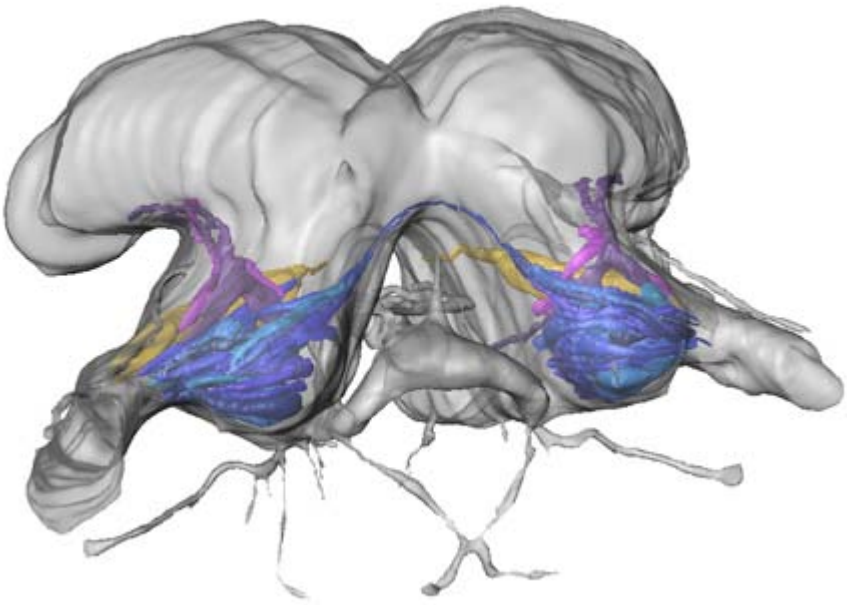
Data from molecular analyses and comparative neuroanatomy ("neurophylogeny") provide increasing evidence that not the Myriapoda but the Crustacea are the closest relatives of the Hexapoda. Hence, as descendents from marine arthropod ancestors, Hexapoda and Myriapoda may represent two taxa that independently conquered land so that the structure of their nervous system could mirror functional adaptations as a consequence of their terrestrial life style. We labeled vibratome-sections with antisera against synaptic proteins and the neuropeptide RF-amide as well as a probe for actin and analyzed the staining pattern using confocal laser scanning microscopy. Furthermore, 3D-reconstructions of its brain were prepared from section series (1.5µm) of plastic embedded tissue using Amira.

Scutigera coleoptrata has olfactory centers which are tube-like instead of spherical glomeruli as the insects have. The mechanosensory input from the antennae is processed in a lamellar neuropil, a structure unknown from any other mandibulate. A pedunculus-complex is present in the dorsal protocerebrum as well as a transverse, midline spanning neuropil with superficial similarities to the insect central body. However, other components of the insect central complex are not present. Our initial results reveal distinct differences to the insect brain so that brain data prove to be useful for obtaining insights into the phylogenetic relationships of the Mandibulata.

Fig.: 3D-reconstruction of the nervous system of *Scutigera coleoptrata* with olfactory and mechanosensory neuropils.



How to change while remaining the same – effects of learning on odor-evoked activity patterns in the antennal lobe

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The antennal lobe (AL) is the insect homologue of the olfactory bulb in mammals. As such, it is the first processing station in the insect olfactory system. It has been shown that odorant representations change during associative odor learning [1], but conflicting findings have also been published [2]. Besides the apparent contradiction in the published results, there is also a conceptual problem: if learning changes odorant representation in the first relay station of the olfactory pathway, how can downstream neuron populations identify those stimuli?

In this study, we present data and a network model that can resolve the apparent contradiction. We recorded Ca^{2+}-activity of uniglomerular projection neurons (PNs) in the AL of the honeybee *Apis mellifera* during differential olfactory reward conditioning. Analyzing single animals, we observed that the activity pattern of PNs in response to odorants changed for the conditioned odor, for the unconditioned odor and for control odors which were not presented during conditioning.

We designed a computational model of the glomerular network that can explain the apparent contradiction between the findings we present here and the results reported in [2] (Fig. 1A). Exploiting the fact that each glomerulus is innervated by several PNs [3], we demonstrate how their individual responses can diverge while their average output remains the same (Fig 1B). In addition, by adding synaptic plasticity into the model, we show how the asymmetric PN response can be acquired during reward conditioning.

Downstream neuronal populations can integrate over all PN responses from one glomerulus and obtain reliable activity patterns, solving the conceptual problem of odor coding in face of learning induced changes. Our model can also bridge the apparent contradiction in published findings: If PN stainings are sparse, i.e. not all PNs in one glomerulus are stained, analysis of a single animal may reveal changes in odorant-evoked activity patterns. These changes may be obscured when staining all PNs in one glomerulus, or when averaging over several preparations.

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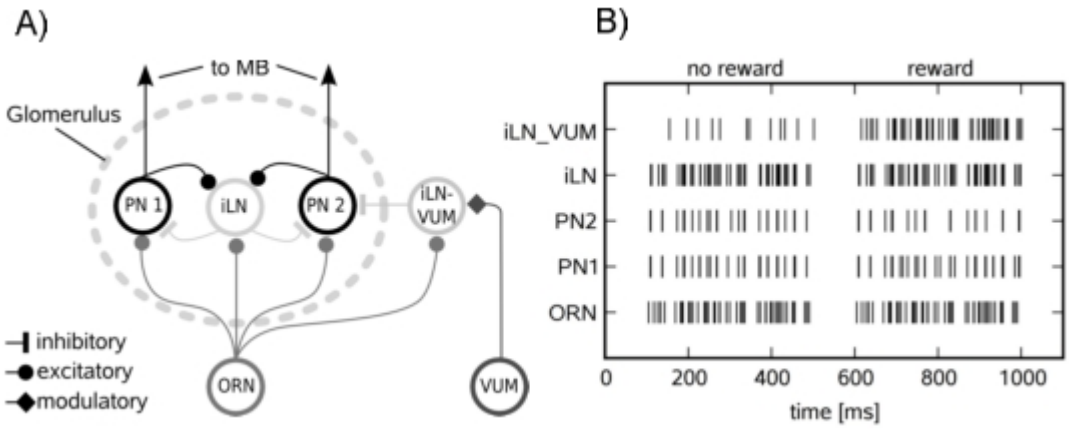
Figure legend:

Figure 1: A) schematic of the model connectivity. B) Spike trains from a simulation run, first without reward (i.e., no VUMmx1 activity) and with reward (VUMmx1 active). The PNs fired 29 (PN1) and 26 (PN2) spikes without reward, and 32 (PN1) and 21 (PN2) spikes with reward, keeping their average spike output constant.


A)
to MB
Glomerulus
PN 1
iLN
PN 2
iLN-
VUM
ORN
VUM
inhibitory
excitatory
modulatory
B)
no reward
reward
iLN_VUM
iLN
PN2
PN1
ORN
0
200
400
600
800
1000
time [ms]

Learning in a parasitoid's brain - a closer investigation of the brain structure of Cotesia plutellae

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Olfaction is essential in the life of most insects as they use odours for foraging, mating, oviposition sites or predator avoidance. To analyse how olfactory learning is processed in the brain, it is necessary to investigate the underlying neural structures. Extensive research has already been conducted with bees, flies and moths, but very little is known about central olfactory processing in parasitoids.

Parasitoids are very important in controlling pest insects, as well as a good model to investigate the properties of learning and memory. They learn through oviposition reward as well as appetitive food or host conditioning and their ecological success relies on the detection of volatiles, which are combinations of odours emitted by plants attacked by crop pests. Their different ecological adaptations may also be represented in differences reflected in their learning and memory abilities.

This work focuses on the structural organisation of the antennal lobes and the glomeruli of *Cotesia plutellae***.**

Brain structures have been stained with a calcium fluorescent dye as well as RH795. The average head size of *C. plutellae* is 645 ± 51µm x 446 ±14µm, with two antennal lobes of each 169 ± 11µm x 147 ± 9µm x 107 ± 28µm (LxWxD). Around 70 glomeruli have been identified, each 23 ± 2µm. A structure similar to a macroglomerular complex (MGC) was found in males, which is a complex of several larger glomeruli involved in pheromone reception. This is similar to the MGC in moths, bees or ants described in the literature. Further study of the neural structure using confocal imaging techniques will provide a better understanding of the underlying mechanisms that enable parasitoids to detect volatiles and, furthermore, will demonstrate how this information is processed in the brain.

A complete understanding of the learning process in terms of ecological and neurobiological activity will be important for future applications in pest control or neuroscience.

This work was supported by an award from the Kerkut Charitable Trust.

Homing in pigeons (Columba livia) induce ZENK activation in piriform cortex

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During homing over an unfamiliar area pigeons use olfactory cues (Gagliardo et al., 2007). Lesion of the piriform cortex (Cpi), which is the main projection field of the olfactory bulb (OB), disrupt the homing performance of pigeons (Gagliardo et al., 1997). To investigate the involvement of the Cpi during the navigation process from unfamiliar sites the expression of ZENK, an immediate early gene, was used to visualize its activation (Shimizu et. al., 2004). Therefore we released one group of pigeons from unfamiliar locations. Two other groups were either transported to the unfamiliar site but not released or were released 200 meters from the loft. The nostrils of the pigeons were either unilaterally plugged or unplugged, to examine whether the ZENK expression in the CPi relies on olfactory input (Gagliardo et al., 2007). Pigeons homed within 120 minutes from the release site were sacrificed, but not earlier than 60 min after release. Immunohistochemical staining was used to visualise ZENK-positive cells (Santa Cruz, Erg-1, C-19). Determination of the density of ZENK-immunolabeled cells was carried out according to Shimizu et al. (2004).

The statistic was conducted by a mixed 2x3x3 ANOVA with hemisphere (left, right) as repeated measure and releasing condition (released (R), transported to the released site but not released (TnR), released in front of the loft (RL)) and the nostril condition (unplugged, left plugged, right plugged) as between-subject factors. Significant main effects of releasing ($F(2.73) = 71.69$, $p<.0001$) and nostril condition ($F(2.73) = 20.22$, $p<.0001$) were found. As expected, R pigeons ($1349.02/mm^2 \pm 448.81/mm^2$) showed a higher ZENK expression compared to the TnR ($840.20/mm^2 \pm 372.15/mm^2$, $p<0.001$) and RL group ($456.03/mm^2 \pm 326.29/mm^2$, $p<.001$). These results indicate that the Cpi as a part of the olfactory system is actively involved in olfactory processing during navigation. Furthermore TnR and RL also differed in ZENK activity ($p<.001$), supporting the finding that pigeons are generally able to orient at the release site using olfactory cues before taking-off (Gagliardo et al., 2001). The groups with no plug ($1193.24/mm^2 \pm 509.67/mm^2$) revealed the highest ZENK expression ($p<.001$), underlining that the ZENK expression in the Cpi is due to sensory input of OB. No differences were found between right ($711.45/mm^2 \pm 469.09/mm^2$) and left ($757.63/mm^2 \pm 539.58/mm^2$) plugged groups ($p=.84$). But the significant interaction between hemisphere and nostril condition ($F(2.73) = 8.14$, $p<.001$) suggested that the hemispheric-specific activation depends on the plugged nostril. While decreased ZENK expression in the right Cpi after plugging the right nostril ($p<0.018$) could be detected, no reduction in the left CPi after plugging the left nostril, was observed. These data support the major importance of the right OB/nostril during navigation. Moreover, the significant triple interaction indicates that the differences in hemispheric-specific ZENK activation between the nostril conditions depended on the releasing conditions. ($F(4.73) = 3.1376$, $p < .05$). This is the first study revealing the neuronal activation of the Cpi during homing, supporting that the olfactory system is strongly involved in navigation over unfamiliar areas.

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The honeybee's mushroom bodies extrinsic neurons - their role in associative and non-associative learning

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The mushroom bodies (MBs) of the insect brain are higher-order centers performing integration of olfactory, visual and mechano-sensory information. They are also known to be involved in neural plasticity underlying associative olfactory learning. In the MBs input region, the calyces, neuronal pathways that convey odour information (CS) and reward information (US) converge allowing a learning induced plasticity to take place. At the output region of the MB, a group of efferent neurons, named extrinsic neurons (EN's), participate in the computation of a learned stimulus too, as some of them change their firing pattern in response to a rewarded odour in an olfactory conditioning. In addition it has lately been shown that some of these neurons display a robust response to the mere administration of a sugar reward, to both the antenna and the proboscis. By means of extracellular recording technique these neurons are further characterized while studying their response to different reward properties (reward magnitude, a delayed reward delivery, reward omission, etc.), their role in non associative forms of learning (habituation), as well as their response to varies forms of CS.

A mass spectrometric approach to determine neuropeptides from defined brain regions of *Apis mellifera*

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The honey bee *Apis mellifera* serves as well acknowledged model to study mechanisms of learning and memory. Among the signaling molecules involved in neuronal communication, neuropeptides present the largest and most diverse group. Functionally, neuropeptides are thought to be involved in processes related to neuronal plasticity, the substrate for learning and memory. The release of the bee genome made it possible to identify putative neuropeptide genes and (subsequently) to study their expression in the brain. Using the method of direct peptide profiling with MALDI-TOF mass spectrometry we were able to routinely obtain mass spectra from defined brain regions of the bee, including antennal and optic lobes, calyces of the mushroom bodies, and central brain. By mass match, we identified a number of peptides which were predicted from the genome data (e.g. tachykinin related peptides, allatostatins). In addition, we found a number of ion signals which could not be assigned to predicted bee neuropeptides. omparing mass spectra of the various brain regions of worker bees revealed spectra typical of each of the brain areas. Currently, we compare these neuropeptide “fingerprints” of the workers, with fingerprints of the same brain areas of nurses, drones, and queens. This approach will eventually provide us with the information whether there are differences in the expression of certain neuropeptides in defined brain areas of these bees, representing different tasks of a bee colony.

Intensity coding in the ant antennal lobe

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Ants use complex olfactory communication systems for social organization within large colonies. Pheromones play a key role and induce specific behavioral tasks such as trail following, alertness or defensiveness and control reproductive behavior. Efficient and accurate recognition of pheromonal and non-pheromonal odors is essential for the survival of ant colonies and requires a precise sensory machinery and neuronal network in the brain of each individual.

We used calcium imaging techniques to analyze the spatial and temporal representation of both odor groups in antennal lobe glomeruli of the carpenter ant *Camponotus floridanus.* Projection neurons (PNs) of large female workers were retrogradely filled by injection of fura-2 dextran into the medial and lateral antennocerebral tracts. We tested whether responses to pheromonal odors differ from responses to general odors regarding sensitivity and/or spatio-temporal representation. Changes in calcium levels were measured during odor stimulation with a releaser component of the trail pheromone (nerolic acid), an alarm pheromone (n-undecane) and two general odors (heptanal, octanol), all applied at dilutions ranging from 10^{-1} to 10^{-12}.

Our functional calcium imaging studies of PN activity revealed reproducible glomerular activation patterns in response to pheromonal (alarm, trail) and general odors at about equally high sensitivity. No obvious spatial segregation among pheromonal and non-pheromonal odor activation patterns was observed for the odors tested. Our results in *C. floridanus* indicate that the spatial activation pattern in response to the trail pheromone component is largely invariant over a rather large concentration range of ~8 log units. Only at the lowest and highest concentrations, glomeruli disappeared or became newly recruited. A very similar response dynamic was monitored in n-undecane, the second pheromone tested. Here the pattern was in most cases stable over at least 5 log units and only at lowest and highest concentrations glomeruli were either added or removed from the existing pattern. In contrast, for both general odors tested in most cases no pattern stability comparable to the responses to pheromones was observed. The response pattern varied between the different odor concentrations and showed a concentration invariance only over small concentration ranges. This indicates that concentration dependence of odor induced activation patterns appears to be odor (group) specific. Whether these differences are persistent in other pheromones and other general odors remains to be tested in future experiments. Response intensities, and especially response durations, were, in most cases, dependent on odor intensities. For all tested odors, response intensities were in some activation spots concentration dependent and, in others, concentration independent. Response durations were only dependent on odor intensities in nerolic acid. In the case of n-undecane, heptanal and octanol for none of the three odors significant concentration dependent signal duration dynamics could be observed.

To summarize, this study revealed odor-specific differences in concentration dependent calcium dynamics in olfactory projection neurons and thus contribute to our general understanding of odor coding. Future investigations of further odor groups, compounds and mixtures are necessary to understand the complex dynamics of the olfactory network in the ant antennal lobe. Supported by DFG, SFB 554, TP A8 and A6 and Evangelisches Studienwerk e.V. Villigst.

Odorant receptors of *Manduca sexta*

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Odorant receptors (ORs) are key elements for odor perception that belong to the family of G protein-coupled receptors. They are activated by specific odorant molecules, thereby triggering a signal transduction cascade. The odorant specificity of olfactory receptor neurons is mainly determined by the specificity of the expressed receptor protein.

In insects, there are 40 to 200 genes encoding OR proteins per species. Due to a pronounced intra- as well as inter-specific sequence diversity, OR-encoding genes are usually identified using complete or partial genomic databases. We here present two methods to identify OR-encoding genes independent of genomic sequence data. As basis for our analysis we used the tobacco hornworm *Manduca sexta*. While there is a wealth of information on the olfactory system of *M. sexta*, due to a lack of a genome project so far no *M. sexta* odorant receptors have been reported.

Using subtractive cDNA-libraries we identified sex-specifically expressed genes that encode putative ORs. The male-specifically expressed genes are expressed below long sensilla trichodea in the male antenna and belong to a comparatively well conserved group of receptors including all known lepidopteran pheromone receptors. In heterologous expression systems these receptors were shown to be specifically activated by pheromone components of the *M. sexta* female pheromone blend. By contrast, the female specific receptor responded to the green leaf odour linalool and may thus be involved in host plant detection.

In a second approach, the combination of cDNA normalization technology and second-generation sequencing led to the identification of further putative *M. sexta* OR proteins. On the basis of available morphological data we expect to have uncovered most of the *M.sexta* ORs. The established techniques are both independent of the presence of genomic sequence information and are less reliant on sequence similarity than PCR-based approaches. Both will permit the analysis of odorant receptor encoding genes and their sequence relationship in distinct, non-model species, leading to a better understanding of the olfactory system.

2-Photon functional imaging of single olfactory neurons in the <*Drosophila* brain

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Most organisms rely on their olfactory system to detect and analyze chemical cues in the environment, cues which are subsequently utilized in the context of behavior. The basic layout of the first olfactory processing centers, the olfactory bulb in vertebrates and the antennal lobe in insects, is remarkably similar. Odors are encoded by specific ensembles of activated glomeruli which constitute the structural and functional units of the bulb-lobe. However, a comparison of the transformation of odor representations between input to the antennal lobe (AL) and output to higher brain centers yields a complex and contradictory picture. The question of how odors are processed is accordingly open. A central problem regarding our present understanding of olfactory processing is the little knowledge about mechanisms that modulate the olfactory information on its way from the first olfactory neuropil, the AL, to higher brain centers. Recently, thorough investigations of projection neuron (PN) morphologies revealed stereotyped projection patterns as well as specific neurite arborizations in higher brain centers as the mushroom body calyx and the lateral horn. Nevertheless, a single neuron analysis of PN activation patterns is missing so far.

The usage of several Gal4-enhancer trap lines in *Drosophila melanogaster* combined with the FLP-out technique allows expression of the calcium-sensitive protein G-CaMP in single neurons. We used two-photon laser scanning microscopy to functional characterize odor-evoked calcium activity at the input site, the AL and the axon terminals in the lateral horn and the mushroom body of single PNs.

The antennal lobes in basal hexapods: characterizing the ancestral insect olfactory system

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Hexapods and myriapods traditionally have been united in the taxon Tracheata. However, recent molecular studies suggest a closer relationship between hexapods and crustaceans, and this new hypothesis is supported by a number of neuroanatomical characters such as structure of certain brain areas, individually identifiable neurons, and neurogenesis (review Harzsch *Species Phylogeny Evolution* 2007, 1:33-57). In the present report we analyse the architecture of the arthropod central olfactory pathway including the antennal or olfactory lobes in order to obtain more characters for phylogenetic inferences. The antennal lobes of insects and the olfactory lobes in crustaceans are the primary olfactory brain centers that receive input from the antenna. In the antennal lobe of most neopteran insects, collembolans and zygentoma spherical dense synaptic neuropils called olfactory glomeruli are present (Schachtner et al.,*Arthropod Struct Dev* 2005, 34:257-300; and Kollmann, pers. comm.;). In decapod crustaceans, the glomeruli display a column-like organization. Elongated cylindrical glomeruli were found in the myriapod *Scutigera coleoptrata* (Sombke, pers. comm.).

In this study, the antennal lobes of the adults of putatively basal insect taxa (Archaeognatha, Ephemeroptera, Odonata) were investigated in order to understand the ancestral organisation of the insect olfactory system. We used histological as well as immunohistochemical techniques including antibodies against synapsin and selected neuropeptides. Spherical glomeruli could not be detected in the antennal lobes of the studied representatives of Ephemeroptera and Odonata, whereas in *Lepismachilis y-signata* (Archaeognatha), similar to *Scutigera coleoptrata,* well defined glomeruli of an elongated cylindrical shape were found. Glomeruli seem to be a plesiomorphic character in hexapods. They might be reduced in Ephemeroptera and Odonata in connection with the optical dominance and the reduction of the antennae. To understand the ancestral shape of glomeruli and to interpret the similarities between *Scutigera coleoptrata* and *Lepismachilis y-signata,* future investigations including more apterygote hexapod species especially diplurans, myriapod and especially basal malacostracan crustacean taxa will be needed. Furthermore, the presence of neuropeptide-ir neurons or nerve fibres in the antennal lobe must be analysed in more detail to extract meaningful information for phylogenetic reconstructions.

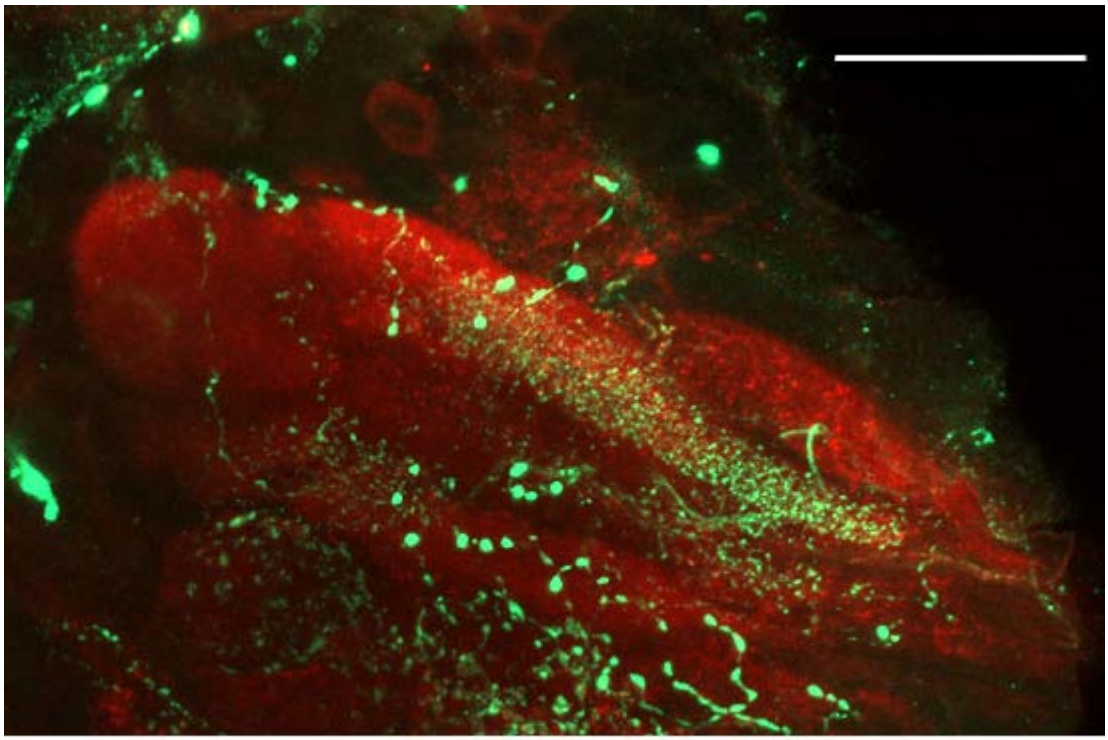

Fig.1. Frontal view of the antennal lobe in Lepismachilis y-signata.Immunolocalization of synapsin (red) and allatostatin (green) imaged by confocal microscopy. Ellipsoid cylindrical glomeruli are innervated by a meshwork of AST-A-ir fibers originating from a dorso- and a ventrolateral cell group. Scale bar 50µm.

Molecular basis of olfactory specialization in *Drosophila melanogaster* siblings

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Species host-specialization entails divergent developmental programs that allow reconciliation of unique physiological, anatomical and behavioural features with the new preferences. *Drosophila sechellia* presents several appropriate characteristics for its particular niche, *Morinda citrifolia* fruit, which is toxic and aversive to other *Drosophila* siblings (*simulans, melanogaster, yakuba, erecta*): resistance to *Morinda* toxic-compounds and a shifted odorant preference to specific fruit components.
To assess the molecular basis of olfactory-behavioural specialization we relied on quantitative differences of gene expression, assayed by micro-array studies on the transcriptome of generalists *Drosophila* siblings (*simulans* and *melanogaster*) versus specialists siblings (*sechellia* and *mauritiana*). Differentially expressed genes at olfactory organs (brain, antenna and maxillary palp) give us information towards candidate genes responsible for particular physiological and anatomical neuronal features in specialist versus generalist flies.
A comparative study of spatial and temporal expression of candidate genes is being carried out between different siblings. Eventually, we will attempt to genetically re-create *sechellia* specialist features in the *melanogaster* generalist by manipulation of the appropriate genes.

In vivo imaging of odor-evoked chloride responses in the *Drosophila* brain

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Natural odors represent an immense variety of chemosensory input to the brain and therefore demand a highly complex system allowing a proper processing to ensure reliable odor discrimination and perception. The neuronal circuits underlying these mechanisms are quite similar among species showing comparable wiring strategies within the primary centers of olfaction and higher brain areas. The gate into this system are the olfactory receptors (ORs) expressed in olfactory sensory neurons (OSNs). In *Drosophila* the OSNs are housed in different sensilla types on the third antennal segment and the maxillary palps. The olfactory information is relayed to the first olfactory neuropil, the antennal lobe, which represents a complex network of neuronal populations. Within the antennal lobe the OSNs are connected through excitatory as well as inhibitory local interneurons (LNs) to the projection neurons (PNs), which represent the output of the antennal lobe to higher processing centers. To understand the network of diversely interacting in particular inhibitory neuronal circuits in *Drosophila*, we analyzed the effect of several GABA-antagonists onto the coding of odors within the antennal lobe. Using a genetically-encoded chloride sensor we visualized inhibitory odor-evoked responses in OSNs and PNs by functional chloride imaging. We observed odor-specific chloride responses on the antenna as well as within the antennal lobes. Applying specific antagonists for $GABA_A$ and $GABA_B$ receptors, the source of odor-evoked chloride signals could be analyzed for the different neuronal processing levels within the antennal lobe. The results will help to decipher the inhibitory interactions underlying olfactory coding and processing in *Drosophila*.

First Order Blend Processing in the Moth Antennal Lobe

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Natural odors are often complex blends of several different compounds. Despite this fact, most studies concerning odor detection and coding in insects have focused on single components. The first olfactory neuropil in the insect brain, the antennal lobe (AL), modifies the final representation of odors in the assembly code carried by projection neurons to higher-order brain centers. The neural representation of odor mixtures in the AL may be simply the sum of its components or non-linear due to odor information processing. The phenomenon of a non-linear response to a mixture that is not predictable from the basis of responses to its components is called a mixture interaction. Our goal is to investigate interactions and different response characteristics in the AL in order to reveal relationships between host odor mixtures and single plant volatile components. Using a novel stimulus delivery system as well as coupled intracellular recording and calcium imaging experiments in the hawkmoth Manduca sexta, we addressed the question: what are the mechanisms of odor information processing at different levels in the antennal lobe? Preliminary results suggesting mixture interactions among host volatile odors in the general olfactory neuropil will be presented.

Mixture interactions on the antenna of *Drosophila melanogaster*

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The fruit fly *Drosophila melanogaster* has become a very important model system for research in olfactory coding. One reason is the availability of genetic and molecular tools to manipulate cell properties. Another reason is the numerical simplicity of the system. With only ~ 45 olfactory receptor types expressed in the adult animal, understanding odor response properties across all receptors appears in short reach. In fact, several studies have reported extensive odor-profile recordings, which form a basis for understanding the whole combinatorial pattern of activity that is elicited across receptor cells when an animal is exposed to an odor.

Most of what we know about single receptor cell response profiles comes from studies which used pure monomolecular odorants as stimuli. In a natural environment, however, odors generally occur as blends consisting of numerous different molecules at varying concentrations. It remains unclear how a receptor cell reacts to such mixtures. Are there situations under which the odor blend is dominated by the component with the highest concentration? What is the contribution of minor components in an odor blend?

In order to study these questions, we used a well characterized odor receptor cell type in *Drosophila melanogaster* (dOr22a), and a typical odor from the fruit fly's environment: banana. We created an artificial banana blend consisting of the 15 major banana odor components that are effective on dOr22a, and analyzed how these components contribute to the odor response in these cells individually, in binary mixtures, and in the full bouquet in which each component is contained in the natural concentration. Interestingly, this mixture does not contain any of the known best ligands for dOr22a, even though banana odor and the artificial banana blend elicit vigorous responses in dOr22a. We made use of the GAL4 expression system to selectively express the calcium sensitive dye G-CaMP under the control of the dOr22a promoter. Using Ca2+ imaging on the antenna of the flies we could then measure the activity of cells expressing dOr22a.

We found that four of the 15 odorants in the banana blend elicited a significant response when presented alone at the concentration in which they naturally appear in banana. The response strength to one of these components alone (isopentyl acetate) did not differ from the response to the mixture of all 15 odorants, whereas the other three elicited significantly lower responses. Thus, for the receptor dOr22a, the single component isopentyl acetate is equivalent to the full odor bouquet. But how do the other components contribute to the bouquet?

We therefore analyzed if any of the other three responsive odorants might interact with isopentyl acetate. We found that for two of these odorants the response to the binary mixture was below the expected response, showing that their presence in the mixture creates hypoadditive mixture interactions.

Uptake of odorant binding proteins in the olfactory epithelium

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The detection of odorants is mediated by distinct chemosensory neurons in the main olfactory epithelium (MOE) of the nose. It is supposed that the hydrophobic odorous compounds are dissolved in the nasal mucus by means of specialized globular proteins, the odorant binding proteins (OBPs) in order to reach the chemosensory cells. To assure the responsiveness to odors of each inhalation, odorant molecules have to be removed rather quickly from the immediate vicinity of the sensory neurons. Therefore, it has been hypothesized that mechanisms exist which remove OBP/odorant complexes from the ciliary environment. To scrutinize this concept, recombinant mouse OBP1a was fluorescently labelled, loaded with odorous compounds and applied to the MOE. It was found that within less than a minute, labelled OBP disappeared from the surface and appeared in the sustentacular cells, glial-like cells which form the apical border of the MOE. This uptake occurred only when the OBP was loaded with appropriate compounds. A candidate system for mediating an uptake of OBP/odorant complexes into sustentacular cells represents the scavenger receptors which are involved in internalizing lipocalin/hydrophobic ligand complexes. RT-PCR and in situ hybridisation studies revealed that only the low density lipoprotein receptor Lrp2/megalin was specifically expressed in the sustentacular cells. Immunohistochemical analyses localized megalin to the microvilli of sustentacular cells. Immunoreactivity was visible throughout the olfactory epithelium; whereas the respiratory epithelium was devoid of megalin. To analyze whether megalin is capable of internalizing OBP/odorant complexes, *in vitro* uptake studies were performed using a megalin expressing cell line. It was found that internalization of OBP/odorant complexes occurred within short period of time and internalized OBP occurred within lysosomes of the cells. Also in the *in vitro* system, uptake of OBP1a/odorant complexes only took place when OBP1a was loaded with appropriate odorous compounds. A pre-incubation of the cells with the “receptor associated protein” (RAP), an inhibitor of Megalin-function, blocked the uptake process; RAP also blocked internalization of OBPs into sustentacular cells of the olfactory epithelium. These data support the notion, that the uptake of OBP/odorant complexes into sustentacular cells mediated by megalin represents an important mechanism for a local elimination of odorants.

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Relating olfactory perception to physiology in Drosophila

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How do physiology and perception relate? Given that sensory processing typically is multi-layered and parallel, the question more specifically is *where* along these various processing streams physiological activity patterns are relevant for perception. We study olfactory physiology and perception in Drosophila. 'Perceived-distance' between odours is determined by a series of odour recognition experiments. Then, using optical imaging of genetically encoded calcium sensors, odour-induced activity patterns in first- and second-order olfactory neurons are measured to derive, for either site of measurement, 'physiological-distance' scores between odours. I will present our data concerning a possible match of physiological distance to perceived distance at second- and/ or first-order olfactory processing stages.

Candidate pheromone receptors of the two silkmoth species *Antheraea pernyi* and *Antheraea polyphemus*

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Females of the silkmoth *Antheraea pernyi* and the sibling species *Antheraea polyphemus* release a sex-pheromone blend containing the same components albeit in inversed ratios. Accordingly, in the sensilla on the antenna of the males the same types of sensory neurons have been found each tuned to one of three sex-pheromone components. Distinct pheromone components are supposed to be transferred by special pheromone binding proteins (PBPs) through the sensillum lymph towards the dendrites of the sensory neurons, where they interact with specific receptors in the membrane. Three types of PBPs have been identified in both species, however, pheromone receptors are still elusive. As a prerequisite to explore the antennal expression pattern of receptor types in the two *Antheraea* species which use the same pheromone components in an inversed ratio and to elucidate a possible interplay between PBP- and receptor types we set out to identify genes encoding putative pheromone receptors. As a first approach we have screened cDNA libraries from male antennae. Using probes according to receptor sequences of *Heliothis virescens* we have identified a cDNA from *A. pernyi* encoding a protein (AperOR-1) with moderate identity to the screening probes; screening an antennal cDNA library of *A. polyphemus* with an AperOR-1 probe led to the identification of a highly related sequence (ApolOR-1). Comparison of AperOR-1 and ApolOR-1 with other moth olfactory receptors assigned them to the relatively conserved group of candidate pheromone receptors from moths. RT-PCR experiments revealed that ApolOR-1 was predominantly expressed in male antennae. *In situ* hybridization experiments demonstrated that ApolOR-1 was expressed in cells located beneath long sensilla trichodea and that these cells were closely associated with cells expressing PBP. ApolOR-1-expressing cells did not co-express sensory neuron membrane protein 1 (ApolSNMP1) and were surrounded by cells expressing ApolSNMP2. The responsiveness of ApolOR-1-expressing T-REx293 cells to the three pheromonal compounds of *A. polyphemus* and *A. pernyi* was monitored by Ca^{2+}-imaging analyses.

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Molecular elements of pheromone reception IN MOTHs

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Many insects use multicomponent pheromone blends for mate attraction. The remarkable ability of male moths to accurately detect lowest concentrations of female-released sex-pheromones is mediated by specific sensory neurons on the antenna, which respond to distinct pheromonal compounds. This specific responsiveness implies that the sensory neurons are equipped with distinct receptors. We have identified candidate pheromone receptors of moths, which form a relatively conserved group of olfactory receptors and are expressed in sensory neurons housed in pheromone-responsive sensilla types. By immunohistochemical approaches the receptor protein could be allocated to dendritic processes of sensory neurons. Receptor expressing cells were found to be surrounded by cells expressing pheromone binding proteins (PBPs), which are supposed to mediate the transfer of hydrophobic pheromones through the aqueous sensillum lymph towards the receptors in the dendrite membrane. In functional studies using cell lines which heterologously expressed definite receptor types it was demonstrated that the candidate receptors indeed recognized pheromonal compounds. Studies employing PBPs revealed an increased sensitivity and specificity of the system, suggesting that both, a distinct PBP and receptor, contribute to the specific recognition of a pheromone component by the moths´ pheromone detection system. In addition to receptors and PBPs, a possible role of “sensory neuron membrane protein” (SNMP) in pheromone reception has been suggested. From several moth species we have identified two SNMP-subtypes, which are expressed in cells of pheromone-sensitive sensilla. Whereas SNMP-1s were expressed in only one of the generally two neurons in a single sensillum hair, SNMP-2s were expressed in cells co-expressing PBP, apparently the supporting cells. Our results indicate that SNMP-1s and SNMP-2s are differentially expressed in cells of pheromone-sensitive sensilla and suggest distinct functions for the two SNMP-subtypes in the olfactory system.

Taste signaling elements in the gastrointestinal tract

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In the gastrointestinal (GI) tract, a variety of digestive processes are continually adapted to the changing composition of ingested foods, which requires a precise chemosensory monitoring of luminal contents. Gustducin-expressing brush cells scattered throughout the GI mucosa are considered candidate sensory cells for accomplishing this task. A large cluster of gustducin-positive cells is located exactly at the boundary between the fundic and the oxyntic mucosa of the mouse stomach, at the so-called “limiting ridge”. In close association with the candidate chemosensory cluster, two populations of enteroendocrine cells were found: one population containing the satiety regulating hormone ghrelin, the other population comprising serotonin-secreting enterochromaffin cells. The particular arrangement of gustducin-expressing cells and enteroendocrine cells at the limiting ridge suggests a direct interplay between these cell types with immediate implications, not only for digestive processes in the stomach, but also for parameters controlling the satiety status.

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Odour concentration learning in Drosophila larvae

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Olfactory discriminative abilities can rely on odour identity, odour intensity or both. While coding of odour identity is often proposed to be combinatorial across range of activated olfactory receptor neurons and the ensuing activity patterns along the olfactory pathway, it seems less obvious how odorant intensity is coded. Using odour-taste appetitive associative learning in larval Drosophila, we first examine how this form of learning depends on odourant intensity. We describe the dose effect curves of learning ability across 10-fold odour dilutions (starting from undiluted odour) for four different odours namely 1-octanol (1-OCT), n-amyl acetate (AM), 3-octanol (3-OCT) and benzaldehyde (BA). Based on these results we now have embarked for intensity generalization experiments; that is, after training with an intermediate odour concentration, we test for retention with either the trained odour concentration, or with respectively higher or lower concentrations.

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Outgrowing olfactory axons contain the Reelin receptor VLDLR and navigate through the Reelin-rich cribriform mesenchyme

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During development, axons of sensory neurons in the olfactory epithelium (OE) grow out and project to the olfactory bulb (OB) in the brain. On their trajectory to the OB, the axons pass the so-called frontonasal (cribriform) mesenchyme which is located between the OE and the OB. Therefore, it has been proposed that cells in the cribriform mesenchyme provide guidance cues for axonal outgrowth although the relevant molecules are largely unknown. To identify such molecules in the cribriform mesenchyme, microarray analyses were performed focusing on extracellular matrix (ECM) proteins that are present in this tissue. Based on these approaches, the ECM protein Reelin was considered as an interesting candidate, since Reelin influences axonal projection in the brain. In the cribriform mesenchyme, Reelin was found to be expressed in a subset of cells in those embryonic stages, when the first olfactory axons navigate through this tissue. By contrast, no expression of Reelin was observed in the OE. The Reelin-positive cells are closely associated with olfactory axons and apparently lack glial and neuronal markers. In the mesenchyme underlying the OE, localization of the Reelin protein was not confined to the Reelin-expressing cells but it was also observed to be widely distributed in the ECM, most prominently in regions traversed by olfactory axons. Importantly, these axons contain the Reelin receptor VLDLR. Reelin expression was also detectable in neuronal cells of the OB which are contacted by VLDLR-positive olfactory axons. In summary, these observations suggest that a Reelin/VLDLR interaction might guide and/or promote projection of olfactory axons to the OB and contribute to the establishment of initial contacts between the incoming axons and neurons in the OB.

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Promotor-motifs governing the spatial expression pattern of olfactory receptors

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Odorant receptors (ORs) of the OR37 subfamily are only expressed in olfactory sensory neurons (OSNs) which are segregated within a small area in the center of olfactory epithelium. The encoding genes comprise highly conserved DNA motifs immediately upstream of the transcription start site which might be candidate elements for governing the spatial expression pattern. To scrutinize this hypothesis, transgenic mouse lines were generated which carry random integrated DNA constructs with the coding region of OR37C and the 5'-region including the conserved DNA motifs. In 6 out of 7 independent mouse lines, the transgene was found to be expressed in cells segregated in the characteristic clustered pattern. The number of transgene expressing OSNs varied considerably between the different lines. The transgene was expressed in a mutually exclusive manner and only one allele per neuron. The axons of transgene expressing OSNs in all mouse lines projected to the ventral domain of the olfactory bulb; those axons of OSNs located within the OR37 area generally co-converged with the axons of cells expressing the endogenous OR37C gene in the same glomerulus. Ectopically positioned transgene expressing cells formed novel glomeruli. These results demonstrate that the major features of the special OR37 topography are recapitulated by the short transgene; thus, indicating that the conserved DNA elements are indeed involved in controling the distinct expression pattern of the OR37 receptor types.

Grueneberg ganglion – a dual sensory organ?

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The detection of odors and pheromones in mammals is mediated by chemosensory neurons in nasal neuroepithelia which generally express the olfactory marker protein (OMP). Recently, we have observed that OMP is also expressed in cells of the so called Grueneberg ganglion (GG), a cluster of neurons present in the anterior region of the nose. GG neurons were found to project axons to the olfactory bulb of the brain, suggesting that the GG serves a chemosensory function. The number of GG neurons peaks in perinatal stages and is significantly reduced in adults. This observation has led to the hypothesis that the GG might be involved in mother/child interactions. To survey potential activation of GG neurons in living animals during the course of mother/child interactions, expression of the activity-dependent gene c-Fos in the GG of neonatal mouse pups was monitored in the presence and absence of the dam. It was found that GG neurons were only activated in the absence of the mother. Moreover, this GG activation was independent from olfactory cues as revealed by naris occlusion. Searching for stimuli eliciting GG activity in pups separated from the dam, cool ambient temperatures were found to induce strong responses in GG neurons whereas warmer temperatures did not. These findings suggest that the GG of neonatal mice is activated by cool ambient temperatures to which they are exposed in the absence of their dam. Moreover, coolness-induced responses were only observed in a distinct subset of GG neurons. Finally, GG responsiveness to coolness was remarkably reduced in older stages. Taken together, coolness-induced responses as well as the expression of OMP and the axonal projection to the olfactory bulb strongly suggest that the GG may act as a dual sensory organ involved in the detection of thermosensory and olfactory stimuli.

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Drosophila olfaction: What makes a good odor what makes a good blend?

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Some monomolecular odors like ethylacetate and butylbuturate have been described to attract *Drosophila melanogaster*, while others like benzaldehyde or hexanol function as repellents (Stensmyr et al. 2003). However, whether an odor is attractive or not does not only depend on its identity but also on its concentration and may additionally be affected by the stimulus duration and frequency. A new stimulus device allows us to produce stimuli that are well defined in the three parameters mentioned. The device also gives us the opportunity to mix a blend of up to eight odors. By tracking freely running flies in miniature wind tunnels we investigate how the attractiveness or repulsiveness of single odors is affected by stimulus concentration, duration, and frequency. We furthermore test whether the appeal of a blend can be predicted by the attractiveness of the blend components.

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Signaling elements in the Grueneberg ganglion

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The Grueneberg ganglion (GG) is a neuronal cell cluster in the anterior region of the nasal cavity. Based on the expression of the olfactory marker protein (OMP) and axonal projections to the olfactory bulb of the brain, it is considered as a chemosensory organ. Recently, we have observed that a subset of GG neurons responds to cool ambient temperatures, suggesting that the GG serves detection of olfactory and thermosensory stimuli. Therefore, in order to elucidate functional implications of the GG in more detail, molecular phenotyping was conducted to identify signaling proteins involved in sensory transduction cascades in the GG. Regarding olfactory signaling, it was found that a particular vomeronasal receptor type (V2r83) is expressed in the majority of GG neurons. GG neurons lacking expression of V2r83 are endowed with trace amine associated receptors (TAARs) which have been identified recently as a novel class of olfactory receptors.

Coolness-induced responses of highly specialized neuronal cells in mammals are supposed to rely on the ion channel TRPM8, whereas in thermosensory neurons of the nematode worm *Caenorhabditis elegans*, detection of environmental temperature is mainly mediated by cyclic guanosine monophosphate (cGMP) pathways, in which cGMP is generated by transmembrane guanylyl cyclases. In terms of thermosensory signaling, the GG was found to be devoid of the ion channel TRPM8 whereas it expresses the transmembrane guanylyl cyclase GC-G and the cGMP-hydrolyzing phosphodiesterase PDE2A. Interestingly, expression of these cGMP-associated signaling elements is confined to those GG neurons which respond to coolness.

In summary, these results suggest that GG neurons are endowed with signaling proteins for both olfactory and thermosensory signaling, further supporting the concept that the GG serves a dual sensory function.

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ODOR PREFERENCE AND SPECIALIZATION IN FRUIT FLIES

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Host specialization and host shifts in the *melanogaster* subgroup provide an excellent opportunity to investigate how habitat and food-choice affect olfactory function and behaviour. By studying closely related species living under different ecological conditions it is possible to understand how extreme specialization drives adaptations in the olfactory system. It is well-known that *D. melanogaster* is able to detect and discriminate between different volatile odors, but much less is known about which compounds induce biological activity or detection thresholds in the other species. In order to specify what odors are detected and preferred, we are currently screening the behavioural responses to different concentrations of several odor compounds in order to characterize olfactory preference variation in the specialists *D. sechellia* and *D. erecta*, and in the generalists *D.melanogaster*, *D. mauritania* and *D. simulans*. In these trials, flies are loaded to a T-maze setup (Tully and Quinn, 1985) and allowed to choose between one odor on one side and mineral oil on the other side (control). The odorants that are being tested are as follows: methyl hexanoate (MEX), hexanoic acid (HEX), ethyl 3-hydroxybutarate (EHB), ethyl acetate (ETA), 3-octanol (OCT) and benzaldehyde (BA). MEX and HEX are compounds present in Morinda fruit and are thereby highly attractive to *D. sechellia*, even at very high concentrations (see Figure). Additionally, these two odorants, as well as EHB, ETA and OCT, have been shown to evoke a strong response in olfactory receptor neurons in all the species that are being tested here (Stensmyr et al., 2003). Furthermore, the behavioural effect of BA, OCT and ETA is well known for *D. melanogaster*. After having characterized for each species whether a specific odor is attractive or not, and with the purpose of further exploring possible differences between the olfactory capabilities of specialist and generalist species, we will conduct positive and negative conditioning experiments to study whether the odor-driven behaviour is hard-wired or not.

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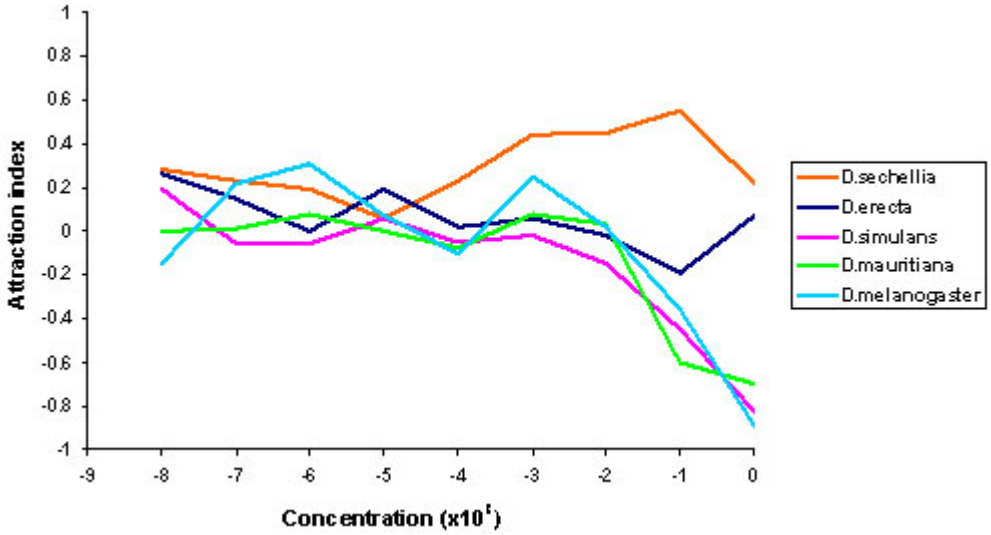
Figure text. Behavioral responses to methyl hexanoate (MEX). Female flies from five species of the *melanogaster* subgroup were tested in a T-maze set up for their behavioural response to different MEX concentrations. Attraction index (AI) was calculated as in Dekker et al., 2006. An AI of 1.0 represents full attraction, whereas an AI of -1.0 represents full avoidance.

Fig 1. Behavioral responses to methyl hexanoate (MEX). Female flies from five species of the *melanogaster* subgroup were tested in a T-maze set up for their behavioural response to different MEX concentrations. Attraction index (AI) was calculated as in Dekker et al., 2006. An AI of 1.0 represents full attraction, whereas an AI of -1.0 represents full avoidance.


Attraction index
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
-9
-8
-7
-6
-5
-4
-3
-2
-1
0
Concentration (x10^{t})
D.sechellia
D.erecta
D.simulans
D.mauritiana
D.melanogaster

Olfactory coding in moths: Evolution *versus* life history

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In insects, odour information is coded in the primary olfactory processing centre, the antennal lobe, as a pattern of activated glomeruli. Among different moth species there are similar numbers of glomeruli (around 60), and thus probably similar numbers of olfactory receptor types. We asked whether this concordance is also reflected in a similar coding pattern within the antennal lobe. Similarities or differences in these coding patterns might either reflect the phylogenetic relationship of moths or might depend on varying life histories, e.g. generalist/specialist larvae or feeding/non-feeding imagines. In order to unravel evolution and life history we measured the coding of a diagnostic set of 14 odours (aromatics, terpenes, alcohols, aldehydes, ketones) by calcium imaging. We investigated moth species with different life histories belonging to the phylogenetically most remote macrolepidopteran superfamilies Bombycoidea (*Manduca sexta*, *Acherontia atropos*, *Bombyx mori*) and Noctuoidea (*Spodoptera littoralis*, *Spodoptera exigua*). We used low odour concentrations (0.5 µg or 5 µg) to determine the position of the most sensitive glomerulus for each odour. To compare the localization of this glomerulus within and between species, the size and orientation of the antennal lobes were normalized and the pair-wise differences between the representations of each odour pair were calculated. Olfactory coding seems to be conserved for some odours (e.g. methyl salicylate and 2-nonanone) even between the two superfamilies while the representation of others (e.g. geraniol and phenyl acetaldehyde) seems to be dependent on the particular life history of the moth.

Synaptic Input and Intrinsic Membrane Properties as Potential Mechanism for Sparsening Cockroach Kenyon Cells

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The insect mushroom bodies are multimodal signal processing centres and essential for olfactory learning. Electrophysiological recordings from the mushroom bodies principle component neurons, the Kenyon cells (KCs), showed a sparse representation of olfactory signals in the mushroom bodies or rather the KCs. It has been proposed that the intrinsic and synaptic properties of the KCs circuitry combine to generate relatively brief integration windows in the KCs, thus causing them to operate as coincidence detectors. We used whole-cell patch-clamp recordings from KCs in the adult intact brain of the cockroach *Periplaneta americana* to analyse a set of voltage or Ca^{2+} dependent inward (I_{Ca}, I_{Na}) and outward currents (I_A, $I_{K(V)}$, $I_{K,ST}$, $I_{O(Ca)}$) to better understand the electrophysiological properties that mediate their special firing properties. In general the currents had properties similar to currents in other insect neurons. Certain functional parameters of $I_{Ca}$and $I_{O(Ca)}$, however, have extreme values suiting them to assist sparsening. I_{Ca} has a very low activation threshold and a very high current density compared to I_{Ca} in other insect neurons. Together these parameters make I_{Ca} suitable for boosting and sharpening the sub threshold EPSP as reported in previous studies. $I_{O(Ca)}$ also has a remarkable large current density and a high activation threshold. In combination the large I_{Ca} and $I_{O(Ca)}$ are likely to mediate the strong spike frequency adaptation during depolarising current injection and the small number of APs during olfactory stimulation.
Finally we were able to show, that constant synaptic inhibitory input hyperpolarizes the Kenyon cells. After application of the GABA blocker picrotoxin the cells depolarised significantly by 2.1 mV. Additionally the input resistance increased significantly by 1.6 GO resulting in an increased excitability.
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Expression of the adiponectin receptor 1 in the olfactory mucosa of mice

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Chemosensory information derived from the olfactory mucosa is a major factor for appreciating the palatability of food, thus influencing food intake and energy homeostasis. However, recent findings indicate that the interaction between the sense of smell and the energy balance status of the body might be mutual: the sensitivity of the olfactory system seems to be reduced if energy stores are well-stocked, whereas the sensitivity is especially high under conditions of malnourishment or hunger. These adaptive alterations in olfactory reactivity have been suggested to be mediated by energy balance hormones. One of the hormones which is, among other metabolic functions, also involved in the regulation of energy homeostasis is the adipocyte proteohormone adiponectin. Serum adiponectin concentration is regulated depending on adipose tissue mass, with a reduction of adiponectin levels seen in obesity. Thus, high levels of adiponectin are a signal for the physiologically challenging state of reduced adipose tissue and starvation, respectively, and might also influence olfactory performance. In fact, AdipoR1 is expressed in mature sensory neurons of the olfactory mucosa of mice, in a pattern reminiscent of the olfactory marker protein OMP. AdipoR1 expression levels in the olfactory mucosa were observed to gradually increase during late embryogenesis until adulthood. No local expression of adiponectin was detected in nasal tissues, indicating that serum adiponectin is the ligand for AdipoR1 in olfactory sensory neurons. Thus, AdipoR1 function in the olfactory epithelium seems to be directly linked to the nutritional status of the body, suggesting a potential modulatory role for AdipoR1 in the adjustment of the olfactory system to energy balance requirements.

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Plasticity of microcircuits in the insect nervous system

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The phenomenon of plasticity underlies many important processes in the development, maturation and maintenance of neuronal connections. Synaptic plasticity in particular must be very dynamic to accommodate behavioural adaptation. Both their dynamic structural abilities and strategic location qualify dendritic spines to play a key role in the formation and stability of synaptic contacts. Dendritic spines are rich in filamentous (f-) actin and exhibit actin-based changes in shape. Most research on spines has focused on vertebrate nervous systems. Our own recent studies have revealed that synaptic complexes labeled with f-actin probes also express developmental and age-related changes in the insect brain, but there is still little information about the subcellular and molecular processes underlying these changes in insects.

In this study we have investigated comparatively the cellular components of neuronal plasticity in two holometabolous insects, the fruit fly Drosophila melanogaster and the honeybee Apis mellifera. In both species, we focused our investigations on cellular components of synaptic complexes within two regions of the brain that are known to contain spiny neurons: the first neuropile or lamina of the visual system; and the mushroom body – a high-order sensory integration centre involved in olfactory learning and memory. Using electron microscopy and immunofluorescence labeling, we visualized dendritic spines of lamina cells and of Kenyon cells, the intrinsic neurons of the mushroom body. We are currently focusing on two major questions:

1) How age and visual experience affects the regular rows of dendritic spines of lamina cells in the visual system; and 2) whether age-dependent volume changes previously reported in flies and bees in the input region of the mushroom body, the so-called calyx, are caused by, or associated with, changes in the numerous dendritic spines of Kenyon cells. The project aims to understand structural plasticity within microcircuits in the insect brain and relate these to changes in spine density and f-actin expression. Preliminary results for Drosophila support the existence of activity-dependent morphogenesis of spines in L2 lamina cells, which are more slender. In the fly's mushroom body, synaptic complexes, called microglomeruli, in the calycal circuitry undergo age-dependent changes in number and size. These alterations involve a reduction within the first 10 days of adult life in the size of the presynaptic bouton. The degree to which plastic processes in the calyx are associated with structural modifications at the postsynaptic site, among the numerous slender spines of the Kenyon cells, is much harder to assess from confocal microscopy, and will need to be resolved at the ultra-structural level.

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Physiological and morphological features of local interneurons in the antennal lobe of *Periplaneta americana*

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Behavioral and physiological studies show that processing of odor information involves neuronal interactions among the glomeruli in the insect antennal lobe (AL). These interactions are mediated by a complex network of inhibitory and excitatory local interneurons (LNs) that regulates the tuning profile of projection neurons. In the cockroach AL, we characterized two LN types with distinctive physiological properties: 1) type I LNs that generated Na^+ driven action potentials upon odor stimulation and exhibited GABA-like immunoreactivity (GLIR) and 2) type II LNs, in which odor stimulation evoked depolarizations, but no Na^+ driven action potentials (APs). Type II LNs did not express voltage dependent transient Na^+ currents and accordingly would not trigger transmitter release by Na^+ driven APs. 90 % of type II LNs did not exhibit GLIR.

In addition to their physiological properties type I and type II LNs were clearly distinguishable by anatomical and morphological features like the location of their somata, the soma size and the branching pattern within the glomeruli. Type I LNs were characterized by multiglomerular heterogeneous innervations, whereas type II LNs showed omniglomerular innervation. So far, we were able to distinguish two subtypes of type II LNs by their glomerular arborization pattern. One subtype was characterized by a homogeneous innervation of all glomeruli. The other subtype showed a zonal innervation of the glomeruli. The physiological and morphological differences between different types of local interneurons suggest functional differences for specific types of local interneurons.

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G alpha protien subtypes in the zebrafish chemosensory systems

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Heterotrimeric G proteins are key signal transduction molecules in animal chemosensory systems that are activated by G-protein coupled receptors (GPCRs), and themselves activate downstream effectors such as adenylate cyclases and phopholipases to elicit the cellular responses to the chemosensory stimuli. In vertebrates, odorants, pheromones, and certain tastants are recognized by GPCRs on the surface of receptor cells. To date, four and two GPCR families are known for the olfactory and gustatory system, respectively: odorant receptors (ORs), trace amine associated receptors (TAARs), vomeronasal receptors type 1 (V1Rs) and type 2 (V2Rs) in olfaction, and taste receptors type 1 (T1Rs) and type 2 (T2Rs) in gustation. Each family employs a distinct G alpha protein to transduce the signals: in mammals these are Golf for ORs and TAARs, Gi2 for V1Rs, Go for V2Rs, Ggust for T1Rs and T2Rs. In contrast, teleost counterparts have not been well investigated so far. We have established the complete zebrafish G alpha protein family by data-mining in publicly available genomic databases. The number of G alpha genes in zebrafish is almost 1.5 times as high as that in mammals. We examined the expression of these genes in the olfactory and gustatory tissues in zebrafish by RT-PCR and *in situ* hybridization. We found cell type-specific expression of several G alpha genes in the olfactory epithelium, confirmed by double labeling with markers for olfactory receptor neurons. We also found G alpha genes expressed in the taste buds, which are identified by staining with PLC-beta2. We are currently analyzing the co-expression of these G alpha genes and chemosensory receptor genes. This study will serve as a basis to elucidate the molecular mechanisms regulating the olfactory and gustatory signaling in teleost species and will further the understanding of the evolution of chemical senses in animals.

Positive selection and the birth of an olfactory receptor clade in teleosts

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Trace amine-associated receptors (TAARs) in mammals recently have been shown to function as olfactory receptors. We have delineated the *taar* gene family in jawless, cartilaginous and bony fish (zero, two, and more than hundred genes, respectively). We conclude that *taar* genes are evolutionary much younger than the related OR and ORA/V1R olfactory receptor families, which are present already in lamprey, a jawless vertebrate. The two cartilaginous fish genes appear to be ancestral for two *taar* classes, each with mammalian and bony fish representatives. Zebrafish *taar* genes are largely arranged according to phylogenetic proximity in two big clusters (both syntenic to the single sarcopterygian gene cluster). *Taar* genes segregate into three classes, with class III being exclusively *teleostean*. Unexpectedly, a whole new clade, class III, of *taar* genes originated even later, within the teleost lineage of bony fishes. *Taar* genes from all three classes are expressed in subsets of zebrafish olfactory receptor neurons, supporting their function as olfactory receptors. The highly conserved TAAR1 (orthologs in shark, mammals and teleosts) is not expressed in the olfactory epithelium and may constitute the sole remnant of a primordial, non-olfactory function of this family. Class III comprises three-fourths of all teleost *taar* genes, and is characterized by the loss of the aminergic ligands motif, a stringently conserved motif in class I and II. Two independent intron gains in class III *taar* genes represent extraordinary evolutionary dynamics considering the virtual absence of intron gains during all of vertebrate evolution. Minimal global negative selection and an unparalleled degree of local positive selection are another hallmark of class III genes. The accelerated evolution of class III teleost *taar* genes conceivably might mark the birth of a new olfactory receptor gene family.

Characterization of Transient Potassium Currents in Identified Olfactory Interneurons of *Periplaneta Americana*

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The insect antennal lobe (AL), the analog of the vertebrate olfactory bulb, is the first synaptic relay that processes olfactory information. The olfactory receptor neurons, each expressing a single functional receptor gene, send their axons to the AL, where they collate by receptor type and converge into specific glomeruli. In the glomeruli they form synapses with projection neurons (PNs) and local interneurons (LNs). The PNs relay information to higher order neuropiles in the protocerebrum. The LNs mediate complex inhibitory and excitatory interactions between glomeruli to restructure the olfactory representation in the AL, which ultimately broadens the tuning profile of projection neurons. The aim of this study is to better understand the intrinsic firing properties of local interneurons and the cellular mechanisms that determine them. In *Periplaneta americana* we recently found two types of LNs with fundamentally different intrinsic firing properties (see accompanied poster), implying that these neurons serve distinct functions in the olfactory system: Type I generated Na^+ driven APs upon odor stimulation. Type II could not generate Na^+ driven APs and accordingly could not trigger synaptic release by APs. Consistent with graded transmitter release the voltage dependence for activation of I_{Ca} was shifted to more hyperpolarized membrane potentials in the type II LNs. Here we used whole-cell patch clamp recordings combined with single cell labeling, to analyse the voltage dependent transient potassium currents (I_A) of the different LN types. Consistent with the distinct electrophysiological characteristics, we revealed differences in the functional parameters of I_A between the different LN types. I_A from both cell types differed significantly in their voltage dependence for steady state activation and in their inactivation kinetics during a sustained voltage pulse.

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Verifying and searching ligands for genetically labeled glomeruli

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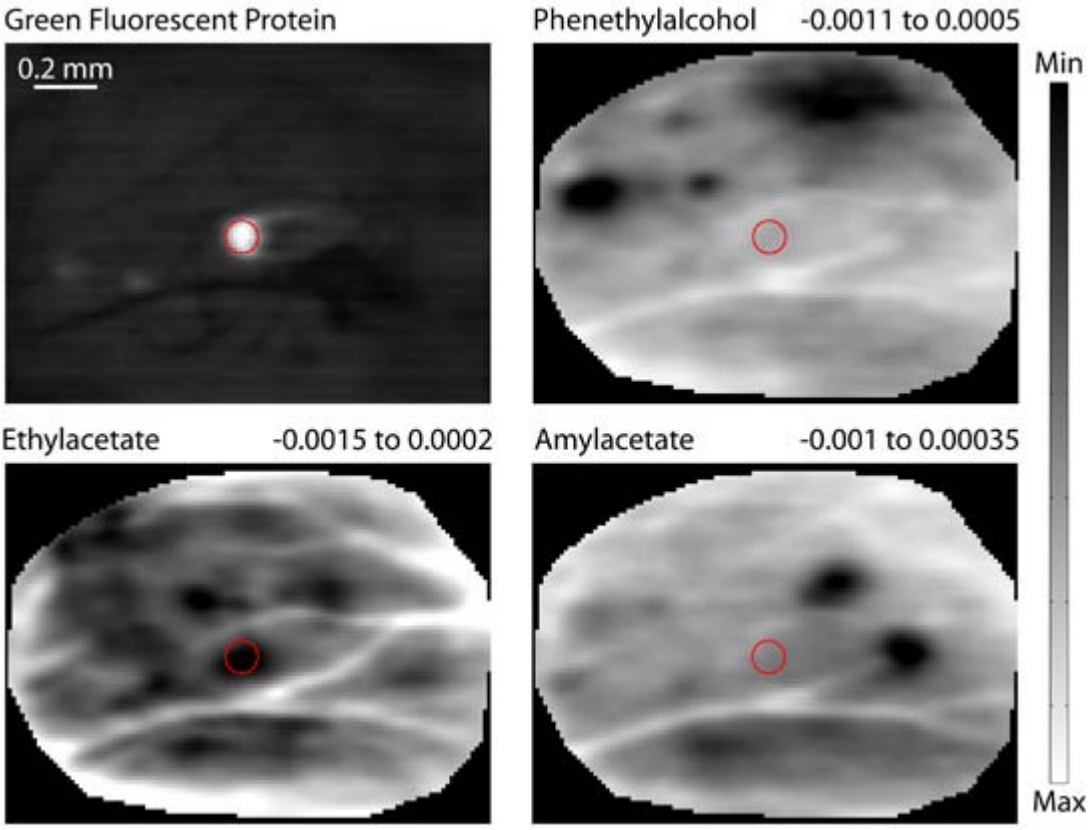
Genetically labeled glomeruli offer substantial advantages in order to study olfactory bulb function and neuronal computations involved in odor processing. Across animals the same functional module can be easily identified. This functional module is defined by the receptor type chosen by the sensory neurons providing input to a glomerulus. Each glomerulus provides sensory input to a group of about 25 projection neurons, so called mitral cells and most of these only receive input from a single glomerulus in mice. Furthermore so called periglomerular neurons can be attributed to their neighboring glomeruli. In order to study the computations in these identifiable neuronal circuits it is crucial to know high affinity ligands for the odor receptor that was used to label the respective glomerulus. This is particularly important since mismatches between *in vitro* and *in vivo* responses have been reported (Oka et al, Neuron 2006). We therefore used *in vivo* imaging of intrinsic optical signals and 2 photon imaging of the genetically encoded activity indicator synaptopHluorin to verify that GFP or RFP labeled glomeruli are indeed activated by the ligand(s) found using *in vitro* screening approaches. Using this method one olfactory receptor could be 'deorphanized'. For a different receptor with a ligand known from *in vitro* ligand screening the corresponding glomerulus responded very faintly while neighboring glomeruli responded strongly to the ligand of choice. Our results demonstrate the need and the suitability of this *in vivo* approach to complement *in vitro* ligand screening.



Background odors specifically change odor discrimination time and accuracy

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Stimulus response times provide important constraints for physiological processes and computations performed by the brain when discriminating odor pairs (Abraham et al, Neuron 2004). Here we ask if odor adaptation changes precision and speed of odor discrimination, since adaptation is a simple way of changing stimulus representations in the brain. In combination with *in vivo* imaging of odor representations with and without adaptation this will allow to test hypotheses of spatio-temporal odor coding. In a first experiment mice were trained in a go/no-go operant conditioning paradigm to discriminate pure odor pairs and 60:40 vs. 40:60 mixtures of the same odor pair.

We introduced a 50:50 mixture of the two odors as background during testing with pure odors and mixtures. In the mixture discrimination task the background decreased either the performance or increased the discrimination time. Higher background concentration led to a more pronounced change. In order to test if the background effect was odor specific we measured discrimination time and accuracy for **two** binary (60:40 vs. 40:60) odor pairs before and after introducing two different constant background stimuli. The two background stimuli were the 50:50 mixtures of the two odor pairs. The performance and discrimination times of the two odor pairs and their mixtures differed. In order to balance for this difference and in order to test all animals with both background odors we chose a cross-over paradigm. I.e. each mouse was tested using an easier and a more difficult odor pair with each of the two background stimuli. Discrimination speed or accuracy decreased significantly more when testing in presence of the background made up of the same two odors as the 60:40 40:60 stimulus pair. I.e. the effect of the background is odor specific.

Dendritic activity in layer 5 pyramidal cells in the somatosensory cortex in vitro during upstates

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Upstates are a large-scale network activity found in the cerebral cortex during sleep and anesthesia. In acute slice preparations upstate initiation is limited, while it can be boosted using a modified artificial cerebrospinal fluid with increased potassium and decreased calcium and magnesium concentrations. Initiation is further supported following a blockade of inwardly rectifying potassium channels with barium, while baclofen as a blocker of $GABA_B$ receptors, in contrast, prevents upstate generation. I am interested whether dendritic activity in pyramidal neurons may initiate, boost, or accompany upstates. These possible relationships were studied in layer 5 pyramidal cells of the somatosensory cortex of P13 mice. The high-affinity calcium-sensitive dye Oregon Green BAPTA-1 was applied intracellularly via a somatic patch pipette. Calcium signals were imaged in the dendrite (600-800 microm from the soma; sampling frequency 1 kHz). At the age investigated, pyramidal cells are not able to generate calcium action potentials in the dendrite. However, two types of dendritic calcium activity can be seen during upstates. Backpropagating action potentials evoke a fast calcium increase without obvious attenuation along the dendritic axis. When upstates do not evoke spikes, subthreshold calcium events (SCEs) can be seen. SCEs are 3-20 times smaller in comparison to calcium increases due to backpropagating spikes and their rise and decay are markedly prolonged. Local, strong activation of NMDA-type glutamate receptors triggers SCEs, while low-voltage-activated calcium channels and metabotropic glutamate receptors are not involved. Upstate activity in the *in vitro* somatosensory cortex can be promoted using changes in ionic concentrations and modulation of intrinsic conductances. When evoked in this way, upstates are accompanied by dendritic calcium transients which are not related to suprathreshold activity and which may reflect a new level of integration in the dendrite.

Infrared sensing in ants

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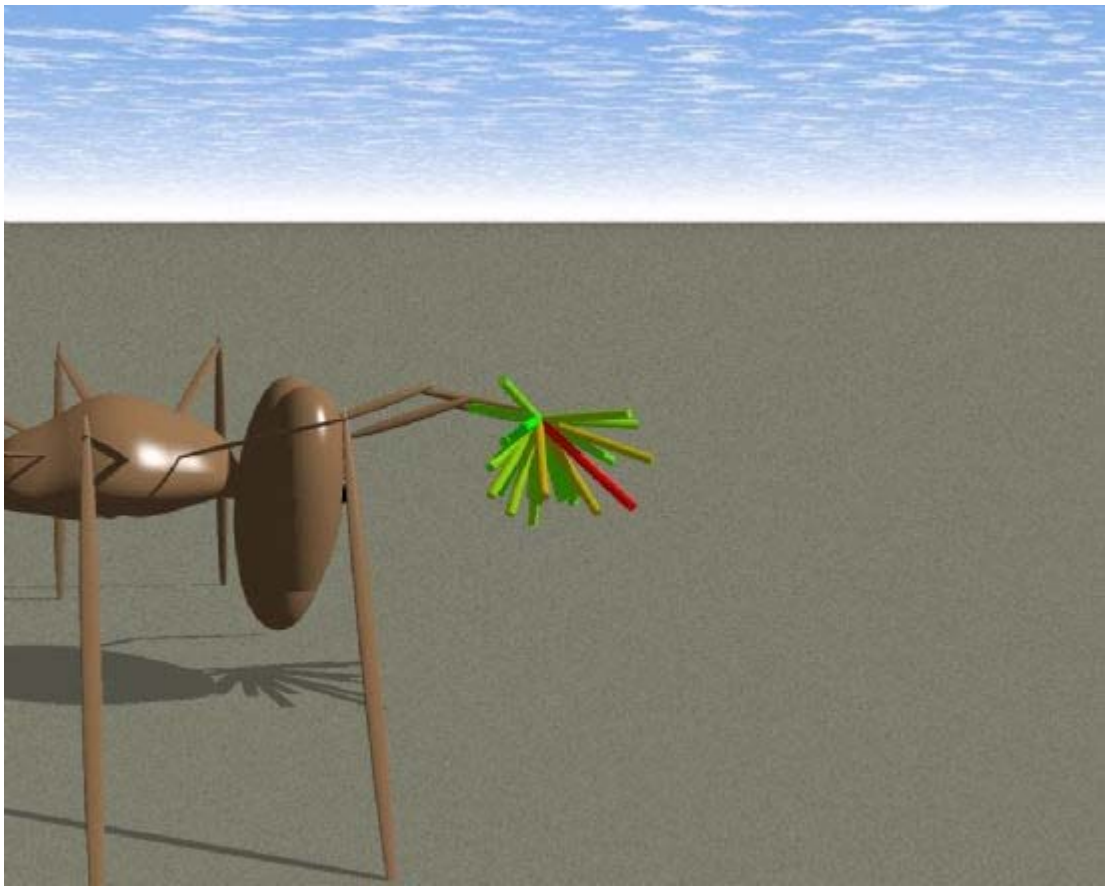
Insects like fire beetles or bugs use infrared radiation (IR) to orientate in their environment. In a recent study we could show that ants as well have the ability to receive IR and use it as orientation cue (Kleineidam 2007). In the present study we investigated the thermo-sensitive sensilla coeloconica on the antennae of the leaf-cutting ant *Atta vollenweideri* in search of morphological and physiological adaptations enabling the described IR orientation behaviour.
Extracellular recordings of the associated neurons revealed a thermo-sensitive neuron with a phasic-tonic response characteristic and a rapid adaptation to prolonged stimulation. Due to the response characteristics, we conclude that the neuron is adapted to track rapid changes in temperature. In addition, IR is a very effective stimulus because a heated peltier element (size: 1mm^2, 2°C above room temperature) positioned at a distance of 3 cm elicits strong responses of the thermo-sensitive neuron.
Besides the response characteristics, we found a morphological adaptation for IR-reception. SEM investigations of the S. coeloconica revealed a peg-in-pit morphology with a sensory peg embedded in a pit, connected to the environment only via a tiny aperture within the apical cuticle. This eye-catching morphology results in a shielding of the peg from side radiation. Subject to the position of the heated peltier the thermo-sensitive neuron responded with a varying sensitivity upon IR-stimulation (directional sensitivity). Based on our investigations, we conclude that the S. coeloconica are morphological IR-sensilla, physiologically adapted to detect rapid changes in IR. These sensilla enable the leaf-cutting ants to assess the position of IR-sources in their environment e.g. for orientation purposes.



Anatomical and neurochemical organization of the sensory system of the earthworm *Lumbricus terrestris*

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The localization and the distribution pattern of the primary sensory cells were determined in the body-wall epithelium applying a fluorescent carbocyanine dye (DiI), as a neuronal tracer. Most of the primary sensory cells were situated in the chaetae rows of the midbody segments forming large sense organs, however, several labelled cells both solitary and grouped ones were randomly located in the anterior and the posterior part of the segments. Central projections of the primary sensory cells located in chaetae rows enter the ventral nerve cord ganglia via the second segmental nerves. The first segmental nerves collected central processes of those cells found in anterior part of own segments and posterior part of the neighbouring ones while the third segmental nerves collected central processes from the posterior parts of own segments and anterior parts of neighbouring ones. These findings are the first experimental anatomical evidence of the existence of overlapping receptive fields in earthworm body wall. The applied tracing method showed the organization of both subepidermal and intramuscular plexus in the smallest details. Several longitudinal connections between segmental nerves were identified as well.

DiI labelling clearly proved that a single ventral nerve cord (VNC) ganglion receives sensory fibres not only from its own but also from the neighbouring segments of the body wall epithelium. It suggests that the central pattern generator of a VNC ganglion is mediated by sensory impulses of several segments.<

Putative transmitters (GABA and glutamate) of primary sensory cells were identified by means of whole mount immunohistochemistry. Both transmitters occurred in distinct sets of primary sensory cells of the body wall but the pathway of their central processes show characteristic differences. Glutamate immunoreactivity was found in five pairs of longitudinal sensory axon bundles of the VNC ganglia but the GABA stained processes were only concentrated in the ventromedial and ventrolateral longitudinal sensory axon bundles. We could propose from these results that the longitudinal sensory axon bundles of the earthworms are neurochemically heterogeneous structures that could transmit various sensory impulses to inter- and motoneurons of the ventral nerve cord ganglia.

Brain Centres involved in the Fruit Flies' Orientation in a Humidity Gradient

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The fly *Drosophila melanogaster* uses its hygrosensation during exploration behaviour to locate spots with comfortable humidity or to avoid dangerous humidity-ranges. The choice for certain humidity has already proven to be dependent on the current needs of the fly. The sense of moist air guiding a thirsty *Drosophila* to a resource of water and it might also be taken into account when the right place for oviposition is to be found. Conceivably, one might think that the humidity detecting system might be used also as an early warning system for unfavourable weather, which signals the fly to hide from dangerous raindrops. This humidity dependent decision-making-system for the flies moving direction requires higher integration centres of the brain and likely also spatial orientation capacities.

Sayeed and Benzer (1996) introduced a binary choice apparatus for testing *Drosophila melanogaster* in a simple humidity-choice paradigm. They described the first screening for mutants based on this behavioural test of humidity preference. For the investigation of the humidity-based decision-making system of the fly we have started a screen for mutant lines aberrant in humidity-choice behaviour. We have tested several mutant lines, which did not behave differently from wild-type strains. In contrast, some structural-brain-mutant lines have shown a significantly different humidity-choice behaviour.

These first results hint towards a role of particular neuropils like the ellipsoid-body of the central-complex and the mushroom bodies to be necessary for orientation in a humidity gradient. Recently we study the flies' actual paths in an almost linear humidity-gradient (Fig.1). The change in relative humidity necessary to provoke the negative hydrotaxis in *Drosophila melanogaster* is from 66 to 76 percent relative humidity. The flies are capable of perceiving the direction of a humidity gradient and turn before the humidity can get from bad to worse. This orientation behaviour is clearly distinct from exploration behaviour in comfortable humidity ranges and is changing with the absence or defects of the mushroom bodies. Further experiments will have to elucidate the particular function of the mushroom bodies and the ellipsoid body in humidity orientation.

The investigation of relevant centres in the fly brain for the action-oriented perception of humidity is one good example, which will be inspiring for computational models and for concepts of information processing in autonomously orienting roving robots.

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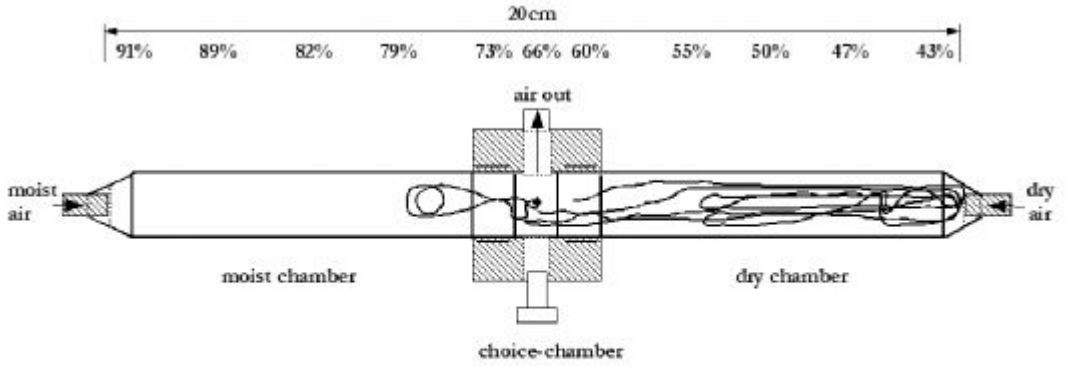
(Spatial-temporal Patterns for Action-oriented Perception in Roving Robots II): an Insect Brain Computational Model;
for more information visit: www.spark2.diees.unict.it

Reference:
O. Sayeed and S. Benzer: “Behavioral genetics of thermosensation and hygrosensation in Drosophila” *Proc. Natl. Acad. Sci. USA* (1996), 93:6079-6084



#47: WT-B 24.04.08; RT: 24°C RrH: 38,6%

Fig.1: Humidity-choice apparatus after Benzer & Sayeed, 1996 as seen from above. One track of wild-type Berlin female fly (#47) in an almost linear humidity gradient (91-43% relative humidity) starting at "♦" initially in the moist chamber.

A targeted induction of mitochondrial dysfunction in the peripheral somatosensory system

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Dorsal root ganglion (DRG) neurons are the first neural transducers of somatosensory input. A tight regulation of their function, activity and excitability is indispensable to avoid loss of sensitivity to external stimuli or to prevent aberrant sensory sensation. Recent studies suggest that mitochondria greatly participate in regulating the above mentioned parameters in DRGs. However, mitochondrial function can be severely impaired by oxidative stress. Such an impairment results in mitochondriopathies, which may induce sensory malfunctions.
Whereas these relationships are widely accepted, only few comprehensive functional studies have analyzed the impact of mitochondrial dysfunction on the physiology of the peripheral somatosensory system, i.e. the DRG neuron and the keratinocytes as interaction partners.
Aiming at a deeper understanding of the interrelationship between oxidative stress-induced mitochondrial impairment and neuronal activity, we have established an *in vitro* model using primary rat DRG neuron cultures and a photodynamic induction of reactive oxygen species.
In the present study we show that oxidative stress induction by the mitochondrial photosensitizer protoporphyrine IX leads to a profound decrease in mitochondrial enzymatic activity as well as a loss in mitochondrial membrane potential after oxidative stress induction, eventually leading to loss of viability at high doses. Moreover we could work out that keratinocytes, when exposed to ROS, release ATP, a well described algogenic messenger.
Now following are studies aiming at the dissection of inter- and intracellular pathways involved in this phenomenon.

Mechanically induced regional changes in free intracellular Ca^{2+}, and the effect of intracellular Ca^{2+} on mechanotransduction in spider sensory neurons

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The compound slit sense organ VS-3 is a cuticular mechanoreceptor in the patella of the spider, *Cupiennius salei*. The organ consists of 7-8 cuticular slits, each innervated by a pair of bipolar mechanosensory neurons. In an isolated, but otherwise intact, preparation the neurons are accessible for intracellular recording, mechanical stimulation and calcium imaging. Previously, we showed that continuous action potential firing leads to a significant increase in free intracellular calcium, $[Ca^{2+}]_i$,in VS-3 neurons. We also found that Ca^{2+} enters the neurons via low voltage activated (LVA) Ca^{2+} channels but not via the mechanically activated transduction channels.

To better understand the roles of calcium increase in mechanotransduction and action potential encoding we measured the changes in time course and amplitude of $[Ca^{2+}]_i$ produced by single, mechanically elicited action potentials in three peripheral regions of VS-3 neurons (soma, dendrite, and axon), and investigated the regional distribution of LVA Ca^{2+} channels in the same parts of these neurons. Modulation of receptor current and receptor potential by $[Ca^{2+}]_i$ were investigated using caged Ca^{2+} release to increase $[Ca^{2+}]_i$while action potential firing was prevented by TTX.

Immunocytochemistry with a specific antibody against the $Ca_V3.1$ isotype of LVA Ca^{2+} channels revealed an even distribution of these channels in all three peripheral regions of VS-3 neurons. The similar time course and amplitude of $[Ca^{2+}]_i$ elevation in all three neuronal regions indicated that mechanically induced action potentials propagate rapidly from the sensory dendrite through the neuron, opening Ca^{2+} channels in all regions.

Release of caged Ca^{2+} in TTX-treated neurons was used to increase $[Ca^{2+}]_i$ during mechanical stimulation. $[Ca^{2+}]_i$ elevation reduced both mechanically induced receptor current and receptor potential, suggesting that Ca^{2+} participates in a negative feedback mechanism that may modulate VS-3 neurons' sensitivity during action potential firing.

Modulatory actions of $[Ca^{2+}]_i$ on the dynamic sensitivity of mechanosensory neurons have been previously described in auditory and vestibular hair cells, where in addition to voltage activated Ca^{2+} channels, Ca^{2+} enters those cells via the receptor channels themselves. There is also evidence that the sensitivity of vertebrate cutaneous mechanoreceptors can be modulated by Ca^{2+} dependent mechanisms.

VS-3 neurons receive extensive peripheral efferent input and receptors for several neurotransmitters are differentially distributed over the neurons. Inhibitory glutamate receptors are present in all regions, while octopamine and $GABA_A$ receptors are primarily located on the somata and axons, and $GABA_B$ receptors are concentrated on the dendrites. $GABA_A$ receptor activation increased $[Ca^{2+}]_i$ and it is possible that other synaptic mechanisms have similar, or opposite, effects, providing additional mechanisms for adjusting the sensitivity of these neurons.

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Antinociceptive effects of the selective COX-2-inhibitors celecoxib and lumiracoxib assessed by functional MRI (BOLD) in rats

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Nowadays different forms of cyclooxygenase-2 (COX-2) selective non-steroidal anti-inflammatory drugs (NSAIDs) are available. Celecoxib is a highly lipophilic, non-acidic compound with a sulphonamide structure and is distributed almost equally throughout the body. Lumiracoxib is a lipophylic phenylacetic acid derivative with a carboxylic group and is weakly acidic which leads to higher accumulation in inflamed tissues.
In order to investigate the analgesic and antihyperalgesic effects of the two COX-2-inhibitors with their different biodistribution on the central pain processing we established a hyperalgesic inflammation rat pain model with repetitive heat stimuli.To directly assess their modulatory effects on cerebral pain processing we have performed functional magnetic resonance imaging (fMRI) experiments in anesthetized rats. Hyperalgesia was induced in the left hindpaw of rats by means of subcutaneous injection of zymosan A. The COX-2-inhibitors in different concentrations (0,5 mg/kg, 1 mg/kg and 5 mg/kg) or the vehicle were applied intravenously during the fMRI measurement. The inflamed left and the non-inflamed right hindpaw were stimulated alternately with four different heat stimuli (46-63 ºC) via peltier elements placed on the dorsal surface of the paws. This noxious stimulation evoked robust changes in the blood oxygenation level dependent (BOLD) effect in several areas of the pain matrix. For the further analysis of the data we focused on the areas which were most consistently activated (53 structures at a 70% incidence threshold throughout thalamus, somatosensory cortex, cingulate cortex, insula, hypothalamus). Heating the hindpaws lead to significant increases in BOLD signal amplitudes and activated volumes in these brain areas. Stimulation of the inflamed paw led to overall higher BOLD signals. With respect to the activation strength celecoxib and lumiracoxib both showed dose dependent analgesic effects - stronger for lumiracoxib. Lumiracoxib produced a stronger BOLD-signal reduction during stimulation of the inflamed paw than celecoxib (mostly pronounced in the limbic system and motoric output regions). Moreover, the lowest dosis of lumiracoxib (0,5 mg/kg) had the strongest analgesic effect. Regarding the size of activated brain structures, only lumiracoxib led to a reduction of the cluster size (especially in limbic structures). Celecoxib led to an increase which was smaller for the non-inflamed, but even larger for the inflamed paw compared to the control. In summary, our study demonstrates that the acidic compound lumiracoxib provides better analgesic and especially antihyperalgesic properties than the other COX-2-selective compound celecoxib.

Nowhere to Go? Fruit Flies in a Bilaterally Increasing Temperature Gradient

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A *Drosophila* brain consists of only approximately 100.000 neurons, but still enough to provide a great richness and flexibility in behavior. For a fly, orientation is essential to find favourable places as well as to avoid dangerous sites. The fruit fly is a well-established model for orientation studies in different environments concerning different senses. Temperature sensing and orientation in a temperature gradient is very important for such tiny fruit flies because they heat up or cool down very fast. Two main issues in this study are the quest for the underlying orientation strategy and the relevant brain centres which play a role in temperature orientation or orientation in general. Obvious candidates are higher brain centres such as the central complex and the mushroom bodies.

The study is based on a novel setup for *Drosophila melanogaster*. A brass catwalk of 14cm length offers a comfortable temperature in the middle and bilaterally increasing temperature gradients towards both ends. Fruit flies are introduced to the catwalk at certain temperature points and the behaviour is described for 10 minutes. In order to analyze the strategy of orientation used by the fly, their trajectories and the sojourn times in different segments are being measured. To this end the catwalk has been subdivided into 14 segments of 1cm length.

Flies are in a conflict between exploration behaviour and the acceptance of hopelessness. The fly has to choose between staying in a place with comfortable temperature or to explore the environment in order to find a potentially more promising place. When the temperature gets too high, the fly has to take the decision to turn around. Furthermore, the chronology of other types of behaviours (attempts to escape, grooming etc.) is being recorded, to fully account for the performance in this setup.

First results obtained for wild-type Berlin flies show high sojourn times in the inner two (24°C) of 14 segments with the most favourable temperature. Sojourn times are decreasing strongly with increasing temperatures (up to 40°C in the outermost segments). On average, a wild-type fly is changing segments 77 times within the 10-minute observation periods and takes 4.5 excursions beyond 2cm path length away from the midline.

Once the wild-type behaviour in this paradigm is fully described, mutant lines with a lack of orientation, with sensing defects, with known defects in decision making, or with structural brain defects will be tested. The comparative approach should allow us to identify brain regions and their network interactions relevant when it comes to navigating in a complex temperature gradient.

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Superior sensory, motor and cognitive performance in elderly subjects with long-year dancing activities

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Our results demonstrate that a long-year schedule of regular dancing activities has a beneficial impact on the overall physical and mental fitness of elderly individuals. From an intervention point of view, dancing appears an ideal therapeutic tool to counteract age-related alterations in elderly subjects.

Housing aged rats under enriched environmental conditions delayed and ameliorated age-related deterioration of somatosensory cortex and sensorimotor behavior. Enrichment is believed to present animals with increased sensory, motor, and cognitive demands and to reinforce a variety of behavior including learning, social interactions, physical activity and exploration. By that a wide range of morphological, molecular and physiologic features of the brain is affected.

In search for equivalent "enriched environments" for elderly humans, we studied the impact of long-year regular dancing in a group of neurologically healthy elderly subjects (aged 65 to 85 years, n=24) and compared the outcome to two control groups of age- and education-matched subjects that had no record of regular dancing, exercising or work out activities. The first control group consisted of subjects with an active and mobile lifestyle (n=12), whereas the second control group consisted of subjects with a passive and sedentary lifestyle (n=12).
Dancing was selected because it comprises beyond simple physical exercise emotional, social, acoustic, musical and affective features. In order to obtain information about a broad spectrum of abilities and levels of performance in different areas and categories, we conducted a study consisting of neuropsychological assessments, psychophysical and sensorimotor tests and recordings of SEPs (somatosensory evoked potentials). In details, we studied touch threshold, tactile 2-point discrimination, haptic object recognition, multiple-choice reaction times, fine-motor and tapping performance, grip strength, posture, balance, attention, life contentment and nonverbal intelligence. The idea was to cover very basic and simple measures of performance as well as rather complex tasks with high cognitive demands.

Surprisingly, in all of the different tests investigated the group of dancers showed a superior performance as compared to the non-dancer control groups. The data indicate that elderly subjects with a long-year regular schedule of dancing show superior performance across a variety of simple to difficult tasks including sensory, motor and cognitive performance. It remains to be clarified whether the superior performance in elderly dancers is due to the dancing activity itself, or whether a specific subpopulation characterized by unusual fitness chooses an active lifestyle including dancing activities, thereby outperforming non-dancers.

Dependency of the negative BOLD signal on stimulus intensity in the human somatosensory cortex

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Introduction:
Stimulation of the somatosensory system elicits activation of neurons in corresponding areas of the sensory cortex. Ascending sensory fibers cross the midline at the level of the spinal cord, thus stimulation of ipsilateral extremities activates neurons in the contralateral hemisphere of the brain. Neuronal activity initiates hemodynamic changes in the brain, which are reflected by the blood-oxygen-level-dependent (BOLD) signal and can be measured with Functional Magnetic Resonance Imaging (fMRI). While a local increase in the MR signal (positive BOLD signal) represents excitatory postsynaptic activation [1, 2], a decreased MRI signal (negative BOLD signal) could be correlated to inhibition [3]. We established a method to induce a negative BOLD signal in the ipsilateral primary somatosensory cortex (SI) during electrical stimulation of a single finger and we show here the dependency of its occurrence on stimulus intensity.

Methods:
Electrical stimulation of a single finger in different frequencies and intensities was performed on 8 healthy subjects. MRI imaging was executed on a 3 Tesla scanner, Data were analysed with BrainVoyager QX 1.9.

Results:
Occurrence of the negative BOLD signal in the ipsilateral SI is dependent on the individual perception of the volunteers. If the stimulation is perceived as being uncomfortable, a negative BOLD signal can be seen in the ipsilateral SI. Stimulation that is experienced as non-uncomfortable does not induce such a negative BOLD signal. Both stimulation conditions elicit positive BOLD signals in the contralateral SI.

Conclusion:
We therefore conclude that the negative BOLD signal in the ipsilateral SI is a correlate of local neuronal inhibition that is linked to an attention shift towards the stimulated region of the ipsilateral hand. Uncomfortable stimulation might focus attention to the stimulated body region and hence cause an inhibition of the cortical areas representing the opposed extremity.

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Age related alterations of response properties of cortical somatosensory neurons after presentation of train stimuli – influence of age on temporal processing.

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We have recently reported that paired pulse suppression is reduced in somatosensory cortex of aged rats (David-Jurgens M. & Dinse, H. R., SfN Meeting 2007). Here we extended this study by investigating neural response behaviour (multi-unit recordings in the hindpaw representation) to trains of 10 successively applied tactile stimuli, whose ISI was varied between 35 and 600 ms.
In contrast to the paired pulse behaviour, which was quantified by the ratios between second to first responses (a2/a1), the ratios between the 10^{th} relative to the first response peak (a10/a1) was smaller in aged animals than in young rats indicative for increased suppression at the end of the train. Closer inspection of the data revealed, however, that in contrast to the young animals, the response behaviour to train stimuli observed in the aged animals showed much greater variability, which allowed to identify two separate groups of aged animals: one group was characterized by only minor age-related changes (unimpaired animals), while the other group showed severe alterations (impaired animals). In case of the impaired old animals we found reduced responses to mid-train stimuli as well as for the last stimulus in the train, although paired pulse behaviour (ratio of second to first response) was comparable to the group of unimpaired aged rats. In respect to the last response in the train, contrary to the impaired old rats, in the unimpaired old animals we found response ratios comparable to the young rats.
Combined, our data demonstrate that age-related changes in response to train stimuli can not be predicted from alterations in paired pulse behaviour. During trains, we found rather complex changes including alterations of response amplitude and response fluctuation. The observation that even animals of high age can show rather normal train responsiveness suggests that aging has no uniform effect on temporal processing of sequences of tactile stimuli.

Differential effects of nitric oxide on the responsiveness of tactile hairs

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Tactile hairs on the legs of locusts of are of two different physiological types depending on their threshold responses to mechanical stimulation, with each type occupying characteristic locations on the leg. Low-threshold hairs respond to deflection in a phaso-tonic way and have low velocity thresholds of less then 3°/s, whereas high-threshold hairs respond phasically and display velocity-thresholds that are about one order of magnitude higher, on average (1). Typically, supra-threshold mechanical stimuli of a given magnitude elicit responses with a high initial spike rate in low-threshold hairs and responses of low spike rate in high-threshold hairs. There is, however, some variation in responsiveness for each type of hair. Here we demonstrate that the neuromodulator nitric oxide (NO) can modify tactile responses in a specific way, depending on the physiological type of response.

In our experiments we perfused the hind legs of locusts with saline to which we added the NO donor 3-(2-hydroxy-2-nitroso-1-propylhydrazino)-1-propanamine (PAPA NONOate). Responses of tactile hairs located on the ventral side of the tibia (low-threshold hairs) as well as on the tibia's dorsal side (high-threshold hairs) were monitored using the 'tip-recording technique', while constant-amplitude ramp stimuli were applied that were well above threshold for both types of hair and had a velocity of deflection of approximately 800°/s. The number of spikes generated by the mechanosensory neuron within each hair was determined over a 50 ms time period starting from the onset of the stimulus.

Application of 0.5 mM PAPA NONOate resulted in a highly significant decrease in the initially high frequency responses of low-threshold hairs, whereas high-threshold hairs showed an increase in their initially low responses. Thus NO has a crucial role in modulating tactile inputs in the periphery and can act specifically and differentially on different classes of exteroceptive neuron.

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ENCODING OF HIGH-FREQUENCY WHISKER VIBRATIONS IN THE RAT'S BARREL CORTEX: AWAKE VERSUS ANESTHETIZED PREPARATION.

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The whisker-to-barrel system of rats and mice is widely used to study coding of surface textures by the somatosensory system during tactile perception and discrimination. Precise control of stimulus presentation over long periods, as required for detailed analysis of relevant stimulus parameters, is only achieved when the animal is not moving, i.e. is anesthetized. In this condition, however, encoding properties of cortical neurons may be altered in an unknown manner. To elucidate these anesthetic effects, we compared recordings obtained under isoflurane anesthesia with those from awake head-fixed rats.

Extracellular multi-unit activities and local field potentials (LFPs) were recorded with up to 9 electrodes placed in the barrel cortex in mechanically ventilated isoflurane-anesthetized rats and in awake head-fixed rats (Stüttgen & Schwarz, 2008). These trained rats tolerated recordings under head fixation for up to 30 min. A feedback-controlled electromechanical stimulator was used for vibration of a single whisker at frequencies of 10-700 Hz for 1 s. The phase-locking to the cycles of the sinusoidal whisker movements was analyzed in phase histograms generated from time stamps of LFPs and spikes. The frequency components of the response activities were analyzed from time-frequency plots obtained by applying FFT to the average of 50 consecutive responses, resulting in a measure of the stimulus-locked (evoked) activity. Non-locked (induced) oscillatory activity was revealed by applying FFT analysis to each single response before averaging.

Whisker vibration elicited tightly correlated LFP and spike activities in the awake barrel cortex and sequences of 1:1 stimulus locking were present throughout the 1 s stimulus epoch for the entire range of frequencies tested (up to 700 Hz). The same vibratory frequencies also elicited responses under isoflurane anesthesia. However, series of 1:1 locked responses were present only up to 320 Hz. Furthermore, the responses to high-frequency stimuli were dominated under anesthesia by prominent On- and Off-activities, and stimulus entrainment began only 50-100 ms after response onset. The increase of stimulus entrainment was associated with the emergence of induced oscillations in the gamma band range (30-80 Hz). In the awake cortex, gamma band activity occurred throughout the stimulus epoch with dispersed foci and much wider spread over neighboring electrodes.

The results show that high-frequency components of whisker movements are encoded in a temporally precise manner in the awake barrel cortex immediately after stimulus onset for up to a second, and thus, are features available for texture discrimination. Under isoflurane, apart from stimulus onset, which is restrained by anesthetic-enhanced GABAergic feedback inhibition at thalamic (Vahle-Hinz et al., 2007) and/or cortical sites, sustained responses during the later stimulus epoch reflect the precision of coding in the awake state. Changes of oscillatory coherence in the gamma band may underlie transmission and spread of sensory signals in the cortical network.

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Subacute exposure of rats to cadmium oxide nanoparticles: electrophysiological and general toxicological effects

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Cadmium is one of the heavy metals with substantial general toxicity, including damage to the liver, kidneys and bones, and inhibition of sulfhydryl enzymes. Its nervous system effects comprise Ca channel blocking, alteration in ion pumps, decreased cholinesterase activity, decreased glutamate-neutralizing capacity of astrocytes etc. In humans, correlation between Cd burden and visual and auditory evoked potentials was described. Cd exposure is mostly occupational, but can also be caused by tobacco smoke and by contaminated food and drinking water. Smoke and metal fumes contain Cd in form of microscopic or submicroscopic solid particles.

In this study, rats were experimentally treated by intratracheal instillation of a suspension of nano-sized CdO_2 (ca. 20 nm diameter) in distilled water into the rats' trachea 5 days a week for 6 weeks. The daily doses were 0.04 (low dose) or 0.4 mg (high dose) Cd/kg b.w. There was an untreated control group and another one instilled with pure distilled water. During the treatment the animals' body weight gain was monitored. At the end, the rats were anesthetized by ip. urethane. After acute surgical preparation, spontaneous and stimulus-evoked activity was recorded from primary sensory cortical areas, and compound action potential from the rat's tail. Finally, the rats were dissected and organ weights were measured.

Instillation itself had an effect on the rats' body weight but the weight gain in the treated groups was significantly reduced vs. both untreated and vehicle-treated rats. The relative weight (on the basis of body weight) of the lungs and thymus was significantly higher in the high dose group. Notably, the relative weight of the brain was not affected.

The most clear-cut alterations of cortical electrical activity were seen in the primary somatosensory area. The spectrum of the spontaneous activity was shifted to higher frequencies: delta activity decreased while beta activity increased significantly. The evoked potentials obtained by contralateral whisker stimulation had increased latency and duration, and impaired frequency following ability, in the high dose group vs. control. Latency and duration of the visual and auditory evoked potential was lengthened also. These functional changes were paralleled by altered body weight, but no altered brain weight.

The above results indicate that the nervous system effects of inhaled submicroscopic Cd particles can be investigated in animal experiments, and that electrophysiological techniques may be suitable also for use on exposed humans.

Direct activation of transient receptor potential V1 by nickel ions

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TRPV1 is a member of the transient receptor potential (TRP) family of cation channels. It is expressed in sensory neurons of the trigeminal and dorsal root ganglions as well as in a wide range of non neuronal tissues and cells of the immune system. The channel proteins serve as polymodal receptors, which can be activated or sensitized by various physical and chemical stimuli, including heat, low pH, touch, and endogenous (e.g. the endocannabinoid anandamide) or exogenous (capsaicin, resiniferatoxin) vanilloids, as well as by interaction with diacylglycerol (DAG) or direct phosphorylation via protein kinase C (PKC). Moreover, it has been shown that divalent cations in concentrations >10 mM are able to directly gate TRPV1, thereby contributing to the nociceptive response of sensory fibers to elevated ionic strength. In general, TRPV1 detects noxious stimuli and prevents tissue damage by inducing painful sensations leading to pain related behaviour. Searching for further activators and modulators of TRPV1, we were interested in the effect of Ni^{2+} ions ($NiSO_4$), known to induce allergic contact dermatitis.

Using Ca-imaging and whole-cell voltage-clamp recording, we observed that micromolar doses of $NiSO_4$ are able to induce Ca^{2+} transients in a capsaicin-sensitive population of cultured mouse trigeminal ganglion neurons. Moreover, we could show that $NiSO_4$ activated currents in recombinant rat and human TRPV1, heterologously expressed in CHO (*Chinese hamster ovary*) cells. Using voltage ramps from -80 mV to +80 mV, we found that the Ni^{2+}-induced currents show a significant outward-rectification. Application of $NiSO_4$ to the intracellular side of the membrane in inside-out recordings did not induce any significant currents. Outside-out recordings revealed a significant increase in open probability paralleled by a decrease in single-channel conductance, resulting in an increased net activity of TRPV1. This increased activity mostly becomes manifest in macroscopic currents in excised outside-out patches. The effect of Ni^{2+} on capsaicin-induced currents is dependent on the capsaicin concentration. Outward currents induced by low doses of capsaicin are sensitized by low concentrations of $NiSO_4$, whereas currents induced by higher capsaicin concentrations were inhibited by Ni^{2+}.

In order to investigate the interaction site of Ni^{2+} and the channel protein, we used TRPV1 mutants with specific point mutations in the extracellular region of the channels pore. Preliminary results indicate that Ni^{2+} ions interact with charged amino acid residues, which are known to be involved in the interaction of TRPV1 and protons as well as polyamine cations. Future experiments will focus on the detailed molecular mechanisms of TRPV1 activation and modulation by Ni^{2+} ions and the impact of TRPV1 in the development of pathophysiological changes in neuronal and non-neuronal tissues.

Neurophysiology of tactile shape recognition in the somatosensory cortex of the Etruscan shrew

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The Etruscan shrew *Suncus etruscus* is one of the smallest mammals. Shrews are excellent hunters that recognise the shape of their prey with amazing speed and accuracy. Behavioural experiments indicate that they have a Gestalt-like representation of objects based on whisker mediated tactile cues (Anjum et al., 2006).

We now seek to characterize the neuronal activity in shrew somatosensory cortex during tactile shape recognition. To this end, we perform extracellular recordings in anesthetized shrews while presenting different objects to the whiskers. Initial experiments suggest that there may be shape-specific responses of neurons in higher order areas of the somatosensory cortex. However, further investigations are necessary to corroborate this preliminary observation.

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Modulation of corticomuscular synchronization during isometric compensation of dynamic forces with different levels of predictability

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The capacity of detecting predictable dynamic patterns in the environment is a critical human ability for survival and well-being at different levels from the biological to the psychological and social one. These abilities are based on implicit learning of predictable traces and the subsequent construction of internal models underlying anticipation behavior and future prediction. In the sensorimotor system, this ability allows human to tune their activities to the environment by coding the 'tempos' and tuning themselves to those external or internal stimulus such as drumming and dancing. However, these predictive abilities are confined to the presence of predictable features, because a truly unpredictable sequence of events prevents us from any construction of internal models of the environment. Nevertheless, recent research has shown that even in such erratic situations the human brain has computational routines to deal with sensorimotor unpredictability. We wanted to add new data to this field of research by studying how the motor cortex controls the muscle. For this purpose, we investigated the CMC, the cortical spectral power as well as the motor performance while subjects compensated for a dynamic force with 3 different predictability levels in its temporal structure, while force amplitude and offset remained the same across conditions. Seven healthy right-handed female subjects were instructed to compensate dynamic forces with predictable (frequency 1 Hz) and with 2 unpredictable frequency patterns (i.e. random sequence of cycles with frequencies 0.6, 1 and 1.6 Hz). EEG was recorded from 52 scalp positions and EMG from the first dorsal interosseus muscle (FDI). A custom-made manipulandum produced the variable force on a ring. The subject had to compensate the force generated by the manipulandum isometrically to maintain the ring in its initial position. Visual feedback about the position of the ring was provided to the subject through a small white circle (representing the ring) that had to be maintained inside a green circle at any time, so that when a given force was applied to the ring the subject had to apply the same force in the opposite direction to keep the ring in its initial position. Preliminary results regarding the subject's performance and the modulation of the CMC by different levels of predictability will be presented.

Functional grouping of descending interneurons that mediate antennal mechanosensory information to motor networks

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Tactile sensors such as vertebrate whiskers or arthropod antennae are important for near-range orientation. The stick insect *Carausius morosus* continuously moves its antennae during locomotion and adapts the antennal searching pattern to the direction of heading (*Staudacher et al. 2005, Adv. Insect. Physiol. 32*). Contact with an obstacle often causes strong passive bending of the antenna, and release of contact results in fast, over-damped return to resting posture (with cut-off frequency near 100 Hz). Contact events can trigger aimed reaching movements of a front leg, and even retargeting of ongoing swing movements within 40 ms.

Here we identify properties of antennal tactile information input to the prothoracic ganglion which is the site of motor networks that control front leg movement. To test for stimuli that are typical for obstacle contacts, we used touch stimuli to three sites along the flagellum, ramp-and-hold deflections of the basal flagellum, and small-range vibration stimuli. The latter two are well suited to excite campaniform sensilla (bending sensors) and Johnston's organ (vibration sensor) in the pedicel. Both antennal joints were fixed. Three kinds of recordings were made: Antennal nerve activity, measured with a fine wire in the pedicel, revealed gross tuning characteristics of afferent input. Neural activity in both neck connectives revealed gross differences to afferent input and spike delayed descending interneurons (DINs). DINs were recorded intracellularly in the prothoracic ganglion.

Activity of antennal nerve and neck connectives increased with the logarithm of ramp velocity. Vibration stimuli caused modulated activity at low frequencies. Modulation frequency was double the stimulus frequency in the antennal nerve, but not in the connectives.

Intracellularly, we characterised 44 DINs that responded to at least one of the three stimulus types. 23 DINs were spontaneously active (spike rate: 0.2-20.5 Hz). Response characteristics of DINs revealed distinct groups: the majority (N=17, group 1) coded for ramp velocity as well as vibration frequency. In 14 of these, increasing stimulus velocity/frequency induced an increased spike frequency, only one showed a bell-shaped response characteristic to vibration stimuli (most sensitive to frequency range 10-20 Hz, with phase-locking to stimulus frequency up to 30 Hz). Finally, 2 DINs of group 1 responded to increasing stimulus velocity/frequency with a decrease in spike frequency. Touch responses of group 1 were often strongest at proximal stimulus sites (N=13), never at distal sites. Velocity threshold revealed two subgroups: 9 DINs responded to antennal deflections at velocities of =0.5 mm/s, while 6 DINs required stimulus velocities of =3.9 mm/s. Thresholds for vibration stimuli varied strongly between 0.5-200 Hz, the majority had a threshold of 5-10 Hz (N=7).

Group 2 DINs showed velocity- but not frequency dependence (N=12). Group 3 DINs showed neither velocity- nor frequency dependence (N=7), however touch responses were significant in 5 of them.

Coding properties of DINs involved a wide range of delays, but spike timing variability was often very low (<1 ms in 26 DINs). The shortest response latency observed was 5.1±0.1 ms.

DINs had their soma either in the brain or subesophageal ganglion.

The response characteristics of some of the described DINs make them likely candidates for the neural substrate mediating tactually elicited reaching behavior.

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Martinotti interneurons control dendritic encoding of tactile stimuli

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The apical dendrites of layer 5 neocortical pyramidal neurons can initiate local spikes which clamp the dendrite to depolarized potentials and cause burst firing at the cell body. While this phenomenon has been well studied in vitro, it is not clear under what circumstances this cellular spiking feature is activated in vivo. Furthermore, in vitro experiments also show that inhibition dampens dendritic calcium activity disproportionately using diverse and specific signaling mechanisms implying that the control of dendritic calcium spikes is fundamental to this form of activity. Till now, their inaccessibility has prevented studies into how dendritic activity relates to spike output in behaving animals.

In this study, we investigated the relationship between dendritic activity and sensory input. To investigate dendritic activity in L5 pyramidal dendrites of the somatosensory cortex we developed a new fiberoptic method for recording dendritic calcium changes. We combined 2 relatively easy and reliable approaches. Firstly, we took advantage of the fact that L5 neurons project dendrites over long distances (> 1 mm) from layer 5 to the pia. This meant that after loadeding the cell bodies with the membrane permeant calcium indicator OGB-1 AM by injecting a bolus of the dye to L5 (~1200 µm from the cortical surface), the only fluorescence in the upper layers came from filled dendrites. Secondly, we developed a fiberscope with a prism that directed and collected light in the horizontal plane of the upper ~700 µm of rat cortex ensuring that all changes in fluorescence recorded represented calcium entry into the apical dendrites of L5 cells. We could apply this technique in both anesthetized and freely moving animals.

We showed that the strength of sensory stimulation is encoded in the combined dendritic calcium response of a local population of L5 pyramidal cells in a graded manner. The slope of the stimulus-response function was under the control of a particular subset of dendritic targeting inhibitory neurons activated by synaptic inputs predominantly in L5. Recordings from single apical tuft dendrites in vitro showed that activity in disynaptically coupled L5 pyramidal neurons directly blocks the initiation of dendritic calcium spikes in neighboring pyramidal neurons and also that this dendritic inhibition was all-or-none phenomena. A simple model of the cortical microcircuitry with ~10-60 dendrites displaying all-or-none calcium spikes showed that feedback inhibition controlled the encoding of sensory stimulus strength in a graded fashion.

The experimental data and the model constitute a functional description of a cortical microcircuit in awake animals that relies on the active properties of L5 pyramidal dendrites and their exquisite sensitivity to inhibition. The microcircuit is organized so that local populations of apical dendrites can adaptively encode the sensory stimuli linearly across their full dynamic range.

Changes in cortical protein expression specifically related to improved learning following transcranial magnetic theta burst stimulation of rats

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Objective:
We could recently show that intermittent Theta Burst Stimulation (iTBS) of the rat brain via transcranial magnetic stimulation (TMS) induced a reduction in the cortical calcium binding proteins parvalbumin (PV) and calbindin (CB), indicative of a reduced activation of certain inhibitory interneurons. Now, we tested whether the same stimulation protocol can affect sensory associative learning, and how the interaction of TMS and learning affects the cortical protein expression.

Method:
In a modified radial arm maze, rats (male Sprague Dawley, 400-600g) learned to distinguish rewarded (food) and punished (unpleasant sound) arms only via a texture at arm entrance to be sensed with the whiskers. Visual, spacial and olfactory cues were prevented as experiments were performed in darkness, arm configurations were randomly changed from trial to trial and an air flow was directed towards the distal end of the arms. Four experimental groups were tested: Two groups received real iTBS (50Hz/5Hz, 3x600 stimuli within 120 min) while further two groups received sham stimulation (coil more distant to head). One group each of the real or sham treated animals was tested in the maze while the others were put back to their home cage using the same time schedule. TMS (real and sham) was applied 3 times daily directly prior to a block of 8 learning trials lasting 10-30 minutes (24 trials per day). Rats were sacrificed for immunohistochemical analysis focussing on frontal, motor, barrel and visual cortex when they achieved more than 75% performance on 6 consecutive days (after 3-4 weeks).

Results:
Rats treated with real iTBS learned significantly faster than the sham-treated controls. Coincident with previous findings obtained from anesthetized animals, iTBS significantly reduced the cortical expression of PV and CB and that of the glutamic acid decarboxylase isoform 67 kD (GAD67) in those rats not subject to the learning task. However, rats trained in the maze showed a different expression pattern: PV, CB and GAD67 expression was significantly less reduced in those cortical regions expected to be involved in the learning task (barrel and frontal cortex and to some degree also the motor cortex), while this effect was not evident in the visual cortex.

Conclusion:
We conclude that the acceleration of learning induced by iTBS is related to a partially weakened cortical inhibition, as indicated by the down-regulation of PV, CB and GAD67 which are specifically expressed by inhibitory interneurons. Stimulation of excitatory and inhibitory cortical activity during learning leads to a compensation of the down-regulation of these proteins in those cortical regions which are involved in the associative tactile learning task. This would finally result in a higher contrast of cortical inhibition between cell assemblies involved or not involved in the task. This could be an ideal condition to accelerate learning and recognition.

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Processing of proprioceptive inputs in the locust: quantitative analysis of the responses of spiking local interneurones in the metathoracic ganglion.

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The performance of adaptive behaviour is dependent on an organism modifying central motor patterns to match information obtained from its environment. Arthropods have proven to be invaluable model systems for these studies due to their accessible nervous systems and the range of diverse behaviours they perform. The femoro-tibial chordotonal organ (FeCO) of locusts (*Schistocerca gregaria*) monitors the position, velocity and acceleration of movements about the femoro-tibial joint of the legs, and contributes to a thoracic neuronal network for which there is detailed information about the input/output characteristics of many of the different neuronal populations that form the network. The FeCO has been shown to control leg reflexes by synapsing onto leg motor neurones, as well as non-spiking and spiking local interneurones. A ventral midline population of spiking local interneurones receives input from FeCO afferents and are part of the control loop that controls leg movements. We used band-limited Gaussian White Noise (GWN) to move the apodeme of the FeCO and recorded the synaptic and spiking activity of the interneurones. A Wiener cross-correlation method was used to identify the dynamic linear and nonlinear properties of the spiking local interneurones. We found that these interneurones fell in two groups depending on whether they were excited by tibial extension or flexion. Most of the interneurones studied had monophasic first order kernels indicating that they were strictly position sensitive. Additionally, we found a second class of interneurone that had biphasic first order kernels indicating that they were more strongly velocity sensitive. Comparison of the synaptic input and spike output showed that for many of the interneurones their response properties changed little despite the highly nonlinear process of spike generation. A detailed characterization and quantification of each of the neuronal classes involved in the production and control of leg motion in the locust is an important step towards understanding how adaptive behaviour emerges from the interaction of these components.

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Cytoarchitectonic mapping and quantitative anatomy of the Etruscan shrew cortex

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The Etruscan shrew, Suncus etruscus, is one of the smallest mammals. Etruscan shrews can recognise prey shape with amazing speed and accuracy, based on whisker-mediated tactile cues. Because of its small size quantitative analysis of the Etruscan shrew cortex is more tractable than in other animals. To assess the anatomy of the Etruscan shrew's brain quantitatively we sectioned brains coronally and stained neurons (Nissl, NeuN). On basis of these stains we estimated the number of neurons, surface area, and volume of seven cortical hemispheres using Stereoinvestigator and Neurolucida (MBF Bioscience) software. On average the neuron number was found to be ~ 1 million, the surface area 11 mm^2, and the volume 4.5 mm^3 per hemisphere. We also identified cortical areas by cytoarchitectonic boundaries (Nissl) and cytochrome oxidase staining patterns in flattened brain sections. In addition, cortical sensory areas were independently delineated using multi-unit electrophysiological mapping of sensory responses. Currently, we evaluate the correspondence of anatomically and physiologically derived cortical maps.

Differing effects of GABA and glutamate on spider *(Cupiennius salei)* mechanoreceptors

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Efferent innervation of mechanosensory neurons occurs in all arthropod species studied so far. The efferents usually modulate centrally located mechanosensory axon terminals, but in arachnids and crustaceans efferent modulation also occurs at peripherally located dendrites and somata. The lyriform slit sense organ VS-3, a strain receptor in the patella of the tropical wandering spider *Cupiennius salei*, is well suited for studying neuromodulation of peripheral parts of mechanosensory neurons. In a semi-intact preparation, a piece of cuticle containing the entire organ, including receptor neurons, can be dissected to allow mechanical stimulation during intracellular recording and drug application.

Previous studies showed that the effects of $GABA_A$ agonists muscimol and GABA on VS-3 neurons depend on the stimulus format. Muscimol caused only inhibition when step-stimuli were used, but with pseudorandom Gaussian noise it caused a triphasic response of brief excitation, then inhibition, and finally excitation for several minutes. Muscimol always caused membrane depolarization and increased intracellular calcium concentration.

Here, we applied glutamate to VS-3 neurons during mechanical and electrical pseudorandom noise stimulation. 1 mM glutamate gave only an inhibitory response, plus minor changes of membrane potential. Spiking recovered quickly after glutamate was removed, without long-term effects on firing rate. Similar inhibition by glutamate was previously shown during step electrical or mechanical stimulation. Therefore, glutamate's effects on firing rate were independent of stimulus paradigm, indicating that glutamate receptors initiate different processes in these neurons than the closely related $GABA_A$ receptors.

To measure the effect of glutamate at different stimulus frequencies, we calculated frequency response functions. Gain, *G*, as a function of frequency, *f*, was fitted by the power law relationship:

$G(f) = A f^k$,

where *A* is a sensitivity parameter, and *k*, the power law coefficient, indicates the level of adaptation, or relative sensitivity to higher frequencies. We also calculated linear information capacity (Shannon 1949).

During glutamate application, sensitivity, adaptation rate and information capacity were all reduced, returning to control levels soon after application. No long lasting changes, as with muscimol application were seen with glutamate.

Although both glutamate and muscimol initially inhibited VS-3 neurons, muscimol's effect ended with excitation, but glutamate's did not. It is possible that these transmitters act to change the overall sensitivity of VS-3 neurons and provide frequency tuning. This could selectively filter stimuli at behaviorally less relevant frequencies.

Inhibitory glutamate receptors and $GABA_A$ receptors are phylogenetically related ionotropic chloride channels. In other preparations, glutamate increases both potassium and chloride conductances. This could contribute to the different effects of the two neuromodulators in VS-3 neurons. Potassium efflux could prevent strong depolarization and hinder an increase in intracellular calcium. Future experiments using calcium imaging are needed to clarify this point.

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Induced plastic changes of tactile perception and somatosensory cortex excitability depend on stimulation frequency and temporal pattern.

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Plastic changes of the somatosensory system can be induced by passive stimulation techniques, like repetitive transcranial magnetic stimulation (rTMS) or tactile coactivation. As with synaptic plasticity mechanisms, the direction of changes depends on stimulation frequency, with low frequencies causing impairment and high frequencies causing improvement of perceptual abilities. TMS studies in the motor system have shown that paired-pulse stimulation with a short inter-stimulus interval (ISI) enhances motor cortical excitability. In the present study we investigated if the somatosensory system also shows plastic changes in response to pairs of stimuli with short ISIs. Six groups of 12 volunteers were studied (18 to 28 years of age). Electrical stimulation was applied to the tip of their right index finger for approximately 30 minutes. Pulse duration was 0.2 ms, intensity was 1.5 times the sensitive threshold. Across groups was matched in pulse number. In groups 1 and 2 we used temporally regular pulses at 1 or 2 Hz, respectively. In groups 3 to 6 we used paired-pulses at a rate of 1 Hz with ISIs of 2, 10, 100 and 200 ms, respectively. As a marker of plastic changes we assessed 2-point discrimination thresholds before and after the stimulation. Furthermore, to get an insight into alterations of cortical excitability and intracortical suppression we also measured paired-pulse behaviour for ISIs of 2 and ISI 500 by means of SEP recordings following paired-pulse median nerve stimulation. Stimulation with single pulses given at 1 and 2 Hz significantly impaired tactile discrimination by increasing the 2-point discrimination threshold by approximately 15 %. In contrast, short interval paired-pulsed stimulation improved tactile acuity, with the strongest effects seen at ISIs of 2 and 10 ms (18 % improvement in both groups). For ISIs of 100 and 200 ms we observed a significant improvement of 11 and 14.5 %. In the SEP recordings, continuous stimulation at 2 Hz increased paired pulse inhibition by 22 % and paired stimuli with 2 ms ISI, reduced paired-pulse inhibition by 32%. The results show that the somatosensory system can be modified by short interval paired stimuli where very high frequencies (500 Hz) evoke maximal improvement. Accordingly, the presence of stimuli in very close temporal proximity appears to be a crucial factor for driving plastic changes.

Impact of thalamus on cortical state change in mouse barrel cortex during whisking

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Mice actively move their whiskers to monitor the surrounding tactile environment. During whisking, barrel cortex neurons undergo a change in state that results in a desynchronisation of cortical activity. Here we investigate the mechanisms underlying this brain-state change in awake head-fixed mice. Extracellular and whole-cell recordings from neurons in layer 2/3 barrel cortex reveal slow oscillations during quiet wakefulness, when the whiskers are not moving. These slow oscillations are enhanced after thalamic inactivation by injection of muscimol or TTX. During active periods, when the mouse is whisking, the membrane potential dynamics are quite different. The slow cortical oscillations disappear during whisking both under control conditions and after thalamic inactivation. However, following thalamic inactivation cortical neurons become hyperpolarised during whisking, in contrast to the depolarised state during whisking under control conditions. The thalamus is therefore responsible for one key aspect of the cortical state change - it appears to provide the depolarising input during whisking. A separate component of the state change, the disappearance of the slow oscillation, is independent of the thalamus.

Mechanosensory Feedback in *Drosophila*

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Flight control in flies provides a model system for neural function in a meaningful context. Flies are able to rapidly maneuver without losing their stability. Flight stabilization and maneuvering is mediated by tiny control muscles, that rely heavily on fast and phased mechanosensory feedback (<2ms delay) from Campaniform Sensilla (CS) on the wings and the halteres. While the halteres have been identified as specialized organs for turning forces detection, the function of the wing blade CS is still unknown **[1]**.
We study the role of mechanosensory feedback in neural flight control by ablating different wing CS of fruit flies (*Drosophila melanogaster*) (Fig.1 A). Free flight experiments showed that CS located on the L3 wing vein are not necessary for stable flight at different airspeeds in a wind tunnel. To investigate fly's flight for more subtle differences, we measure the wing movements of tethered flies in detail using a custom build high speed (6 kHz) vision system **[2]** (Fig.1 B,C) and analyze the recorded wing positions with nonlinear time series techniques: Wavelet analysis and Autocorrelation function **[3]**.
We observe changes in flight kinematics as transient changes in wing amplitude and phase. We identify different flight regimes with the use of discrete wavelet analysis. We quantify regularity of flight with the global measure of correlation time and the local calculation of intrinsic phase coherence. Ablation of the L3 CS leads to a significant more regular flight, showed both as an increase in phase coherence and correlation time (Fig.1 D).
With the combination of modern measurement tools with data analysis methods from nonlinear dynamics we are able to identify different flight regimes and even subtle differences from normal flight.

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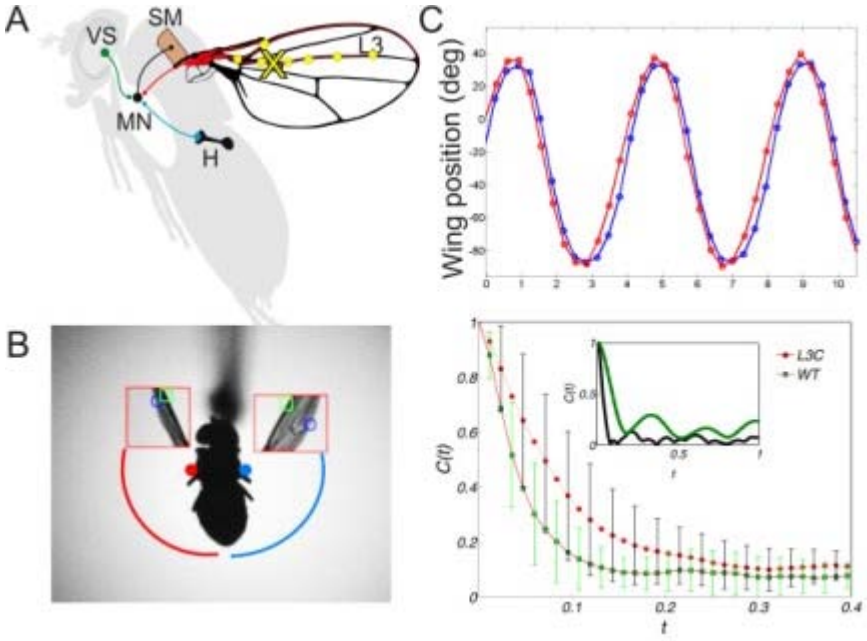
FIG. 1: (**A**) Schematics of flight control circuit in Drosophila, information from the visual system (VS), the halteres (H) and wing mechanosensors (yellow dots) are fused in the motor neurons (MN), which control the steering muscles (SM). Yellow cross indicates cutting position on L3 vein (L3). Adapted from Dickinson 2006. (**B**) Sample of wing position extracted from regions of interest. (**C**) An example interval of the recorded time sequence of the wings positions (in total 60 sec). (**D**) Auto–correlation averages across nWT = 5 of WT and nL3C = 5 of L3 cut flies (mean±std). (Inset) Two examples of auto-correlations are shown for for WT (black) and L3C (green) individuals.


A
VS
SM
L3
MN
H
B
C
Wing position (deg)
C(t)
t
L3C
WT

Investigating the effects of proprioceptive feedback on a central pattern generator with a real-time computer model

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One of the most fascinating properties of the nervous system is the capability to handle sensory feedback and to form adequate responses.

This is particularly interesting since many sense organs possess complex intrinsic properties and it is not yet understood how these properties affect the processing of sensory information and the response of the nervous system.

To address this issue we investigated an identified sensory neuron in the crab stomatogastric nervous system. The muscle tendon organ AGR (anterior gastric receptor) provides sensory information about the tension of a muscle that protracts a tooth in the gastric mill chamber of the crab foregut (Smarandache & Stein, J. Exp. Biol. 210, 2007). AGR is activated during tooth protraction and it affects the central pattern generator that drives tooth movement. Here, we demonstrate the complex intrinsic properties of this proprioceptor and the use of a real-time computer model to test their influence on the motor output.

We first used intracellular recordings in isolated preparations to characterize active membrane properties in AGR that caused spike frequency adaptation, postinhibitory rebound and plateauing. The contribution of these properties to the motor response, however, can neither be estimated in the isolated nervous system (since the muscles that activate AGR are missing) nor in more intact preparations (because of poor accessibility). We thus built a real-time computer model which simulates the muscle activity and the resulting AGR activity. In this model, motoneuronal activity is used to calculate muscle tension via a set of low pass filters. The time constants used for these low pass filters were derived from an exhaustive search analysis of experimental data. The calculated muscle tension is then fed into a single compartment Hodgkin-Huxley model that is based on the intrinsic properties of the biological AGR. To achieve adequate responses of the model, I_{Na}, I_{Kd}, I_h, I_{Ca} and $I_{K(Ca)}$ were hand-tuned to match experimental data. Both - the muscle – and the receptor model were integrated into the simulation environment madSim (www.neurobiologie.de/madSim) which allows real-time computation. This enabled us to construct a closed-loop system between model AGR and the nervous system by feeding the calculated activity back into its biological counterpart via current injection. We are currently testing the effects of AGR's intrinsic properties on the motor output by selective manipulation of single ionic conductances in the real-time model.

Influence of movement signals from the Femur Tibia-joint in front-, middle- and hindleg of the stick insect during forward and backward walking

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Processing of proprioceptive information from the legs is important to perform coordinated locomotion in terrestrial animals. Sensory feedback from movement and force sensors influences the magnitude and the timing of neural activity generated in the central neural networks driving individual joints of a leg. Present evidence from a variety of animals suggest that one important mechanism by which sensory feedback contributes to the generation of the motor output for walking is through reinforcement of movement [1,2]. In stick insects for example, flexion in the Femur-Tibia (FT-) joint is signalled by the femoral chordotonal organ (fCO) and is known to reinforce stance phase motor output of the FT-joint when the locomotor system is active [3]. Flexion signals promote flexor and inhibit extensor motoneuron (MN) activity. This represents a *reflex reversal* the so-called "active reaction" (AR) [3]. Here we investigated, whether processing of movement related feedback from the fCO is segment specific and altered between for- and backward walking, as was shown for load feedback [4].

Using a semi-intact preparation of the stick insect walking on a slippery surface [5], we studied the generation of the AR in front-, middle- and hindleg when the animal was walking for- or backward with the other five legs. The animals were mounted on a balsa stick and the leg in question was fixed for surgery and stimulation of its fCO (range: 110°- 30°). Simultaneously the activity of the extensor tibiae MNs was recorded extracellularly from nerve F2, while the activity of the flexor tibiae was recorded by an EMG.

Tibial MN activity in each of the legs differed in general between forward and backward walking. In frontlegs elongation signals from the fCO during forward walking elicited an AR in 71% of the trials (n=79 (sample size); N=5 (number of animals for all conditions). As well in middle legs, in 48% of the stimuli an AR was generated (n=122). Interestingly, in frontlegs (9%, n=56) and middlelegs (26%, n=99) fCO elongation was not found to induce reliably ARs during backward walking. In hindlegs fCO elongation neither elicited an AR during forward (n=150) nor during backward walking (n=135).

Our results indicate firstly that the processing of movement related feedback from the fCO is segment specific, a finding which differs from previous reports [6]. Secondly, our results suggest that the processing of movement related feedback depends on walking direction. While in forward walking front- and middlelegs flexion signals from the FT-joint can contribute to generating flexor activity during stance phase no such influence is detectable, when the animal walks backwards.

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Expression of FoxO transcription factors in peripheral nerves undergoing Wallerian degeneration *in vivo* and *in vitro*

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In mammals the FoxO transcription factors FoxO1, -3a, -4 and -6 are important downstream targets in the PI3K-dependent gene regulation and they are regulators of cell fate. Among many other functions these factors regulate cell proliferation, differentiation and apoptosis and they are regulated by growth factors (insulin-like growth factor), hormones (insulin) and their receptors. They play a role in cancer, diabetes, and longevity and have also been found to be expressed in the central nervous system. Depending on the state of phosphorylation, these factors either stay in the cell cytoplasm or activate downstream cell death signals.

Wallerian degeneration occurs in the distal part of neuronal cells in the nervous system, when a traumatic insult has severed the axon from the cell body. In peripheral nerves which have been axotomized, such an injury is accompanied by an invasion of peripheral macrophages which phagocytose cellular and myelin debris. Here we investigate two paradigms of Wallerian degeneration (in vitro and in vivo) in order to show the presence of FoxO transcription factors and how they can be blocked during peripheral nerve degeneration.

For in vitro experiments we injected i.p. mice of the C57/BL6N strain with thioglycollate solution and harvested peritoneal macrophages 4 days later and co-cultured them with sciatic nerves. Cultures were treated with an antibody against the insulin receptor, with a synthetic inhibitor or with an IGF-analog. After 10 days of culture, nerves and macophages were harvested, separated and examined for the expression of FoxO1, FoxO3a and FoxO6. Some of the nerve pieces were processed for semithin sections and scrutinized for different parameters of invading macrophages and for preserved myelin and axons. In other nerve pieces, we applied immunohistochemistry to find the number of macrophages or Schwann cells and determined the number of Foxo3a coexpressing cells. Co-expression was also examined in cryostate sections of degenerating sciatic nerves which had been axtomized.

In co-cultured nerves and macrophages, we found a weak signal of FoxO1 and a moderate signal of Foxo3a, but Foxo6 was not detectable. Due to these findings, the immunohistochemistry was restricted to Foxo3a, which was found coexpressed in higher numbers in Schwann cells than in macrophages. Application of inhibitor JB-1 showed the most potent effect by inhibiting the migration of invading macrophages. The inhibition by insulin receptor antibody was less effective and the IGF-analog showed no effect. However, the application of all substances had a diminishing effect on the remaining myelin in cultured nerve pieces and they also reduced the number of preserved axons within the myelin sheaths in comparison to controls. The verification of FoxO3a signal in axotomized nerves confirmed an overall weak signal of this transcription factor, but it increased up to 6 days after axotomy. We also confirmed that a higher signal in Schwann cells is present than in macrophages.

From these experiments we conclude that FoxO3a transcription factor signal is consecutively expressed in peripheral nerves at low levels and is regulated after injury also on low levels. The signal seems to be partially regulated by insulin/IGF receptors but not only dependent on this pathway. Therefore it is not unlikely that other regulatory pathways, apart from the PI3K-pathway, play a role in the activation of FoxO transcription factors as is already being discussed in the literature.

Mechanisms in the Control of Walking Speed in the Stick Insect

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How exactly walking speed and the corresponding changes in coordination of the six legs are controlled in insects, in particular *in vivo*, is still largely an unresolved issue. It has been shown for the stick insect single leg preparation that mechanisms to control stepping velocity only become effective during an already ongoing stance phase motor output (1,2). We performed electrophysiological and behavioral experiments in reduced preparations and intact animals of the stick insect *Carausius morosus* to understand mechanisms underlying the control of walking speed on different levels of the nervous system.

On the level of the single leg, stepping velocity arises from modification in flexor MN activity as previously shown (2). We found no additional significant correlation between spike frequency of the fast extensor tibiae motor neuron (FETi MN), which is active during swing, and stepping velocity. At the transition from stance to swing phase, there is, however, a correlation between the pause between motoneuronal stance and swing activity and stepping velocity. This pause becomes shorter with increasing speed and completely disappears during fast stepping sequences. These results corroborate previous findings that stepping velocity in the single leg is solely dependent on stance phase MN and not on swing phase MN activity.
We then tested whether the stepping speed in one leg has an influence on the general excitation in other legs by means of extra- and intracellular recordings in reduced stick insect preparations. On the inter-leg level, no systematic linear relationship was found between the velocity of a stepping front leg and the motoneuronal activity in contralateral mesothoracic protractor and retractor MNs. Furthermore, no correlation was found between the stepping frequency of the front and middle legs and the frequency of oscillation in the active ipsilateral hind leg ThC-joint CPG.
The observations on the lack of coordination of stepping velocity between legs under normal walking conditions were confirmed in behavioral experiments with intact stick insects tethered above a slippery surface, thereby effectively removing mechanical coupling through the ground. In this situation, there were no systematic correlations between the stepping velocities of different legs.
However, when the tethered animal increased walking speed due to a short tactile stimulus, provoking an escape-like response, stepping velocities of ipsilateral legs were found to be correlated for several steps.
These results show that neuronal influences exist which coordinate stepping velocities between legs under certain circumstances, but that these are usually weak in the slowly stepping animal.

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Muscle Activity of Antagonistic Leg Muscles in the Turning Stick Insect

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The stick insect *Carausius morosus* has long been an organism used to study the neuronal control of locomotion and much is known on straight walking and it's sensory and central control, down to the level of the single middle leg (1). Very little, however, is known on the neuronal control of adaptive behaviors such as turning. For the understanding of locomotor control it is necessary to understand both, the kinematics of leg movements and the neuronal activity underlying the movements. Stick insect leg kinematics change drastically from straight walking to turning and recent studies have shown that leg movements also change for tethered stick insects displaying turning behavior on a slippery surface (1,2). These turning kinematics are also largely conserved in the turning two-leg and one-leg preparations (2). Knowing the activity of the muscles underlying the kinematics and their timing under different behavioral contexts is the first step towards understanding how these changes are brought about and controlled.

Here, we describe muscle activity of the three antagonistic pairs of muscles that largely control the leg movement during stick insect stepping under the conditions of straight walking and turning. The latencies and phase diagrams of EMG activity in the *pro-/retractor coxae*, the *levator/depressor trochanteris* and the *extensor/flexor tibiae* muscles of the middle leg are reported with respect to the precise, electrically measured touch down signal of the leg's tarsus (2). Generally, the beginning of muscle activity associated with stance phase, that is the activity of the depressor and flexor muscles, is tightly coupled to touch down with average timing of 95ms prior to, and 15ms after touch down, respectively. On the other hand, activity of muscles associated with swing phase, i.e. levator and extensor activity, is coupled to lift off to a much smaller extent, with timings for the first spike in the levator up to several hundred Milliseconds before lift off and extensor spike timing varying over a range of more than 100ms around lift off. During turning the most drastic changes in muscle activity compared to straight walking occur in the inside leg activity. Especially the pro- and retractor activity of the inside leg in the turning animal are altered in that no consistent phase coupling of either pro- or retractor activity to stance or swing phase exists anymore, reflecting the occurrence of forwards and backwards steps in this context. In addition, the *depressor trochanteris* is activated later and the *flexor tibiae* muscle is activated more strongly. We compare the data between the intact animal and the reduced two-leg- and one-middle-leg preparations.

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Morphology of motor neurons innervating labral muscles of *Locusta migratoria*

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Previous studies indicated that the innervation of the labral muscles might be complex and also rather unconventional. We investigated this innervation in migratory locust (*Locusta migratoria*) in a systematic fashion in order to address two questions: First, how many neurons innervate the muscles that move the labrum and where are their somata located? Second, does the motor innervation yield any further hints that might help to decide the controversy whether the labrum represents a structure that developed during evolution by the fusion of paired appendages. Using Neurobiotin as a retrograde neuronal tracer, we specifically stained the motor nerves of individual labral muscles. We show that all labral muscles receive innervation from both the tritocerebral lobes of the brain and the suboesophageal ganglion, except for M1, the labral compressor muscle. M1 is innervated by two motor neurons. Both tritocerebral lobes contain one soma. The axons of both neurons branch in the periphery to innervate ipsi- and contralateral muscles. The labral retractor muscles, M2, are innervated by 6 tritocerebral motor neurons, with 3 somata located in each tritocerebral lobe. Their axons cross the midline in a distinct commissure between the two muscles. The other two labral muscles, M3 and M38, are innervated from the ipsilateral tritocerebrum only. There is still some uncertainty as to the number of motor neurons innervating these two muscles. Both muscles together receive 8 motor axons and there might be common motor neurons that innervate both muscles. In addition, the labral muscles M2, M3 and M38 also receive innervation from neurons located in the suboesophageal ganglion (SOG). In relation to the muscle, their somata are located on the ipsi- and the contralateral sides of the SOG. Their axons ascend through the circumoesophageal connectives and proceed into the frontal connectives without forming ramifications in the tritocerebral lobes. These axons also cross the midline in the periphery to reach muscles on both sides of the head. They either cross within the commissure between muscles M2, or through the frontal ganglion.

These results indicate that labral muscles are innervated from both sides of the CNS. The innervation pattern is bilaterally symmetric as that of other appendages. The participation of both tritocerebral and suboesophageal ganglion elements supports the notion that the labrum is innervated by the so-called intercalary segment. The innervation pattern may support the idea that the labrum derives from fused appendages: There are two symmetrical sets of motor neurons. Some of them cross the midline in the periphery. This might indicate that during the evolution of labrum its motor innervation pattern was reconfigured in the periphery, while the original bilateral sets of central neurons remained unchanged.

Targeting Modules in the Gap-Climbing Control of *Drosophila melanogaster*

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The fruit fly *Drosophila melanogaster* shows an astounding maneuverability and agility during walking. Even with shortened wings, the flies can overcome gaps in their walkway with widths of up to 1.6x their body length (Pick and Strauss, 2005; Curr. Biol.). Furthermore, this behavior is visually adapted to the width of the gap, i.e. if the gap gets insurmountably broad, climbing will not be elicited. In a climbing attempt, the fly will try to reach the opposite wall with its front legs first. To this end the fly moves its body out into the gap, whereby the hind legs keep contact to the proximal edge of the gap. The middle legs, which are attached to the proximal side wall of the gap, lift up the body and by that are giving the front legs a better working area. After the fly has ultimately reached the opposite side with its front legs, it is forming a bridge. Next the middle legs are released and transferred and finally the hind legs give up contact to the proximal side.

We are analyzing this complex behavior in detail under a set of two orthogonal high-speed video-cameras. In a comparative approach we found mutant lines that fail at specific phases of this behavior, hinting strongly at the modular structure of climbing control. Some lines hardly initiate climbing at easily surmountable gaps, whereas the novel *sisyphus* mutants do so even at clearly insurmountable gap widths. Other lines fail in lifting up the body with their middle legs or fail to move into the gap before engaging in climbing. The latter flies virtually increase the distance to be overcome by half a body length. A set of mutant lines takes a normal decision to climb which is correctly based on the visual estimation of gap size. However, on approaching the gap they are losing direction to the opposite side and will start climbing attempts in arbitrary directions.

The latter lines all show interruptions of the protocerebral bridge, one of the four neuropilar regions of the central complex amidst the fly brain. In an attempt to test for the causal structure-function relation we partially rescued one of those genes, *tay bridge*, in the *tay bridge*[1] mutant background such that the protocerebral bridge has been structurally restored. The rescue flies can compensate for their initial problem. While still producing wider-spread targeting angles than wild-type flies, their attempts are nevertheless almost all pointing to the opposite side. Their success rate reaches wild-type levels. We conclude that we have restored a visual targeting system sufficient to hit the opposite side. Restoration of the protocerebral bridge in the given manner was not sufficient to rescue the presumed short-term storage of the climbing direction that enables wild-type flies to stay within a narrow range of ±5deg around the optimal climbing direction. Conversely, it takes the loss of two staggered systems to bring about the full-fledged mutant phenotype observed, which seriously lowers the success rate of the flies.

Coordinated locomotion via a gradient of synaptic strength

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We are using the crayfish swimmeret system to understand the cellular mechanisms of coordinated locomotion. Swimmerets are the abdominal limbs used for forward swimming. They are innervated by a chain of ganglia in the central nervous system, abdominal ganglia 2 (A2) through 5 (A5), with A5 being the most posterior in the chain. Each swimmeret is driven by its own local pattern generating module in cycles of alternating power-stroke (PS) and return-stroke movements. During active expression of the swimming motor pattern, the most posterior swimmeret module starts each cycle with a PS burst and the others follow with a phase lag of 0.25, independent of the frequency of the rhythm. One coordinating neuron in each module ASC_E projects to more anterior ganglia, and a second one DSC projects to more posterior ganglia. These neurons are necessary and sufficient to maintain the posterior-to-anterior progression of the PS bursts. Each ASC_E and DSC fires a burst of impulses at a characteristic phase in each cycle. Targets of these coordinating neurons are named Commissural Interneuron 1 (ComInt 1). A single ComInt 1 neuron is situated in each module and influences the local pattern generator via graduated transmitter release.

We used sharp microelectrodes to record from ComInt 1 at the midline (where it receives its synaptic input) or in the lateral neuropil (where the synaptic output is located). ComInt 1 integratedexcitatory postsynaptic potentials (EPSPs) elicited by all coordinating neurons. In each ganglion, ComInt 1 received a unique pattern of coordinating input. Usually the biggest EPSP was elicited by the ASC_E from the neighbouring posterior ganglion. We compared the EPSP sizes and got the following results. ComInt 1 in A2 integrated ASC_E inputs from all posterior modules. In A2 the largest EPSP was from the neighbour, ASC_E3(N=8). EPSPs from two ganglia (ASC_E4) away were 60% smaller (N=6) and those from three ganglia away were 95% smaller (N=1) or could not be detected (N=4). ComInt 1 in A5 received only DSC input, with the biggest EPSP from the neighbouring DSC4 (N=5). The EPSPs from two ganglia away were 20% smaller (N=3) and those from DSC2 were 85% smaller (N=2). ComInt 1 in A4 and A3 integrated a mixed input of descending and ascending coordinated information. In A3 the biggest depolarization was always elicited by the neighbouring ASC_E4 (N=8). The EPSPs from the neighbouring DSC2 were 70% smaller (N=3) and those elicited by ASC_E5 (two ganglia away) 90% smaller (N=2). The biggest EPSPs in A4 were from the neighbouring ASC_E5 (N=8), but here there was no significant difference of the averaged amplitude from DSC3 (neighbouring ganglia, N=8). The EPSPs from two ganglia away (DSC2) were 60% smaller (N=3).

We also measured attenuation of EPSP amplitude from the input at the midline to the synaptic output in the lateral neuropil. Amplitudes of EPSPs recorded in the lateral neuropil were 50% smaller than those recorded at the midline (N=4).

For the first time, these experiments demonstrate a gradient of synaptic strength in a system that coordinates locomotion.

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Modulation of corticomuscular synchronization by different frequencies of dynamic force output

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Current neurophysiological research has focused on the elucidation of mechanisms by which cortex drive the muscles in a dynamic environment. In particular, corticomuscular synchronization or coherence (CMC) has been shown to occur in the gamma-range (30-45) during isometric compensation of dynamic (periodically modulated) low-level forces during a visuomotor task. Although these results have consistently pointed to gamma-range CMC as an effective corticospinal communication process, they have been confined to experimental paradigms where only the amplitude of the dynamic force or its offset has been manipulated. Thus, the critical role of different time scales or 'tempos' on the visuomotor process has remained unexplored. We addressed this question by investigating the CMC, the cortical spectral power as well as the motor performance during a visuomotor task, where 3 different frequencies of the dynamic force were used. Seven healthy right-handed female subjects were instructed to compensate dynamic forces at frequencies 0.6, 1 and 1.6 Hz while EEG was recorded from 52 scalp positions and EMG from the first dorsal interosseus muscle (FDI). A custom-made manipulandum produced the variable force on a ring. The subject had to compensate the force generated by the manipulandum isometrically to maintain the ring in its initial position. Visual feedback about the position of the ring was provided to the subject through a small white circle (representing the ring) that had to be maintained inside a green circle at any time, so that when a given force was applied to the ring the subject had to apply the same force in the opposite direction to keep the ring in its initial position. Preliminary results regarding the subject's performance and the modulation of the CMC by different force frequencies will be presented.

Sonic hedgehog regulates Cadherin-20 expression by motor neurons during spinal cord development

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During development of the spinal cord, distinct classes of neurons express defined transcription factors in a dorsoventral pattern, which is determined by a gradient of Sonic hedgehog (Shh) secreted from the floor plate. Cadherin-20 is expressed by motor neurons of the lateral motor column and other cells in the basal and alar plates. In the present study, we investigate whether Cad20 expression in motor neurons of spinal cord is also regulated by Shh signaling. After Shh signaling was altered in chicken spinal cord and hindbrain by in vivo electroporation, the regulation of Shh on the cadherin-20 expression was investigated by immunohistochemistry. Our results show that, at an early embryonic stage 12, Shh induces cadherin-20 expression by motor neurons. Later, at stage 24 when the sorting of motor neuron pools begins, cadherin-20 expression is induced in the neural progenitors of the ventricular zone but not in motor neurons. Blockage of Shh signaling inhibits cadherin-20 expression in the motor column. Therefore, cadherin-20 expression in motor neurons is regulated by Shh in a time-dependent manner.

The Preoptic Area in Anuran Amphibians

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In anuran amphibians the preoptic area (PO), localized in the ventral anterior telencephalon, plays a crucial role in reproductive behavior, e.g., in initializing call readiness and call triggering in males. In the past, different approaches were used to characterize the function of the PO mainly by electrical stimulations, recordings, and lesion experiments. A prominent locus in the PO is the anterior part (APO): electrical stimulations in this area induced mating call behavior and even egg-laying postures in males. These reactions depend strongly on the hormonal state of the animal. The neurohormone arginine vasotocin, e.g., has a strong influence on the reproductive behavior. It increases the call frequency in males and stimulates the phonotaxis in females. Moreover, neurons within the PO were found to have steroid hormone receptors and, in turn, neurosecretory granules.

Numerous questions remain to be answered concerning the APO: Which subdivisions or neural subpopulations trigger calling activity? Which forebrain areas control or modulate the activity of APO? How are the hormonal influence and auditory input integrated? How act hormones and neuromodulators on APO cells?

In a first step, we adapted the c-fos staining method, a marker of activity-dependent immediate early gene expression. Labeling of neurons in the tel- and diencephalon was compared between males that were exposed to conspecific calls and those that were not stimulated. In regard to the auditory and vocalization related pathways labeled neurons were found in following higher brain centers: the septal area, predominantly in the lateral site, the medial and lateral pallium, some in the striatum, the amygdala and inside the bed nucleus of the stria terminalis. The APO showed staining in the rostral part and mainly in the nucleus suprachiasmaticus. The diencephalon contained some labeled neurons in the ventromedial and posterior thalamus, whereas the midbrain torus semicircularis, mainly the nucleus principalis and laminaris, were profoundly marked by activated neurons. The nucleus secundarius isthmi contained just a few labeled cells as well as the reticular formation.

In addition to the c-fos studies we started to characterize synaptic inputs to APO cells via whole-cell patch-clamp recordings in an isolated half-brain preparation which has the advantage of semi-intact interconnections of brain nuclei. First results will be presented.

Stick insect tarsi – surface structures and muscle recruitment in posture control

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While attachment structures of insect tarsi have received considerable attention in recent years, less is known about their use in posture maintenance and walking. We thus tried to reveal the recruitment of relevant tarsal muscles in attachment-related behaviours. First, we characterised structure and mechanical properties of tarsal pad structures in two stick insect species (*Carausius morosus*, *Cuniculina impigra*). Second, we recorded the activity of the tarsal claw retractor muscle during posture control. Finally, we scrutinised electrophysiological data in relation to the properties of attachment pads.

The tarsal pads of both species are similar in size and number per tarsus. They differ, though, in their surface microstructure, especially in the euplantulae. *C. impigra* euplantulae have rather smooth surfaces, while those of *C. morosus* possess pronounced nubby surfaces, with the nubs measuring up to 5 µm in length, and the diameter tapering from 1.5µm at the base to 0.5µm at the tip.

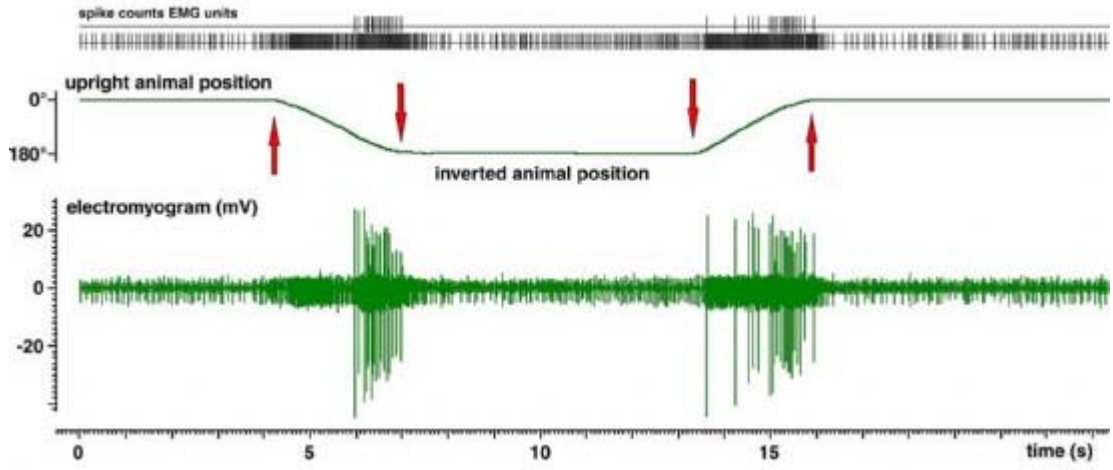
We tested both frictional and adhesive forces in the two species on smooth and rough surfaces (roughness 3µm). *C. impigra* had better **adhesion** on smooth surfaces ($p<0.05$, smooth: $n_s=30$, rough: $n_r=17$), whereas *C. morosus* showed no considerable differences on different surface textures ($n_s=17$, $n_r=13$). In a species comparison, *C. impigra* had better adhesion on the smooth surface compared to that measured in *C. morosus* ($p<0.05$), whereas there was no species difference on the rough substrate. When considering **friction** forces, *C. impigra* exerted larger forces on smooth substrate than on the rough one ($p<0.05$, $n_s=25$; $n_r=22$), while *C. morosus* showed stronger friction on the rough than on the smooth surface ($p<0.05$, $n_s=27$, $n_r=22$). In a species comparison, *C. impigra* had larger friction on the smooth surface than *C. morosus* ($p<0.05$), while there was no considerable difference between species on the rough surface.

Euplantulae of neither species showed anisotropic frictional properties, which corresponds to the symmetry of the surface structures on the tarsal pads. Structural E-moduli of the pads were about 5.8fold lower in *C. morosus*, probably as a result of the compliancy of the surface microstructures upon initial substrate contact.

Our results suggest an advantage of smooth pads on smooth surfaces, and a better adaptation of microstructured pads to cling to rough surfaces. The overall adhesive forces of the euplantulae were in all animals insufficient to hold the animal weight on a smooth ceiling. This appears to indicate that the euplantulae serve primarily the generation of frictional, rather than adhesive, forces.

In posture control, discharges of the claw retractor muscle were observed mainly during substrate movements, such as tilting the support platform from horizontal into vertical positions. Tonic muscle activity in upright, vertical or inverted animal positions was usually lower than during movement (**figure**). Nevertheless, there was a tendency for mechanically less demanding postures, such as upright standing, to produce lower maintained discharges in the claw retractor than more demanding situations, such as clinging to the ceiling in inverted position.

Figure. Activity of the claw retractor muscle. Position of animal platform, top trace; retractor unguis electromyogram, bottom trace. Arrows mark beginning and end of two 180°-turns, from horizontal to inverted position and back. Traces at the very top indicate counted spike events of the two muscle units


spike counts EMG units
upright animal position
0°
180°
inverted animal position
electromyogram (mV)
20
0
-20
0
5
10
15
time (s)

A Tracing Study of Mesothoracic Leg Motoneurons and DUM Neurons in the stick insect *Carausius morosus*

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During walking in stick insects legs are moved by contractions of antagonistic muscles. For understanding how the nervous system controls muscle activity it is essential to know all motoneurons and modulatory neurons that innervate the leg muscles. Neuroanatomical studies based on cobalt backfills of nerves that contain axons of leg motoneurons in stick insects already revealed that some leg muscles are innervated by suprisingly many motoneurons [1]. However, these studies suffer from a lack of detail. Backfills of nerves with up-to-date dyes in combination with high-resolution optical imaging systems largely improve the reproducability of labeling structures and the richness in detail of labeled structures. Therefore, we re-investigate number and anatomy of motoneurons and modulatory dorsal unpaired median neurons (DUM neurons) that innervate the leg muscles in the stick insect *Carausius morosus*.

In the mesothoracic ganglion, lateral nerves or nerve branches of nervus cruris, the main leg nerve, were backfilled with fluorescence dextran dyes. Additionally, all lateral nerves were filled with dye to display the whole DUM neuron population within the mesothoracic ganglion. Labeled cells were visualized by using a confocal laser scanning microscope (Zeiss LSM 510Meta).

Our tracing studies revealed motoneuron-pools sizes which differ from previous findings and besides we were able to stain DUM neurons for almost all dye filled nerves. For example, we found for nl5, the retractor coxae innervating nerve, 23-26 motoneurons and at least 5 DUM cells instead of previously reported 18 motoneurons. Stainings of nerve C1, which innervates the levator trochanteris muscle, showed 12 motoneurons and 2-3 DUM neurons instead of previous 10 motoneurons. Preparations in which all lateral nerves were dye labeled displayed a maximum number of 9 DUM neurons around the ganglion's midline.

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Audio-vocal integration within the Medulla oblongata of anurans

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The musculature involved in vocalization and respiration in discoglossid anurans is controlled by trigeminal nerve (N. V), facial nerve (N. VII), vagal nerve (N. X), and hypoglossal nerve (N. XII). The motor patterns of breathing (i.e., lung inflation) and inspiratory call generation are similar: levator muscles of the mouth floor press air into the lungs while the glottis is opened and depressors of the mouth floor generate the expiratory air stream. Vocal behaviour and call timing can be elicited or modulated, respectively, by auditory stimulation so that, e.g., calls are uttered antiphonally in a chorus to avoid acoustic overlap. Accordingly, in an *in-vitro* preparation of the isolated whole brain, motor patterns similar to those of respiration and vocalization can be elicited by stimulation of the posterior (auditory) branchlet of the statoacoustic nerve (N. VIII).

To elucidate the mechanisms of audio-vocal integration we used isolated brain preparations of the Chinese fire-bellied toad, *Bombina orientalis*, and the painted frog, *Discoglossus pictus*, to record responses of single neurons intracellularly within the Medulla oblongata while stimulating the auditory branchlet of N. VIII electrically by a suction electrode. Concomitantly, compound potentials of the motor output of Nn. V, X, and XII were recorded by suction electrodes.

The typical motor pattern after auditory nerve stimulation in the isolated brain starts with the activation of N. XII motor neurons, which *in-vivo* control the depressor muscles of the mouth floor, with an average latency to the onset of the N. VIII stimulus of 104ms. 250 to 350ms later, the N. XII is less active for a short period (<80ms) and then levator motor neurons of Nn. V and XII are activated. Concomitantly, N. X motor neurons fire which control the fictive opening of the glottis. These activities last up to 400ms and after this phase, N. XII motor neurons fire again for 300 to 500ms to activate fictively the depressor muscles of the mouth floor.

Recordings of 185 neurons in areas of motor nuclei of Nn. V, X, and XII (Vmot, Xmot, and XIImot, respectively) revealed that motor neurons showed mean latencies of 413 and 470ms in Vmot and XIImot, respectively, and 1440ms in Xmot. However, 19% of motor neurons in XIImot showed latencies below 100ms. These 'fast motor neurons' could not be found in Vmot and thus can be interpreted as part of the motor neuron population that activates the depressor muscles of the mouth floor. Shorter mean latencies could be found in interneurons (Vmot: 99ms, Xmot: 299ms, XIImot: 48ms). 32.5% of recorded interneurons in XIImot showed latencies below 30 ms (Vmot 16.7%, Xmot 17.1%).

The high proportion of short latencies (<30ms) in XIImot interneurons demonstrate that audio-vocal integration does not exclusively involve higher brain centres such as Torus semicircularis[1] (Colliculus inferior) but is partly realized within the Medulla oblongata. These fast interneurons in XIImot together with the fast activation of the N. XII motor neurons suggest that the XIImot plays a crucial role in controlling the other motor nuclei involved in vocalization and may be an important interface of audio-vocal integration.

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Revealing Excitable Subcortical Networks by Microstimulation-fMRI of the Deep Cerebellar Nuclei

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Electrical stimulation, combined with functional magnetic resonance imaging (es-fMRI), is proving to be an important tool to study the functional properties of spatially distributed neuronal networks of the brain. Here, we want to understand how information is propagated between the two major cortices of the primate brain, the neocortex and the cerebellar cortex. We therefore electrically stimulated the deep cerebellar nuclei of rhesus monkeys. So far we have electrically stimulated 19 different sites in different parts of the deep cerebellar nuclei. Electrical stimulation of the DCN leads to reliable transsynaptic responses in the neocortex. Surprisingly, the BOLD responses can be observed in multiple neocortical sites extending beyond classical cerebellar targets (such as primary motor cortex) and also extending to the hemisphere ipsilateral to the stimulation site. An analysis of the BOLD amplitude in cortical and subcortical structures indicated that the bilateral spread of activity is already present at subcortical levels, i.e. the thalamus. Currently we cannot exclude the possibility that we stimulated fibres of passage that then activated the contralateral DCN and hence contributed to the bilateral neocortical activation patterns. However, the observation of wide-spread BOLD responses in thalamic regions outside the known thalamic termination sites of the DCN indicates that the DCN are able to drive brainstem circuits effectively that then reach neocortex through several thalamic nuclei. These results indicate that, apart from the direct DCN -thalamic projection, indirect routes exist by which the cerebellum can mediate information to the neocortex that may be equally important and effective despite requiring the additional passage through synapses in met- and mesencephalic structures.

Identification of genes mediating the dorsal/ventral choice of sensory and motor axons in the limb

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During development of neuromuscular circuitry in vertebrates, motoneurons (MN) initially extend their axons towards peripheral targets like limb muscles before sensory axons from the dorsal root ganglia (DRG) join the projectory. The axons then navigate an important choice point at the base of the limb to select either a dorsal or ventral trajectory into the limb. Known guidance factors like Sema3F/Neuropilin-2 and ephrinAs/EphA4 control the divisional decision of motor axons, but the molecular mechanism that guide sensory axons remain elusive. Since DRG axon growth is disturbed if motor axons are eliminated it was hypothesized that interactions between these two fiber populations lead to correct targeting of sensory projections. To get a hand on the molecular basis of this behaviour we examined the differential gene expression of ventrally versus dorsally projecting MN and DRG neurons using microarray analysis. We separated these neurons by backfill tracing techniques and FACS analysis. Out of more than 200 candidate genes which display at least a two-fold changed gene expression between ventrally and dorsally projecting neurons, interesting genes were verified by in situ hybridisation and/or immunohistochemistry. Amongst many proteins with unknown functions but predicted cellular localisation important for axon guidance, several transcriptions factors known to be involved in neuronal development displayed a differential gene expression. Additionally, several integral membrane proteins and proteins involved in prominent signalling cascades are differentially expressed as well as factors involved in cytoskeleton formation and stability. Using *in vivo* models and *in vitro* systems, promising candidates found in the microarray and verified by ISH will be examined in detail concerning their role in axon guidance in order to identify distinct key players important to establish the correct sensory-motor circuit in vertebrate limbs.

Analysis of the intersegmental sensory influences in the stick insect walking system

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Walking movements result from a complex interaction of central pattern generating networks (CPG), local sensory feedback about movements and forces generated in the legs and coordinating signals from neighboring limbs. Studies on the behavioral level have shown that coordination between legs of a walking animal rely both on the action of neural signals between neighboring segments as well as mechanical coupling between legs, thereby establishing various rules for coordination between the legs [1]. As yet, the neural basis for the intersegmental interaction in coordination is barely unraveled.

We addressed this topic using a semi-intact preparation of the stick insect with single legs stepping [2, 4] and/or stimulation of specific leg sensors in neighboring segments, i.e. the campaniform sensilla (CS) and the femoral chordotonal organ (fCO). Motor activity was either induced by tactile stimulation of the animal with a paint brush or by means of pharmacological activation of individual segmental ganglia by application of pilocarpine. In the present study we focused on intersegmental influences from the front leg on the activity generated in retractor and protractor motoneurons (MNs) of the middle leg thoraco-coxal (TC-) joint.

Our study yielded the following results:
1. Front leg stepping entrains the active middle leg TC-joint CPG, most probably due to sensory signals emanating from the front leg [3, 4]. The front leg retraction coincides with middle leg retractor activity. We characterized the influence of front leg sensory signals on the active middle leg TC-joint CPG by phase response curves (PRCs). The PRC for front leg steps reveals a strong influence on middle leg TC-joint CPG. The PRCs for front leg CS stimulation and front leg fCO stimulation on the active middle leg TC-joint CPG show a weaker but similar course as the PRC for intact front leg steps. This indicates that signals from both front leg CS and fCO contribute to the observed entrainment of the middle leg TC-joint CPG by front leg steps.

2. We investigated the interplay of intersegmental signals from front leg stepping and local load signals on the middle leg TC-joint CPG by adding stimulation of local sensory feedback to the single front leg preparation [3]. Middle leg CS were stimulated during front leg stepping sequences while middle leg protractor and retractor MN activity was monitored. Protractor and retractor MN activity was influenced both by front leg stepping and middle leg CS stimulation. Front leg stepping induced alternating activity in protractor and retractor MNs. Stimulation of middle leg CS during front leg steps mimicking local load increase induced or increased retractor MN activity, while protractor MN activity was terminated or decreased. Alternating protractor and retractor MN activity could be entrained by CS signals, when the stimulation frequency was similar to front leg stepping frequency.

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Temporal patterning of a vocal pacemaker circuit in fish

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Sound communication is not a trait unique to terrestrial vertebrates, but rather is shared with teleost fish, the largest group of living vertebrates. Many teleosts produce sound by contracting a pair of vocal muscles attached to the lateral walls of their gas-filled swim bladder. Male midshipman fish (*Porichthys notatus*) use this mechanism to generate both broadband and multiharmonic vocalizations with widely varying durations in agonistic encounters and during courtship. Previous studies identified three hindbrain nuclei associated with vocal patterning. Among these, single neuron recording and staining studies have characterized a vocal pacemaker nucleus (VPN) and a vocal motor nucleus (VMN) that ipsilaterally innervates each vocal muscle. The VPN bilaterally innervates the VMN and sets its firing frequency that determines, in turn, the fundamental frequency of natural calls. Here, the functional attributes of a vocal pre-pacemaker nucleus (VPP) that innervates the VPN is characterized for the first time using single neuron recording coupled to neurobiotin filling to investigate its role in generating natural vocalizations.

The VPN-VMN firing pattern determines the temporal attributes of a vocal motor volley that is readily recorded intracranially from vocal/ occipital nerve roots. The motor volley is referred to as a fictive vocalization because its firing frequency and duration directly determines, respectively, the fundamental frequency and duration of natural calls. Fictive vocalizations are induced by either electrical microstimulation or glutamate microinjections in the midbrain. A preliminary analysis of intracellular recordings with sharp electrodes (~40MO) showed a strong temporal relationship between VPP activity and each fictive sound pulse. VPP neurons fired just prior to and hence activated the VPN-VMN circuit that fired, in turn, immediately before the fictive call. VPP activity appeared to further determine the duration of VPN-VMN firing and hence, the duration of natural calls.

Intracellular injection of neurobiotin showed that the dendritic trees of VPP neurons have a bipolar organization, extending both lateral and ventromedial of VPP somata. VPP axons mainly targeted the dendritic area of VPN neurons and some extended to the spinal cord. A subset of VPP neurons projected to the region of the facial and trigeminal motor nuclei, and the octavolateralis efferent nucleus that innervates the inner ear and lateral line organs and is known to be modulated by the vocal motor system. Together, the results suggest that the VPP activates and determines the duration of VPN-VMN firing and hence natural vocalizations, and also informs other hindbrain motor and sensory nuclei of the onset and duration of vocalizations.

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High frequency random noise stimulation modulates levels of cortical excitability in the human motor cortex

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A random oscillating electrical spectrum applied over the human cortex is able to induce sustainable levels of excitability, outlasting the duration of stimulation. 10mins transcranial random noise stimulation (tRNS) applied to the primary motor cortex of over 80 healthy subjects, in combined physiological and behavioural studies, induced an excitability increase of up to 50% lasting 60mins post- stimulation. Further investigation revealed that tRNS is also polarity- independent and relatively focal. tRNS had no effect on paired- pulse measures of cortical excitability (SICI, LICI and recruitment curves) indicating a cortico-spinal level of action. Higher oscillating frequencies (100-640Hz) appear to be responsible for the observable after- effects. Behavioural studies (SRTT) showed that tRNS facilitated early- stage implicit motor learning manifested in reduced response times to the task. The potentiation of voltage- gated ion channels is hypothesised to underlie the mechanism of tRNS action. In addition, the input of 'noise' into a physiological system may provide evidence for the role of stochastic resonance in synchronising oscillators in the human body. Our results indicate that tRNS remains a powerful new tool in the induction of neuroplasticity in the human motor cortex; following other neurostimulation techniques like rTMS and tDCS, and may be a useful therapeutic measure in neurological disorders characterised by disruptions in central processing.

Physiological characterisation of a simply-innervated insect muscle

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Aimed scratching movements of a locust are well compensated against loading, and it has been argued that this compensation cannot be due to high levels of joint stiffness (Matheson & Dürr, 2003, *J Exp. Biol.* 206: 3175-3186). Instead, it has been argued that sensory feedback must modify the motor pattern driving the movements and may modulate joint stiffness during loaded and unloaded movements. This study aims to analyse, both experimentally and through biomechanical modelling, the role of joint stiffness in aimed limb movements of an insect.

We have focussed on the femoro-tibial joint of the locust (*Schistocerca gregaria*) metathoracic leg, and in particular the extensor muscle. We have characterised the active and passives force responses (1) by passive stretch and relaxation of the muscle and (2) through independent stimulation of the single fast and single slow motor neurones innervating the extensor tibiae muscle under isometric and isotonic conditions. The passive forces generated by the muscle revealed visco-elastic muscle properties, with workloop cycling showing increased energy loss at higher cyclic rates. Linear ramp stretches of the muscle elicited phasic-tonic force profiles, with an initial peak followed by relaxation. The tonic force increased non-linearly with increasing muscle length. The muscle generated increasing active force with increasing length over the physiological range of muscle length. Peak force was generated at lengths at or above the physiological range for both fast and slow motor neurone innervation. Tetanus was reached at around 16 and 40 Hz respectively for fast and slow extensor tibiae motor neurone activation. Our results show that a commonly used muscle model (Zajac, 1989, *CRC Crit. Revs. Biomed. Eng.*, 17: 14: 359-411) is not ideal in this case.

Physiological characterisation of the extensor tibiae muscle has permitted further development of our computational model (Zakotnik et al. 2006, *J. Neurosci.* 26: 4995-5007) of the neuromuscular system of the femoro-tibial joint. In additional work we will extend the model to incorporate the effects of the common inhibitor motor neurones and their effects on joint stiffness. Our goal is to use the model to calculate joint stiffness during aimed limb movements based on electrophysiological recordings of motor neurone activity.

Temporal synchrony as critical factor for facilitation and interference of action recognition by self-generated movements

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It has been postulated that the recognition of body movements might be influenced by synchronous internal simulation of the observed motor behaviour. Furthermore, it has been proposed that during self-generated movements efference copies of descending motor commands in conjunction with internal forward models supports the prediction of our own movement's consequences (e.g. Wolpert, 1997). Finally, recent experiments suggest a dependency of self-awareness, i.e. the sense of agency, on proprioception (e.g. Balslev et al, 2007). All these results suggest that the facilitation of action recognition by executed motor behaviour should critically depend on the temporal synchrony between the observed and the executed motor behaviour.

METHOD: To test this hypothesis we presented in a real-time VR setup point-light figures that showed waving movements of the subject's dominant arm, represented by five dots. The displayed movement was either synchronous with the executed movement or temporally delayed by 280 or 550ms. In an additional control condition the same movements were displayed without concurrent motor activity. Recognition performance of 15 subjects was determined in a signal detection task presenting the target action embedded in a scrambled mask or the mask alone. The applied masks consisted of a varying number of scrambled noise dots (seven levels). In a second experiment observers had to discriminate between a left and a right point-light arm embedded in a noise mask, while waving only with their dominant right arm. The shown stimulus was either their actual waving arm ('right') or a mirrored version of it ('left'), both in synchrony with subject's self-generated movements.

RESULTS/CONCLUSION: Consistent with our hypothesis, we found a significant improvement of biological motion detection when the observer executes a compatible action in synchrony with the visual stimulus (facilitation, $t = 3.19$, $p = 0.003$, compared to control condition). If the visual stimulus was delayed relative to the executed action, instead detection performance decreased significantly with increasing delay (ANOVA: $F = 12.77$; $p < 0.001$). For the highest delay (560ms) the detection performance was even worse than without motor activity (interference, $t = 2.48$, $p = 0.01$). Both modulations, facilitation and interference were specific for the ipsilateral arm and could not be observed for the conditions where subjects saw a mirrored version of their actual moving arm. Therefore the detection of simple features like rhythm as an explanation for modulations of the detection performance could be ruled out.
These results support a critical role of relative timing for the interaction between action execution and recognition and are compatible with the hypothesis of a dynamic internal model that is activated synchronously with the visual stimulus and whose predictions interact with the visual stimulus information.

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Role of an inhibitory motor neurone in aimed limb movements

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The limb muscles of the locust, *Schistocerca gregaria*, are innervated by both excitatory and inhibitory motor neurones. The extensor tibiae muscle of the hind leg is innervated by just two excitatory motor neurones, the fast and the slow extensor tibiae (FETi and SETi) and one inhibitor, Common Inhibitor 1 (CI1). In a walking locust, CI1 has been shown to increase the rate of tibial movement during the swing phase of the step (Wolf, *J. Exp.Biol*. 152: 281-304, 1990). We have previously analysed the roles of the excitatory motor neurones in aimed scratching movements, but focus here on the role of CI1. The activity of this single identified neurone was recorded in the central nervous system to understand its role in generating a complex aimed movement. We have developed an experimental protocol that permits us to record and manipulate the activity of CI1 while simultaneously recording the patterns of excitatory motor neurone activity of two key leg muscles (the extensor and flexor tibiae) in animals that carry out natural aimed scratching movements. The movements were characterized using video tracking and kinematic analysis software so that patterns of excitatory and inhibitory motor activity could be precisely related to the resulting movements.

CI1 was active during all recorded scratching movements. It started firing before the excitatory motor neurones SETi and FETi that innervate the same muscle, and before the antagonistic flexor tibiae motor neurones. Firing typically began before the tarsus (foot) lifted off from the substratum, and diminished during the course of a scratch. During cyclical scratching movements consisting of alternating tibial extensions and flexions, there were generally discrete bursts of spikes in CI1.

We report the results of experiments in which the firing rate of CI1 was controlled through the injection of depolarising or hyperpolarising current while the effect on aimed leg movements was monitored.

Our initial findings suggest that CI1 must play an important role in the control of aimed limb movements. Our ongoing research will incorporate the patterns of CI1 activity into a biomechanical model (Zakotnik et al, *J.Neurosci.* 26(19):4995-5007, 2006) of locust aimed limb movements to better understand how regulation of joint stiffness affects movement kinematics.

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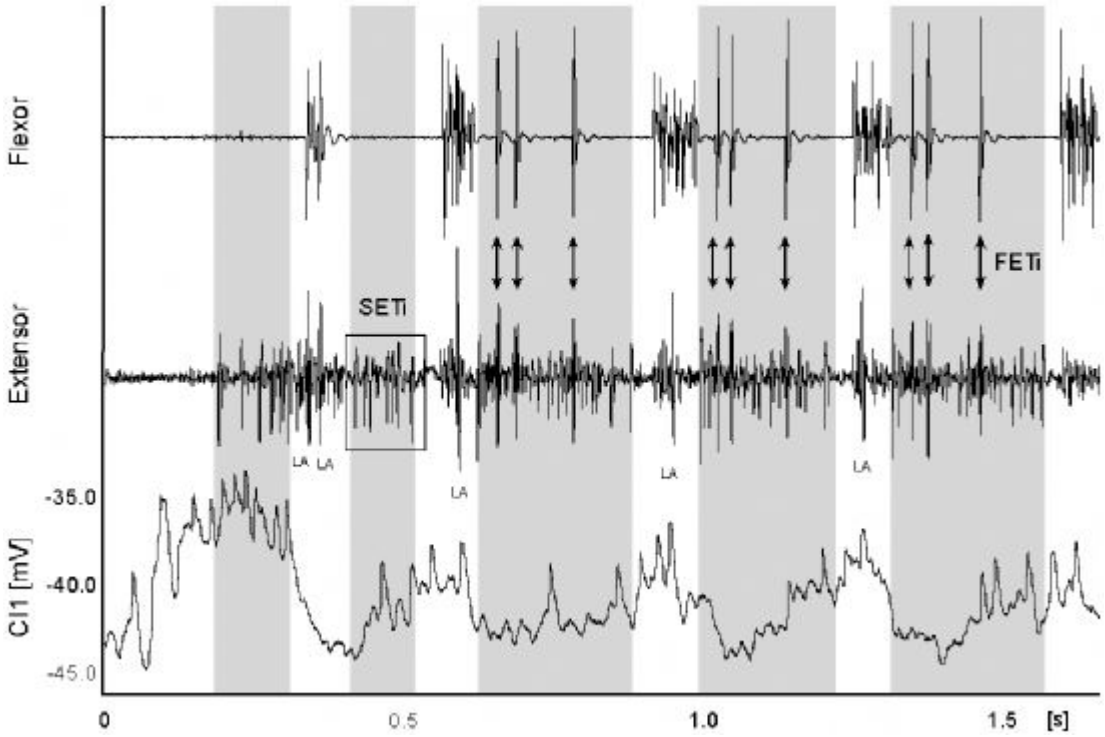
Figure: Sample intracellular recording of CI1 and electromyograms from flexor and extensor muscles during a cyclical scratching movement. Grey shading marks extensor burst activity, with SETi (e.g. box) and FETi (double-headed arrows) firing. FETi spikes are identified by their consistent crosstalk onto the flexor channel. In this particular recording, some flexor crosstalk is evident in the extensor channel coincident with bursts of flexor activity in the flexor channel. One large-amplitude flexor motor spike apparent in this crosstalk is identified by 'LA'. The membrane potential of CI1 is depolarised prior to the first excitatory motor activity, leading to a burst of spikes that overlaps with the first burst of extensor spikes. Subsequent oscillations of CI1 membrane potential lead to further bursts of spikes that generally peak in frequency out of phase with the bursts of extensor activity that drive tibial extension.


Flexor
Extensor
SETi
FETi
LA
LA
LA
LA
LA
-35.0
-40.0
-45.0
CI1 [mV]
0
0.5
1.0
1.5
[s]

Measuring and modelling biomechanical parameters using individual stick insect extensor tibiae muscles

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In Guschlbauer et al. 2007 (*J Exp Biol.* 210 (Pt 6)), we examined about 100 extensor tibiae muscles in order to gain understanding of principle muscle characteristics. In another study, naturally occurring extensor muscle contractions revealed to be highly variable which is not only reflected in the motoneuronal firing patterns during walking but in the muscular output of different animals as well (Hooper et al. 2006, *J Neurophysiol.* 96 (4)). Due to the variability in muscle properties of different animals (e.g. maximal isometric force varies from ~90-180 mN, i.e. ~100%), it is unclear how the complete property set of a specific muscle would look like. To address this problem, one could either use advanced numerical model optimization techniques (e.g. ISOFIT method, Wagner et al. 2005, *Biomech Model Mechanobiol.* 4 (1)) or measure muscle properties directly from experiments on single muscles. We preferred the latter option because the muscle´s activation dynamics are complex and only partly understood. *A priori*, these measurements would be impossible because of the large number of stimulations needed to describe the muscle properties sufficiently. However, our prior work (mostly Guschlbauer et al. 2007) had defined the muscle's basic characteristics. We could therefore reduce the number of required muscle stimulations to a value (37) small enough to perform on individual muscles. Determining all relevant muscle parameters can thus be accomplished by measuring in most significant regions only and using averaged data as a modelling template.

We investigated six extensor tibiae muscles and calculated parameters like maximum isometric force (across-animal range from 121 to 196 mN) or maximum contraction velocity (across-animal range from 5.8 to 7.3 mm/s). These experiments confirmed that the previously observed variation is a reproducible and natural property of the extensor tibiae muscle. In a second step, we adapted model equations derived from the average data to reconstruct each muscle's active and passive force-length characteristic, the relationship between force and contraction velocity, the influence of activation (motoneuron stimulation frequency) on these relations and other crucial properties.

The approach presented here is valuable for direct determination of individual muscle parameters. We are now incorporating these findings into a modified Hill-type muscle model as part of a dynamic simulation of insect locomotion (Ekeberg et al. 2004, *Arthropod Struct Dev.* 33 (3)). The model performance will be evaluated by comparing it with isometric and isotonic muscle responses to real spike patterns recorded during stepping movements, which were also part of the stimulation protocol. We expect the model based on individual muscle data to be superior in performance to the one based on averaged data (a similar issue was discussed in Yu et al. 1999, *Biol Cybern.* 81 (5-6); for a discussion of this problem with respect to building neuron models from averaged ion channel conductance data, see Golowasch et al. 2002, *J Neurophysiol.* 87 (2)).

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Cholinergic currents in identified leg motoneurons isolated from stick insects.

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Acetylcholine (ACh) is an important neurotransmitter in the sensory-motor pathways of many insect species. Bath application of muscarinic agonist pilocarpine on the thoracic nerve cord of the stick insects *Carausius morosus* elicited a tonic depolarization in leg motoneurons (Büschges 1998; Brain Res. 783:262-271). During walking a tonic depolarization in those leg motoneurons was blocked by applications of the muscarinic antagonist atropine (Westmark et al. 2008, submitted). These data indicate that ACh might be a transmitter mediating the tonic depolarization in leg motoneurons of stick insects during walking. So far, it is unclear whether ACh affect the motoneurons directly. To answer this question, whole-cell patch-clamp recordings from acutely dissociated leg motoneurons of stick insects were performed and their cholinergic currents were characterized.

Backfilling the motoneuron axons in the main leg nerve (*nervus cruris*) with a fluorescent dye solution (dextran tetramethyrhodamine) prior to cell dissociation promoted an easily and unequivocal identification of leg motoneuron cell bodies in short term cell culture. In 95 % of the stick insect leg motoneuron cell bodies studied application of ACh elicited inward currents, the amplitudes of which varied among cells. The response to ACh of these motoneurons remained stable for long periods (up to 180 min). Two types of ACh induced currents were observed. Type 1 currents were the most frequent (found in 90 %) and consisted of a transient followed by a sustained component. In type 2 currents only a sustained component was observed (found in 10 % of the ACh responsive cells). The ACh induced currents were abolished in a concentration dependent manner by the muscarinic antagonist atropine or partially nicotinic agonist imidacloprid. The muscarinic agonists pilocarpine, oxotremorine and muscarine, even in high concentrations, did not elicit any currents. ACh induced current was mainly carried by sodium ions. Our results demonstrate that the classification of the cholinergic receptors expressed on isolated stick insect leg motoneuron cell bodies can neither be termed as being classical nicotinic nor muscarinic. This study shows for the first time that ACh has direct effects on stick insect leg motoneurons, suggesting possible role of ACh in the generation of the tonic depolarization on these motoneurons during walking.

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Gain modulation of reach related neurons in the parietal reach region and dorsal premotor cortex of monkeys.

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How does the frontoparietal reach network create a motor-goal representation from spatial sensory information flexibly depending on the current behavioral context? Computational models suggest that an adaptable sensorimotor mapping can be achieved with a context-specific gain-modulation of spatially selective neurons similar to gain-field mechanisms for multi-sensory integration (Brozovic et al. 2007[1]).

To test the predictions of this model we investigated how spatial sensory and motor-goal representations in the parietal reach region (PRR) and the dorsal premotor cortex (PMd) of macaque monkeys are modulated by context-specific spatial transformation rules during reach planning. Spatial transformation rules could be represented either explicitly or implicitly, leading to two different types of context modulation: explicit modulation of neuronal activity independent of spatial tuning, and quasi-multiplicative context-gain modulation of spatially selective neurons (implicit). Both types of context modulation are predicted from our context-gain model, but for different functional layers. In a memory-guided pro/anti-reach task with partial context pre-cuing subjects had to reach toward a previously shown spatial cue (pro-reach) or to an opposite position (anti-reach). The context cue (pro/anti) and the spatial cue were presented simultaneously or at different points in time before and/or after the memory period. In the conditions were both cues were presented before the memory period we could analyze the influence of context on the spatial motor-goal tuning during movement planning. In the condition with only the context cue being shown before the memory period we could analyze explicit context modulation.

We found both types of context-related modulation in PRR and PMd during reach planning in the memory period. While context-gain modulation of spatially selective neurons was very prominent in both areas, explicit context modulation was less common. PRR showed stronger motor-goal tuning for pro-reaches. Motor-goal tuning in PMd and explicit context modulation in either area did not show a preference for a specific context. Context-gain modulation and explicit context modulation were uncorrelated in PMd neurons and only weakly correlated in PRR.

The prevalence of context-specific gain-modulation in PRR and PMd suggests that in the frontoparietal reach network flexible spatial sensorimotor remapping is achieved by a mechanism equivalent to gain-modulation.

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Subcellular localizations of intraflagellar transport (IFT) molecules indicate differential ciliary and novel non-ciliary functions in retinal neurons

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Intraflagellar transport (IFT) proteins are required for the assembly and maintenance of cilia and flagella. Nevertheless, the spatial distribution of IFT proteins (IFTs) within distinct ciliary and other subcellular compartments remained so far elusive. We evaluated the subcellular localization of individual IFTs in the mouse retina using a combination of high resolution immunofluorescence and immunoelectron microscopy. All IFTs were expressed in the ciliated retinal photoreceptor cells. The IFTs were differential localized in sub-compartments of the photoreceptor ciliary apparatus; some IFTs appear in the axoneme in the outer segment and in different parts of the connecting cilium, all were highly concentrated at the periciliary region around the basal body within the inner segment where they were associated with transport vesicles. IFT20 was additionally associated with the Golgi apparatus of the photoreceptor inner segment and with Golgi stacks of bipolar and horizontal cells. Furthermore, a set of IFTs was localized in dendritic processes of non-ciliated neurons of the retina, providing evidence for cellular functions of IFTs other than ciliary function.

Recruitment pattern of motoneurons and interneurons in the spinal locomotor network of adult zebrafish

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The flexibility of locomotor networks allows animals to move over a large range of speeds. In zebrafish (Danio rerio), the myotomal musculature is innervated by two types of motoneurons: large, dorsally located primary motoneurons are recruited during fast swimming, whereas the smaller, more ventrally located secondary motoneurons are recruited already at low swimming frequencies (Liu & Westerfield, 1988). In larval zebrafish a topographic recruitment of both interneurons and motoneurons has been proposed, and different populations of interneurons drive the swimming pattern at different frequencies (McLean et al., 2007). We were interested in the maturation of this pattern and investigated how intrinsic properties of motoneurons and the synaptic drive by different groups of premotor interneurons contribute to motoneuron recruitment in juvenile and adult zebrafish.

Using an in-vitro brainstem-spinal cord preparation, we performed whole-cell patch clamp recordings from identified neurons. When fictive locomotion was elicited by electrical stimulation of the rostral spinal cord, the membrane potential of both primary and secondary motoneurons displayed a tonic depolarization and superimposed phasic modulations. For a given swimming frequency, the membrane potential modulations had a larger amplitude in secondary than in primary motoneurons and increased in both types of motoneurons during faster swimming. While the modulation amplitude increased linearly with swimming frequency in secondary motoneurons, it did not increase in primary motoneurons until higher swimming frequencies were reached.

While the modulation amplitude can be influenced by intrinsic properties (e.g. input resistance), the asymmetry in the recruitment most likely reflects a difference in synaptic drive from premotor interneurons to primary versus secondary motoneurons. We investigated this by recording from identified glycinergic and glutamatergic interneurons. Here, a similar recruitment pattern could be observed, which shows that different groups of interneurons provide the synaptic drive to specific motoneurons at different swimming speeds.

Arm Stiffness and Movement Control

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The organization of unconstrained arm movements in humans appears to be determined essentially by the biophysical properties of the limb [1] such that the effects of the underlying neural control cannot be discerned without an interference with the natural movements. The stiffness of the arm with respect to a perturbing force [2] is an established way to reveal information about the control mechanisms. We approach the problem by analyzing experimental measurements of stiffness in human subjects and by simulations of emergent control of a model of the human arm.

We studied properties of human arm movements in a four-DoF (wrist-restrained) setting in three spatial dimensions using a high-performance haptic device (SensAble Phantom 3.0 6DoF). Stiffness was measured based on the equilibrium point hypothesis by the motor response to a perturbing force and in dependence on the spatial position of the arm. A detailed statistical analysis of the data at each position provided us with reliable estimates of the stiffness tensor in the sagittal plane that was chosen in order to complement existing measurements of 2D stiffness [2]. The obtained stiffness estimates in three-dimensional workspace can be related to inertial properties of the particular arm configuration. In a second experiment constant force fields of various strengths have been applied in addition to the perturbing forces. The resulting stiffness ellipsoids appear as a near-linear superposition of the force field with the force-free stiffness for the main workspace of the subject. Further experimental work has been devoted to the evaluation of the experimental paradigm. If the perturbing force is ramped up to its final value with a time constant between 500 and 1000 ms a robust estimation of the stiffness is possible provided that the resulting displacements remain small. For hard ramps or very slow ramping the stiffness became less reproducible.

In the model control is achieved by a standard PID control algorithm that is used to control a four DoF model of a human arm. Perturbations of the controlled system can be applied result in the same way as to the human subjects, although the time scales had to be readjusted. The resulting stiffness measurements lead to comparable results both for the force-free and the force-field situations. The measurement of the stiffness allows us to derive a common explanation for the control-related aspects of movements in experiment and simulation which is potentially applicable to prosthetics and compliant robot control.

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Tyramine and octopamine, a transmitter pair of specific action in insects.

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Evidence from studies on *Caenorhabditis* and *Drosophila* suggest that tyramine and octopamine play their own independent role as transmitter. As octopamine has to be synthesized from tyramine, and as the octopaminergic system in insects is well known, we used respective antibodies to study the distribution of tyramine and octopamine in the locust nervous system. In the brain, the suboesophageal ganglion, and all fused thoracic and abdominal ganglia we found a small number of neurons that were only labeled by the tyramine antibody under all behavioural treatments that we tested. In contrast, the known efferent octopaminergic neurons of thoracic ganglia show various amounts of tyramine depending on how animals were treated before the experiments. Those kept undisturbed before dissection expressed roughly equal amounts of tyramine- and octopamine-immunoreactivity in their cell bodies and varicose terminals, whereas those that underwent stressing stimuli for about 10 min expressed high levels of octopamine-immunoreactivity and hardly any tyramine-immunoreactivity in their cell bodies. In the latter group of animals, however, the primary and secondary neurites expressed high levels of tyramine-immunoreactivity. Thus, behavioural treatment can alter the level of tyramine and octopamine in identified neurons that play a role in modulation of motor systems. In order to test specific effects of tyramine and octopamine on a well studied motor behaviour we induced fictive flight in an isolated *Manduca* central nervous system by chlordimeform, an octopamine-receptor agonist. When applying yohimbine, believed to be a tyramine-receptor antagonist, only the depressor system was affected while the elevator system continued to function almost unaffectedly. Consequently, application of tyramine itself increases the activation of depressor neurons that now fire in bursts rather than single spikes. Thus, we conclude that octopamine and tyramine may have different targets in motor networks and, thus, can be used in differential modulation of specific subsystems of networks.

Dopaminergic Signaling in the Central Complex Promotes Sound Production

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The catecholamine dopamine belongs to the group of biogenic amines. Dopamine is a potent neuromodulator that affects various behaviors in invertebrates. Another important function of dopamine is the regulation of arousal states. It could be shown for invertebrates, that increasing dopaminergic signaling leads to hyperactivity and increased sexual arousal). Studies on *Drosophila* used systemic injections of drugs that alter dopamine transmission and therefore no conclusion about the anatomical location of the circuits in the brain that underlie these arousal mechanisms could be made.

The song production of grasshoppers has been proven to be a suitable model to investigate the neurochemical basis of arousal at distinct anatomical locations. Grasshoppers use their song for mate find as well as during courtship and agonstic behavior. Studies on the grasshopper*Chorthippus biguttulus* identified a number of different neurotransmitters and second-messenger pathways that contribute to the cephalic control of sound production. These studies identified the central complex (CX) as a potential major control neuropil that determines the timing and selection of sound patterns.

This study shows that dopaminergic signaling in the central complex of the grasshopper*Ch. biguttulus* increases sexual arousal. This could be shown by the fact that injections of dopamine into the central body induce courtship songs. Furthermore, dopamine seems to be constantly released into the central body, which is indicated by the finding that blocking of dopamine transmission in the central body reduced muscarine-dependent sound production. Dopamine containing cells could be detected in both subdivision of the central body and originated from three types of tangential neurons.

Grasp context representation in macaque parietal area AIP

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When grasping an object under visual guidance, a sensorimotor transformation is performed to shape the hand according to the form of the target. In addition, there is often a choice between several ways to grasp a given object, a selection usually made according to the purpose or context of the action. The anterior intraparietal area (AIP) of macaques has been shown to represent 3D-features of graspable objects that are important for the hand preshaping. Here we investigate whether AIP also represents abstract context information that is relevant for the choice of grip type.

Two macaques were trained to grasp a single handle with one of two grip types and in one of five orientations. The grip type was instructed to the animals using a non-spatial cue (colored LED), which served as context information. Different target orientations represented an object-inherent spatial variable. In a first paradigm, the *delayed grasping task*, the two cues were given simultaneously, followed by an instructed delay period (dividing the task into *Cue, Memory and Movement* epochs). In a second paradigm, the *cue separation task*, the two instructions were presented sequentially; either orientation first, followed by grip type (OT task) or vice versa (TO task).

We recorded 571 single cells in the first paradigm. A 2-way ANOVA showed different patterns for the two variables: orientation was represented by a constant number of cells across all epochs (cue: 357, memory: 337, movement: 360), whereas the number of grip type selective cells increased during the course of a trial (c: 199, p: 268, m: 369). More detailed analysis of the time course (moving window, width 200ms, step 50ms) revealed that the cell population represents orientation approximately 150 ms before grip type. Furthermore, grip type selective cells fall into two distinct groups: one becoming active after cue presentation, the other only during movement execution.

120 cells were also recorded in the cue separation task, which revealed some differences between grip type and orientation representation: orientation was fully encoded when presented as first cue (OT-task). However, in the TO-task, the abstract grip type instruction (in absence of target orientation) was represented in AIP only in few neurons, until the addition of the orientation information strongly increased the representation of grip type.

Together, these results demonstrate that AIP represents different grip types when performed on the same object. Furthermore they indicate that the abstract instruction of a grip type can be represented by AIP, but that this representation is reinforced when an actual grasp target is presented in succession to the context instruction.

Mating behavior of female *Chorthippus biguttulus* and its modulation by juvenile hormone

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Earlier studies with female grasshoppers of the species *Chorthippus biguttulus* (L.) suggested that they mate infrequently and generally very rarely during their life. According to these studies, the first copulation occurs in a time window between six and eleven days after imaginal molt in order to fertilize eggs before the first oviposition at about two weeks after imaginal molt. After this first mating, females were thought to remain unreceptive to males' courtship and mating attempts for several cycles of oviposition.
We observed groups of males and females beginning with the females' imaginal molt until the first oviposition and found a clearly different mating behavior than previously reported. Females generally signaled copulatory readiness by song production starting at six days after imaginal molt and copulated multiple times before their first oviposition. All females reassumed copulatory readiness including song production independently of copulation duration and spermatophore reception, some already within one to two days after preceding copulations. Juvenile hormone (JH), an important regulator in insect development that is released from the corpora allata, has also been demonstrated to influence mating behavior and ovarian development of adult females. Chemical allatectomy with precocene I suppressed JH-production *Ch. biguttulus* females and reduced their stridulatory activity. Radioimmunoassays detected lower hemolymph titers of JH in recently mated compared to virgin females of the same age. But directly after the imaginal molt when females are not receptive, the JH titer was higher than in older virgin females that signalled copulatory readiness through song production. Thus it appears that high JH titers are not always correlated with high receptivity of females and the contribution of JH to the receptive behavioral state may be more complicated than previously thought.

Pituitary adenylate cyclase-activating polypeptide (PACAP) modulate the activity of coelomocytes during the regeneration of the ventral nerve cord ganglia in the earthworms

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Recently we have shown the accumulation of PACAP-like compound expressing coelomocytes (thought to be homologous with vertebrate white blood cells) in severed and renewed body segments of the regenerating earthworm *Eisenia fetida*. Coelomocytes are believed to be involved in the regeneration process. Coelomocyte are helping tissue remodelling by phagocytosis of debris materials and transporting certain hormones and nutritive materials to regenerating segments. The regeneration of the ventral nerve cord ganglion and peripheral tissues was investigated by histology, immunohistochemistry and radioimmunoassay in *Eisenia fetida*. The presence of PACAP-like compounds and the expression and ultrastructural distribution of PAC1-receptors in severed and regenerating ventral nerve cord ganglia and in coelomocytes located in severed segments and rest of the body have also been investigated. PAC1 receptor-like molecule was detected on the surface of a distinct coelomocyte subgroup by flow cytometry. Related to functional analysis freshly isolated coelomocytes from intact animals were loaded with Fluo-3AM and treated with ionomycin and different concentrations of PACAP38 for Ca^{2+}-influx measurements. We observed an increase of intracellular Ca^{2+} level after the PACAP38 administration.

The most characteristic morphological feature during caudal regeneration was the accumulation of coelomocytes and neoblasts (thought to be totipotent stem cells in earthworms) in the injured segments. High number of coelomocytes accumulated in the damaged segment and partly attached to severed surface of the body wall, gut and ventral nerve cord ganglion. Attached coelomocytes showed phagocytotic activity and tissue debris was often found their phagosomes. Radioimmunoassay (RIA) revealed an up-regulation of PACAP-like compounds in both ventral nerve cord ganglia and coelomocytes during regeneration. The concentrations detected showed a decreasing rostrocaudal gradient in coelomocytes, suggesting that they could synthesise and transport PACAP-like compounds to regenerating tissues. PAC1-receptors were found on a distinct type of coelomocytes (amoebocytes characterised by phagocytotic activity). By means of western blot and far western blot analysis PAC1-receptor proved to be a 45 KDa molecular weight protein. Electron microscopic immunocytochemistry showed that PAC1-receptors are located on distinct set of coelomocytes (amoebocytes and some granulocytes). Immunogold particles attached to plasma membranes and intracellular membrane fractions (endoplasmic reticulum and limiting membranes of vacuoles and granules). The present results reveal a link between PACAP and coelomocytes suggesting that it modulate the function of amoebocytes and certain granulocytes that play a role in tissue remodeling of regenerating earthworms.

Is the medial neurosecretory brain region of the earthworm the anatomical correlates of the pars intercerebralis?

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Hormonal control of reproduction and regeneration in *Eisenia fetida* (Annelida, Oligochaeta) has been experimentally demonstrated suggesting that the cerebral ganglion (so-called brain) synthesises certain peptides namely gametogenic factor and the cephalic regeneration inhibitor. By means of immunohistochemistry occurrence of some vertebrate neurohormone-like compounds, like substance-P, cholecystokinin, pituitary adenylate cyclase activating polypeptide (PACAP) in various neurons of the earthworm brain has also been described. Recent work focuses on the histological and ultrastructural characterization of those neurons of the earthworm brain that synthesises FPRLamids and FVRLamids resembling insect neuropeptide families such as periviscerokinins (PVK) and pyrokinins (PK). In insects PVKs and PKs typically expressed in neurosecretory cells and released by neurohaemal organs into the hemolymph. Earthworm brain is comparatively simple in structure containing high number of neurosecretory cells located in various brain regions but no anatomical correlates of pars intercerebralis, characteristic of polychaetous annelids and arthropods, has been identified yet.
A distinct neuron group stained for FPRL- and FVRLamides was situated dorsoposteriorly on the brain - named medial neurosecretory brain region (MNS) - close to the dorsal cerebral blood vessel. Each cell had round or polygonal, medium size soma and a short process that passed into deeper brain region and reached the wall of capillaries identified as the neurohemal region of the brain. Ultrastructural observations revealed that the perikarya contained numerous dense granules that often occurred in clusters separated by granular endoplasmic reticulum cisternae. A few dilatated cisternae of smooth endoplasmic reticulum, well developed Golgi complex and a few autophagous bodies were typically appeared. Cytoplasm was rich in free ribosomes, mitochondria and glycogen rosettes. High number of granules was seen in axon hillock and the end foot of neurons was tightly packed with dense and grey granules. Exocytosis from perikarya seems to be a release mechanism for only some granules, however most of them released through synapses or was liberated extrasinaptically from neural profiles to the neuropile. Omega profiles, thought to be characteristic of neurosecretory cells, frequently occurred also in end foot of neurons attached to blood vessels. Based on the morphological characteristics of the stained neurons we could propose that medial neurosecretory brain region of the earthworms is the anatomical correlates of pars intercerebralis found in polychaetes and arthropods.

Expression of small heat shock proteins in the rat brain

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Heat shock proteins play a major role in the development of stress tolerance, which is essential for the survival of an organism. The family of small heat shock proteins (sHsps) comprises 11 members in mammals (HspB1 to HspB10 and Hsp16.2) characterized by low molecular weight (12 - 30 kDa) and displaying chaperone function. Their cytoprotective function in heart and skeletal muscle is well documented. Evidence for a neuroprotective function of sHsps comes from investigations of neurodegenerative diseases as Alzheimer and from some peripheral neuropathies caused by mutations of HspB1 and HspB8. The expression pattern of all 11 sHsps has not been investigated systematically in the mammalian brain. Thus, we measured their mRNA level in cortex, cerebellum, striatum and hippocampus of adult rats by real-time RT-PCR. HspB5 and HspB6 were found to be expressed at highest level (6 - 47 % of the geometric mean of the reference genes CycA and Rpl13A) in all brain regions. Lower expression (0.4 – 1.7 %) was found for HspB1, B8 and Hsp16.2 in all brain regions. HspB3 was only expressed in cortex (0.19 %) and cerebellum (0.48 %). HspB2, B4, B7, B9 and B10 were not expressed in any brain tissue tested. In addition, we investigated the expression of all sHsps in the developing rat hippocampus. HspB1, B5, B6, B8 and Hsp16.2 were expressed in embryonal (E18 - E20) and postnatal (P4 + P6) stages. The expression of HspB1, B5, B6 and HspB8 was significantly higher in adult animals (9 weeks) than in embryonal and postnatal stages while the Hsp16.2 expression was unchanged. In conclusion, we could show that six sHsps are expressed in various regions of the rat brain and therefore may play a role in neuroprotection. Their precise function needs to be further investigated.

Paternal care is critically involved in the development of Corticotropin Releasing Factor (CRF)-expressing neurons in the rodent orbitofrontal cortex, amygdala and hippocampus

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In a variety of species including humans, both, the mother and father engage in the upbringing of the offspring. So far, the majority of experimental studies have focussed on the contribution of maternal care on the development of the brain and behavior of their offspring. We have established the biparental rodent *Octodon degus* to test the hypothesis that paternal care exerts a critical impact on brain development of his offspring, in particular prefrontal cortical and limbic areas, which are essential for cognition and emotional regulation.

Here we show that the development of central neurons, which express the corticotropin-releasing factor (CRF), is in a region- and age-specific manner affected by the experience of paternal care in the orbitofrontal cortex, the amygdala and the hippocampus. Comparing the density of immunocytochemically identified CRF-expressing neurons revealed that juvenile preweaning degu pups (postnatal day 21) which were raised with their father display reduced densities of CRF-containing neurons in the orbitofrontal cortex (OFC) (lateral OFC: -69%, ventromedial OFC: -69%) and in the basolateral amygdaloid complex (-44%). In contrast, in the dentate gyrus (+115%), and the innate dentate granule cell layer (+52%) preweaning animals reared with their father showed increased densities of CRF-containing cells. In all analyzed regions these neurodevelopmental effect of paternal care appear to be *transient* since they are no longer detectable in adulthood (postnatal day 90). Additionally, irrespective of age biparentally reared animals displayed a) lower CRF cell densities in the lateral and ventromedial OFC and in the BLA and b) enhanced CRF cell densities in the hippocampal dentate gyrus as well as the pyramidal and oriens cell layer of the CA1 region compared to father-deprived animals. Also, regardless of rearing condition, in mostly all analyzed regions juvenile animals showed higher CRF cell densities compared to adults. The central amygdala, where we found dense clusters of CRF-immunopositive neuropil, was not altered in consequence of the availability of paternal care.

Taken together, this is the first evidence that paternal care induces temporary but not long lasting changes of stress-related CRF neurons in central prefrontal and limbic neuronal systems in the brain of his offspring.

Peptide quantification in direct mass spectrometric tissue profiling: a case study in prohormone convertase 2-deficient *Drosophila*

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Matrix-assisted laser desorption ionization- time-of-flight mass spectrometry (MALDI-TOF MS) is increasingly used to quantify changes in the abundance of peptides and proteins in proteomic studies. Although prone to inherent variabilities, several studies have convincingly demonstrated that it can be used to (semi)quantify peptides and proteins in tissue extracts if specific standards are met and stable isotope-labelled standard peptides are used as calibration standards.

In previous studies, we have used direct MALDI-TOF MS profiling of nervous and endocrine tissue to characterize the neuropeptidome of the fruitfly Drosophila. The preparation and embedding of whole tissues in matrix salts, however, adds a further source of sample variability that is not encountered in the analysis of tissue extracts. Nevertheless, direct MS profiling of tissues holds significant advantages over the standard analysis of tissue extracts. Perhaps most important, it can be used to analyse tissues from single flies and does not rely on pooled samples.

We now investigated whether it is possible to combine the advantages of direct MALDI-TOF MS tissue profiling with peptide (semi)quantification. We relied on fruitflies deficient in the gene amon that encodes prohormone convertase (PC) dPC2. These flies show defects in growth and carbohydrate metabolism, suggesting that a deficiency in amon interferes with the production of adipokinetic hormone (AKH) in glandular cells of the adult corpora cardiaca (CC) [1].

Standard curves with dilution series of isotope-labelled AKH show that the native AKH peptides can be (semi)quantified by direct peptide profiling of the CC. With this method, we next quantified the amount of AKH and AKH intermediates in control and amon-deficient flies. We found that the level of AKH is significantly reduced in amon-deficient flies, a phenotype that can be rescued by heatshock-induced ectopic expression of dPC2.
In our study, we observed higher standard deviations than reported for extracted peptides. These deviations appear to only partly be caused by differences in the preparation. We are currently testing the contribution of naturally fluctuating AKH levels between individual flies to the observed variability.

Our results so far suggest that amon is a key enzyme in the production of Drosophila AKH and other neuropeptide hormones. Direct mass spectrometric profiling of tissue appears to be a quick and suitable technique to investigate the role of PCs in the production of neuropeptide hormones. It still has, however, to be investigated whether it will also be suited to quantify physiological fluctuations or RNAi-induced concentration changes.

[1] Rhea et al. (2007) 48th Annual Drosophila Research Conference, Philadelphia, PA, USA. Poster 619A.

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LOW DOSE HEXABROMOCYCLODODECANE SUPRESS THYROID HORMONE RECEPTOR- MEDIATED TRANSCRIPTION.

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Hexabromocyclododecane (HBCD) is a nonaromatic, brominated cyclic alkane used essentially as an additive flame retardant in thermoplastic polymers, textile coatings, cables and unsaturated polyesters. It may bioaccumulate and persist in the environment.We investigated the effect of HBCD on thyroid hormone receptor (TR)- mediated transcription using transient transfection – based reporter gene assays. Doses as low as $\mathbf{10^{-8}}$M HBCD suppressed T3 – induced transactivation by TR in CV-1 cells by 30%. We further examined the effect of HBCD on TR- thyroid hormone response element(TRE) binding, and found a partial dissociation of TR from TRE . Interestingly, HBCD also recruited co-activators (steroid receptor co-activator 1; SRC-1) to TR in CV-1 cells but did not recruit co-repressors(nuclear co-repressor; NCoR, silencing mediator for retinoid and thyroid hormone; SMRT) to TR in CV-1 cells. The effect of HBCD on developing brain using primary culture of rodent brain cells are under investigation. In summary, our results shows that low dose HBCD can potentially disrupt TR – mediated transactivation, suggesting that HBCD can interfere with thyroid hormone homeostasis in target organs including the developing brain.

Individual cortisol profile associates with performance in academic examinations

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Enhancement of glucocorticoid levels impairs memory retrieval. Because performance in academic examinations depends in part on the successful retrieval of declarative memory, we hypothesize that individuals with a strong cortisol response are at risk of getting bad grades in examinations.
We tested this hypothesis in a sample of psychology students taking a final examination in an introductory statistics course. Prior to the examination, students were tested for their basic algebraic abilities (one-digit multiplications, two-digit subtractions, multi-digit divisions, calculation of percentages) and mathematics anxiety. Saliva was collected (i) two months before, (ii) a few minutes before, and (iii) a few minutes after the examinations and cortisol levels were quantified. We distinguished four different cortisol profiles depending on the change in saliva cortisol levels before and after the examination.

Most participants responded either with an increase in cortisol before and a decrease after the examination (A-profile, 52%) or with a decrease in cortisol level before as well as after the examination (D-profile, 42%). A minority of students showed either a decrease before and a minor increase after the examination (L-profile, 4%) or non-significant increases before or after the examination (C-profile, 2%). Predictors of performance in the examination depended on the cortisol profile. Only in participants with an A-profile, mathematics anxiety and arithmetic abilities predicted statistics performance. The relationship between the cortisol increase before the examination and performance in the examination was tested in participants with an A-profile. We found indications that the relationship between statistics performance and the cortisol increase in participants with an A-profile cannot be modelled by a linear, but by a quadratic relation.

ELUCIDATION OF MELATONIN METABOLIC PATHWAY INVOLVEMENT ON AGING OF *ACHETA DOMESTICUS* IN INSECT LINES SELECTED FOR FAST- AND SLOW-DEVELOPMENT

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Melatonin (MEL) regulates a wide range of physiological and behavioral events including aging in vertebrates. MEL has a geroprotective effect on *Drosophila melanogaster*. When introducing MEL with culture medium Izmaylov et al. (1996) observed that life span of wild type fruit fly significantly increased. In the following studies Bonilla et al., (2002) has shown an increase in life span of more than 30% in *D. melanogaster* if the insects were fed with MEL. Those reports are therefore instructive and lead us to test the hypothesis that MEL may be beneficial in aging in animals.

For the past six years at the University of Silesia we were able to select two lines of house crickets, *Acheta domesticus* that differ in their life span. Selection for early and delayed reproduction has given rise to lines with substantial differences in longevity. Those exclusive lines are therefore valuable model to study aging in insects.

The aim of this study was to understand the nature of the biochemical and physiological variations between different lines of *A. domesticus*. Using fast- and slow development lines of crickets we challenged to clarify whether *N*-acetyltransferase (NAT), MEL, and MEL receptor (MT) may vary in lines of insects with different life span.

Ageing can have deep effect on the brain, however the defined causes of the deceleration are not known. The immunocytochemical comparison of brains from two lines of insects with long-and short development was done. We analyzed cellular distribution of NAT, MEL and MT antigens and observed difference at the selected protein level.

We will also indicated the possible direct involvement of MEL when applied with water on rhythmic activity of adult crickets from both lines. To analyze the differences in rhythmic activity we used the open field method and recorded movement of animals at chosen time intervals after the transfer to measurement chamber. We measured the distance of insects movement. Application of MEL induced the rhythmicity in animals from slow-development line.

Izmaylov D., et al. (1996). Geoprocector effectiveness of melatonin: investigation of lifespan of *Drosophila melanogaster.* Mechanisms of Aging and Development, 155-164.

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The role of noradrenaline within the perirhinal cortex in stress-induced potentiation of the startle response.

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Exposure to a stressor elicits physiological and behavioral changes. These changes are accompanied by noradrenaline release in multiple brain regions. The present study examined the potential relevance of noradrenaline in the rostral perirhinal cortex (rPRh) for the behavioral changes following electrical foot shock-induced stress. In the first experiment, the rPRh of rats was lesioned bilaterally by local microinjections of 10 mg N-methyl-D-aspartic acid (NMDA) before foot shock administration (0.7 mA, 1 s). The effects of these lesions on foot shock-induced potentiation of the startle response was tested 30, 90 min, 3, 6 and 9 h after foot shock administration. Sham-lesioned rats showed a potentiation of the startle amplitude. The strongest potentiation could be observed 30 and 90 min after foot shock administration. This potentiation was blocked by neurotoxic lesions of the rPRh. Furthermore, rPRh lesions did not affect the startle amplitude in unstressed condition.

The second experiment focussed on the release of noradrenaline and serotonin in the rPRh following foot-shock stress measured by microdialysis technique. After the quantification of the basal extracellular monoamine concentrations in the rPRh, the rats received 10 electric foot shocks (0.7 mA, 1 s).The stress administration increased the noradrenaline release with a maximum 20 min after foot-shock administration. In contrast, there were no changes in the serotonin release during the whole test time (2 h).

In summary, the data indicate that the rPRh is crucially involved in neurophysiological mechanisms that mediate the potentiation of defensive reactions induced by foot-shock stress. Furthermore, foot-shock stress administration elicits an early noradrenaline release within the rPRh.

Insulin in the brain promotes locomotor activity in lean

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Insulin is required for a variety of regulatory functions in multiple tissues such as induction of glucose uptake in skeletal muscle, suppression of hepatic glucose production, and inhibition of lipolysis in adipose tissue. In addition to the classical insulin-sensitive tissues, the brain emerged as a major target of insulin action in rodents as well as in humans. There is sufficient evidence from mouse models that alterations in the insulin signaling pathway in the cerebrum are accompanied by an obese phenotype. Recent work of our group demonstrated by magnetoencephalography (MEG) measurements that insulin modifies cortical activity in lean humans, while obese subjects displayed insulin resistance. Despite extensive research on insulin action in the periphery, the impact of insulin resistance in the brain with regard to cortical activity and behavioural aspects and its modulation by adiposity-related signals are largely unknown.

Here, we show that intracerebroventricular insulin application into lean mice was accompanied by a profound increase in cortical activity in the slow frequency range estimated by electrocorticogram (ECoG) recordings, while diet-induced obese mice displayed insulin resistance. In parallel, insulin application into the brain was accompanied by an increase in locomotor activity in lean, whereas a phosphatidylinositol 3 kinase (PI 3-kinase) inhibitor or adiposity-related factors abolished insulin-mediated locomotion performed by the animal. In parallel, the insulin-induced desire to move was tightly correlated with body weight and cortical activity as determined by magnetoencephalography in human subjects that underwent a hyperinsulinemic-euglycemic clamp. Mechanistically, a potential candidate that links insulin action to locomotion is the voltage-gated, margatoxin-sensitive potassium channel Kv1.3 that is activated by PI 3-kinase. In accordance with this concept, pharmacological inhibition of Kv1.3 channels bypassing insulin receptor activation promoted activity in lean and obese mice, and therefore represents a novel target to increase locomotion. In conclusion, our data provide functional evidence for a direct effect of insulin on brain activation patterns associated with locomotor activity in lean, while in obese, insulin-dependent locomotion is blunted and further aggravates physical inactivity.

This research has elucidated the insulin signaling cascade as a key mediator that couples the metabolic state to electrical activity, and finally locomotion, and therefore closes the gap between insulin-mediated changes in electrocortical activity and its behavioural consequences to prevent obesity.

Gender- and genotype-dependent differences in stress reactions in mice deficient for the serotonin transporter

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Serotonin (5-HT) is an important modulator of many physiological, behavioural and developmental processes and is discussed to play an important role in coping with stress. Anxiety disorders and depression are stress-related disorders associated with disturbances of the serotonergic system, where the serotonin transporter (5-HTT) plays an important role. 5-Htt knockout (KO) mice represent an artificially hyperserotonergic environment and show an increased anxiety-like behaviour. Furthermore, it could be shown that female 5-Htt KO mice display exaggerated adrenomedullary responses to stress. Therefore, these mice seem to be a good model to investigate the role of the serotonergic system in the context of stress reactions and anxiety disorders.

As we recently showed acute immobilization resulted in significantly increased plasma corticosterone levels in mice of all 5-Htt genotypes compared to unstressed mice, and in general higher corticosterone levels were detected in females compared to males, as it is known from the literature. Interestingly, we revealed genotype-dependent differences of corticosterone levels only in stressed males but not in stressed female mice.
Based on these gender- as well as genotype-dependent differences in stress reactions in the periphery we looked for possible expression differences in the brain. As synaptic proteins modulate the release of neurotransmitters into the synaptic cleft and are shown to be involved in stress reactions we studied the effects of acute immobilization stress on the expression of synaptic protein such as Synaptotagmin (Syt) I, Syt IV and Syntaxin (Stx) 1a. In addition, we studied the expression of the two immediate early genes (IEGs) FBJ osteosarcoma oncogene (c-Fos) and fos-like antigen 2 (Fra-2) as markers for neuronal activity. We performed a quantitative real time-PCR study in five different brain regions of female/male 5-Htt KO and wildtype (WT) control mice. Acute immobilization stress primarily resulted in increased expression of Stx1a in hypothalamus of male 5-Htt KO mice and in hypothalamus and hippocampus of unstressed compared to stressed female WTs. Interestingly, Stx1a is discussed to interact with the 5-Htt and to modulate the cell-surface expression of this transporter. There were also gender-dependent differences in the expression of Stx1a and Syt IV, mainly in cortical areas. Regarding the expression of c-Fos we found stress- as well as gender-dependent differences primarily in the amygdala, hypothalamus and raphe. Gender-dependent expression of Fra2 was mainly found in cortex and hypothalamus. In general, expression differences were mainly found in brain regions which are involved in anxiety circuits and / or emotion.

In conclusion, besides the increased anxiety-like behaviour of mice deficient for the 5-Htt, there exist not only 5-Htt-genotype-dependent differences but also gender differences regarding the responses to acute stress in the periphery (as demonstrated by plasma corticosterone levels) and in the brain (as demonstrated by altered expression levels of synaptic proteins and IEGs).

The phylogeny of blattopteran insects: neuropeptides as a new character set

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To establish neuropeptides as a novel character set, we examined a number of neuropeptides, namely Capa-peptides, adipokinetic hormones, sulfakinins and pyrokinins (altogether 13 peptides) from more than 100 insect species. The sequences of these peptides are highly conserved due to the constraint of fitting into their receptors. For that reason they are very good suited for phylogenetic analyses.

After dissecting single neurohaemal organs (abdominal perisympathetic organs, corpora allata, and corpora cardiaca), all sequences were identified by using MALDI-Tof mass spectrometry.

The phylogeny of cockroaches is highly disputed and several authors also dealed with the arrangement of cockroaches, termites and praying mantids within Dictyoptera. Therefore our taxon sampling represents the major lineages of cockroaches (Polyphagidae, Cryptocercidae, Blattidae, Blaberidae, Blattellidae), a Praying Mantid *Popa spurca* and the termite *Mastotermes darwiniensis* (Mastotermitidae). The alignment resulted into more than 150 characters (ClustalX) and the following topologies of the phylogenetic analysis (MP) are in general agreement with the formerly published relationships, based on molecular analyses of different gene loci, but show also differences which are well supported with bootstrap-values (1000 repeats).

In addition, we identified apomorphic characters in mass fingerprints for most of the higher and a number of lower taxa that are easily detectable.

Acute stress induces increased oxytocin expression in brain regions important for emotional regulation only in male wildtype mice

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The neuropeptide oxytocin is primarily produced in the hypothalamus and released centrally and peripherally in response to stimulation of other neurotransmitter systems like the serotonergic, noradrenergic and dopaminergic system. Oxytocin acts as a neuromodulator within the CNS and is critically involved in mediating social bonding and parental care. In humans, early parental separation seems to induce changes in central oxytocin systems and thereby increases the risk for emotional disorders in adulthood. Stress has been shown to increase oxytocin secretion and oxytocin seems to be involved in pain modulation. Serotonin and selective serotonin reuptake inhibitors (SSRIs) stimulate the release of oxytocin and likewise, serotonin is proposed to stimulate oxytocin expression in the paraventricular and the supraoptic nuclei of the hypothalamus. Furthermore, specific behavioral and autonomic evidences demonstrate anxiolytic-like effects of oxytocin in males and females.

Serotonin transporter knockout (5-Htt KO) mice represent an artificially hyperserotonergic environment and show an increased anxiety-like behaviour. Furthermore, it could be shown that female 5-Htt KO mice display exaggerated adrenomedullary responses to stress. Therefore, these mice seem to be a practical model to investigate the interplay of serotonin and oxytocin especially in the context of stress reactions.

In this study, we investigated the expression levels of oxytocin in different brain regions of interest (cortex, hippocampus, amygdala, hypothalamus and raphe nuclei) from female and male wildtype and 5-Htt KO mice with or without exposure to 1 h of acute immobilization stress. Quantitative real time PCR studies were done using a 384-well plate, which permits an all-in-one-run, meaning in one single PCR run all tested groups (male/female, wildtype/5-Htt KO and stressed/unstressed) could be included. After normalization of the quantitative values with four different housekeeping genes and subsequent statistical evaluation, we found significant higher expression levels of oxytocin in brain regions which are involved in regulation of emotional stimuli (amygdala and hippocampus) of stressed male wildtype (WT) mice, whereas male 5-Htt KO as well as female WT and 5-Htt KO mice lack these stress-induced changes. Therefore, we found gender- as well as 5-Htt-genotype-dependent differences in oxytocin expression after stress exposure. Our results are in accordance with the hypothesis of oxytocin being necessary for protection against stress, depressive mood and anxiety in wildtypes and provide novel theories about altered responses of 5-Htt KO mice to stress and anxiety inducing stimuli.

Protocadherin7: Isoform Specific Function and Signaling Pathway

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Protocadherins (Pcdhs) are members of the rapidly growing cadherin superfamily and are thought to be involved in cell-cell recognition in the central nervous system. We have previously identified a novel member of the Pcdh superfamily, Pcdh7. Its mRNA was uniquely expressed highly in the brain and heart. We have also determined three species of alternatively spliced cytoplasmic tail sequence for Pcdh7. Here we focused on analyzing the functional diversity of Pcdh7 isoforms to elucidate the molecular basis of neuronal circuits.

Results

By employing a yeast two-hybrid to screen the human brain cDNA library, we identified protein phosphatase type I isoform alpha (PP1alpha) as an interactor of isoform 7c of Pcdh7, which has longest cytoplasmic tail compared to other isoforms 7a and 7b. The interaction between Pcdh7 isoform 7c and PP1alpha suppressed the PP1alpha activity towards glycogen phosphorylase. Moreover, mouse fibroblast L cells stably overexpressing the Pcdh7 isoforms 7a and 7b, but not 7c, showed a morphological change and Ca ion-dependent cell adhesion.

Conclusions

Pcdhs are a family of cadherins considered to play an important role in the cell-cell adhesion of specific neurons in the central nervous system; however, relatively little is known about the functional role of Pcdh7, and there is no evidence of Pcdh7 mediated cell-cell adhesion. From the present data, Pcdh7 isoform c could interact with PP1alpha and suppress it. This could be one reason to account for Pcdh7-mediated on/off switch of cell adhesion.

Tonic activation of presynaptic NMDA receptors during seizure development in the amygdala

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Fear and anxiety-related behaviors are commonly reported in patients suffering from temporal lobe epilepsy (TLE). Moreover, experimental models of TLE have revealed a regulation of intrinsic as well as synaptic neuronal properties by seizure activity. Most studies have been focusing on the hippocampal formation, and therefore we thought it timely to investigate neuronal properties in the amygdala, a region of critical importance during TLE.

For this purpose, we have used the pilocarpine-mouse model of TLE in which 7 to 9 weeks old C57Bl6/J mice underwent single subcutaneous administration of pilocarpine, a muscarinic agonist, at a dose adjusted to 340 mg/kg. Diazepam 4mg/kg was injected once s*tatus epilepticus* stopped. Three months thereafter, slices were prepared from pilocarpine-treated mice and from age-matched saline-injected mice as a control. Electrophysiological recordings using the whole-cell patch-clamp technique were performed *in vitro* from projection neurons in the lateral amygdala (LA), which were identified as such by an established battery of electrophysiological and morphological criteria.

The results obtained after comparing experimental data from saline-injected mice with those from the pilocarpine-injected mice outlined three major lines of findings in LA projection neurons. First, electrotonic but not electrogenic membrane properties were affected. Second, there was a significant increase in excitatory synaptic activity, which selectively involved the NMDA receptor subtype at presynaptic sites. Importantly, NMDA receptor-driven activity appeared at the network level until spontaneous seizure development, after which it was restricted to an increase in frequency of miniature events. Thirdly, an increase in amplitude of GABAergic synaptic events was found to be correlated to the increased network glutamate activity in projection neurons from mice that did not develop seizures. On average, the excitation-inhibition ratio in LA projection neurons displayed a threefold increase in LA projection neurons in pilocarpine injected mice.

These results indicate a NMDA receptor-mediated remodeling of synaptic networks in the amygdala after s*tatus epilepticus* leading to a dramatic imbalance between the excitation and inhibition levels in projection neurons, which in turn favors seizure activity.

Dendritic architecture of pyramidal neurons in the rat infralimbic cortex affected by diurnal activity and stress

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Pyramidal neurons of the rat medial prefrontal cortex (mPFC) have been shown to react to chronic stress by retraction of their apical dendrites and spine loss. Here we focused on a specific subarea within the mPFC, the infralimbic cortex and analyzed the dendritic tree of layer III pyramidal neurons in both hemispheres. Animals were subjected to daily restraint stress for one week (6 hours/day), during either resting or activity periods. We digitally reconstructed the complete dendritic tree and counted spine density of GolgiCox-stained neurons. The left and right hemispheres analyzed separately. We observed the following: In control rats, there was an inherent hemispheric asymmetry in the morphology of the apical dendritic tree and both the dendritic tree and spine density displayed a diurnal variation. Furthermore, chronic restrain stress affected the dendritic tree on a hemisphere and activity dependent manner. Our results show dynamic hemisphere-dependent structural changes in pyramidal neurons of the rat infralimbic cortex, which are tightly linked to the periods of resting and activity. These morphological alterations reflect the capacity of the neurons to react to external stimuli and mirror presumptive changes in neuronal communication.

Functional role of intralaminar thalamic neurons during spike and wave discharges in a genetic rat model of absence epilepsy

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Absence epilepsy, a non-convulsive type of epilepsy, occurs predominantly during childhood and is characterized by a loss of consciousness. During seizures, electro-encephalogram (EEG) recordings show 3Hz bilaterally synchronous spike and wave discharges (SWDs). Previous studies showed that the thalamo-cortical network, which generates oscillations during sleep, also produces absence seizures. As opposed to the sleep-rhythms, these oscillations can show a dramatic increase in synchronization, leading to SWDs. The seizure starts by a concerted interaction within the thalamo-cortical network. The initiation of this occurs in regions of the somatosensory cortex. It is noteworthy, that nearly all studies of SWDs focused on the nucleus reticularis or the specific nuclei of the thalamus (e.g. the lateral geniculate nucleus), although the unspecific intralaminar thalamic nuclei (ILTN) are classically assumed to resemble the centrocephalic pacemakers. Therefore, in our study we aimed to experimentally test the hypothesis that ILTN functions as pacemaker of SWDs.

Experiments were performed in vivo under neurolept anesthesia on rats of the WAG/Rij strain, an established genetic rat model of absence epilepsy. Experimental procedures include intra- and extracellular recordings and microstimulation of ILTN neurons.

Our results from intracellular recordings indicate that the activity in ILTN cells depended on the membrane potential with tonic-firing and Ca^{2+}-mediated burst firing at potentials positive and negative from rest, respectively. The occurrence of SWDs was correlated with an inhibition of tonic firing or induction of burst-firing in ILTN neurons. The determination of the reversal potential revealed that SWD-related activity of ILTN cells depended on GABAergic mechanisms. Extracellular measurements supported the findings from intracellular recordings, and, in addition showed differences in SWD-related firing in different types of ITLN nuclei: Neurons in the paracentral nucleus (PC) displayed an inhibition of tonic firing during a SWD. By comparison, ILTN cells of the centrolateral nucleus (CL) displayed burst-like discharges, which are correlated to SWDs. Therefore, we propose that there is a functional difference between CL- and PC-neurons. Indeed, microstimulation of PC-neurons could depress or recruit SWDs on the EEG, depending on the used stimulation-frequency.<

In conclusion, these results are difficult to reconcile with the proposed traditional role of ILTN neurons as pacemakers of SWD, but rather indicate that ILTN activity has to be suppressed to allow occurrence of SWDs.

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An uncommon gain control – amplification instead of inhibition

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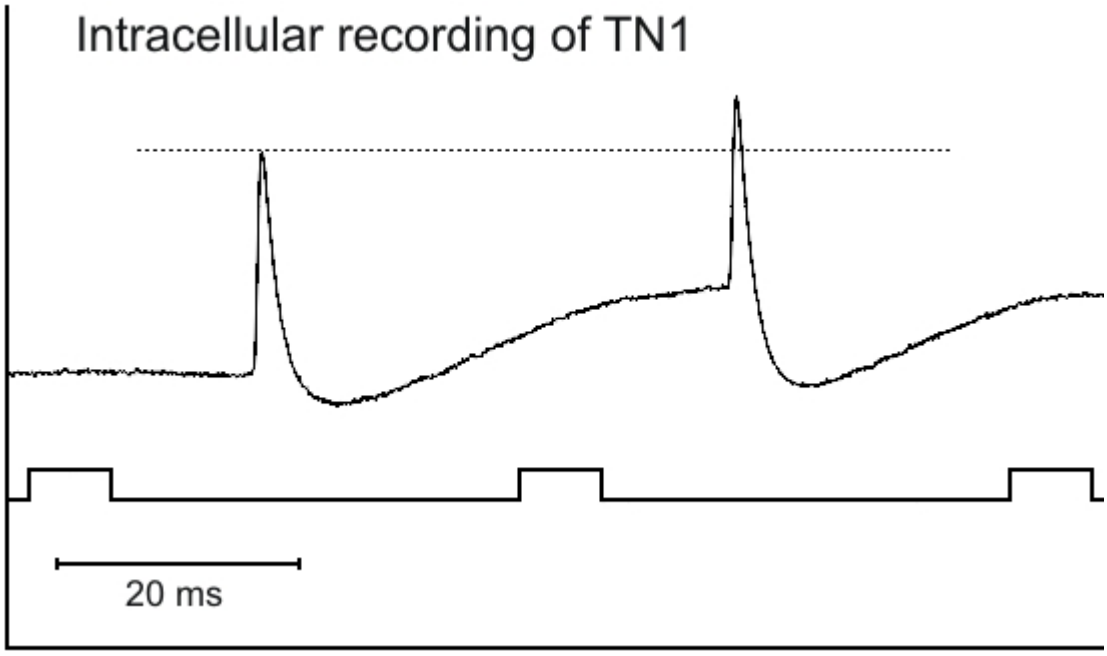
The axonal output of a nerve cell can not only vary on the postsynaptic side, but also presynaptically, e.g. by presynaptic inhibition. This mechanism known as PAD (presynaptic afferent depolarization) has already been extensively described (e.g.: Burrows & Laurent, 1993; Clarac & Cattaert, 1996). Here we present a different kind of gain control where the target neuron's activity is not inhibited but at least partially amplified.

T-shaped neuron 1 (TN1) is an intersegmental auditory interneuron in the central nervous system of the bush cricket *Ancistrura nigrovittata*. Its dendrites are located in the prothoracic ganglion and receive excitation from the ipsilateral ear and additionally inhibition from the contralateral ear. The main axon ascends to the brain and arborizes in the deuto- and protocerebrum. When stimulated with sound, intracellular recordings of TN1 in the brain reveal large depolarizations with long latencies. We were not able to inject sufficient positive current to find the equilibrium potential of this graded potential. The currents are therefore likely to be sodium or calcium driven. Since no extra action potentials are released, we conclude that the origin is situated at a section of the axon where spikes are passively propagated. The axon of TN1 therefore exhibits a switch from active to passive transmission before arborizing in the protocerebrum. When thoracic spikes and depolarizations simultaneously occur a summative effect becomes evident (see figure). The absolute spike amplitude increases and an increased transmitter release at the synapses is likely.

The physiological properties of the observed depolarizations closely correspond to data drawn from a different ascending neuron – AN3 (ascending neuron 3). Due to long latencies AN3 is likely to be polysynaptically linked to TN1. The timing of the depolarizations might enhance the response to certain pulse repetition rates which may be relevant for intraspecific communication.



Dysfunction of thalamic adenylyl cyclases in an animal model of human absence epilepsy

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Thalamocortical networks can generate both normal and abnormal patterns of synchronized network activity, such as spindle waves and spike-and-wave seizures. It has already been shown that the intracellular second messenger cyclic AMP (cAMP) modulates ionic currents which are involved in the generation of synchronized discharges. cAMP induces changes in the opening kinetics or the current amplitude of channels like the hyperpolarization- (I_h) or the high-voltage activated (HVA) current.

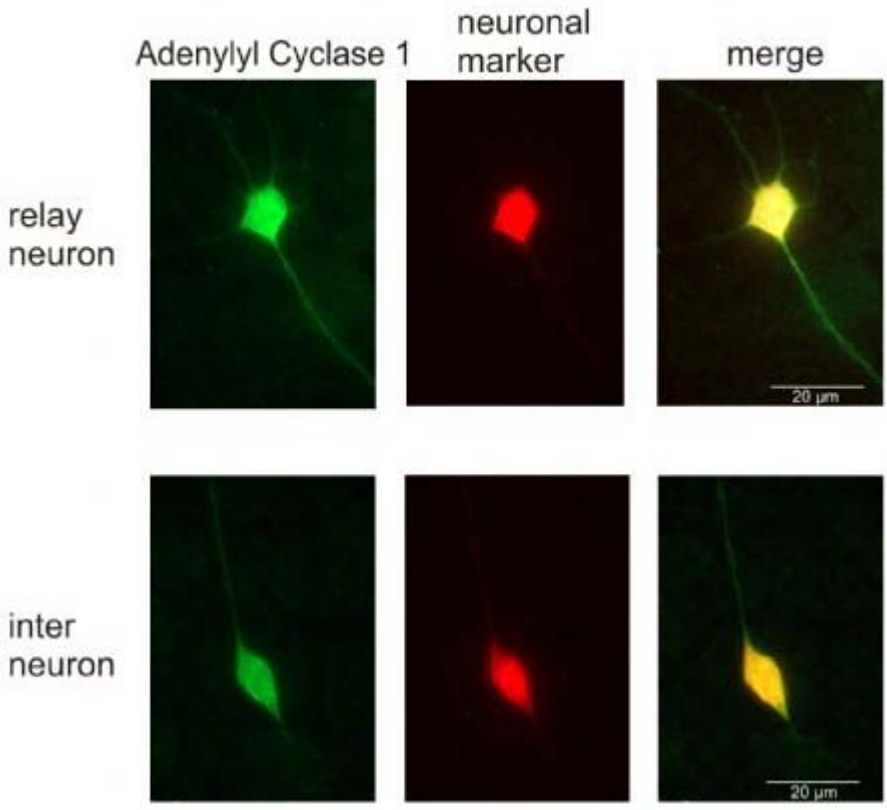
Adenylyl cyclases (ACs) synthesize cAMP upon activation via a series of extracellular and/or intracellular signals. Thus they act as on/off switches in the cell. This study aims to analyse the role of AC in the generation of thalamic activity pattern. Dysfunctions of these proteins may lead to abnormal EEG pattern accompanied by spike-and-wave discharges which are characteristic for absence epilepsy.

WAG/Rij rats were chosen as model organisms for human absence epilepsy. These animals develop spike-wave discharges at the age of 3 months. In addition they respond to classical antiepileptics. Non-epileptic ACI rats were taken as control. Experiments focused on the dorsal lateral geniculate nucleus (dLGN) of the thalamus. Standard methods were used for qPCR (TaqMan®), immunocytochemistry, and whole cell patch-clamp recordings.

qPCR data showed a significant reduction of AC isoforms 1, 6 and 8 expression in dLGN of epileptic rats at postnatal day 21. In comparison to AC6 and AC8, AC1 was highly expressed and thus seemed to be the prominent isoform in the dLGN. In order to check that the reduction in gene expression leads to reduced intracellular cAMP levels, an assay was used to quantify cAMP in lysates of different brain tissues. Under basal conditions there were no differences in cAMP concentrations between the strains under investigation. After stimulation with a specific AC-activator the cAMP increase in lysates of epileptic rats was smaller. In addition double immunfluorescent staining confirmed expression of AC1 in relay and interneurons of the thalamus and of the dLGN in particular. Finally electrophysiological recordings were done to investigate the consequences of diverging AC expressions on HVA Ca^{2+} currents in relay cells of the dLGN. A $ß_2$-specific agonist was used to induce receptor-dependent AC-activity. Neurons of epileptic rats showed a significantly smaller increase in HVA Ca^{2+} current amplitude. In addition current clamp recordings revealed that $ß_2$-mediated AC activation induces membrane depolarization.

It is concluded that ACs fulfil an important function in the generation of thalamic activity pattern and dysfunction may contribute to epileptogenesis.

Immuncytochemistry of thalamic neurons



Monoamines block kainate- and carbachol-induced gamma oscillations but augment stimulus-induced gamma oscillations in rat hippocampus in vitro

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Monoamines are implicated in cognitive processes in variety of brain regions including the hippocampal formation, where storage and retrieval of information is facilitated by synchronous network activities. We have investigated the effects of norepinephrine, serotonin and dopamine on carbachol-, kainate- and stimulus-induced hippocampal g-oscillations employing combined extra and intracellular recordings. Monoamines dose-dependently and reversibly suppressed kainate- and carbachol-induced g-oscillations while increasing the frequency. The effect of serotonin was mimicked by fenfluramine, which releases serotonin from presynaptic terminals. Forskolin also suppressed kainate- and carbachol-induced g-oscillations. This effect was mimicked by 8-Br-cAMP and isoproteronol, an agonist of noradrenergic beta-receptor suggesting that the monoamines-mediated suppression of these oscillations could involve intracellular cyclic AMP. By contrast, stimulus-induced g-oscillations were dose-dependently augmented in power and duration after monoamines application. Intracellular recordings from pyramidal cells revealed that monoamines prolonged the stimulus-induced depolarization and membrane potential oscillations. Stimulus-induced g-oscillations were also suppressed by isoproteronol, the D1 agonist SKF 38393 forskolin and 8-Br-cAMP. This suggests that the augmentation of stimulus induced g-oscillations by monoamines involves – at least in part - different classes of cells than in case of carbachol- and kainate-induced g-oscillations.

Analysing the central pattern generator for cricket stridulation

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Male crickets (*Gryllus bimaculatus*) produce acoustic signals for intraspecific communication. They rhythmically open and close their front wings and generate a short sound pulse during each closing movement. In the calling song pattern three to six of these sound pulses are grouped to chirps that are stereotypically repeated with a rate of 2-3 Hz. The singing behaviour is controlled by an identified descending brain neuron that functions as a calling song command neurone (Hedwig 2000) and activates the central pattern generator (CPG) for stridulation. Although the motoneurons that drive the corresponding wing-opener and wing-closer muscles are located in the mesothoracic ganglion, lesion experiments as splitting thoracic ganglia in combination with cutting thoracic connectives (Henning & Otto 1995) point to the metathoracic ganglion housing the singing CPG. However, the exact location and cellular organization of the CPG generating the singing motor pattern in crickets is still unknown.

In order to identify the interneurones participating in the central generation of the stridulatory motor pattern, we intracellularly recorded and stained individual neurons in the thoracic ganglia of fictively singing crickets with all thoracic motor nerves cut. Singing was elicited by pressure-injection of the acetylcholine esterase inhibitor Eserine into the region of the protocerebral neuropile where the dendrites of the calling song command neuron are located, while the singing pattern was monitored by extracellularly recording of the corresponding motoneuron activity in the truncated wing nerves. The spike activity of the neurones was modulated by intracellular current injection to examine if experimentally elicited spike bursts modulate the opening-closing cycle of the pattern generator. A neurone that is rhythmically active in phase with the stridulatory motor pattern can only be referred to as part of the singing CPG if transient distortions of its neural activity can stop and reset the CPG, or if it even would initiate singing from a resting state.

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A study of neuropeptidergic circadian coupling pathways in the cockroach *Leucophaea maderae*

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The cockroach *Leucophaea maderae* is a well established model organism for the characterization of circadian clocks in insects. Lesion and transplantation studies identified the peptidergic accessory medulla (AMe; plural abbreviation: AMae) with associated pigment-dispersing factor (PDF)-immunoreactive (-ir) neurons as circadian pacemaker center in the insect's optic lobes. The about 16 PDF-ir medulla neurons branch in the AMe as well as in optic lobe and midbrain neuropils. With backfill studies from one AMe combined with immunohistochemistry it has recently been shown that at least three of the PDF-ir medulla neurons directly connect both bilaterally symmetric AMae, apparently as a circadian pacemaker synchronization pathway. However, it remained unclear, whether also other peptidergic neurons, such as FMRFamide- and Asn^{13}-orcokinin-ir neurons, contribute to circadian coupling pathways between both pacemaker centers. Here, we examined circadian coupling pathways with neurobiotin backfill experiments combined with double-label immunohistochemistry employing antisera against PDF, FMRFamide, and Asn^{13}-orcokinin.

Previously to this work three groups of contralaterally projecting medulla neurons were known and discussed as coupling pathways between the bilaterally symmetric AMae. One group contained the contralaterally projecting PDF-ir medulla neurons. In this work, a fourth group emerged, thus probably adding a new level of complexity to the pacemaker synchronization pathways. Up to four PDF-ir medulla neurons were shown to project to the contralateral optic lobe. One of them, the largest PDF-ir cell, neither colocalized FMRFamide- nor Asn^{13}-orcokinin-immunoreactivity. It appeared to connect both AMae apparently via both the anterior and posterior optic commissure. In addition, three medium-sized PDF-ir neurons appeared to connect both AMae via the anterior optic commissure. These neurons colocalized both FMRFamide and Asn^{13}-orcokinin immunoreactivity. No additional FMRFamide or orcokinin-ir pathways were found parallel to the contralateral PDF-ir pathway.

In the immunohistochemical double label studies all medium-sized and most small PDF-ir medulla neurons were additionally FMRFamide- and orcokinin-ir. Interestingly, PDF- and FMRFamide-ir colocalization was observed in only few termination sites of PDF-ir medulla neurons, which included the AMe. PDF/orcokinin-ir colocalization was never observed in terminals. Furthermore, backfill labeling was so far not observed in terminals with PDF/FMRFamid-ir colocalization. Therefore, we assume co-expression of at least three different modulatory neuropeptides in all medium-sized and most small PDF-ir cells, while co-release appears to occur only in a small portion of fiber terminals. In summary, we suggest the existence of a peptide sorting mechanism in terminals of PDF-ir circadian pacemaker neurons as an important mechanism for output of timing signals to output organs as well as for the synchronization of insect pacemaker neurons. [This work was supported by DFG grants STE531/15-1 and 18-1 to Monika Stengl]

Alteration of brain states with high phase-coherence and transient states indicate the intermittency information processing in brain dynamics

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Phase-coherence between the local field potential (LFP) recorded from auditory and visual cortex of awake Mongolian gerbils was studied during spontaneous ongoing activity as well as after stimulation with auditory stimuli (pure tones) and visual stimuli (flashes). Oscillatory frequency bands describing the global dynamics of all measured trials were extracted by considering only their intra-areal (auditory or visual cortex) in-phase synchronization periods between different band frequencies, in the range between 7Hz and 200Hz, using short time Fourier transform analysis. These brain states showed three different modes of inter-areal (between auditory and visual cortex) phase coherence: in-phase synchrony, anti-phase synchrony, and a different but stationary phase relation. Brain states were found to continuously change from one to the other inter-areal high coherence state via a transient state of low inter-areal phase coherence. Several invariant features of the coherent and transient states could be determined. In addition, states of high phase coherence could demonstrate significant variation of the energy distribution across the auditory and visual channel indicating effective modulation of neuronal activity under maintenance of a stable phase relationship. The implications of these findings for sensory stimulus processing and crossmodal integration will be discussed.

Self-organized criticality of developing artificial neuronal networks and dissociated cell cultures

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Self-organized criticality (SOC) (Bak et al., 1987) was first described in neuronal cell cultures by Beggs & Plenz (2003). Neuronal networks being in a critical state produce avalanche-like discharges that are power-law distributed. The assessment of avalanches in neuronal networks is a new way of looking at neuronal activities apart from bursts, synchronization etc. The main novelty of our approach is to assess the avalanche distribution at different developmental stages of neuronal networks. For this, we used dissociated post-natal cell culture taken from the rat cortex (Experimental data was provided by the Ulrich Egert group, BCCN Freiburg, Germany). We found that different network states as subcritical, critical or supracritical specify a time and spatial activity profile that is linked but not equivalent to low, moderate or high levels in neuronal activity, respectively. We are the first who show that the activity profile in cell cultures develop from supracritical states over subcritical into critical states. To shed light to the dependency of SOC on network development, we used a self-organizing artificial neuronal network model based on a previous model by Van Ooyen and Abbott (Van Ooyen & van Pelt, 1994; Van Ooyen et al., 1995; Abbott & Rohrkemper, 2007). An important novelty of our model is that it is more detailed with respect to representing seperate axonal and dendritic fields (Butz et al., 2008; Butz & Wörgötter, 2009). The model network aims to develop towards a homeostatic equilibrium in neuronal activity which is achieved by growth and retraction of axonal and dendritic fields. This abstract model already reproduces the transient behaviour as seen in cell cultures from supracritical over subcritical to critical states. However, we found that some cell cultures remain in a subcritical regime. The model offers a simple explanation as depending on the strength of inhibition, equivalent to the friction in self-organizing systems (Lauritsen et al., 1996), neuronal networks may or may not reach criticality even though they are homeostatically equilibrated.

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State-dependent patterns of spatiotemporal coupling in rat visual cortex

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Oscillatory population activity is ubiquitous in the mammalian neocortex. Oscillations are present across many frequency bands, and are postulated to reflect the integration of information over neural populations [1]. Such oscillations can be evoked by sensory stimuli, and neocortical populations can exhibit distinct forms of resonance to the stimuli. For instance, when subjects are exposed to flickering light at certain frequencies, their EEG-waves are prone to entrain, i.e. phase lock, to the stimulation frequency [2].

First, we address the state-dependence of such entrainment using a rat-model of sleep [3]). Under urethane anesthesia, the electrocorticogram (ECoG) alternates spontaneously between a low-voltage desynchronized state, which resembles the ECoG during rapid eye movement (REM) sleep, and a synchronized state, which resembles the ECoG during slow-wave sleep. This biphasic state represents an ideal model system to investigate state-dependent changes in the network responses to sensory stimuli. As expected, frequency coupling varied with both stimulus frequency (2.5 Hz to 15 Hz) and cortical state. Overall, the desynchronized state, indicative of a more activated cortex, is more susceptible to entrainment than the synchronized state, which is in agreement with previous reports (e.g. [4]). Furthermore, in some trials, the entrainment outlasts the stimulus train, giving way to poststimulus illusory responses resembling a dampened oscillation. Next, we use voltage-sensitive dye imaging (VSDI) with high signal-to-noise ratio [5] to study the spatiotemporal characteristics of single trials of this phenomenon, and describe the spatial coupling by cortical area and cortical state.

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Multi-scale modelling of cortical population bursts

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One of the challenges of current neuroscience research is to understand the relationships between multiple levels of neuronal organisation: from ion channels to single cells to neuronal networks to behaviour. In particular, the relationship between single neurons and population activity has recently gained much interest. Combining experimental and modelling work we investigate here how these two levels are related in a phenomenon called population bursting. This term refers to short trains of action potentials which are synchronous over large population of neurons and thus are rendered visible in field potentials (LFP, EEG). Such responses were identified in many brain areas, among others in hippocampus [1] and cortex [2]. Here we focus on bursts elicited by neurons in primary somatosensory cortex after peripheral nerve stimulation. They were shown to correlate well with population bursts seen in high-frequency EEG oscillations (hf-EEG, >600 Hz) recorded from scalp [3] or dura [4] (Figure 1). We review briefly the main experimental findings on cellular substrates and physiology of hf-EEG and their sensitivity to neuronal parameters, such as vigilance. Next, we show how single-unit bursts can be reproduced in a simple biophysical neuron model implementing well-known properties of cortical cells and their connectivity. Finally, we discuss how the high-frequency EEG properties could be related to the parameters of the neural model. Since high-frequency EEG can be recorded from human scalp we conclude that it may provide non-invasive access to basic properties of thalamocortical circuitry such as synaptic dynamics, neuronal excitability and interneuronal correlations making it an invaluable tool for studying large-scale brain phenomena.

Figure 1 Population bursts in primary somatosensory cortex of macaque monkeys. Median nerve stimulation applied at time t=0 ms evokes primary somatosensory EEG response (A, epidural EEG averaged over 956 trials). High-pass filtering (400-1200 Hz) reveals small amplitude deflections repeating at very high rates (> 600 Hz) on top of the primary somatosensory response (B, the maxima of the deflections are marked with dashed lines). Single neurons recorded extracellularly from primary somatosensory (Brodmann area 3b) fire bursts of spikes (D, sample extracellular traces of single-cell activity) which align to the late peaks of high-frequency EEG response (hf-EEG) giving rise to multi-peaked single-unit post-stimulus time histogram (C, PSTH, bin width 0.25 ms).

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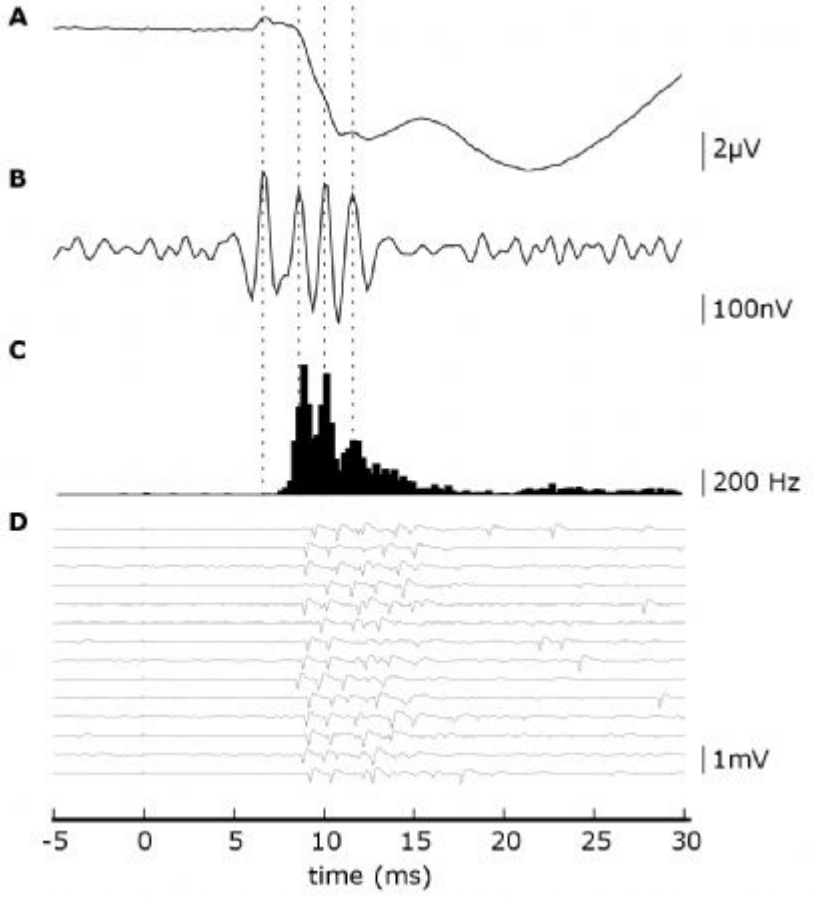
A
2μV
B
100nV
C
200 Hz
D
1mV
-5
0
5
10
15
20
25
30
time (ms)

Identification and characterization of circadian clock molecules in the circadian pacemaker network of the cockroach *Leucophaea maderae*

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What we know about the circadian system of insects today is mainly based on knowledge acquired from experiments in *Drosophila melanogaster*. Work done on several other insect species in recent years showed that the circadian clock of insects is not homologous as previously expected. Also, the identity of circadian pacemaker cells and their interactions are still not fully understood. Since electrophysiological studies in tiny *Drosophila* are extremely tedious and difficult, we turned to another larger insect model organism, the cockroach *Leucophaea maderae*. To locate and characterize the circadian pacemaker cell groups, we used molecular genetics and immunocytochemistry. With RT-PCR the full length sequence of *Leucophaea maderae period* (*Lper*) were acquired and immunostainings with an antibody against *Lper* suggested a widespread expression all over the brain. In addition, Westernblots of whole brain extracts showed two peaks of *Lper* expression near dusk and dawn, suggesting a complicated pacemaker network.
Timeless and the two main types of *cryptochrome* in insects, insect type 1 and vertebrate like *cryptochrome type 2* are not present in all insect species that have been examined for circadian genes so far. Thus, it was of particular interest to determine wether these genes are present in the cockroach *Leucophaea maderae*. Whereas first attempts to clone *timeless* in *Leucophaea* weren't successful, now we succeeded with degenerate primers derived from published insect timeless genes. We obtained a partial sequence of *timeless (Ltim)*. Also a vertebrate like *cryptochrome type 2* could be identified using this method. Insect like *cryptochrome type 1*, which is also present in *Drosophila* could not be found so far. Using fluorescence *in-situ* hybridization we started to identify the cell groups expressing circadian genes in the brain. Preliminary results of *cryptochrome type 2 in-situ* hybridizations show staining in the optic lobes near the accessory medulla and in the lamina. Antibodies against the proteins of those genes are also being generated to investigate circadian oscillation and localisation on the protein level. In future experiments we want to identify and characterize further circadian genes such as clock and cycle, to learn to understand the molecular and cellular circadian network in the cockroach. [Supported by DFG grant STE531/15-2 and 18-1 to Monika Stengl]

Layering of the dentate gyrus is crucial for homogeneous hilar mossy cell input

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Layered arrangement of neurons is a common feature of cortical regions. Although disrupted layering has been found to be associated with pathological conditions such as epilepsy, the functional relevance of cortical layering is still poorly understood. To investigate how changed neuronal layering influences synaptic properties in a small neuronal network we used reeler mutant mice as a model. In these animals the lack of reelin results in altered layering due to defects in neuronal migration. We focused our investigations on the dentate gyrus, the input region of the hippocampus. Dentate gyrus granule cells and most its interneurons receive entorhinal input via the perforant path in the outer molecular layer. Hilar mossy cells in turn receive their main input primarily from neurons of the dentate gyrus. We simultaneously recorded the extracellular activity of the dentate granule cell population and the intracellular activity of single mossy cells in response to stimulation of the perforant path, thus monitoring a well-defined disynaptic pathway.

In wild-type mossy cells, synaptic responses elicited by perforant path stimulation were uniform and dominated by a short-latency, long-lasting inhibition and a longer-latency, brief excitatory component corresponding to a disynaptic EPSC. In reeler, the synaptic responses were heterogeneous. In the majority of the cells, inhibition was markedly enhanced and the disynaptic EPSC was reduced. Additionally, in many cells a short-latency monosynaptic EPSC could be observed. As a consequence, action potentials (APs) were generated over a broader temporal window. While in some reeler mossy cells APs could be evoked monosynaptically with high temporal precision, in others, APs were generated with long latency and low temporal precision. The probability for disynaptic APs, but not for monosynaptic APs, was overall reduced.

Consistent with the electrophysiological findings, visualization of the intracellularly labeled reeler mossy cells revealed marked changes in the morphology and a high degree of heterogeneity in the localization and the distribution of dendrites. While in the wild-type hippocampus mossy cells and their dendrites were confined to the hilus, in reeler the cell bodies and their dendrites were often found in the molecular layer.

In summary, changes in localization and morphology of reeler mossy cells enable them to receive direct synaptic inputs from the perforant path. However, the disynaptic excitatory input via dentate granule cells is reduced and feed-forward inhibition is increased. Thus, changes in the connectivity associated with the altered lamination in reeler mice result in a reduced efficiency and higher temporo-spatial variability of synaptic activation in the dentate-hilar network.

Independence of Functional Neuronal Network Architecture from Cholinergic and GABAergic Modulation: no Escape from the Small World

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The topological structure of anatomical and functional neural networks has repeatedly been shown to possess properties indicative of a small-world architecture on various spatial scales, supporting fast signal transmission, coexistance of local and global computations and robustness against lesions. Recently, simulations demonstrated that spike timing dependent plasticity can convert a globally connected network into a functional small-world network that arises when the excitatory and inhibitory connection strengths between the neurons have reached a distinct bimodal distribution [1]. So far, it was unclear how stable small-world properties of real neural networks are with respect to potential changes in this distribution through neuromodulation.

To investigate these issues, we analyzed single unit activity from acute slices of rat visual cortex under varying conditions including no pharmacological interference, saturation of muscarinic receptors by application of 20-50 µM Carbachol, and complete blockade of $GABA_A$ receptors while synergistically enhancing the action of $GABA_B$ receptors by concurrent application of 30 µM Bicuculline and 10 µM CGP13501. Recordings were performed with a 32-channel extracellular electrode matrix ("Utah probe") yielding on average about 90 isolated single units per session. To assess their functional connectivity, spatiotemporal firing sequences were detected using sliding windows with lengths of 5-50 ms and evaluated statistically by Monte Carlo methods. The resulting functional neuronal networks were based on only the significant spike patterns without any further thresholding.

It turned out that indeed the functional neuronal networks emerging in vitro exhibit a small-world architecture: The clustering coefficients range roughly from 0.6 to 0.75, clearly contrasting with the values of randomly rewired networks that have the same number of vertices and edges (0.02 to 0.2). In addition, the average shortest path fluctuates closely around 1.8, a number even below those obtained from the randomly rewired networks. Finally, the degree distributions revealed many sparsely connected neurons and a long tail of increasingly interconnected units, suggesting the existence of "hubs" in the network. Surprisingly, neither a massive change in cholinergic modulation nor a pronounced imbalance of GABAergic transmission has any substantial effect on the topology of the network, although spike rates and firing patterns change considerably following drug application or washout.

These findings demonstrate a remarkable stability of functional neuronal network structure with respect to diverse modes of synaptic integration, providing extensive freedom to visit a multitude of functional states while seamlessly maintaining a small-world architecture. As a consequence, the ability to rapidly process and integrate information both locally and globally – a highly desirable property for neuronal computations – arises naturally and appears to be an inherent feature of cortical connectivity that is independent of cholinergic and GABAergic modulation.

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Combining Experiments with a Percolation Model to Study Connectivity in Neural Cultures

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We introduce a novel approach for the quantitative assessment of the connectivity in living neuronal networks, based on the statistical mechanics of percolation on a graph. Our network consists of rat hippocampal neurons cultured in glass coverslips. Neurons are excited by a global electrical stimulation applied to the entire network through bath electrodes. The network's response is then studied for gradually lower synaptic coupling between neurons, which is achieved through application of CNQX. We consider a percolation model that takes into account the requirement of multiple inputs to excite a neuron, so that a neuron fires and passes the signal only if it receives at least m inputs. Application of CNQX reduces the connectivity strength between neurons, effectively increasing m. Initially, all the neurons in the culture are connected through a network-spanning cluster termed *giant component*. As the synaptic coupling is reduced and m grows, those neurons having less than m inputs get disconnected from the network and, in turn, reduce the number of inputs into their target neurons. The giant component decreases in size as m grows. The connectivity undergoes a percolation transition at a critical m, where the giant component disappears and the network breaks down into isolated clusters. By monitoring the disintegration of the network as a function of m we are able to extract relevant statistical information of the neural network. Among other quantities, we are able to estimate the average number of connections per neuron and the distribution of those connections. By additionally blocking of $GABA_A$ receptors in inhibitory synapses we quantify the amount of inhibition in the network. Finally, we also consider developmental aspects. We study how the formation and maturation of synapses influence the connectivity, and at which moment a giant component emerges in the culture. Our approach provides a method to extract detailed information on the connectivity in neuronal cultures. Such global information is, at present, inaccessible using electrophysiological or other techniques.

Reference:

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Trial-to-trial variability of interaction dynamics between auditory and visual cortex during asynchronous audiovisual stimulation

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Crossmodal integration of sensory input requires large-scale coordinative interactions between distant cortical areas. Here we investigate how states of coordination change continuously over time and across trials in periods with and without external stimulation.
Auditory pure tone and visual flash stimuli with fixed interstimulus onset asynchrony were presented continuously to awake Mongolian gerbils while local field potential activity was recorded from depth electrodes implanted in the primary auditory and visual cortex, respectively.
The frequency and direction of coordinative interactions between auditory and visual cortex was analysed in single trials using the Directed Transfer Function (DTF, Kaminski & Blinowska, 1991). In the prestimulus interval the dominant frequency of interaction showed a high variability in that it changed constantly. In contrast, the overall rates of occurrence of certain dominant frequencies were however highly invariant across sessions and animals. The distributions of rates of occurrences of certain dominant frequencies differed depending on the direction of interaction.
In the poststimulus interval these distributions changed: certain dominant frequencies occurred more often at certain time points during the course of the trial. We show that the frequency-dependent amplitude of the trial-averaged DTF is shaped not only by the amplitudes of the single-trial DTF at these frequencies but also by the rates of occurrence of trials with significant amplitude values at these frequencies.
The implications of these findings for (1) the putative mechanism of crossmodal interaction, and (2) for theories conceptualizing stimulus-evoked cortical responses as processes "adding" neuronal activity to the ongoing cortical activity will be discussed.

Insect neuronal cell culture on multi electrode arrays

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Our project concentrates on the development of biohybrid systems composed of defined neuron populations and semiconductor chips, which allow for simulataneous extracellular stimulation and the non-invasive recording of their activity *in vitro*. We integrate sputtered iridium oxide film (SIROF)-electrodes for single cell stimulation and field-effect transistors (FET) for recording on one chip. The main goal is the bidirectional coupling between stimulation system, neuron, and recording system. In our study, neurons from thoracic ganglia of the locust, *Locusta migratoria*, were used as biological test system. Insect neurons are suitable as test system because, first, the neurons have a size range of 10-120 µm and, second, allow for culture at low densities. In the long run, the investigations will result in a new approach for the investigation of synaptic connections and analysis of communication patterns in simple neuronal networks. Special emphasis is on coating chemicals that provide a means to fix the cells to the electrodes. In addition it is essential to find growth promoting chemical modifications which allow for a directed growth of neurons. In this context coating with specific antisera yielded promising results. In the course of our studies we found that insect neurons - compared to vertebrate cells - are very unselective as far as the surface material they grow on is concerned. For this reason it became important to also find growth inhibiting substances. In first experiments some very promising candidate chemicals could be identified. A combination of structured substrates consisting of structured areas that allow for cell adhesion and growth with areas with cell-aversive coatings may provide the means to, first, fix insect neurons to MEA electrodes and, second, to directly guide the outgrowth of their neurites for the formation of defined neuronal networks *in vitro*. Beside investigations of the surface modifications to guide neuronal growth, it is important to focus investigations on the isolation of defined cell types from intact animals and cultivate them *in vitro*. In this context, certain locust neuron populations were labeled with Rhodamine conjugated dextraneamine and subsequently prepared for cell culture. Our results show that i) specific cell populations can be selectively excised from the CNS and ii) maintained in long-term culture. Further investigations will address the question if certain neuron types will exhibit distinct properties in culture.

Local field potential oscillations are reduced at high-beta and high-gamma frequencies in basal ganglia regions in an animal model of epilepsy

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The basal ganglia, especially the substantia nigra pars reticulata (SNr), are known to be involved in the propagation and manipulation of limbic seizures. Local field potential (LFP) oscillations were investigated in basal ganglia brain regions of amygdala- kindled rats as a model of temporal lobe epilepsy.

Adult female Wistar rats were kindled once daily by stimulation of the right basolateral amygdala (BLA). LFP activities of fully kindled rats and two groups of non-epileptic controls (sham-kindled rats and naïve rats) were recorded bilaterally from SNr and globus pallidus (GP) and unilaterally from the right BLA. Recordings were obtained from freely moving rats one hour, one day, two weeks, and four weeks after a generalized kindled seizure. Artifact-free sweeps of 1 sec each were analyzed by the Fast Fourier Transform in different bands over a broad frequency range.

Preliminary data revealed a suppression of synchronization in high frequency bands in kindled rats. Oscillatory LFP activities were significantly reduced at high-beta (20.5-35 Hz) and high-gamma (60-90 Hz) frequencies in the right SNr in kindled rats versus naïve controls ($p<0.05$; unpaired t-test) one day after a kindled seizure. Four weeks after a kindled seizure, oscillatory LFP activities were significantly reduced in the left GP at high-gamma frequencies (60-90 Hz) in kindled rats versus naïve controls ($p<0.05$).

The data support previous single-unit recordings showing kindling-induced changes in the right SNr one day after a kindled seizure. Basal ganglia motor and limbic loops are thought to be separated anatomically and functionally processed by independent oscillatory frequencies. The data indicate that the motor loops rather than the limbic loops experienced seizure/kindling-induced modulations over time.

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Pigment-dispersing hormone-immunoreactive neurons in the optic lobe of the marbled crayfish appear to be homologous to insect circadian pacemaker neurons

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Crustacean and insect species have been extensively studied in chronobiology. Although these taxa are closely related, their circadian systems differ in degrees of centralization of pacemaker centers in their brains. Since in many insect species ablation of optic lobes, or even distinct parts of them, resulted in permanent loss of circadian rhythm in locomotor activity, similar experiments in crustacea lead to inconclusive results. However, also in Crustacea circadian pacemakers reside in the optic lobes.

Insect optic lobe pacemaker neurons wire up in a specialized neuropil, the accessory medulla (AMe). The insect AMe is thought to be an integration center for circadian timing information, and is heavily innervated by neurons expressing various neuropeptides. Among them are distinct medulla neurons that express the neuropeptide pigment-dispersing factor, the PDFMe (In *Drosophila* known as ventral group of lateral neurons, LNv). The insect PDFMe are thought to be circadian pacemaker and/or output neurons that arborize in large areas of the central brain. Parts of them apparently co-express additional neuropeptides as FMRFamide-related peptides (FaRPs). While in insects pigment-dispersing factor (PDF) is distinctly expressed only in the PDFMe and, in a variety of species, in one or two clusters at the lamina, its crustacean ortholog pigment dispersing hormone (PDH) is expressed in a variety of dispersed neurons in the optic lobes and the central brain. However, it was recently shown that PDH appears to play a role also in the crustacean circadian system (Verde et al. 2007; Comp Biochem Physiol A Mol Integr Physiol 147:983–992).

In this study, we attempted to identify neurons corresponding to the insect PDFMe in the marbled crayfish, *Procambarus* spec. With anti-PDF/PDH antibodies we labeled four groups of PDH-immunoreactive (-ir) neurons in the optic lobe of the crayfish. One of them situated near the lobula, the PDH-C, consisted of about 20 neurons, and appeared to provide most of the tangential PDH-ir fiber projections of lamina, medulla, and lobula. Some of these neurons could be additionally labeled with an antiserum recognizing members of the FaRP family. With backfill experiments we could show that the PDH-C appears to be the only group of PDH-ir optic lobe neurons that project into the central brain, as is the case for the insect PDFMe. In summary, we propose that the PDH-C group is homologous to the insect PDFMe according to the homology criteria of similar position and specific quality.

We further tried to identify a neuropil corresponding to the insect AMe in the crayfish. In insects, the AMe and conspicuous neuron clusters of the AMe can be distinctly labeled by antisera against serotonin and the neuropeptide allatotropin. In the crayfish however, both antisera labeled neurons dispersed throughout the optic lobe, partly with colocalization of labeling, but no peculiar soma clusters or a peculiar allatotropin-ir neuropil could be identified. Hence, we so far assume that the insect AMe may be either lost in decapod crustacea, or it is a newly attained structure in the evolution of the insect optic lobe, which was not present in the common ancestor of crustaceans and insects. This work opens first insights into the evolution of the arthropod circadian system and might be a further step to identify crustacean circadian pacemaker neurons.

Motor patterns during the initiation of different walking directions in stick insects

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During walking the phasing of the motor patterns driving adjacent leg joints has to be adapted to the task at hand, e.g. straight forward, backward or curved walking.
In stick insects the different walking directions can be elicited by specific tactile stimulation. Touching the abdomen induces forward walking. Backward walking can be induced by gently pulling the antenna. Both situations require distinctly different motor patterns, as e.g. the functional swing and stance muscles are inverted. Thus, different intersegmental ascending or descending information is able to organize the concerted activation of the premotor and motor neuron pools in a task dependent way.
Here we investigated the starting behavior of forward or backward walking and how the motor patterns of the different legs are initiated and concerted and which role can be attributed to the sensory status of the leg (e.g. standing either with ground contact or lifted off).
Motor patterns were analyzed by recording the protractor and retractor coxae and extensor and flexor tibiae in front, middle and hind legs of animals held tethered above a treadwheel. The posture of the examined leg was set either to be lifted off or to be at different standing positions within the action range of stance movement. Is the animal able to immediately adapt the motor pattern to the different starting positions of the leg? Are there differences in motor patterns between the initiation of forward and backward walking?
The results show that all legs without ground contact always started with a swing movement, i.e. the respective swing muscle (forward: ProCx, backward: RetCx) was immediately activated.
However, the data obtained from different legs starting with ground contact show different behavior. When inducing forward walking in front legs with ground contact the respective stance muscle (RetCx) started with activity. The initiation of backward walking by stimulating the ipsi- or contralateral antenna reveals differences. Stimulating the contralateral antenna led to a clear-cut activation in the protractor coxae (stance muscle in backward walking). In about 50 % the stimulation of the ipsilateral antenna led to an activity of the retractor coxae. In the remaining cases the protractor started first or co-contractions could be observed.
When initiating forward or backward walking in middle legs with ground contact always the respective stance muscle (forward: RetCx, backward: ProCx) was activated first, independent from the starting position. The duration of this activity was dependent on the starting position.
For the hind leg this was generally true only for backward walking. In contrast, when inducing forward walking in the hind leg, the activation of the muscles depends on the starting position. In an anterior or middle position in more than 50 % of cases the retractor coxae was activated first. In the remaining initiations the protractor starts with activation or co-contractions could be observed. Hind legs in a posterior position started in about 70 % of cases with a premature swing phase, i.e. the protractor coxae was activated first.
Experiments where we tested if the campaniform sensilla or the tarsal sensors measure the sensory status of the leg showed that both sensory organs play an important role.

These results indicate that already before the start of the movement the motor neuron pools in all legs are appropriately coordinated depending on the task by the different incoming information.

Perisomatic inhibition mediated by axo-axonic cells in CA3 area of the hippocampus

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Hippocampal perisomatic-targeting GABAergic interneurons have a pivotal role in driving network gamma oscillations. However, it remains to be resolved how distinct perisomatic interneuron subtypes contribute to pyramidal cell excitability. To determine effect of activation of parvalbumin-expressed axo-axonic cells on the membrane of innervated principal cells we obtained dual patch-clamp recordings of monosynaptically coupled cells in CA3 area of the hippocampus from 3-4 week old mice. To leave the intracellular contents of pyramidal cells unchanged the cell attached experiments were performed in both in current and voltage clamp mode. Short burst of action potentials in presynaptic axo-axonic cell at 100 Hz which simulate the firing frequency of these cells in the active network triggered small membrane potential hyperpolarization in postsynaptic pyramidal cell. In addition, puff application of GABA (100 µM) at the axon initial segment of pyramidal cells resulted in a transient hyperpolarizing voltage deflection at the soma. Hyperpolarization evoked by application of GABA was reversible reduced by the bath-applied $GABA_A$ receptor antagonist bicucullin, indicating that the generation of these events required $GABA_A$ receptor activation. These experiments show that axo-axonic cells elicit hyperpolarizing inhibitory responses at axon initial segment of hippocampal CA3 pyramidal cells.

Electrophysiological characterization of circadian pacemaker candidates of the cockroach *Leucophaea maderae in-vivo* and at the level of single cells *in-vitro*

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In the cockroach *Leucophaea maderae* lesion and transplantation studies located the circadian pacemaker center which controls locomotor activity rhythms to the accessory medulla (AMe). The AMe is a small neuropil at the ventromedial edge of the medulla in the optic lobe, which shows nodular instead of retinotopical organisation. It is densely innervated by a multitude of peptidergic neurons, among them 12 pigment-dispersing factor immunoreactive (PDF-ir) neurons. These neurons are circadian pacemaker candidates, controlling locomotor activity rhythms in *L. maderae* and *Drosophila melanogaster*. PDF was shown to act as a non-photic input signal into the clock, delaying the phase of wheel-running activity at the late subjective day. Extracellular recordings of the excised AMe revealed circadian as well as ultradian rhythms produced by circadian pacemaker candidates. The ultradian oscillating cells produce very regular interspike-intervals and are coupled via synaptic (mostly GABAergic) and nonsynaptic interactions, forming different assemblies of cells that share the same frequency and the same phase (timing of spikes). Cells belonging to different assemblies differ in phase, but can share the same period. Application of PDF was shown to phase lock and thereby synchronize different assemblies mostly via inhibition of electrical activity.

To support the findings of the *in-vitro* model we established a new technique that enables extracellular as well as intracellular recordings *in-situ* in intact animals. Furthermore, we developed long-term primary cell cultures of fully differentiated adult neurons of AMe explants. This allows the characterization of individual presumptive pacemaker neurons with different patch-clamp techniques. In both approaches we test the effects of PDF, other neuropeptides and classical neurotransmitters on the electrical activity of AMe neurons. So far, we found physiological responses of AMe neurons to light pulses, to PDF, and to GABA. Some AMe neurons show ON/OFF sensitivity to light pulses, but no neurons with reaction to moving stimuli were found. PDF-sensitive neurons of the AMe apparently do not show reactions to light-stimuli. We hypothesize that responses to PDF-application can be simulated by cAMP but not cGMP. We are convinced that the combination of these electrophysiological methods offers further insights into circadian clock function. [Supported by DFG grant STE531-18].

MOUSE CENTRAL AND PERIPHERAL CIRCADIAN CLOCKS ARE ENTRAINED BY THE PHOTOPERIOD

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Under natural conditions the circadian rhythms are entrained to the 24-h day by the light-dark (LD) cycle, mostly by the light period of the day. In temperate zones duration of daylight, i.e., photoperiod, changes with the season. The molecular core clock mechanism generating the rhythmicity within the suprachiasmatic nucleus (SCN) is entrained by the photoperiod. Circadian rhythms expressed within the peripheral tissues are entrained via signaling from the SCN as well as via different mechanism, independent of the SCN.

The aim of the study was to characterize the effect of photoperiod on the molecular timekeeping system within different parts of the suprachiasmatic nucleus as well as peripheral tissues such a liver, heart and lungs. The effect of the long photoperiod and short photoperiod on daily profiles of clock gene Per1, Per2, Rev-erba, Cry1 and Bmal1 were studied.

Mice were maintained under the long (LD18:6; 18h of light and 6h of darkness) or short (LD6:18; 6h of light and 18h of darkness) photoperiod with rectangular light-to-dark transition and free access to food and water. On the day of the experiment, mice were released into constant darkness and sacrificed every 2h throughout the whole circadian cycle. Daily profiles of clock gene mRNA within the rostral, middle and caudal parts of the SCN were determined by *in situ* hybridization. In the peripheral tissues, the profiles of clock gene expression were determined by RT-PCR.

Photoperiod affected phase, waveform, and amplitude of the rhythmic profiles of gene expression as well as the phase relationship between the profiles within the rostral, middle and caudal mouse SCN. Moreover, the rhythms in clock gene expression within the liver, heart and lungs were also affected. The data demonstrate that photoperiodic modulation of the circadian system is complex and affects not only the structures within the brain but also in various peripheral organs of the body.

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Frequency processing by the identified vibratory interneurons in a non-hearing Ensifera (*Troglophilus neglectus*; Rhaphidophoridae) and its behavioural correlates

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In the wingless and non-hearing cave cricket *Troglophilus neglectus* (Rhaphidophoridae) twenty-six types of vibration-processing interneurons of the prothoracic ganglion have been identified anatomically and physiologically using intracellular recording and staining. The majority of the studied neurons express highest sensitivity to vibrational frequencies between 50 and 400 Hz. In a part of these "low frequency" neurons threshold curves at the high frequency side follow approximately the lines of constant displacement; in addition several of these units express inhibitory side-bands at frequencies above 400 Hz. This neuron class also includes the most sensitive of all recorded units with lowest thresholds between 0.005-0.01 m/s^2. Other "low frequency" neurons have broader tuning curves and receive no inhibition specific for high frequencies. Only six of the identified neurons represent "high-frequency" units, being most sensitive in the range between 400 and 2000 Hz. We have investigated the putative importance of the determined central frequency processing in the cave cricket within two behavioural contexts; mating and escape. Lateral body vibrations of males were observed during the courtship – communication mode of these animals that was previously unknown. The spectra of the produced vibration signals have not been recorded yet; however, the extremely low-frequency bias of the known tremulatory signals from other Orthoptera imply the importance of (a part of) low-frequency processing neural elements in intraspecific communication. Furthermore we investigated frequency-intensity characteristics of a stationary startle response, elicited by vibrational pulses applied to the unrestrained animal sitting on a loudspeaker membrane. The response movements, recorded by the laser-Doppler vibrometer from the back of the animals, express a sharp tuning to 50 Hz with lowest thresholds around 0.7 m/s^2 and latencies around 30 ms. We present a class of neurons with corresponding frequency characteristics and discuss their putative inclusion into the startle response pathway. The behavioural role of the identified "high-frequency" neural elements stays unclear.

Monoaminergic innervation of NPY-immunoreactive neurons in the rat amygdala: a neuroanatomical study

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INTRODUCTION The amygdala is a telencephalic nuclear complex playing a central role in emotional stimulus processing, especially for fear- and anxiety-related stimuli. Specific peptides such as the corticotropin releasing factor seem to have an anxiogenic function, whereas Neuropeptide Y (NPY) presumably has anxiolytic effects to keep up an „emotional homeostasis". NPY is a highly evolutionarily conserved neuropeptide and acts as a neuromodulator in the mammalian brain. Earlier investigations in experimental animals have suggested morphological and functional interrelations between monoaminergic and peptidergic amygdaloid systems. In double immunolabelings for light- and electron microscopy, we have previously documented occasional contacts of tyrosine hydroxylase (TH) immunoreactive (ir) dopaminergic afferents on NPY-ir neurons. In the present study the dopaminergic and, particularly, the serotonergic innervation of NPY-ir neurons in the lateral and basolateral nucleus of the rat amygdala (L+BL) were analysed in detail. **METHODS** Sequential double immunolabeling was carried out on serial 45 µm vibratom sections of perfusion-fixed rat brains. Immunoreactions for TH and the serotonin transporter (5-HTT) as marker for dopaminergic and serotonergic afferents, respectively, were detected by nickel- or silver-gold intensified 3,3´-diaminobenzidine (DAB), subsequent NPY-labeling only by DAB. **RESULTS** In our study the highest number of NPY-ir neurons (85%) counted in the whole amygdala was found in the L+BL. 76% of the NPY-ir neurons possessed oval and 24% round somata. 98% of these neurons displayed close perisomatic appositions by serotonergic afferents. Three appositions per NPY-ir soma could be enumerated, on avarage, regardless of the geometrical form of the somata. Two different morphologies could be distinguished in serotonergic fibers contacting NPY-ir neurons: the majoritiy (76%) showed thick varicosities, 24% displayed a narrow and smooth morphology. It was frequently observed that both axonal forms built pericellular baskets around NPY-ir somata. The proximal neurites of the NPY-ir neurons also showed serotonergic innervation, which seemed to be less prevalent than on the somata. In contrast, we found only sparse dopaminergic perisomatic innervation of NPY-ir neurons. **CONCLUSION** This neuroanatomical study provides a detailed description of the contacts of monoaminergic afferents on NPY-ir neurons in the L+BL. While dopaminergic perisomatic contacts appear sparse, our quantitative analysis show a dense perisomatic innervation by serotonergic axons. The high number of serotonergic appositions, sometimes in form of pericellular baskets, indicates a direct influence of serotonin on NPY-ir neurons. The exact nature of the contacts (synaptic vs. non-synaptic) will be determined by further analysis on an electron microscopic level. Moreover, the analysis of the receptor expression of NPY-ir neurons will provide more detailed information on the functionality of the dopaminergic and serotonergic afferents in the L+BL.

The accessory medulla in the optic lobes of butterflies: Towards the solution of a homology problem in a putative circadian pacemaker neuropil

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The main mass of the insect optic lobe is formed by three to four large neuropils, which process visual information such as object and movement detection. Additionally, one or two small neuropils reside in the optic lobes and are called, according to their positions, accessory lamina (ALa) and accessory medulla (AMe; ALae and AMae, respectively, for plural abbreviation). In larval brains of Holometabola, ALae and AMae are present before the other neuropils develop during pupation, and process visual information of larval eyes, the stemmata. In many Holometabola, these larval neuropils and stemmata remain in the optic lobes of adult insects. In hemimetabolous insects, AMe-like structures were only occasionally described until they attracted attention as probable integration centres for circadian timing information. They are densely innervated by medulla neurons expressing the neuropeptide pigment-dispersing factor (PDF). These neurons, generally known as PDFMe, or LNv in *Drosophila*, are involved in the insect circadian pacemaker system.

The generally assumed homology of AMae in hemi- and holometabolous insects is problematic, since despite their similar position they differ considerably in important respects. First, the AMae of hemimetabolous insects apparently do not receive direct input from photoreceptors. Second, the AMae of hemimetabolous insect are clearly not retinotopically structured and thus, unsuitable for image and movement detection. Third, nymphs of hemimetabolous insects generally do not possess specialised larval visual systems.

To trace the evolutionary origins of AMae in hemimetabolous insects and Holometabola, I started to investigate AMae in two lepidopteran species (*Pieris brassicae* and *Vanessa cardui*) by immunohistochemistry with antisera known to label typical AMe-neurons of hemimetabolous insects. The optic lobes of adult butterflies showed a prominent, compact AMe without any apparent stratification in an anterior position between medulla and lobula. Near the AMe lied somata of apparent PDFMe-neurons, which branched in the AMe. An antiserum against the neuropeptide allatotropin labelled a distinct cluster of smaller neuron somata in direct vicinity to the AMe that gave rise to compact and dense arborisation in the AMe. Allatotropin-ir neurons very similar in soma arrangement and arborisation profile were also described in cockroaches and locusts, and as is the case there, no PDF/allatotropin-ir colocalisation was observed in the butterflies. Also in L5-caterpillars of *V. cardui* an AMe with PDF- and allatotropin-ir terminals was observed, but a part of this AMe receiving visual input appeared to be largely devoid of immunostaining. Therefore, I propose that one part of the lepidopteran AMe, which is innervated by neurosecretory fibres (= neuropeptidergic AMe core), is homologous to the AMe of hemimetabol insects, and may belong to the ground plan of pterygote insects. In larval Holometabola, an early developing part of the medulla serves as secondary visual centre for the stemmata, and fuses with the neuropeptidergic AMe core. Thus, the whole AMe of Holometabola would be only partly homologous to the AMe in hemimetabolous insects. To test this hypothesis, the lepidopteran AMe will be further explored with other peptide antisera and backfill experiments in developing and adult butterflies.

Protein kinase C dependent connectivity and activity dynamics in developing cortical networks

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In early brain development, cortical circuitry and activity dynamics evolve on the basis of activity-regulated structural differentiation processes in neurons. In this scenario, a central regulator of neuronal morphology is the protein kinase C (PKC), which is activated via metabotropic glutamate receptor downstream signalling pathways. In a simplified model, activation of the PKC phosphorylates and mobilizes cytoskeletal proteins, thereby promoting structural plasticity. Antagonistic pathways involving the NMDA receptor mediated activation of protein phosphatases in turn promote cytoskeletal assembly and stabilization (Quinlan ‘96). The differential regulation of this structural homeostasis in the course of development is crucial for the establishment of proper connectivity statistics and the adaptive modulation of synaptic plasticity in neuronal networks.

We study this fundamental feature of neuronal systems in dissociated cortical cell cultures grown on micro-electrode arrays. These generic random networks display a self-regulated maturation process with similar phases as in the developing cortex. Within this period of network formation we interfered with the structural homeostasis by inhibiting PKC activity. Previous studies showed that inhibition of PKC activity in cerebellar slice cultures promotes dendritic outgrowth and arborization (Metzger ‘00) and that climbing fiber pruning is impaired in PCK deficient mice (Kano ‘95). Further *in vitro* data demonstrate the importance of PKC activity for experience-dependent modulation of synaptic weights on the basis of AMPA receptor trafficking (Zheng ‘08), suggesting reduced synaptic plasticity with PKC inhibition.

To assess the functional consequences of these dependencies, we chronically inhibited PKC activity in cortical cell cultures and compared network activity and connectivity characteristics. Applying new morphometrics, we found significantly increased arborization and extent of dendrites as well as increased synapse density, indicating increased connectivity in these networks. Spike activity remained organized in network-wide bursts that characteristically emerge in cortical cell cultures. Bursts were, however, more synchronized across the recording area and contained more spikes, suggesting faster propagation of activity through the network and longer reverberations due to increased connectivity. By further analyzing the temporal stability and the diversity of spatio-temporal activity patterns, we assess possible consequences of reduced PKC activity on the formation of functional pathways during early network development.

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Two independent cortical subnetworks control spike timing in layer 5 neurons during dynamic oscillation shifts

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Human brain oscillations occur in different frequency bands that have been linked to different behaviors and cognitive processes. In EEG recordings, oscillations in the beta and gamma frequency band (15-90 Hz) are associated with attention and working memory. Even within specific frequency bands oscillations fluctuate in frequency and amplitude. Such frequency fluctuations most likely reflect changing states of neuronal network activity, as brain oscillations arise from the correlated synchronized activity of large numbers of neurons. However, the neuronal mechanisms governing the dynamic nature of amplitude and frequency fluctuations within frequency bands remain elusive.

Here we show in rat brain slices that frequency fluctuations are generated by two distinct cortical subnetworks within the same cortical column. Upon application of the muscarinic agonist carbachol superficial layers 3/5 showed a higher oscillation frequency than deep layer 6 (L3/5: 16.6±1.0 Hz, L6: 11.2±0.5 Hz, n=14, $p<0.01$). In layer 5 both low and high oscillations frequencies were present in the power spectrum. The frequency of oscillations was temperature-dependent. Linear extrapolation suggests that at physiological temperatures, both oscillation frequencies would fall within a range of 25 – 40 Hz. By recording from individual cortical neurons during oscillations, we show that layer 5 pyramidal neurons and interneurons alter their spike timing upon shifts between episodes of low and high oscillation frequencies. Frequency and phase information is encoded and relayed to layer 5 neurons through timed excitatory and inhibitory synaptic transmission. In addition, we find that in EEG of humans and in intracranial EEG of awake, freely moving rats at rest, episodes of high (~23 Hz) and low (~18 Hz) oscillation frequency in the beta band (15-30 Hz), lasting 100 to 400 ms, occur independently. These latter observations suggest that frequency fluctuations in brain oscillations could be a general phenomenon.

In conclusion, our data indicate that frequency fluctuations reflect synchronized activity in distinct neuronal networks, and suggest that cortical subnetworks can process information in a parallel fashion.

Identification of long-range calcium waves in the mouse cortex

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We had previously reported the presence of wave-like calcium signals in the cortex of newborn mice in vivo (Adelsberger et al., Nat. Neurosci., 2005, 8:988-990). Here we report the identification of large-scale Ca transients in the cortex of adult mice. In order to analyze these Ca waves we developed a new imaging method for the analysis of large cortical regions of the mouse cortex. This method involved the use of a high speed CCD camera (up to 1000 frames/s) and a new variant of the multi-cell bolus loading technique of calcium sensitive dyes, like OGB1-AM (Stosiek et al., PNAS, 2003, 100:7319-7324). First, we detected wave-like Ca transients that occurred spontaneously at a rate of about 0.5 waves/s in isofluorane-anaesthetized mice. These spontaneous Ca waves were in general first observed in the frontal cortex, from which they propagated distally. By using optical fiber-based measurements, we found that these waves also crossed to the other hemisphere. The rate of occurrence (1-2 Hz) and the speed of propagation of 50-60 mm/s identified the new responses as Ca signals associated with the classical slow wave activity, known to be particularly prominent during non-REM sleep (Steriade et al., Neuron, 2003, 37:563-576). We found next that similar Ca waves could be triggered by brief sensory stimuli, like for example a 50 ms duration light flash or an 100 ms auditory sweep stimulus. The evoked Ca waves triggered by either visual or auditory stimuli were first observed in the corresponding primary sensory cortical regions, the visual or auditory cortex, respectively. From there, the Ca waves spread radially and recruited eventually the entire cortex. The speed of propagation of the spontaneous and the triggered Ca waves were similar. Furthermore, we found that during a period of several hundred milliseconds following the onset of a spontaneous Ca waves, no sensory-evoked Ca waves could be triggered. This refractoriness and the similarity in both kinetics and speed of propagation indicated that the spontaneous and the sensory-triggered slow Ca waves involve the activity of the same neuronal population.

In conclusion, our results demonstrate that slow wave-associated Ca transients represent a global cortical signal. Our results suggest that the spontaneous and the evoked slow waves are initiated in highly distinct brain regions. These waves may serve the long distance transmission of information within the cortex.

Impaired gamma frequency oscillations in the entorhinal cortex in a mouse model of mesial temporal lobe epilepsy

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Mesial temporal lobe epilepsy (mTLE) is the most common form of epilepsy, characterized by recurrent complex partial seizures and hippocampal sclerosis. Since the entorhinal cortex (EC) occupies a pivotal position in gating hippocampal input and output the dysfunction of this region may contribute to epileptogenesis in humans. To identify the anatomical and functional changes in the EC, we performed field potentail recordings and immunohistological analysis in a new model of mTLE 3-4 weeks following KA-injection into the entorhinal cortex. Field potential recordings were done from different region of the EC. Morphological analysis of lateral and neighboring perirhinal cortices revealed no clear differences in the lamination between control and epileptic group. In contrast, the medial EC display significant shrinkage that could be attributed to neuronal loss in this area. According to expectation, in epileptic mice field gamma oscillatory activity in this area has lower power than in control mice. In contrast, the network gamma oscillations in the lateral EC have significantly higher power in epileptic than in control mice. We conclude that a structural and functional reorganization in the medial EC network following KA-injection may contribute to altered rhythmogenesis and the development of seizure activity in mTLE.

Transient Oscillations in Ongoing Activity

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Ongoing activity in the brain is receiving increased attention in recent years. Unlike event related experiments, that require extensive averaging of signals to uncover conditional changes, this research focuses on the nature and functional role of ongoing activity. Previous work in electroencephalography (EEG) has focused on recurring scalp topographies and rapid transition processes amongst others. Recent fMRI studies reveal several networks of correlated BOLD signals in ongoing activity that are linked to e.g. a default mode of the brain. These experiments have found ongoing activity to play a role in a range of cognitive functions and pathologies.

In contrast to existing EEG studies, that investigate large changes in amplitude of ongoing signals, we focus on transient oscillations (TO) at various frequencies in the ongoing signal. Using a time frequency decomposition of a data-set from a bistable perception task, band-pass filtered between 2 and 40 Hz, we isolate transient relative power changes (increases) in ongoing oscillations. After dipole fitting and multi-dimensional scaling these TO's are clustered into several classes each comprising distinct source activities. These clusters occur across the whole spectrum, however are more prevalent in Alpha, Beta and Theta ranges. They have clear and individual frequency characteristics. Each is present in one or more distinct frequency bands only, e.g. in the Theta range at 5 Hz and low Gamma around 30 Hz. The relation between TO clusters and their underlying brain areas is shown using beamforming source localisation. The classes show activity in discrete regions of the brain individually, e.g. prefrontal cortex, various areas in the motor cortex and occipital regions. Overall these activity patterns complement each other and involve most of the brain. The transient nature of TO's results in sequential re-occurrence of the TO classes. We show the transitions between clusters and their relation to perception and cognition.

We introduce transient oscillations as a promising tool to gain new insight into ongoing activity. In particular we present its application on a bistable perception task.

Modulation of Stimulus Efficacy by Ongoing Activity and Reproducibility by Online-interaction with Neuronal Networks *in vitro*

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The neocortex receives massive sensory input, processes this information and generates output commands of astonishing precision and reliability. In-vivo responses to the same stimuli are, however, highly variable with respect to number of spikes and phase-locking to the stimulus. In some cases, response prediction is possible only when the cortical activity state at stimulus onset is taken into account [1]. Neuronal interactions during natural inputs are thus arising as dynamical processes and very little is known about the mechanisms that govern them. We want to understand how neuronal networks respond to incoming stimuli, which interactions arise and how these influence their responses. We aim for a user-defined interaction with ongoing activity that controls for the activity state during stimulation and thereby increases response reliability and reproducibility.

We use in vitro cortical cell cultures grown on microelectrode arrays as generic neuronal networks to address these issues. Neuronal activity can be recorded from 60 sites simultaneously over months under controlled conditions. Multi-site electrical stimulation enables to study the spatio-temporal processing of input patterns.

We show that stimulus efficacies are network-state dependent. Ongoing activity, consisting of synchronized, network-wide bursting, modulates response length and delay. High bursting activity prior to stimulation results in short responses and large delays. Low bursting activity before stimulation results in long responses and small delays. Stimulus efficacy was modulated by 20-60 seconds long periods of 3-4 folds increased firing above baseline, so called superbursts. Responses were longest and efficacy was maximal during superbursts. Responses were shortest or stimulation even failed to elicit spikes directly after superbursts.

Network-state dependent stimulus efficacies confine the examination of input/output dynamics and demand for a user-defined interaction with ongoing activity. Phase-coupled input, that is, stimulation during a pre-defined network state minimized the interference between spontaneous and induced activity. Response reliability and reproducibility was increased for phase-coupled compared to random, un-coupled stimulation.

Modulation of stimulus/response dynamics by ongoing activity gives thus rise to state-dependent processing of external inputs. Interaction with ongoing activity can improve response reliability and reproducibility and thereby contribute to an understanding of the processing capabilities in neuronal networks in vitro, and other, physiological more realistic systems in general.

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How ionic conductances affect the temporal precision of action potentials

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Although for bare spike generation only very few ionic conductances would be needed, neurons express a large variety of ion channels with diverse properties. It can hence be assumed that these conductances play an important role in shaping neural responses. One relevant characteristic of neural responses is the timing precision of action potentials, which is known to depend on the stimulus as well as on properties the responding neuron. Here, we analyze conductance-based model neurons and present two mechanisms by which ionic conductances can influence spike-timing precision. First, ionic conductances can set the stimulus frequency for which the spike-timing jitter is lowest. We illustrate that this mechanism allows for fast dynamic regulation of spike-timing precision via neuromodulation. Second, ionic conductances can set the overall sensitivity of spike timing to noise. In this case, ionic conductances can widen or narrow the range of stimulus frequencies that are transmitted with high temporal precision. Moreover, we show that such modifications of the sensitivity to noise are reflected in neuronal phase-response curves, which characterize the impact of perturbations on the timing of the following spike as a function of firing phase. In particular, we show mathematically that the jitter of action potential timing can be derived from phase-response curves in a quantitative fashion. This type of analysis allows to predict the influence of individual ion channel types on spike precision and leads us to conclude that temporal precision is, for example, increased by slow potassium channels while it is impaired by persistent sodium channels. As malfunctions of individual ion channel types can lead to serious neurological diseases affecting network synchronization, such as epilepsy, it is important to further explore the role of ionic conductances for signal processing in individual neurons as well as neuronal networks.

Structural and functional embedding of individual neurons into cultured neuronal networks

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We investigated the local connectivity and individual activity of neurons as key features of their embedding into larger generic networks. In culture, dissociated cortical cells obtained from neonatal rats form such simple networks. When maturing, the neurons make synaptic connections in apparently random fashion and the networks display a characteristic bursting behavior (Maeda *et al.*, 1995; Wagenaar *et al.*, 2006) with simultaneous onset timing in a range of 10-100 ms. We recorded activity from networks with a local density (*i.e.* in a 100 µm radius) of 1,000-2,000 cells per mm^2 with 60-site microelectrode arrays (MEA; 200 or 500 µm electrode distance, 1.4x1.4 or 2.5x4.5 mm array size) and dual patch-clamp electrodes.

Based on the intracellular patch-clamp recordings, we determined the pairwise connection probability at up to 250 µm distance. We identified excitatory (exc) and inhibitory (inh) postsynaptic potentials in response to presynaptically evoked action potentials (AP) as well as unidirectional (UD) or bidirectional (BD) connections. Of all neuron pairs (n=94), 38% were connected and 62% were unconnected, in agreement with reports in Nakanishi & Kukita (1998). We further found that 18% of all pairs had UD exc and 4% UD inh connections. 12% of the pairs had BD exc-exc, and 2% both BD inh-nh or BD exc-inh connections. The connection probability decreased with distance. The observed connection probability for UD and BD connections lies above the expectation values described by Song *et al.* (2005) for the connectivity in native cortex.

We further characterized the functional embedding of individual neurons into the global bursting activity. Individual neurons followed bursts in the local network recorded extracellularly at nearby MEA electrodes with sharp onsets and narrow temporal jitter (±10 ms). Local population activity onsets are thus predictive for the timing of activity onsets in individual neurons. Activity onsets of individual neurons with respect to distant network regions ranged from near-simultaneous to preceding or delayed timing, but likewise with only a small temporal jitter between pairs. In contrast, MEA electrodes that repeatedly fired before burst onsets in many network bursts, and thus reliably predicted burst onset in the network, only weakly predicted the spiking of individual cells, largely irrespective of their absolute distance. Individual delays to these predictors were broadly distributed, with a peak at approx. 50 ms. The overall activity within bursts was typically higher than average at these sites and their peak rate coincided with intracellular activity onsets.

The low probability for direct connections at distances of several hundred µm suggests that this narrow timing of burst onsets at most sites in the network can be maintained via polysynaptic connections, which could be supported or mediated by bottlenecks or hubs in the propagation pathways within the network, such as described recently (Shahaf *et al.*, 2008).

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Concurrent expression of two mutually exclusive locomotor patterns - walking and flight in the locust

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A key problem in motor control is how the central pattern generators (CPG) for behaviour are organised. While similar forms of locomotion (e.g. gaits) may be controlled by a common oscillator, we ask to what degree the CPGs for mutually exclusive behaviours share common circuitry. In locusts, flight and walking are such incompatible behaviours, that are each driven by a CPG.

Minute-long stepping sequences (0.05-0.2 Hz) of leg levator and leg depressor motor units corresponding to walking can be elicited by applying the muscarinic cholinergic agonist pilocarpine (Ryckebusch and Laurent, 1993 J Neurophysiol 69(5):1583-1595). Interestingly, pilocarpine also initiates bouts of locust flight (10-13 Hz), which is typified by alternating activation of wing elevator and wing depressor muscles (Buhl et al., 2008 J Exp Biol 211(14):2346-2357). This response lasts several minutes and typically comprises fictive flight sequences alternating with silent periods that vary throughout a sequence and between preparations from seconds to minutes. In the present study, simultaneous recordings from leg and wing motor units revealed that both the flight and walking patterns can be executed by the locust deafferented thoracic nervous system simultaneously. Seconds after bath application of pilocarpine (1-5 mM) a previously silent preparation produced motor activity in the flight muscles, resembling the co-ordination of a normal flight pattern, and in parallel activated the leg motor units alternately corresponding to the walking pattern. To date, we have found no indication that pauses or activity sequences in the one rhythm results in changes in the temporal timing pattern of the other rhythm. However, close inspection of concurrently active sequences often revealed tight temporal coupling of action potentials in wing depressor- and fast trochanter depressor motor units at fictive flight frequency during the stance phase.

These findings, at the whole system level, verify observations from single unit recordings (Ramirez and Pearson, 1988 J Neurobiol 19(3):257-282) favouring the existence of distinct pattern generators for flight and walking that operate largely independently, without prohibiting each other, and having only weak synaptic connections between the two.

Comparison of morphological and electrophysiological cell properties in different segments of the medicinal leech

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The nervous system of the medicinal leech *Hirudo medicinalis* consists of 21 sequentially connected segmental ganglia and two "brains" at the anterior and posterior end of the animal. Most mid-body ganglia are extremely similar in structure and function. They contain approximately 400 individually characterized neurons, which can be found in each ganglion in approximately the same place with approximately the same morphological and electrophysiological properties. However, the ganglia in segments 5 and 6 are considerably different from the other mid-body ganglia. These ganglia contain several additional neurons, some of which are known to be involved in the control of the reproductive organs, which are located in these segments.

The Retzius cell (RZ), probably the best-studied neuron in the nervous system, is present in each ganglion twice. This cell is known to have different functions in reproductive and other mid-body ganglia [Wittenberg et al., 1990]. Segmental differences can already be seen morphologically (compare Fig. B: Rz in ganglion 8 and A: Rz in ganglion 5, both filled with neurobiotin). Retzius cells in most mid-body ganglia send processes to both adjacent ganglia and to the body wall of their own segment. These long-range processes are missing in Retzius cells of segments 5 and 6, which were shown by Glover & Mason 1986 to innervate the genitalia instead.

Differences in Retzius cell properties between segments 5/6 and the other midbody-ganglia can also be seen electrophysiologically (compare Fig. C: double recording of Rz cells in ganglion X and D: double recording of Rz cells in ganglion 5). In most ganglia the electrical coupling between Retzius cells is extremely strong, yielding a 1:1 coupling between presynaptic spikes and EPSPs with most EPSPs resulting in a postsynaptic spike. In ganglia 5 and 6, however, the electrical coupling is much weaker, as it would already be expected from the absence of staining in the second ganglion cell when one of the Retzius neurons is filled with neurobiotin (Fig A).

For other well-known neurons like the mechanosensory cells, however, segmental specialization should not be expected. Their main function to enable the leech to move away from harmful tactile stimuli should be present in reproductive as well as in other mid-body ganglia. Since the mechanosensory cells are also paired, electrically coupled neurons, we will compare in this study their morphological and electrophysiological properties in different leech ganglia to properties of the Retzius cells.

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Dopaminergic silencing of spontaneous network activity in the developing zebrafish spinal cord

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Neural network assembly is a complex process that involves a suite of tightly orchestrated developmental events. One hallmark event that occurs during network ontogeny is the onset of spontaneous, synchronous patterns of activity that persist for a transient period of development. Such activity is unique to developing networks, being later replaced by more mature forms of activity that resemble those observed in the adult nervous system. Output generated by immature networks is thought to be important for maturation of nascent nervous tissue.

Whilst the developmental roles of early spontaneous network activity have been studied in considerable detail, less is known of the mechanisms that regulate the transition from immature to mature forms of network output. In order to address this issue, we have examined putative mechanisms controlling the termination of embryonic network activity in the zebrafish embryo, a popular model for developmental studies. During early life, neurons of the zebrafish spinal cord transiently generate slow, synchronous bursts of network discharge. This activity only occurs between around 17 and 30 hours in development, after which the network acquires the ability to generate rhythmic locomotor output.

We demonstrate here that the neuromodulator dopamine (DA) acts as a potent inhibitor of embryonic network activity in the developing zebrafish spinal cord. Using whole cell patch clamp electrophysiology, we show that exogenous application of 1-20µM DA completely abolishes spontaneous network activity in embryonic spinal neurons. We further show that the inhibitory actions of DA can be blocked by pre-incubation with DA receptor antagonists. Our findings suggest that developing dopaminergic systems may be causal in silencing embryonic network activity and could help facilitate the subsequent transition to more mature forms of network output.

Multi-transistor array recording of field potentials in acute hippocampal slices at high spatial resolution

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The analysis of epileptiform signals and oscillations in acute brain slices often rests on simultaneous extracellular recordings at different sites. Planar metal electrode arrays (MEA) were used previously to probe the CA3 region of hippocampal slices at a resolution of 100um [1]. To investigate network dynamics in its complexity it is desirable to record 2D maps of field potentials at higher spatial resolution.
To overcome this problem we used silicon based field effect transistor (FET) chips. For understanding the principle of the recording, hippocampal slices of mice and rats were coupled to simple field effect transistor chips. We found that the FET recordings were identical to micropipette electrode signals. In spite of inactive cells caused by cutting processes the recorded signals at the bottom of the slice were about 40% of their maximum in the center of the tissue [2].
To record 2D maps of high spatial resolution we used CMOS fabricated multitransistor arrays (MTA), which provide a spacing of 7.8um within an area of 1mm^2 [3]. The acute brain slice could be displaced to probe multiple areas of the slice. We performed following experiments: (i) After stimulation by an tungsten electrode the pathways mossy fibers–CA3 and Schaffer collaterals–CA1 could be mapped at high resolution. (ii) Epileptiform signals were evoked by application of Mg(2+) -free medium as well as bicuculline to resolve 2D maps of field oscillatory activity. Sharp wave complexes and phase-locked fast ripples in CA3 could be monitored in space and time. (iii) Carbachol was applied to induce spontaneous field oscillations in the gamma and theta range. Highly synchronized oscillatory patterns along cornu ammonis and near the dentate gyrus could be resolved by the MTA at high spatial resolution.

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Determination of Singular Activity Patterns of Functional Neuronal Networks on Microelectrode Arrays

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Neuronal networks of primary dissociated cell cultures grown on microelectrode arrays (MEA) are an interesting alternative for substance screening in drug development and safety pharmacology. The activity pattern changes obtained following chemical stimulation of these networks are both reproducible and substance-specific, so that they are applicable as a new read-out system for cell-based drug screening.

This report is going to present results about the finding of new singular activity patterns which might be of more general interest. This presentation describes data analysis and classification methods of activity patterns of neuronal network cultures on MEA neurochips. The spike train parameters that describe the activity patterns of spontaneously active neuronal cell cultures are different from those needed for stimulation experiments of brain-slice cultures. The parameters which are most suitable are evaluated by performing classification experiments based on our activity pattern data base of 80 neuro-active substances. Since the substance effects depend on the concentration, it is being investigated whether a classification approach with different concentrations is possible.

>On the base of these investigations we discovered a very singular activity pattern caused by baclofen and gabapentin. They reveal a unique activity pattern for their bursting behaviour. These results will be discussed and compared to results from computational neuroscience which also show similar effects on general activity and burst structure.

How Neural Responses to Supra-Threshold Inputs affect Circuit Dynamics: Desynchronization via Partial Reset

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The response of a neuron to synaptic input strongly depends on whether or not it has just emitted a spike [1]. An excitatory stimulus that causes a neuron to spike will maximally shorten the inter-spike intervals (ISI) in which the stimulus is applied. Additionally the following ISI is typically affected as well, e.g. due to charge transfer among different neuronal compartments. Spike time response curves (STRC) encode the change of the (ISI) following an excitatory input at different phases of a periodically firing neuron. For instance in a two-compartment conductance based neuron model (type I) we observe a shortening of the second ISI, i.e. a positive second STRC, after supra-threshold stimulation.

We propose a simple point neuron model taking this effect into account by introducing a partial reset to supra-threshold inputs [2]. It allows for rapid numerical integration and analytical study of its collective networks dynamics. We uncover a novel desynchronization mechanism that causes a sequential desynchronization transition: In globally coupled neurons an increase in the strength of the partial response induces a sequence of bifurcations from states with large clusters of synchronously firing neurons, through states with smaller clusters to completely asynchronous spiking.

We indeed observe similar desynchronization transitions in networks of the two-compartment conductance based neurons when varying the coupling between soma and dendrite which in the approximate partial reset model controls the partial reset strength.

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Evidences for nitric oxide/cyclic guanosine monophosphate (NO/cGMP) signalling in putative circadian clock neurons of the cockroach *Leucophaea maderae*

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For most animals, the daily light cycle is the principal zeitgeber for synchronisation of the endogenous clock to the physical day. This entrainment is performed by time-dependent phase shifts of the endogenous rhythm in reaction to the daily rhythmic changes in the presence and physical quality of ambient light. Further, endogenous clock elements such as single pacemaker neurons must constantly synchronise each other. Entrainment and mutual synchronisation are generally performed by time-dependent phase shifts, which can be experimentally described by so-called phase response curves (PRCs). Here we report evidence of a role for NO as a novel candidate for pacemaker entrainment and/or synchronisation in the circadian system of the cockroach *L. maderae*.

In the cockroach and other insects, neurons expressing the peptide pigment-dispersing factor (PDF) are thought to be the central circadian clock's pacemaker and output neurons (reviewed by Homberg et al. 2003, Chronobiol Int 20:577). PDF-immunoreactive (-ir) neurons of the medulla (PDFMe; known in *Drosophila* as ventral group of 'lateral neurons', LNv) arborise densely in the accessory medulla (AMe), a small neuropil region within the optic lobe of the cockroach, which is suggested to be an integration centre for circadian timing information. Using anti-citrulline immunolabelling and NADPH-diaphorase histochemistry as markers for activity of the NO producing enzyme nitric oxide synthase (NOS), we labelled fibres in the AMe neuropil. This NOS activity arose mostly from a distinct set of about a dozen neurons closely attached to the AMe. Among them was a subgroup of the PDFMe. Further, with anti-cGMP immunohistochemistry we labelled about 40 mostly peptidergic neurons of the AMe. These also included a subpopulation of the PDFMe, but there was little overlap with the citrulline-ir PDFMe. Since the cGMP-ir neurons are presumed targets of NO signalling in the AMe, our results suggest that the NO/cGMP pathway is involved in the cockroach's circadian system, and may convey synchronisation signals to and between clock neurons.

To test this hypothesis, we have begun to investigate the function of NO/cGMP signalling in the circadian clock of the cockroach with combined pharmacological and behavioural experiments. An NO-donor with short half-life (PAPA-NONOate) was applied at different subjective times to the AMe of cockroaches living in constant darkness. Their free-running circadian locomotor activity was assessed with running wheels. Circadian period lengths before and after operation as well as resulting phase shifts were measured. Preliminary results indicate a significant NO-dependent mean phase delay of 1.5 h at CT 12, the beginning of the subjective night. In this ongoing study, we are attempting to establish a complete PRC for NO application, which will help to identify the role of NO/cGMP signalling in pacemaker entrainment and synchronisation in the insect circadian system.

A single dose of ketamine impairs attentional set-shifting in rats: an animal model of schizophrenia-like cognitive impairment?

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Cognitive impairment is a core symptom of schizophrenia, with deficits in executive function particularly relevant to the quality of life and everyday functioning. Schizophrenic patients develop neuropsychological dysfunctions across a range of cognitive domains, including reduced flexibility in modifying behavior in response to changing relevance of stimuli. This aspect of executive function is commonly assessed in humans using Wisconsin Card Sorting Test (WCST). In fact, poor WCST performance is an important diagnostic feature of schizophrenia. Cognitive inflexibility might be also modeled in healthy humans after a single exposure to ketamine, the noncompetitive antagonist of N-methyl-D-aspartate (NMDA) receptor. Although many studies have demonstrated ketamine-induced working-memory deficits in rats, there has been no evaluation of the drug's effect on attentional flexibility in rodent task.

The purpose of present study was to investigate the effect of acute ketamine (3 and 10 mg/kg) treatment on male Sprague-Dawley rats' performance in the attentional set-shifting task (ASST). In this paradigm, animals have to select the bowl containing the food reward; the pairs of bowls are distinguished by either an odor or a medium that covers the bait. Rats perform series of discriminations including simple and compound discrimination, an intra-dimensional shift (IDs), an extra-dimensional shift (EDs) and reversals. This task requires rats to initially learn a rule and to form an attentional set within the same stimulus dimensions (IDs). During the EDs, the crucial part of the task, subjects have to switch their attention to a new, previously irrelevant, stimulus dimension (e.g. odor to medium). The number of trials required to achieve a criterion of six consecutive correct responses is recorded for each rat and for each discrimination problem and serves as an index of cognitive performance.

The results of the present study have demonstrated that control rats required significantly more trials to reach the criterion on the EDs stage, than on the IDs stage of the task, indicating the development of an attentional set. Ketamine administrated at a dose of 10 mg/kg significantly and specifically impaired rats' performance at the EDs stage of ASST. There was no significant drug effect during any other discrimination stage. In sum, these data provide a link between preclinical and clinical models based on ketamine-induced set-shifting deficits. This rodent model may be useful in the evaluation of potential treatments of schizophrenia-related cognitive deficits.

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What is 'anti' about anti-reaches? -- How reference frames affect reach reaction times.

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Reaches can be aimed either directly towards visual objects, or the reach goal can be inferred indirectly from spatial information about visual objects. Anti-reach paradigms are characterized by this difference and typically show that reaches to spatially inferred targets are initiated slower than reaches towards directly cued targets. Kornblum (1990) suggested a dimensional overlap model to account for such effects of stimulus-response congruency. However, this model can not identify the stimulus-response features that are critical for the time delay. This makes it difficult to relate the model to the underlying neural mechanisms which account for the behavioral findings.

In a standard anti-reach task the spatial cue and the associated motor goal are either are either congruent (pro-reach) or incongruent (anti-reach). This congruency can be interpreted either as the coincidence of the cue with the reach-endpoint position (location congruency) or the equivalency of the directional information contained in the cue with the direction of the reach (direction congruency). We developed a generalized anti-reach task to find out why anti-reaches are slower than pro-reaches. Our new task design let us define reaches in the same direction but to a different location than a spatial instruction cue ('pro'-reach, by definition), and reaches in the opposite direction but to the same location as the spatial cue ('anti'-reach). Variable eye and hand starting positions allowed us to differentiate the effects of direction and location congruency on reach reaction times. Results show that reaches are faster whenever the location of the visual cue matches the endpoint of the reach. In contrast, direction congruency has no impact on reach reaction times, despite the fact that this feature is used in the behavioral instructions to dissociate pro- from anti-trials.

We present a conceptual model that explains the location congruency effect based on previous neural findings. In this view visually instructed reach goals are initially represented in an eye-centered frame of reference, and the planning of pro-reaches benefits from an easier direct mapping of visual cue representations onto visuospatial, eye-centered motor-goal representations. We also observed an effect of eye-hand congruency on reaction times. Our model explains this by an additionally required transition from eye- to hand-centered motor goal representation prior to movement initiation. Our data shows that neural motor goal representations in different frames of reference not only affect reach kinematics during motor control, but also reach planning.

No evidence of emotional modulation of consolidation in sequence learning

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Memory consolidation may be defined as a stabilizing process occuring after the end of the exposure to the new experience. Consolidation of declarative memory may be modulated by emotions and the amygdala plays a crucial role in this process. However, less is known about the modulatory effects of emotions on implicit processes, such as in consolidation of a motor memory. In the present experiment we tested if emotions modulate the consolidation of serial reaction time task (SRTT) depending on the dimensions of emotional valence and on the time interval of the emotional interference with respect to the termination of the motor learning task. The results do not support the hypothesis that consolidation of procedural skills is modulated by timed emotional interference. This finding thus differs from previous studies demonstrating emotional modulation in the consolidation of tasks of explicit learning. Although methodological factors cannot be ruled out, it is possible that the emotion system is differently involved in different memory domains. Because consolidation of procedural skills were previously shown to depend on the dorsal striatum, it is conceivable that the failure of emotional valence to modulate consolidation of procedural tasks is related to the absence of strong amygdalo-striatal projections.

The African spitting cobras *Naja pallida* and *Naja nigricollis* adjust their spitting pattern to target distance

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Spitting cobras defend themselves by ejecting their venom towards the face of a human harasser. Circulating head movements of the cobra ensure that the venom is distributed on the face. To assure an optimal distribution of the venom the amplitudes of the head movements should decrease with increasing target distance. To find out whether cobras (*Naja pallida* and *N. nigricollis*) indeed adjust their spitting act to target distance we induced spitting from different distances. For data analysis the venom was collected on a plastic screen, situated between the snake and the harasser, where it formed spitting patterns. We found that the horizontal and vertical angle of venom ejection decreased when the human harasser was further away. Thus, spitting cobras adjust their spitting act to target distance.

Song features as a basis of mate choice in a grasshopper - do they correlate with the condition of the males?

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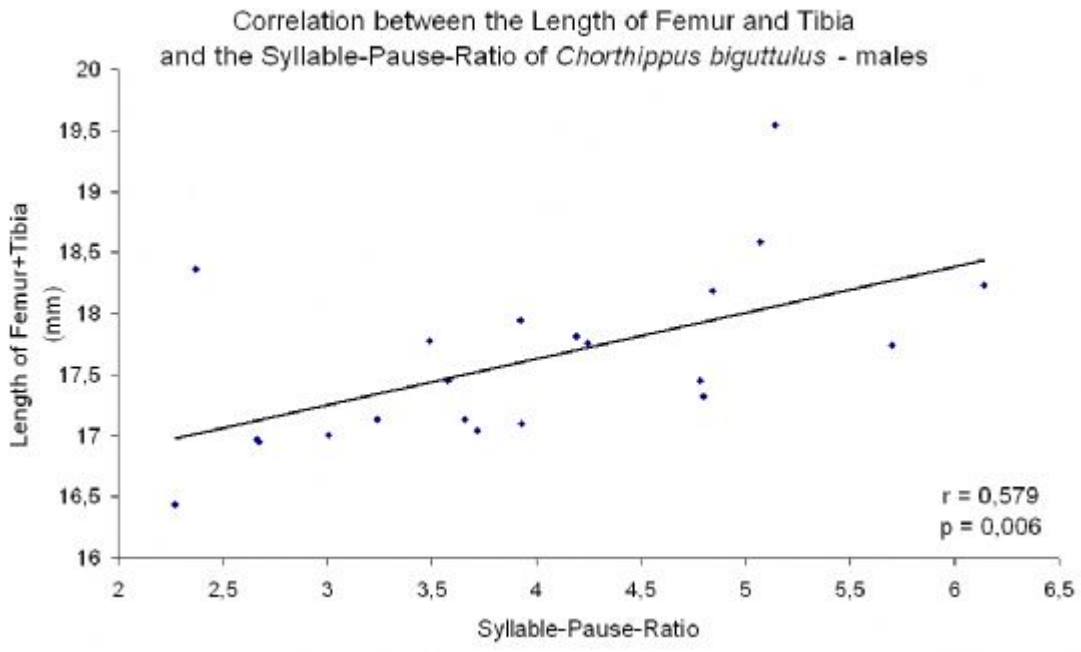
Many gomphocerine grasshoppers use acoustic communication to attract and to localize their mates. The "songs" are produced by rubbing the hind legs against a vein on the forewings. The temporal pattern of leg movements is driven by a central pattern generator which leads to a species-specific pattern of sound amplitude modulations. Typically males stridulate to attract females, but in several species a duetting mode of communication has evolved: females respond to the song of a conspecific male if they are inclined to mate. As males provide only sperm while females invest heavily in large eggs, the situation is typical for sexual selection. Females of the grasshopper *Chorthippus biguttulus* were indeed found to be choosy, and to reject songs of males that have lost one hind leg (Kriegbaum 1989, Naturwissenschaften 76: 81). Additional evidence exists that females evaluate males on the basis of their songs, i.e. the presence of certain attractive features (e.g. Klappert & Reinhold 2003, Anim. Behav. 65: 225). What acoustic features confer attractiveness to a song is currently investigated in a combination of behavioral and neurophysiological experiments. Here we take a different viewpoint and ask what information about the potential quality of a male singer a female can extract from a heard song? In order to be useful indicators songs should be an honest signal. Does song provide reliable cues about morphological features that could be used by a female to infer the condition or even the genetic quality of a male? To this aim we searched for correlations between characteristic song features and morphometric characters of individual males. Special attention was given to features that are known to be decisive for acceptance of the song as stemming from a conspecific male. Important song features in this context are the syllable-to-pause ratio of the song pattern and the accentuation of syllable onsets. We found significant correlations between both song characteristics and the length of the femur and tibia of the hind legs ($p = 0.006$; see Fig.1). Hence females could exploit these song features to assess a male's size and potential quality.



Cognitive binding during goal directed hand movements

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In daily life a large amount of skilled movements can be observed like picking up an object or hitting a ball. At first, the target has to be selected for action and subsequently attention has to be focussed on the target throughout the move. During the move the control error is reduced by perception-action-cycles. Selection for action is based on the phenomenon that various different optical cues are combined to form a unified cognitive impression. Such visual binding is based on the assumption that a selection of cues activates a population of neurons, a cell assembly coincidentally. It is quite natural to focus on the selection of a target. However, it is strange to be forced to choose a valid effector. Subjects are able to control cursor movements on a screen easily. Thus the question comes up how visual binding occurs during goal directed movements if there are different potential effectors and cursors, respectively. During the move the subject has to explore the position of the valid effector. He or she has to bind to a certain effector. This allows to analyse how binding is affecting eye-hand-coordination if attention is divided between target and effector.

Eye-hand-coordination of 9 subjects during two subsequent sessions was recorded. The task was to hit a small moving target circle by means of a cursor. The position of the target and of the cross-hair cursor were displayed on a screen. The target moves along a circular path 24 cm in diameter with a marked centre. The single target and the cursor cloud appeared when the marked centre was hit. The cursor cloud consisted out of a single valid cursor in a cloud of 18 distracting invalid cursors arranged randomly within a circle of 7.2 cm in diameter. While moving a pen on a digitizer board the conspicuity of a valid cursor resulted from its faster speed in relation to the moving cursor cloud. Three different relative speeds between valid and distracting cursors and three different target speeds were investigated in a full factorial statistical design.

At the start of a single experimental trial the cursor had to be moved to the marked centre of the circular path of the target in order to make it appear. Without a distracting cursor cloud the randomized initial target position and its velocity was detected in the periphery of the eyes and resulted in an immediate saccade towards the target in order to fixate it. However, the application of a distracting cursor cloud forced the subject to detect the valid cursor at first to be able to hit the target. Now, the strategy of the eye movements changed. During the fist part of the trial the moving cursor cloud was fixated and not the target. Obviously the target was detected in the periphery during the phase of looking at the cursor cloud. The required time to hit the target increases significantly with decreasing relative speed between valid cursor and cursor cloud. However, even during high relative speed it rarely occurred that a saccade towards the target is performed. Obviously the distracting cursor cloud was still too effective to allow for dismissing the fixation of the valid cursor for the fixation of the target.

Expression of immediate early genes in limbic brain areas of male DA rats in territorial aggression

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Aggression is one of the significant symptoms in antisocial behaviors, anxiety or depression disorders. The underlying neuronal basis of offensive aggressive behavior is investigated using the resident-intruder (RI) paradigm to provoke territorial aggression in male Dark Agouti rats. Aggressive behavior occurring in a single RI-test defines state aggression; aggressive behavior averaged over three encounters characterizes trait aggression. Antibodies against the transcription factors c-Fos and Egr-1 were used as markers of neuronal activation. Analyses was undertaken in limbic cortices, accumbens nucleus, amygdaloid complex, and raphe nuclei. Rats performed only the RI-test or were treated with S15535 and performed the test. Serotonin is linked to aggression in a wide range of species, and S15535 acts as a 5-HT_{1A} autoreceptor agonist and postsynaptically as a 5-HT_{1A} receptor antagonist.

In all rats investigated, Egr-1 labeling included maximally 85% of total cell number in respective brain areas, while maximally 40% of neurons were c-Fos labeled. In non-pharmacologically treated rats, Egr-1 was highly activated in the ventral and lateral orbital cortex and lateral amygdala, during state aggression. An increase of Egr-1 in the dorsal and paramedian raphe nucleus was correlated with an increase of aggressive behavior. C-Fos was highly activated in the ventral orbital cortex, infralimbic cortex, nucleus accumbens and basolateral amygdala. A negative correlation was found between aggressive behavior and c-Fos staining in the dysgranular insular cortex and caudal linear raphe nucleus. Trait aggression positively correlated with Egr-1 staining in the paramedian raphe nucleus, whereas c-Fos staining in the dysgranular insular cortex and median raphe nucleus correlated negatively with trait aggression. Rats were divided into highly aggressive (attack latency <200 s) and less aggressive (latency >200 s). A significant increase of Egr-1 activation in the paramedian raphe nucleus and of c-Fos activation in the lateral amygdala was observed in brains of highly aggressive rats, whereas c-Fos activation in the caudal linear raphe nucleus was significantly increased in brains of less aggressive rats.

In rats treated with S15535, the aggressive behavior was significantly reduced compared to untreated rats. In treated animals, significant higher levels of Egr-1 labeling were found in 22 out of 31 brain areas investigated; c-Fos labeling was significantly increased in the lateral septum, nucleus accumbens and dorsal raphe nucleus.

C-Fos and Egr-1 staining are complementary tools for examining the neuronal basis of aggression. The results demonstrate a high cortical and amygdaloid influence on aggressive behavior (Hasen & Gammie, 2006). Aggressive encounters are accompanied mainly by increased activation of limbic centers, but decreases in activation of the raphe nuclei or limbic cortices also occur. Activation of the nucleus accumbens suggest a rewarding effect on the resident during execution of aggressive behavior. Together with the findings after application of a serotonergic substance, the results indicate the importance of the rostral raphe nuclei and the relevance of the serotonergic system in aggression.

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Activation of cortical brain areas

	C-Fos		Egr-1	
Brain areas	RI-Test	S15535 prior to RI-Test	RI-Test	S15535 prior to RI-Test
Medial orbital cortex	++	++	++	+++

Ventral orbital cortex	+++	+++	+++	+++
Lateral orbital cortex	++	+	+++	+++
Prelimbic cortex	++	++	++	+++
Infralimbic cortex	+++	+++	+	++
Agranular insular cortex	++	++	++	+++
Granular insular cortex	+	+	++	++
Dysgranular insular cortex	++	+	++	+++

Very intense activity +++ c-Fos > 24 % of total cell number / Egr-1 > 50 %; intense ++ > 14 % / > 30%; moderate + > 5 % / > 10%; weak ± < 5 % / < 10 %

The inhibition of oxytocin-induced grooming in rats by i.p. application of amide of a specific oxytocin receptor antagonist

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Grooming in rodents (the face washing, body and genital grooming, body and paw licking and scratching) may serve many functions, from cleaning of the fur to temperature regulation or the spread of chemical substances. It has long been related to dopamine receptors in the brain (1). For example administration of the dopamine receptor agonist apomorphine caused grooming in a dose-dependent manner. Grooming was also induced by i.p. injection of different doses of nicotine to rats. Further, grooming is induced by novelty stress and by many peptides like prolactin, ACTH, melanotan II and mainly by oxytocin (OXY). Larger doses of OXY induce excessive grooming even after intraperitoneal (i.p.) application. Recently we synthesized amide (AORA) of the OXY receptor antagonist (desGly-NH_2-d$(CH_2)_5$[D-Tyr^2,Thr^4] OVT), which was originally synthesized by Manning et al. (2). The aim of this study was investigation of the effect of specific OXY receptor antagonist (AORA) on spontaneous behavior of rats in the open field device, including grooming, which was induced by i.p. application of higher doses of OXY. We used male Wistar rats (200-220 g b.w.) that were injected i.p. with OXY and/or AORA 60 min before the start of behavioral test. A circular arena with the diameter of 150 cm and inner zone 130 cm was used as an open field device. Locomotion of rats was video-monitored by an automated activity monitoring system (AnyMaze, Stoelting, USA). Total movement distance and distance in the inner zone, as well as several other parameters, were recorded automatically; an experimenter measured the total number of rearing (vertical activity) and the total time spent in grooming. OXY in dose 1 mg/kg b.w. induced very large increase of grooming that was potently antagonized by AORA given also at dose 1 mg/kg b.w. or 0.3 mg/kg b.w. Other behavioral parameters, horizontal and vertical activities, were antagonized much less potently than grooming. Our findings from experiments using selective OXY antagonist show that OXY-induced grooming is specifically mediated by OXY receptors. The attenuating effect and not full antagonism of other behavioral parameters of OXY-induced behaviors by AORA suggest that these behaviors may utilize some other mechanisms in addition to OXY receptors.

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Cortical Networks of Attention in Humans and Macaques

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Spatial attention in motion-detection and motion-discrimination tasks is known to modulate motion-selective area MT in both macaques and humans (TREUE S & MAUNSELL JH (1996); O`CRAVEN KM et al. (1997)). The use of different behavioural paradigms in humans and monkeys and of different experimental techniques, mostly single-unit recordings in macaques and functional MRI in humans, has rendered direct comparisons of the neural mechanisms of attention between these species difficult. Here we attempt to overcome these limitations by using the same attentive motion-discrimination task in both species and by studying the cortical networks of attention with the same technique, fMRI. Subjects had to pay attention to one of two random dot surfaces (RDSs), positioned to the left and right of a central fixation spot, while foveating this spot. The target RDS was foveally cued. RDSs were randomly changing translational direction every 60ms for a random duration, with constant direction thereafter. Subjects payed attention to the target and indicated the end of its direction changes by a saccade in the direction of the constant motion. We scanned humans and monkeys in a 3T horizontal head-scanner (SIEMENS Allegra), using an EPI sequence and, in macaques, contrast agent Sinerem (Guerbet). Eye positions were monitored with a custom eye-tracking system. In both humans and monkeys, spatial attention was found to modulate a network of areas including motion-selective area MT. Differences, similarities and potential homologies between the species will be discussed.

TREUE S & MAUNSELL JH. (1996): Attentional modulation of visual motion processing in cortical areas MT and MST. Nature 382(6591):539-41

O`CRAVEN KM, ROSEN BR, KWONG KK, TREISMAN A & SAVOY RL (1997): Voluntary attention modulates fMRI activity in human MT–MST. Neuron 8(4): 591-598

Two loudspeakers and a rat: An ultrasonic dialogue.

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The notion that rats emit different types of ultrasonic vocalizations in a variety of motivationally relevant contexts has received increasing experimental attention, since these calls might serve as indices of the animals' subjective state and/or as social signals. For example, we recently observed that rats emit 50-kHz calls after separation from their cage mate, possibly to (re)establish, or to keep contact (Wöhr et al. 2008, Physiol Behav 93'4-5).

To find experimental support for the hypothesis that 50-kHz calls serve communicative purposes, we started to conduct playback studies. Using a radial maze we tested how rats respond to playback of natural 50-kHz calls, or to artificial sine wave signals with the same length, frequency and amplitude as the natural calls. Both stimulus types induced locomotor activity and approach behaviour towards the loudspeaker in group-housed animals (Wöhr & Schwarting 2007, PLoS ONE 2'12).

The intention of our present study is to directly compare such stimuli; therefore, we placed two speakers at the opposing sides of the radial maze. In addition, we tested A) the role of certain stimulus features in eliciting approach behaviour, and B) the test's sensitivity to changes in social motivation, i.e. differences between group- and singly housed animals. For the former, we created artificial 50-kHz stimuli, similar to Ehret (e.g. Ehret & Haack 1982, J Comp Physiol 148).

We found that animals entered those arms more often, that were proximal to the source of 50-kHz stimulation: both the natural and artificial calls were preferred over noise. When the two test-stimuli were presented simultaneously, the animals spent a comparable amount of time in both areas of the radial maze. However, when comparing the reactions to natural or artificial calls, each presented against noise, we found differences due to the animals' state of social motivation: singly housed animals spent more time in those arms in which the natural calls were presented, whereas group-housed animals showed no preference.

After six trials of stimulus-presentations, the animals were placed in an empty cage and ultrasonic vocalizations were recorded. We observed that rats emitted a high number of 50-kHz calls, similar to the amount of calls measured when separated from cage mates. Again, we observed differences due to the animals' status of social motivation: calls of singly housed animals had a greater mean frequency-modulation and these rats also emitted calls of longer duration.

Measuring animals' responses to playback of 50-kHz calls provides a rather unique opportunity to study certain communicative determinants of social motivation by using a standardized non-social test, i.e. without confounding effects of actual partner presence.

In general, the study of social approach in laboratory animals can help to reveal biochemical, genetic and environmental factors underlying psychiatric disorders such as autism, schizophrenia, and depression, characterized by deficits in social behaviour and loss of desire to engage in social interactions.

The Simon effect in rats: A combined behavioral and PET study

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The Simon effect is a neuropsychological interference effect in which reaction times are longer and errors more frequent when spatial features of the stimulus (although task-irrelevant) are inconsistent with spatial attributes of the response. There is evidence from studies in humans that the dorsal anterior cingulate is responsible for the monitoring of such a conflict while prefrontal areas are involved in resolving it (Kerns 2006), but the nature of the brain regions involved in conflict detection and resolution is still unknown.
Four Lister hooded rats (*Rattus norvegicus*) were trained to perform an auditory Simon task in an operant chamber. In compatible trials (C), auditory stimuli (7 or 15 kHz) and correct response (go to left or right pellet trough) were on the same side. In incompatible trials (I), stimulus and required response were on opposite sides. Compatible and incompatible trials were presented in a pseudo-randomized fashion with a probability of 0.5 each.
To assess sequence-dependent modulations of the Simon effect both compatible and incompatible trials were further subdivided into whether they had been preceded by either compatible or incompatible trials (cC,iC,cI,iI; preceding trials in lower case). Reaction times and error rates were analyzed. As predicted, the error rate in incompatible trials was significantly higher than in compatible trials. The analysis of the sequence-dependend modulations showed shorter reaction times in condition repetitions (cC;iI) compared to condition alternations (iC;cI). A significant difference was found between iI and iC.
Furthermore, in two rats the Simon task was combined with metabolic ^{18}F-FDG PET imaging. For each rat, four scenarios were measured: (1) naive rat (i.e. before training=control); (2) Simon task as described above; (3) Simon task with compatible trials only; (4) Simon task with incompatible trials only. The images were normalized for intensity, and differences between Simon scenarios (2) to (4) and control (1) were calculated.
Relative to control, metabolic activity in motor, somatosensory, and auditory cortices were higher (by approx. 15 %) in all Simon scenarios. By contrast, metabolic activity was lower compared to control (by approx. 15 %) in the most rostral part of the anterior cingulate cortex (prelimbic area Cg3). A strong activation in the incompatible, but not in the compatible condition occurred in the left rostral Cg1-region. In the compatible condition, activity was reduced in the left rostral Cg2.
The present study demonstrates that rats show a Simon effect in an auditory operant chamber paradigm, including sequential effects. As in humans, the cingulate cortex is activated during the task.

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Kerns, J. G. (2006). Neuroimage 33(1) :399-405.

Single-cell activity and local field potentials in monkey prefrontal cortex during a spatial proportion discrimination task.

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Primate brains are equipped with evolutionarily old and dedicated neural circuits to grasp absolute quantities. In many conflicting situations, however, an assessment of quantity ratio is required to guide decisions. We report that rhesus monkeys can judge length proportions in a delayed match-to-sample task. The monkeys had to judge the length of a test line relative to the length of a reference line. Ratios of 1:4, 2:4, 3:4 and 4:4 were shown. To test whether the animals could also discriminate proportions they had not been trained on, we applied transfer tests showing novel proportions (3:8 and 5:8). The animals reliably discriminated transfer tests, thus, demonstrating that they had generalised the concept of proportionality.

Subsequently, we recorded single cell activity of 526 neurons in the lateral prefrontal cortex (PFC). During sample presentation, many of the tested neurons (131/526 or 25 %) were significantly tuned to proportion, irrespective of the absolute lengths of the test and reference bars. A similar fraction of proportion-selective neurons (126/526 or 24 %) was found in the delay period during which the monkeys maintained the length ratios in memory. Thus, different populations of neurons coded proportions both during sample presentation and maintained this derived quantitative information online during the memory period. Importantly, the responses of selective neurons were unaffected by the absolute magnitude of the stimulus components and responded irrespective of lengths variations of the test and reference lines. Each of the selective neurons preferred one of the four tested proportions; neurons preferring 1:4 were most frequent. We calculated population neural filter functions by averaging the normalised activity for all neurons that preferred a given proportion. The neural activity in the PFC correlated well with the behavioural performance of the monkeys. Both the behavioural and neural discrimination curves showed similar tuning selectivity.

Moreover, we simultaneously recorded local field potentials (LFPs) and investigated their powerspectra with a spectral multitaper analysis. Analysis of the temporal structure of LFP activity throughout the task showed modifications of energy in the alpha- and beta-range in different trial epoches. On average the energy in the alpha and beta frequency band was increased during sample presentation. Classification into correct and error trials revealed that the increase was only observed during correct trials. In addition, we analysed the phase relations between spikes and LFPs. Interstingly, strong coherence was only observed in the beta frequency band.

Our data emphasise the importance of the PFC in integrating absolute quantity information to derive relational quantity. Single cell activity as well as local field potentials contribute to the information processing related to spatial proportions in the monkey brain.

Coding of abstract quantitative rules in the monkey prefrontal cortex

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In many everyday-situations, decisions are based on magnitude comparisons and quantitative rules. During shopping for a given product, we pick the vendor that offers it for the least price. When looking for a job, however, we choose the one that provides more salary. Flexible "more than – less then" decisions are essential for goal directed behaviour in both man and animal. The prefrontal cortex (PFC) has been implicated in controlling rule-based decisions. At the same time, electrophysiological recordings in rhesus monkeys have shown that neurons in the PFC are also involved in processing numerical information. We thus explored the role of PFC neurons in guiding most abstract quantity judgments based on constantly changing decision rules.

We used a rule switching quantity comparison task in which two rhesus monkeys (macaca mulatta) had to indicate whether a given number of dots is greater or smaller than a previously shown sample. A trial started with a pure fixation period of 500 ms, after which the sample numerosity was displayed for 500 ms. Next a delay period with no stimulus lasting for 1000 ms was followed by a presentation of the rule cue for 300 ms and another delay period lasting 1000 ms. Finally, a test stimulus was presented that showed either a larger or a smaller number of dots than the sample display. Depending on which rule ("more than" or "less than") was cued on any given trial, the monkeys had to release a lever to chose the corresponding response. To force a most abstract quantitative decision, sample and corresponding test numerosities were greatly varied.

The monkeys' average performance in this task was around 85%, indicating that they were indeed able to understand the general concept of "more than" and "less than" and could apply it based on changing rules. In total, we recorded the activity from 484 PFC cells while the monkeys performed this task. Based on a multi-factorial statistical analysis, we found 90 cells (18.60%) whose activity only showed a significant main effect for the rules "greater than" or "less than", respectively, irrespective of the set size in the sample display and the sensory features the rule cues. Among these, 50 cells responded significantly to "more than" and 40 cells responded to "less than".

Our data expands the concept of rule application and combines it with numerical competence. The frontal lobe seems to be a cardinal processing stage in providing cognitive control by most abstract numerical information, functions that do also play a major role in logical and mathematical reasoning.

Will you still come if I call you? – How rats' approach-behaviour towards 50-kHz vocalisations is affected by striatal DA depletion

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After having discovered rats' ultrasonic vocalisations in the past century, research has been constantly progressing in this area. In the meantime several distinct classes of calls have been set, of which the current study's focus is on 50-kHz vocalisations, which are associated with positive affective stimuli. Our previous studies have shown that these 50-kHz calls induce socially motivated approach-behaviour (Wöhr & Schwarting, 2007, PLoS ONE 2'12) and additionally, it has been established that dopamine plays an important role in the processing thereof. Also, it is known that 50-kHz calls can be elicited by enhancing dopaminergic activity in the striatum.

Using the neurotoxin 6-hydroxydopamine (6-OHDA), this study investigates how striatal dopamine depletion affects approach-behaviour induced by playback of 50-kHz ultrasonic rat vocalisations and the according functional distinction of neo- and ventral striatum. In general, motor-processing and stimulus response learning are associated with the neostriatum, whilst the ventral striatum is rather associated with reward learning and motivational behaviour.

In separate experiments 6-OHDA lesions were placed bilaterally either in the neostriatum or in the ventral striatum/nucleus accumbens. Lesions in the neostriatum led to a subtotal loss of dopamine there (i.e. around 50-70%). Our previous work (Domenger & Schwarting, Neurosci Lett 444' 08) has shown that this kind of lesion is sufficient to impair instrumental behaviour. However, it was not sufficient to impair approach-behaviour towards 50-kHz calls. Therefore, neostriatal dopamine seems not to be critical for social approach as elicited by ultrasonic vocalisation. In our ongoing study, we are testing whether the same holds for dopamine in the ventral striatum.

Dad matters, too! Paternal care stimulates behavioral development and neuronal maturation in the orbitofrontal cortex of his offspring

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Although paternal care can be observed in many species, the majority of investigations address their attempts to maternal care effects. However, the involvement of both parents seems to be the optimum for the child's well-being and health. Human studies revealed that an absent father is clearly associated with a higher prevalence rate of drug and alcohol abuse, poor educational achievements, affective, anxiety-related, impulsive and/or personality disorders. While research in humans has given novel insights in behavioural outcomes and psychological effects of paternal investment, the "biological" impact is poorly understood.
Thus, the aim of this study was to investigate the significance of paternal investment on emotional (anxiety, impulse control), cognitive (play behavior, learning) and neuronal development in a biparental animal model (Octodon degus).
Two groups of degus were compared at the age of 21 and 90 days: 1) offspring raised by both parents and 2) offspring raised by a single mother, i.e. the father was removed from the family one day after their birth. For analysis of social behavior degu families of both groups were videotaped during the first 21 postnatal days (PND) and analysed for allogrooming and play-fighting behavior between the pups. At PND 90 adult degus were tested for anxiety-related behaviour in the Open-field (e.g. running activity covered by each animal in the whole arena and the center of the arena, entries and time in the center of the arena), reward learning in the Skinner-Box (animals had to learn a sequence of lever presses to maintain a food pellet) and impulsivity (fixed-consecutive number protocol in the Skinner Box). For neuromorphological analysis dendritic length and ramification and spine density of pyramidal neurons in layer II/III in the orbitofrontal cortex (OFC) and in the lateral amygdala (LA) were measured.
The quantitative comparison of preweaning biparental and single-mother families revealed that pups which were raised in biparental families showed decreased frequencies of play-fighting episodes, but no changes in allogrooming. Furthermore, adult biparentally reared animals displayed lower levels of anxiety, an enhanced learning performance in a reward task and less impulsive behavior compared to father-deprived animals. Furthermore, compared to offspring raised by a single mother, degus raised with both parents displayed elevated spine densities of dendritic spine on pyramidal apical and basal dendrites in the OFC. This difference was maintained until adulthood on the apical dendrites, which in addition were elongated in animals raised with father. In the LA elevated basal spine densities and numbers (dendritic length remained unchanged) was found in three week old biparentally raised degus. These synaptic differences were no longer evident at adulthood, where the complexity of apical dendrites was reduced in biparentally raised degus.
These lasting structural synaptic and dendritic changes might be causally linked to the behavioral differences as the observed reduced anxiety, improved reward learning and enhanced impulse control in animals which were raised with their father, compared to father-deprived animals. It remains a future challenge to identify and characterize the neuronal mechanisms (release of growth factors, opiates/dopamine, epigenetic changes?) which are triggered by paternal care, and which cause the observed changes in neuronal development.

Splitting the spotlight of attention during multiple-object tracking

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Behavioral studies in humans have suggested that during multiple-object tracking, the focus of visual spatial attention splits into multiple foci that can individually track different items (Cavanagh and Alvarez, 2005). Furthermore, fMRI studies have demonstrated that directing attention to multiple stationary objects produces a modulation of BOLD signals in human extrastriate cortical maps that is compatible with the existence of multiple attentional spotlights (Morawetz *et al*, 2007, McMains and Somers, 2004). All together, these results suggest that the spotlight of attention can split into multiple foci that simultaneously modulate responses of neurons in visual cortex. However, no electrophysiological evidence of such a modulation has been reported. We examined this issue by recording the responses of direction selective neurons in middle temporal area (MT) of two macaque monkeys to moving random dot patterns (RDPs) during a multiple-object tracking task.

We trained the animals to direct gaze (fixate) to a colored spot at the center of a projection screen where three RDPs were simultaneously presented at different locations. One RDP remained stationary inside the RF of the recorded MT neuron (RF-RDP), the other two (the flankers) moved following parallel trajectories along which a virtual axis joining the two flankers crossed the RF-RDP and therefore the neuron's RF. We recorded responses from 108 single MT neurons to identical stimulus configurations when the animals attended to: a) the fixation spot (sensory condition), b) the RF-RDP, or c) the flankers. We found that relative to the sensory condition, a) attending to the RF-RDP produced a response increase (~40%), and b) attending to the flankers had almost no effect on sensory responses, even when the virtual axis joining them crossed the RF at multiple levels. We conclude that during flanker tracking the spotlight of attention divided into two foci attached to the flankers and excluding the RF area. In a control experiment (n=45), where flanker trajectories entered the RF boundaries, attending to the flankers produced an increase in neuronal responses that resembled the one evoked by attending to the RF-RDP. The latter findings rules out the alternative hypothesis that the lack of attentional enhancement when attending to the flankers was due to an effect of spreading attention across the visual field. We conclude that during multiple-object tracking the attentional spotlight splits into multiple foci that differentially modulate neuronal responses within cortical maps in extrastriate visual areas of primates.

Principal Components Factor Analysis of Different Behavioural and Neurochemical Items in Mice - a Method to Reveal Contextual Relations of Behaviours and Neurochemistry

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A variety of equipments allows more and more detailed observations and descriptions of animal's behaviour. Commonly a lot of parameters is measured to analyze the frequency and the duration of items or to score the intensity of a behaviour by a graded system. But the increasing lot of parameters is difficult to handle especially deciding which of the parameters is relevant and should be analyzed further. The principal components factor analysis is an effective method to reveal interrelationships between measured items assembling parameters into factor groups by means of a multicorrelative procedure.

The study reports mice data collected by the following behavioural tests: magnetic impulse based running wheel test measuring 90^{o} movements of the wheels; light beam based open field and plus maze tests measuring place and time characteristics of movement sequences; video recorded social intruder test with subsequent manual based evaluations measuring frequency and duration of different social and neutral behaviours. Additionally neurochemical items are reported which were obtained immediately after the social intruder tests.

All parameters collected are subjected to test-related principal components factor analyses. Important steps and appropriate results (K-M-O quality criteria, communality, anti-image matrix, rotated factor matrix, factor loadings) are described for each test performed. Furthermore the result of an overall factor analysis including the most important behavioural and neurochemical data is presented. A functionally relevant differentiation into behavioral Clusters as drive, curious eager and irritability is revealed regarding activity parameters directed to explore an environment. Behavioural items measured during the social intruder test are to distinguish into social affine respective social aversive behaviours; additionally drive, curious eager and irritability as relevant behavioural axes are also determined. Finally, exemplary behavioural measurements of each test are shown to demonstrate housing and sex effects on mice's behaviour. Interrelationships between the revealed behavioural axes and neurochemistry as well as the power of the multicorrelative principal component factor analysis are discussed.

Behavioral and electrophysiological changes in rats following nine weeks intratracheal exposure to manganese dioxide nanoparticles

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Inhalational manganese exposure of occupational or environmental origin causes a syndrome similar to parkinsonism. Submicron particles have a huge specific surface area and are much more mobile throughout the organism than microscopic-sized particles. In the nervous system, Mn interferes, among others, with Ca channels, glutamate metabolism, and mitochondrial energy production.

In this work, adult male Wistar rats were treated with a nanosuspension of MnO_2 of ca. 23 nm nominal particle diameter, instilled into the trachea for 3, 6 and 9 weeks with the daily doses of 2.63 and 5.26 mg Mn/kg. There was an untreated and a vehicle-treated control group. The animals' body weight was checked weekly. At the end of treatment, the rats' spontaneous motility was tested in an open field box. Then, spontaneous and stimulus-evoked activity of the somatosensory, visual and auditory cortical area, and action potential of the tail nerve, were recorded in urethane anesthesia. The rats were finally dissected, organs weights were measured, and the presence of excess Mn in lung and brain samples was determined using scanning electron microscope with energy dispersive X-ray spectroscopy.

Control rats had normal weight gain, but the body weights of the treated rats ceased to grow from the 6th week on, indicating significant toxicity. In the open field activity, the time spent in ambulation and rearing decreased in a dose- and time-dependent way, while local activity and immobility increased (see Table). In the spontaneous cortical activity (electrocorticogram, ECoG) the ratio of slow/fast waves (ECoG index) decreased, indicating a shift of ECoG to higher frequencies. The latency of the evoked potentials, and partly also their duration, increased, and the conduction velocity of the tail nerve decreased. Decreased frequency-following ability in all evoked activity forms was also detected. The functional alterations seen were similar to those observed previously with water-soluble forms of Mn. The relative weight of the lungs increased, while that of the liver decreased, in the treated rats in a dose-and time-dependent manner, and Mn could be detected in the lung and brain samples (frontal lobe) of the high dose rats.

Our results indicated that: 1/ The Mn content of instilled nanoparticles had access from the airways to the brain; 2/ The changes of spontaneous open field behavior and cortical electrical activity were similar to those obtained earlier with a administration of $MnCl_2$ solution; 3/ The applied neuro-functional methods were suitable for detection of nervous system damage due to nanoparticle exposure.

Table: Data of open field behavior and electrophysiological records from the 9th week.

*, **, ** $p<0.05$, 0.01, 0.001 vs. untreated control.

Groups		Untreated control	Vehicle control	Low dose group	High dose group
Dose (mg/kg b.w.)		--	--	2.63	5.26
Open field results	Ambulation (s)	240.60 ± 55.19	250.20 ± 63.03	204.50 ± 28.18	113.83 ± 50.70**
	Local activity (s)	162.20 ± 38.75	171.60 ± 63.01	220.00 ± 61.06	288.00 ± 38.11**
	Rearing (s)	136.60 ± 42.89	143.40 ± 64.89	116.333 ± 81.29	48.67 ± 31.09
	Immobility (s)	61.60 ± 50.16	35.80 ± 19.68	60.17 ± 32.11	150.50 ± 69.36*
El. phys.	SS ECoG index	2.34 ± 0.28	1.98 ± 0.59	1.73 ± 0.30*	1.61 ± 0.16***

results				
SS EP latency (ms)	7.56 ± 0.17	7.62 ± 0.26	7.84 ± 0.19*	7.96 ± 0.24**
Tail nerve cond. vel. (m/s)	18.98 ± 1.35	18.94 ± 0.81	16.89 ± 1.02**	16.35 ± 1.28**

Distinct neuronal subtypes of the intercalated cell masses of the amygdala provide novel intra- and extra-amygdala GABAergic connections

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Intercalated cell masses of the amygdala (ITC) are clusters of densely packed cells or thin strands of neurones interspersed among the various amygdaloid nuclei and in particular between the central nucleus (Ce) and the basolateral complex. Whereas it is becoming increasingly clear that the neurones of the ITC play an important role in fear learning and extinction, the cellular configuration and connectivity of this extensive population has received so far little critical attention. We have therefore investigated the structural relationships of the neurons in the intermediate capsular region of the mouse. To this end whole cell recordings of ITC neurons were obtained in coronal slices and after electrophysiological recording one neuron per slice was filled with biocytin for anatomical characterization. We have analyzed 118 filled neurons of which 48 were confirmed as ITC and 7 as neurons of the central paracapsular nucleus (CeC). All ITC neurons displayed a characteristic bipolar shape with the primary dendrites oriented parallel to the intermediate capsule, whereas CeC neurons were multipolar with their axon mostly restricted to the Ce. In our preliminary analysis of the ITC neurons we could identify 3 principal distinct neuronal subtypes, which differed in their axonal projections. The largest group consisted of 24 neurons and was characterized by an intense innervation of different sectors of the Ce, and in particular of the CeC. The vast majority of these cells were medium spiny although a few showed smooth largely aspiny dendrites. No obvious differences could be observed in the axonal pattern between spiny and poorly-spiny neurons. The second group (n=14) was composed of medium spiny neurons with their soma located in a cluster laying between the fundus striati, the basolateral complex and the Ce and highly immunoreactive for mu-opiod receptors. The axon of these neurons prevalently innervated other ITC neurons within the same cluster. In some instances a major axonal branch travelled ventro-medially along the intermediate capsule to reach the ITC nucleus. The third group (n=6) gave rise to a primary axon that run alongside the border of the Ce nucleus to reach the internal capsule. In some cases these axons entered the internal capsule, but whether they projected outside the amygdala through this bundle could not be established. All three groups displayed conspicuous axonal projections coursing both ventrally and caudally in the intermediate capsule. Axonal branches were also observed to travel dorsally to the basal ganglia, or medially to the stria terminalis from where they could project to other forebrain areas.

Our data provide compelling evidence for the existence of distinct ITC neuronal subtypes possessing different axonal patterns. We also corroborate our previous findings demonstrating that ITC neurons give rise to extrinsic amygdaloid projections.

OPTOGENETIC INVESTIGATION OF NERVOUS SYSTEM FUNCTION USING WALKING BEHAVIOUR IN *DROSOPHILA*

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The classical method of investigating neuronal function has been to inactivate neuronal populations and analyze a possible loss of function. However, the results may not be sufficient to establish the roles of the inactivated neurons due to compensatory mechanisms that may exist in animals or the disturbance of homeostatic equilibrium which may lead to a phenotype. The uncertainty of inactivation experiments has lead to the development of new optogenetic tools that enable temporally precise activation of selected subgroups of neuronal populations.

The neuronal mechanisms mediating the rewarding or punishing events in the insect brain are currently not clear and require further investigation. To this end we have established a new paradigm which employs the walking behaviour of *Drosophila* as a behavioural readout. In this paradigm the flies are hooked to a manipulator by their thorax and are free to walk on a styrofoam ball which is suspended on an air cushion. The movements of the ball are electronically monitored and thus the preferred direction of the animal can be ascertained. To establish this paradigm we have employed heat punishment which has been operantly linked with a walking direction. The animal thus is confronted to the punishment when it walks in a particular direction and now requires to operantly choose between the punished and unpunished direction. From our experiments we demonstrate that animals associate the punishment to a particular direction and avoid walking in the punished direction. Also, the preference of the animal can be quantified by our current analysis procedures. Having established this paradigm with heat punishment we are currently using channelrhodopsin-2 as a tool to activate populations of modulatory neurons (octopaminergic and dopaminergic) and sensory neurons (gustatory and olfactory). The goal is to gain insights into the function of modulatory neurons as a *mediator* of a response or a *reinforcer* of a stimulus. The studies involving the activation of sensory neurons should enable us to investigate whether behaviour can be driven by just activating certain neurons or whether it requires the direct interaction with sensory stimuli. We have been successful in optically activating modulatory neurons and sensory neurons by expressing channelrhodopsin-2 while quantifying the activation using the walking ball paradigm. First results will be presented.

Application of neuron mean field models in the study of psychophysiological behavior: the case of selective attention and habituation

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The functional behavior of the human brain is encoded in spatio-temporal structures that have been succesfully modelled by means of a non-linear dynamics and a spatial interconnection between different populations of neurons. It is well-accpeted that one of the most direct ways to quantify such pattern formation is by means of the dynamics of macroscopic quantities such as the EEG and MEG. These macroscopic measurements represent the synchronized post-synaptic activity of many ensembles of neurons. In this respect, neural-mass models offer the possibility to simulate these interactions in a more efficient and realistic way. In this paper, we make use of neural large-scale models together with novel stochastic approaches so as to study the phase dynamics of neural population responses reflected in evoked potentials during states of focal and non-focal human attention as well as habituation. It is concluded that our approach enforces experimental and theoretical results regarding the degree of neural synchronization and its relationship to neural correlates of attention and habituation processes.

Representations of large numerosity in humans and monkeys on the behavioral and neuronal level.

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Several lines of evidence suggest that abstract numerical competence is a sovereign faculty independent of language. However, whether non-verbal numerical representations in human and nonhuman primates are quantitatively similar and whether the coding of abstract quantity judgments is accomplished by summation coding (typical for sensory magnitudes) or by labeled-line coding remains elusive. Moreover, it is not clarified whether linear or nonlinearly compressed scaling underlies such magnitude judgments in both species. To resolve these issues, we tested the numerical discrimination performance of 36 human subjects and two rhesus monkeys (Macaca mulatta) in an identical delayed match-to-numerosity task for a broad range of numerosities from 1 to 30. In addition, we recorded single neurons in the monkey prefrontal cortex. We analyzed the representation of the numerical information and the scaling scheme of numerosity-selective neurons and compared them to the behavioral data. The behavioral results revealed performance peak functions for all tested numerosities in both species. Numerosity discrimination in humans and monkeys showed similar precision (Weber fractions: 0.55 and 0.60) and demonstrated the numerical size and distance effects. Moreover, numerosity-selective neurons in the monkeys pointed out a clear and behaviorally-relevant labeled-line code for all numerosities. Both the behavioral and neuronal tuning functions were best described on a logarithmic scale. However, in humans, the difference between linear and logarithmic scaling was less pronounced. The reason might be a gradual transformation of a logarithmic to linear magnitude scale in human adults caused by the course of mathematical education.

Influence of the Dopaminergic System on Emotional Acoustic Processes of Evaluation in the Brain of the House Mouse (*Mus musculus domesticus*).

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Primiparous house mice show maternal behaviour towards pups who vocalize ultrasounds (40 - 80 kHz tones) when they accidently get outside the nest. The mothers react to these emotional acoustic stimuli in a special way: They search for the lost pups (appetitive component), find them by phonotaxis and carry them back to the nest (consummatory act). Such maternal instinct is influenced by the dopaminergic system with its receptor types D1 and D2; in general, dopaminergic antagonists reduced the occurrence of maternal behaviour. (Hochleiter, Diploma-thesis, University of Ulm, 2005). These receptors seem to be involved in the regulation of behaviour control mechanisms: They modulate attention, motivation/emotion, cognition, and motor activity. Where in the brain is the regulation of the elements of this pup caring instinct affected by the dopaminergic system? How does dopamine influence this kind of behaviour? Are the main effects on the evaluation and processing of the stimuli found in the fields of the auditory cortex (AC) or in the emotional network in the depth of the limbic system?
Virgin female mice were anaesthetized and the auditory cortex was electrophysiologically mapped and labelled for histological identification of cortical fields. After having recovered from the surgery the females were mated. Then, the experiments were conducted with the mothers and their 2-5 day old pups. Receptor agonists (D1: SKF 38393; D2: Quinpirole) and antagonists (D1: SCH 23390; D2: Raclopride) were injected while the mothers were caring for their pups and the mice were exposed to 50 kHz tone bursts (model ultrasounds) for 15 minutes. Afterwards the mother's brain was cut in 30 µm slices and activated neurons were visualized via c-Fos immunocytochemistry.
D1 and D2 antagonist-treated females showed a significant decrease in the number of activated neurons in the auditory cortical fields AII, UF, and DP, whereas agonists had only little effect. This means that dopamine has a modulating influence on sound processing in primary (UF) and higher-order fields (AII, DP) of the auditory cortex and thus may affect sound recognition via both D1 and D2 receptors. Also in some parts of the limbic system dopaminergic influence led to changes in numbers of Fos-postive cells, e.g. in the right-side cortical amygdala neuronal activation was suppressed by the D2 dopaminergic antagonist, which shows that D2 receptors in the amygdala are involved in regulating the emotional response of the females. In the right-side piriform cortex there was a significant increase in the number of Fos positive cells caused by the D2 agonist which suggests that dopamine supports via D2-receptors multimodal integration in the piriform cortex and, thus, may support the release of maternal behaviour. In summary, D1 and D2 receptors take part in the process of sound evaluation in the auditory cortex. Processing in the limbic system is influenced mainly via D2 receptors.

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Are gender-specific orientation strategies universal among mammals? - New clues from a bat model.

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From recent studies on bats it can be concluded that microbats, primates and rodents share the same basic neuronal equipment for spatial cognition, i.e. hippocampal place cells (Ulanovsky & Moss 2007, Nat Neurosci. 10(2): 224-33). Within the class Mammalia, microbats belong to a different superorder (Laurasiatheria) than primates and rodents (Euarchontoglires), so that bats can be considered as being phylogenetically distinct from the latter. To investigate whether the similarities between the orders that have been found on the neuronal level manifest themselves in commonalities on the level of complex behaviour, we examined the utilisation of *acoustic* landmarks by the microbat species *Phyllostomus discolor* (n = 6 females, 6 males). It is known from some polygamous rodent and primate species that male individuals preferentially use Euclidean cues when it comes to orientation, whereas females rely more on landmarks. Based on three independent lines of evidence we could show that, indeed, the same is true in microbats for the use of *acoustic* landmarks:

(I) In the absence of *acoustic* landmarks, female individuals of *P. discolor* need significantly more training sessions than males (one-tailed M-W-U-test, $p < 0.5$) to learn the position of a fixed landing platform at the end of a flight tunnel.
(II) In a transformation test, the manipulation of a learned landmark constellation (removal of one from a total of four acoustic landmarks) leads to a significant reduction in the landing success in female bats (one-tailed M-W-U-test, $p < 0.5$), but not in male bats.
(III) The same modification to the landmark configuration also leads to variations in the animals' flight paths to the landing platform. In females these flight-path deviations are significantly more extreme (Hollander-Extreme-Test, $p < 0.5$) and show a higher inter-individual variability than in males.

Two conclusions that can be drawn from our results are that the phenomenon of sex-specific orientation strategies is widespread within the class Mammalia, and that it seems to be independent of the modality the animal primarily uses for sensing its environment. Therefore, our findings may provide valuable information for scientists developing acoustic orientation aids for the blind and strongly militate in favour of utilising microbats as model organisms for neuroethological studies on *three-dimensional* spatial cognition in mammals.

Notation-independent encoding of proportions in the human frontoparietal cortex determined by fMRI adaptation

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The ability to encode magnitude, irrespective of the modality and format of presentation, is a pre-requisite for human cultural evolution. Simple enumeration or quantification, however, is often insufficient to make decisions and guide behavior. In many instances, we need to relate two quantities, generating a new composite construct: a proportion or magnitude ratio. For example, the introduction of fractions, the ratio of two integers, has substantially broadened the range and flexibility of the number system, circumventing the frequent restriction of absolute numbers in quantifying features of interest.

In two sets of experiments using non-symbolic (ratios of line lengths and numerosities) as well as symbolic stimuli (fractions and proportion words), we investigated the neural representation of number ratios in humans with fMRI adaptation protocols to tag interspersed cortical neuron populations at sub-voxel resolution. Following adaptation to visually presented constant proportions, we introduced novel, deviant magnitudes to examine the tuning characteristics of the population of stimulated neurons. With non-symbolic stimuli, we found that BOLD recovery from adaptation in bilateral parietal and frontal cortex was a function of numerical distance between the novel proportion and the adaptation stimulus. Strongest effects were observed in the intraparietal sulcus (IPS). The same regions were involved in processing stimuli encoding proportions via the relation of two lines (continuous quantity) and by the number of items in two dot groups (discrete quantity), perceptually entirely distinct stimuli. Control experiments verified that the effect of proportional distance was independent of non-numerical novelty and not influenced by low-level visual features. Rearranging the images in the dot experiment, we found substantial overlap of cortical structures dedicated to the processing of non-symbolic proportions and whole numbers. We then extended the experiments to examine the encoding of symbolic proportions, fractions and proportion words. Despite strong variation of the nominator and denominator (while maintaining constant the actual fraction), there was a pronounced adaptation effect in parietal cortex. As for the non-symbolic stimuli, a graded BOLD recovery to novel proportions was observed in bilateral IPS. Importantly, this effect was reproduced even when we changed the deviants' notation (fractions or words) *within* the same experimental run. Control conditions contrasting identical proportions presented either as fractions or words failed to elicit a response, arguing that the analysed areas were exclusively sensitive to novel *ratios*.

These findings demonstrate that populations of neurons in the human parietal and frontal cortex are tuned to preferred magnitude ratios using the same non-verbal analog code to create abstract concepts of both absolute and relative number. Our results add to the magnitude system a remarkable level of sophistication by suggesting automatic access to a composite, derived quantitative measure.

Interaction between expectation and reward in the human visual system

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Much of the past work on visual attention has addressed issues concerning the allocation of attention to spatially distributed visual information (e.g. Treisman and Gelade, 1980; see review by Kincha, 1992). One way is to modulate attention with cue validity. Another way is to modulate attention with the predictability of reward.

Our aim in two experiments was to gradually modulate the endogenous orientation of attention with the expected value of distributed reward in the environment. The expected value is given as the predictability of the reward and the amount of reward associated with each location. Shifts in attentional focus were induced by linking one location with a higher expected value than other locations. Larger expected values should then increase the likelihood that subjects would focus their attention on one location rather than distribute it across the entire display.

The discrimination performance at a target location is a close indicator of attention. We setup a discrimination task and measured the behavioral discrimination performance and event-related potentials (ERP). The task included two possible target locations, where subjects were responsible of reporting the target status on the vertical periphery of the central fixation and received negative feedback for incorrect discrimination.

In Experiment 1, the feedback and therefore the value for all target locations was constant. With no difference in the associated value, subjects allocated their attention only according to the predictability of the target location and increasingly discriminated the target status. Grand average ERP waveform sites showed an increase in components as early as the N1 component correlated to the predictability of the target location.

In Experiment 2, the subjects could not only acquire the probability of future target locations, but were also location-dependently rewarded for correct detection of the target status. Here the subjects distributed their attention with the associated expected value and increasingly discriminate the target status with larger expected value. Similar to Experiment 1, ERP amplitudes for the N1 component increased and followed the expected value.

Models based on Bayesian updating (Sharma et al. 2003), on a moving average over past target locations and on a hidden Markov model account quite closely for basic properties of the behavioral and EEG results. Increasing predictability of the reward leads to increased discrimination performance and elevated ERP amplitudes. However, a close analysis on a trial-by-trial basis showed that only a model based on a moving average is likely to account for the subject performance.

Neural correlates of consciousness – insights from sleep imaging

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Introduction: One of the most challenging goals in neuroscience is to evaluate the neural correlates of consciousness. Common categorizations distinguish between more basal perceptual and higher-order aspects of this multi-faceted concept. Studies evaluating categorical shifts in consciousness have to date been confounded by shifts in vigilance levels, e.g. from awake to asleep. There is one case in which such a categorical shift occurs within the same vigilance state. In rapid eye movement (REM) sleep, ordinary dreaming comprises only basal aspects of consciousness. In contrast, the phenomenon of lucid dreaming is characterized by full-blown consciousness, including all higher-order aspects like meta-awareness. Here we show that neural activity related to the genesis of higher-order consciousness can be revealed by contrasting ordinary REM sleep with physiologically verified lucid REM sleep, using a combined fMRI/EEG approach.

Methods: Four experienced lucid dreamers repeatedly slept in the MRI scanner under concurrent polysomnographic monitoring. They were instructed to communicate the state of lucidity by quick left-right-left-right (LRLR) eye movements that create an unambiguous pattern on the EOG. To allow for continuous tracking of lucidity, subjects were asked to repeatedly signal the LRLR code. Dream reports were collected immediately after awakening to confirm the LRLR coding. Imaging data (GE Signa 1.5 T, 25 slices, FOV 22x22, TR 2s, TE 40ms) were recorded in continuous data acquisition of up to 22 min with continuous sleep EEG recordings (Brain Amp Systems, 32 channels including EOG and EMG). After postprocessing, data derived from the EOG (LRLR eye movements) was utilized to mark the short periods of lucidity within unambiguous REM sleep.

Results: Increased activation related to lucid vs. non-lucid REM sleep was observed in a range of neo-cortical regions, including bilateral precuneus, cuneus and parietal, prefrontal and occipito-temporal cortices. Precuneus and fronto-polar activation have been repeatedly associated with self-referential processing, while the fronto-parietal network activation is likely a correlate of working memory demands. Activation in the bilateral cuneus and occipitotemporal cortices overlaps with the ventral stream of visual processing. Interestingly, increased activity was found in right dorsolateral prefrontal cortex, which is deactivated in normal REM sleep – something associated with the bizarre characteristics of normal dream thought.

Discussion: Our results suggest that higher-order consciousness is a purely neo-cortical phenomenon. Cerebral regions showing increased activity also show extensive expansion in humans as compared to non-human primates, providing neuroanatomical evidence for the hypothesis that the presence of higher-order consciousness is richest in humans. While the concept of higher-order consciousness has received much theoretical attention, our results provide direct evidence for its neural correlates and show that experiments on lucid REM sleep can help to refine the conceptualisation of this elusive phenomenon.

Feature-based Attention Shifts the Directional Tuning Curves of MT Neurons towards the Attended Feature

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Visual attention is a mechanism that enables the brain to focus on a small amount of information which is behaviorally relevant. Previous studies in feature based attention show that attention changes the gain of the neural responses in a multiplicative manner but has little effect on the shape of the tuning curves. The strength of this modulation depends on the similarity between the attended feature and the cell's preferred feature (Treue et. al, Nature, 1999). To investigate the effect of feature-based attention on full tuning curves for attention to various direction of motions and to test if the gain modulation responses created by feature-based attention follow the feature similarity gain model, we recorded from more than 120 single cells in macaque area MT. In particular we wanted to test if directing attention to a direction slightly off the preferred direction of a neuron would shift the tuning. This shift is predicted by the model and has not been studied before. The full tuning curves were extracted while the monkey was attending to one of 12 directions of motion (Target) in each trial, outside the receptive field (RF) of the neuron. The distracter was presented inside the RF, which its direction was continuously changed every 250 ms, randomly selected among 12 possible directions, enabling to extract the tuning curves in a fast way by analyzing the responses for the 50ms to 250ms for each direction of motion. The monkey task was to keep his attention toward the target and to release a lever as soon as a speed or direction change happened in the target surface and ignore changes in the distracter. In line with our previous findings, the attentional modulation observed, while the monkey attended to the preferred direction of the neuron was about 13% and for the null condition was about 3% with respect to the fixation condition. This modulation is multiplicative between the tuning curves in these two attentional conditions. In agreement with the hypothesis outlined above, we found that attention to a particular direction of motion off the preferred-null axis, shifts the tuning curve of neurons in area MT toward the attended direction (about 2-3 degree on average). Our findings show that the feature-similarity gain model can account for non-multiplicative attentional effects on tuning curves.

OPTOGENETIC INVESTIGATION OF NERVOUS SYSTEM FUNCTION USING WALKING BEHAVIOUR IN *DROSOPHILA*

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The classical method of investigating neuronal function has been to inactivate neuronal populations and analyze a possible loss of function. However, the results may not be sufficient to establish the roles of the inactivated neurons due to compensatory mechanisms that may exist in animals or the disturbance of homeostatic equilibrium which may lead to a phenotype. The uncertainty of inactivation experiments has lead to the development of new optogenetic tools that enable temporally precise activation of selected subgroups of neuronal populations.

The neuronal mechanisms mediating the rewarding or punishing events in the insect brain are currently not clear and require further investigation. To this end we have established a new paradigm which employs the walking behaviour of *Drosophila* as a behavioural readout. In this paradigm the flies are hooked to a manipulator by their thorax and are free to walk on a styrofoam ball which is suspended on an air cushion. The movements of the ball are electronically monitored and thus the preferred direction of the animal can be ascertained. To establish this paradigm we have employed heat punishment which has been operantly linked with a walking direction. The animal thus is confronted to the punishment when it walks in a particular direction and now requires to operantly choose between the punished and unpunished direction. From our experiments we demonstrate that animals associate the punishment to a particular direction and avoid walking in the punished direction. Also, the preference of the animal can be quantified by our current analysis procedures. Having established this paradigm with heat punishment we are currently using channelrhodopsin-2 as a tool to activate populations of modulatory neurons (octopaminergic and dopaminergic) and sensory neurons (gustatory and olfactory). The goal is to gain insights into the function of modulatory neurons as a *mediator* of a response or a *reinforcer* of a stimulus. The studies involving the activation of sensory neurons should enable us to investigate whether behaviour can be driven by just activating certain neurons or whether it requires the direct interaction with sensory stimuli. We have been successful in optically activating modulatory neurons and sensory neurons by expressing channelrhodopsin-2 while quantifying the activation using the walking ball paradigm. First results will be presented.

Attentional alteration of direction tuning of neurons in macaque area MT to transparent motion

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An important aspect of processing visual information is the capability of analyzing visual motion. Our visual system is able to distinguish multiple motion directions in the same part of visual space, the motion transparency problem. At this, transparent random dot patterns (RDPs) are of peculiar experimental interest, as they are the only stimuli that allow a highly controlled, yet very flexible combination of different stimuli in one spatial location. Recording neuronal responses to unattended bidirectional transparent stimuli showed the presence of two peaks in population tuning curves if the directions were separated by more than 90 degrees (Treue et al., 2000). A mechanism of focusing on the behaviorally relevant objects in a complex scene, thus solving the transparent motion problem, is attention. In this project we investigated the effects of attention on the representation of the direction components of transparent motion by recording neuronal activity from neurons of the middle temporal area (MT).

Two rhesus monkeys were trained on the behavioral task, whereupon a cue - indicating the location and the direction of motion that the animal was later required to attend to- is followed by a delay period. In the subsequent attentional period, two RDPs moving within one stationary aperture as a transparent pattern, sized and positioned to fit within the classical receptive field (RF) and whose component directions moved with a relative angle of 120 degrees, were presented (attend in condition). While maintaining gaze on a fixation point, the animals were required to attend to the cued RDP and to respond to a speed increment within this surface. In a sensory stimulation condition (attend fix), the monkeys were asked to respond to a luminance change occurring on the fixation point, while ignoring the transparent patterns. By systematically varying the directions of the two motion components, neuronal responses to 12 different RDP direction combinations were measured.

The activity profile of the attend in condition of a population of more than 60 units showed an overall enhancement relative to the sensory condition which was most pronounced around the peak where the preferred direction is attended. That is shifting attention inside the receptive field boosts neuronal responses compared to pure sensory stimulation. This spatial effect of attention is additionally modulated by a feature-based attention effect by the cell's response to the directions of the RDP components.

In another study (see poster by Kozyrev *et al.*), we placed the same stimulus configuration non-transparently within two spatially separated apertures inside the RF. The results showed an attentional enhancement for the condition where attention is applied on the preferred direction inside the RF and a suppression for attending to the null direction. One possible explanation for the difference of the findings might be, that attending to one stimulus inside the RF leads to a shift and shrinkage of the RF around the stimulus, thus causing a disproportionate representation of the unattended stimulus inside the RF.

Agonistic communication calls trigger amygdaloid neurons in the bat species *Phyllostomus dicolor*

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The inter-individual transfer of emotional information is vital for group-living (i.e. social) mammals. The relevance of this information is processed in the limbic system with the amygdala playing a pivotal role in the analysis of emotional content in a context-specific manner.
Phyllostomus discolor, a group-living neotropical bat, has both a rich and divers repertoire of social calls and a comparatively large amygdala. Hence, P. discolor is well suited to study auditory responses of amygdaloid neurons on the single-cell / few-cell level.
In the present study, social calls from a digital call library (20 call types, up to 50 paratypes per call) are presented to the awake animal.
To enable recordings from the amygdala, a metal rod is attached to the animal's skull for immobilization and a small craniotomy is performed. Subsequently, an electrode drive containing an electrode array (both custom-made) is attached to the skull with dental cement. Alternatively, in some of the experimental animals, a syrinx is implanted to allow for a more variable electrode positioning. The stereotactic orientation of the electrodes is determined with the help of a 3D-model of the bat's skull and brain respectively in combination with pilot tests using a trepanned skull. During the experiment, the trunk of the awake bat is placed into a comfortable body holder while the head is fixed (see above) and acoustic stimuli are presented under free field conditions. Amplified neuronal responses are digitized and recorded to disc for off-line analysis. After termination of chronic recording, electrode positions are verified histologically using metal deposition followed by Berlin-Blue staining.
Neurons in the amygdala robustly respond to aversive sound stimuli (i.e. species-specific aggression calls). These responses are often accompanied by pronounced muscular activity of the animal.
Furthermore the present study intends to test for responses to affiliative playback stimuli as well as to systematically investigate the response properties of neurons within different (sub)nuclei of the amygdala.

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Lateralized category-specific cognition in a "people-present/people-absent" discrimination task by pigeons

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Herrnstein and Loveland (1964) showed that pigeons are capable of people-present/people-absent concept discrimination. Yamazaki et al. (2007) suggested that the use of concept-based strategy is lateralized in birds. In their study birds showed categorization superiority with the right eye (left hemisphere). To further our understanding of the role of each hemisphere in categorization behaviour, we examined concept discrimination in birds while one hemisphere or the other was deactivated. Pigeons were trained in a go/no-go procedure to discriminate pictures of humans and to transfer to novel exemplars. To examine which hemisphere was involved in categorization, an additional transfer test was conducted while the left or the right entopallium was blocked with Tetrodotoxin. On the transfer test, the birds were tested with both previously learned as well as novel people-present/people- absent instances. Discrimination of learned stimuli was not impaired when either the left or the right hemisphere was blocked. However, concept discrimination of the novel instances was impaired when the left hemisphere was blocked, although they still performed the discrimination above chance. This suggests that the left hemisphere was superior in category-specific processing, whereas the right hemisphere is just capable of discriminating between the known stimuli, thus relaying on a memorization strategy. Our findings of an asymmetrical cognitive architecture in birds are largely shared with humans and might have a long phylogenetic history.

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In search of the ideal rat model: A comparative study on ultrasonic calls in three strains of male rats

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Rats emit three types of ultrasonic vocalizations (USV's), which can be assigned to different motivationally relevant situations. The 40-kHz-distress calls of rat pups occur in isolation from the mother and the nest. They help the mother to localize the pups in order to carry them back to the nest. The 50-kHz calls emitted by adult animals often appear in appetitive situations, but also when rats are separated from their cage mates and are placed in a fresh cage (housing cage test, for details see Schwarting et al. Behav Brain Res 2007). In an aversive environment adult rats emit 22-kHz calls. Panksepp and coworkers suggested that these three types of vocalizations might serve as an index of the animal's subjective state (Panksepp Science 2005 and Knutson et al. Psychol Bull 2002).

For research on ultrasonic calls, different rat strains are used in various labs. Due to this variability it is difficult to compare results between labs, although similar tests are used. To provide a basis for comparison we recorded all three types of USV's that occur in rats. We tested male rats of the three outbred strains which are most common for ultrasonic experiments, namely Long Evans, Sprague Dawley and Wistar Unilever.

To elicit 40-kHz calls, we isolated the pups from their mother, nest and siblings. We used a tickling paradigm and the housing cage test for recording of the 50-kHz calls. To cause 22-kHz calls, we tested rats in a fear conditioning paradigm (Wöhr et al. Neurobiol Learn Memory 2005). Results of these comparative experiments are going to be presented. They will address both quantitative and qualitative features of ultrasonic vocalizations.

Learning-induced changes of an attentional check-up mechanism of interval timing in non-human primates

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The human ability to precisely judge temporal durations is critical for many behavioral, cognitive, or motor functions, as demonstrated in studies involving patients with Parkinson's disease, or Attention-Deficit-Hyperactivity-Disorder (ADHD). Despite a substantial body of research, the neural mechanisms underlying timing are still poorly understood. The presentation of distractors within a critical to-be-timed interval is known to attract attentional resources (away from the timing task), which induces temporal misperceptions in humans and animals. Here, we conducted psychophysical experiments in monkeys to investigate the potential impact of attentional distraction on precise reproduction of learned time intervals in non-human primates. In addition, learning induced changes were measured after several sessions of distracted time-reproduction. Before the experiments, two rhesus monkeys were trained to memorize time-criterions in the range of several seconds. After successful training, the monkeys' ability of precise time-reproduction was tested in baseline trials without any distraction, and in trials with presentation of distractors within the to-be-timed interval. In a first experiment, modality (visual vs. acoustic) and duration (1s vs. 2s) of distractors (presented in the middle of the to-be-timed interval) were varied. Both monkeys showed precise time-reproduction in baseline-trials without attentional distraction, which was in accordance to predictions from scalar expectancy theory and Weber's law. However, a constant extension of subjective time-reproduction of about 1s was observed in distractor-trials. This misperception of time was independent of the modality and duration of distractors, and suggests a checkup-mechanism (in contrast to a stop- or reset-mechanism of an internal clock), which might be responsible for re-allocation of attentional resources away from and back to the timing task. In follow-up sessions distractor-presentation was continued to investigate the stability of induced misperceptions. Our results revealed an increased ability of both monkeys to ignore task-irrelevant distractors, and to establish precise time-reproduction only after a few sessions. This cognitive ability was previously reported exclusively in humans, and might constitute a unique characteristic of temporal processing in primates. After both monkeys acquired the ability to ignore task-irrelevant distractors, we varied the duration, position, and perceptual complexity of distractors in a final experiment. Misperception of subjective time was only observable after introduction of extended distractor-durations of 3s or 4s. Variation of distractor-position within the to-be-timed interval, as well as an increase in perceptual complexity of the distractor was insufficient to produce any temporal misperceptions in the highly adapted state. Our data contribute to a better understanding of timing mechanisms and their interaction with attentional processes of task-dependent re-allocations of attentional resources, which is especially important for the development of new therapeutic treatments in the context of ADHD.

A Computational Framework for Theories of Negative Priming

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The priming effect refers to a sensitivity of reaction times in psychophysical experiments resulting from repeated usage of neural pathways. The reduction of reaction times in positive priming is experimentally well understood. The opposite effect – negative priming – is, however, experimentally less tangible, but nevertheless of pronounced interest in research on memory, selective attention, and aging effects. Consequently, several theories (cf. e.g. Refs. 1-3) have been proposed to explain the effect by emphasising different underlying mechanisms. The complex pattern of empirical results does not clearly favour one theory over the others and is an incentive for the computational implementation of negative priming theories. In order to assess the theories quantitatively and on an equal footing, we constructed a model for stimulus-based action selection that accommodates all of the respective mechanisms. Reaction time differences in various priming conditions emerge by the interplay of all model components. The architecture comprises six layers, five of which are explicitly modelled by neuro-dynamical systems consisting of rate-coded units with exponential decay and adaptive thresholds. The sixth layer acts as a symbolic central executive and represents the externally given instructions. Visual stimuli are processed by the feature layer that consists of sublayers for the relevant modalities (colour, shape, words). Features belonging to the same stimulus object are bound by a binding layer by flexible activity markers that decay slowly when the stimulus is no longer present. A semantic layer matches words with object shapes when their joint representation is stabilized by the binding layer. The semantic layer actives an action layer if an adaptive threshold is surpassed. The action layer finally triggers a behavioural response that result from a competition among the different options including a void response. The layers are modulated by inputs from an episodic memory module temporarily storing previous episodes that are triggered to be retrieved by a matching percept. The system presents itself a unified theory based on existing approaches that allows us to obtain quantitatively testable predictions even from qualitative theories. Although it was formulated as a description of a specific set of experiments it may serve also as framework for artificial cognitive systems such as in mobile robots.

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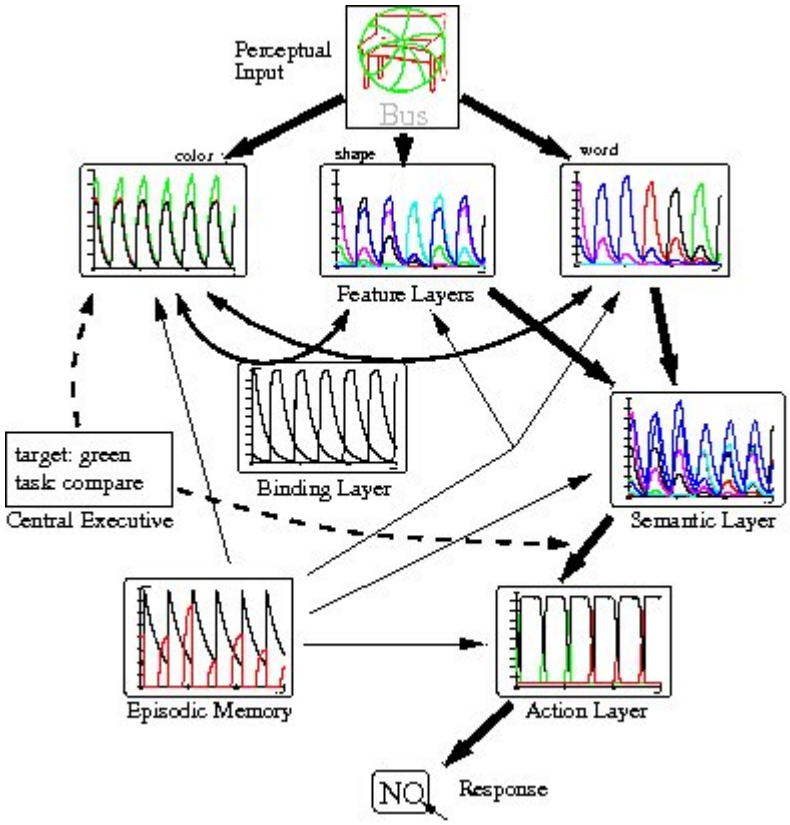
Perceptual Input
Bus
color
shape
word
Feature Layers
Binding Layer
target: green
task: compare
Central Executive
Semantic Layer
Episodic Memory
Action Layer
NO
Response

On the insights of cognitive neuroscience for economic theory and research.

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This study analyses, if and how cognitive neuroscience ay contribute to scientific progress in economic theory and research. We argue that the application of neuroscience methods and findings as well as the consideration of findings of cognitive neuroscience can help to solve some of the current problem of economic (and social science) research.

Standard economic theory assumes that (a) the concepts of objective rationality and objective irrationality exist, (b) that they are separate from each other (c) that the objective rationality and objective irrationality of an actor's behaviour can be defined and identified by others and that (d) objective irrationality is less desirable than objective rationality for actors.

Based on current neuroscientific findings we show that economic theory needs to distinguish between objective (i.e. observer-independent) and subjective (observer-dependent) phenomena and judgements. The core variables and phenomena of economic theory are both observer-dependent and subjective(e.g. money). By integrating the observer-dependent and subjective perspective, we show that the concept of irrationality as currently defined in economics is devoid of meaning. By considering the findings of cognitive neuroscience and by arguing that it is impossible in an observer-dependent way to conceptually differentiate between rational and irrational economic behaviour, we show how neuroscience can help to solve some of the current problem of economic research.
We use neuroscientific methods in an experimental setting to show that the central phenomena of economic theory (i.e. contract, money, success, property) are observer-dependent and subjective and are constituted by the concept that names the phenomena. For example, in order for something to count as money, actors involved have to have certain appropriate thoughts. "Money" refers to whatever people use as and think of as money. In order for A to pay B with money, both actors have to agree that the items that are exchanged are in fact money. Thus, the central phenomena of economic theory are self-referential.
We conclude by examining the consequences of this feature for economic theory.
As the defining principles of economic phenomena set no physical limits whatever on what counts as physical realisation of them, there cannot be a systematic distinction between objective and subjective rationality in economic actions. The main argument rests on the mental character of the social phenomena. In the neuroscientific experiments we will show that one can describe the neurophysiological conditions for certain visual experiences, e.g. for seeing blue, but that it is impossible to give inter-individual invariant accounts of the neurophysiology of seeing something as money, since in order for it to be money, one has to *believe* that it is money.
The aim of this study is to bridge the gap between mind and brain research in the context of economic theory.

Synchronized theta burst stimulation in the CA1 reduces freezing in a mouse model of fear extinction

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Synchronized theta (4-12Hz) activities between amygdala and hippocampus have been shown to be key elements of fear memory consolidation and reconsolidation. After consolidation, fear memory may vanish by a process termed extinction, which –at least partly- involves formation of a new memory trace. Pathways from the prefrontal cortex (PFC) to the amygdala modulate the fear expression patterns during fear memory extinction. Recently we observed that the balance in network oscillation at theta range between the amygdala, hippocampus and prefrontal cortex predicts the consolidation of fear memory extinction. Moreover, it has been shown that the hippocampus can regulate prefrontal cortical activity. At this point, we tried to answer the major experimental question, i.e., the influence of theta on PFC during extinction. In our experimental approach, we implanted bipolar stainless steel electrodes in the CA1 area of the mouse hippocampus bilaterally for theta burst stimulation. After surgical recovery, all implanted animals underwent a well established Pavlovian fear conditioning (cued) training and 24h later the retrieval and extinction sessions. During retrieval and extinction, one group (Stimulation group) was exposed to theta burst stimulations in the CA1 and the other group (Sham group) performed extinction trials without theta burst stimulation. The extinguished fear memory was reinstated by a set of footshocks. Fifteen days later, the spontaneous recovery of freezing was assessed in both groups. Data show that the extinction training with theta burst stimulation resulted in a decrease of freezing behavior compared to the sham controls. The stimulated group also showed reduced freezing during reinstatement and spontaneous recovery to the cue after 15 days. However, when they were exposed to the initial shock context, stimulated animals showed comparable freezing to the sham animals indicating that the original background contextual memory is still intact while the cued memory is unexpressed. In conclusion, our preliminary data show that bilateral CA1 stimulations specifically facilitate extinction learning, which is more likely through an establishing of a strong reconsolidation memory trace.

Synaptic inhibition of Purkinje cells guides consolidation of motor memories

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Studies on learning and memory have focused largely on the role of plasticity at excitatory synapses onto projecting neurons. Here we examined the role of synaptic feed-forward inhibition from molecular layer interneurons onto Purkinje cells in cerebellar motor learning. We investigated adaptation of the vestibulo-ocular reflex in a mouse model (PC-Δγ2), in which the GABA-A receptor γ2 subunit was selectively deleted from Purkinje cells, resulting in a loss of GABA-A receptor mediated synaptic inhibition. While baseline motor performance in these mice was only mildly impaired, motor learning was severely disrupted. Specifically, phase reversal learning as well as consolidation of both gain and phase adaptations of the vestibular ocular reflex were strongly compromised. The simple spike activities of Purkinje cells in PC-Δγ2 mice showed abnormal temporal patterns, both during and in the absence of naturally evoked compensatory eye movements, whereas mean firing frequencies were unaffected. We propose that feed-forward inhibition from molecular layer interneurons, by controlling the temporal patterns of Purkinje cell activity, is necessary for the consolidation of cerebellar learning in downstream neurons of the cerebellar and vestibular nuclei.

Trace conditioning in harnessed honeybees – behavior and physiology

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Our understanding of the neuronal mechanisms of classical conditioning, where a neutral stimulus (CS) is associated with a temporally overlapping reinforcing stimulus (US), has made substantial progress in recent years. However, when the two stimuli are temporally disjunct, a non-associative trace of the first stimulus is necessary to build up a transient "bridge" allowing the nervous system to form an association with the second stimulus. The neuronal substrate for stimulus traces is as yet unknown, as are their physiological mechanisms. We aimed at investigating these mechanisms by studying appetitive olfactory trace conditioning in honeybees. In trace conditioning the US is presented after the CS has terminated, but the CS is nonetheless associated with the US and learned to be predictive.

We studied the time course of the odor trace by varying the interval between CS and US. We found that an odor trace maximally lasts between 6 and 10 s. Next, we studied the odor specificity of the odor trace. We found that the associative odor memory formed during trace conditioning is more odor specific than the one formed during conditioning with overlapping CS and US. We then searched for a physiological substrate of odor trace by optically imaging olfactory projection neurons in the first olfactory brain area, the antennal lobe. We found that an initial odor specific glomerular response patterns was followed by a persistent and odor specific activity pattern. This pattern developed within 3 to 4 s after odor offset, lasted for more than 20 s and was different from the initial activity pattern. However, we found no correlation between the perceived odor similarity during trace conditioning and the similarity of the persistent activity in the antennal lobe projection neurons.

Sleep in honeybees: Searching for the role of sleep in memory consolidation

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Sleep, though much better characterized in mammals, has also been found in insects like the fruitfly (Drosophila melanogaster; Hendricks 2000) and the honeybee (Apis mellifera; Kaiser 1988). In mammals sleep seems to be important for long-term memory consolidation (Gais et al. 2006). Since honey bees consolidate early forms of memory into long-term memory, we studied their sleep behavior in the context of learning and memory consolidation. A natural form of learning is foraging. To test if sleep behavior and memory consolidation are correlated, we compared the duration of foraging flights of individually RFID-marked bees trained to a nearby feeder, and that of naturally foraging bees, and monitored their respective sleep behavior inside the hive. Furthermore we tested navigational learning in RFID-marked bees. In a second step we deprived sleep in single bees by shaking them over night and compared their navigational memory with bees that spend a normal night in their hive.

We found that sleeping time is independent of the difficulty of the navigational tasks and of foraging behavior. Sleep deprivation does not change foraging performance at a previously trained feeder, but impairs navigational memory. These findings suggest that undisturbed sleep might play a role in the consolidation of navigation memory in honeybees even though differences in normal sleep behavior do not affect the navigation performance.

In addition we recorded extracellularly from the alpha-lobe of harnessed honeybees, looking for correlations between neuronal activity and sleep. Since honeybees display different sleep signs and not all of them are easily detectable, we decided to define sleep phases by the antennal mobility. We used olfactory PER (proboscis extension response) conditioning in harnessed bees, using sugar water as the unconditioned stimulus (US) and different odors as conditioned stimuli (CS). After conditioning we monitored the sleep behavior over night and tested for memory consolidation the following day. We found correlations between neuronal activity and sleep but could not find correlations between sleep behavior and memory consolidation.

Knowledge transfer depending on task difficulty

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The ability of NMRI mice to discriminate pairs of pure tones (PT) or amplitude modulated (AM) pure tones was tested in an aversive shuttle-box GO-NOGO paradigm. Whereas mice easily learned to discriminate PT of different frequencies within about two training sessions, discrimination learning of AM tones took longer (5 to 10 sessions) to reach significant discrimination performance. When transfer of knowledge was tested in animals that were consecutively trained to both PT and AM tone discrimination (transfer between stimulus classes), we found that (1) beneficial transfer of knowledge was only seen when the demanding AM tone training was followed by the easy PT training but not when order of training was reversed, and (2) confusion of conditioned stimuli occurred when the AM tones used shared spectral components with the PT training. We conclude from these results that the type of knowledge transferred between training paradigms is likely to be procedural knowledge about shuttle-box training rather than possible stimulus generalizations. Obviously, as the benefit of knowledge transfer was small, it was only seen when an easy task followed a demanding one. It was not sufficient to over-compensate for the increased stimulus processing difficulty when a demanding task followed an easy one.

Inhibitory mechanisms may be involved in the control of the sensitive period for sexual imprinting in the zebra finch.

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Inhibitory circuits of GABAergic neurons, in particular those also Parvalbumin – immunoreactive, play a major role in the control of sensitive periods, as has been shown for the development of ocular dominance in the visual cortex of cats and mice (Hensch 2006). To test whether the scenario developed for the control of visual cortex plasticity may also apply for other early learning paradigms with sensitive periods, we examined in the zebra finch the density of inhibitory neurons within brain areas relevant for sexual imprinting, an early learning paradigm characterized by a sensitive period and irreversible storage of the imprinted information.

According to our quantitative measurements, the density of GABAergic and Parvalbumin – immunoreactive neurons within such areas increases significantly when, by a one week exposure, a male zebra finch is imprinted on a female. Other brain areas are unaffected, and no effect can be seen in nonimprinted controls of the same age.

This result is a first hint for a participation of inhibitory control of sensitive periods in sexual imprinting, and it contributes to the view that such control mechanisms may also apply for early learning phenomena other than visual cortex plasticity.

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Improvement of auditory discrimination learning by Ginkgo biloba extract EGb761®

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The effect of oral application of Ginkgo biloba extract EGb761® on auditory discrimination learning in young adult male Mongolian gerbils was investigated in an aversive shuttle-box GO-NOGO paradigm using discrimination tasks with three different degrees of difficulty (cf. Schulze and Scheich, 1999, Neurosci. Lett. 261: 13-16; Kaernbach and Schulze, 2002, Neurosci. Lett, 329: 37-40), and two protocols for drug administration starting 2 weeks prior to or at the beginning of training. In comparison to placebo-treated controls we observed statistically significant improvement of learning performance in treated animals both in easy as well as in more demanding discrimination tasks. EGb761® has been reported to increase the extracellular concentration of dopamine in the prefrontal cortex of rats (Kehr et al., 2006, Planta Medica 2006; 72: 1083 (P 347)) which plays a major role in the type of learning used in the present study. We, therefore, suppose that EGb761® improves discrimination learning through its effect on the dopaminergic system.

Need for Speed: Conditions for the Formation of an Implicit Memory in *Drosophila melanogaster*

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Drosophila melanogaster can improve a motor sequence, and learn, store and retrieve the improvements after extended practice. This is shown in a novel paradigm in which flies are walking on a rotating narrow ring with eight gaps. In an effort to compensate for the optic flow elicited by the passing-by surrounding, the flies walk on the ring preferentially against the direction of rotation and thereby cross the gaps. The training comprises five 60-sec runs which are interrupted by 20-min breaks. The short-term memory can open out into a long-term memory after 50 or more successful transitions. Motor skill learning is a two-stage process.

Neither improvements during the training session nor long-term learning effects after 24h were found in *rutabaga*^{2080} flies. The genetic defect is in the adenylyl cyclase I, a key protein of the cAMP signalling cascade, which is proven to be necessary for proper olfactory and visual learning. The defect is being used to map memory functions by partial rescue experiments. Short-term and long-term improvements in climbing could be restored by a wild-type *rutabaga*-transgene. It was expressed either pan-neuronally or just in parts of the CNS by means of the Gal4/UAS-system.

Gap crossing improves during training on a regular succession of gaps in terms of the success rate and the time needed to cross a single gap. The underlying kinematic changes can be proven under a high speed camera (method: Pick and Strauss 2005). Training on a non-rhythmic succession of gaps or on a succession of gaps with variable widths did not result in motor skill learning. The formation of a long-term memory after successful training is dependent on a sleep phase (cf. vertebrates: Walker et al. 2002). This has been shown with deprivation techniques. After consolidation the memory is stable for 1, 2 or 3 days without noticeable decay.

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Effects of Astemizole on rat hippocampal network oscillations

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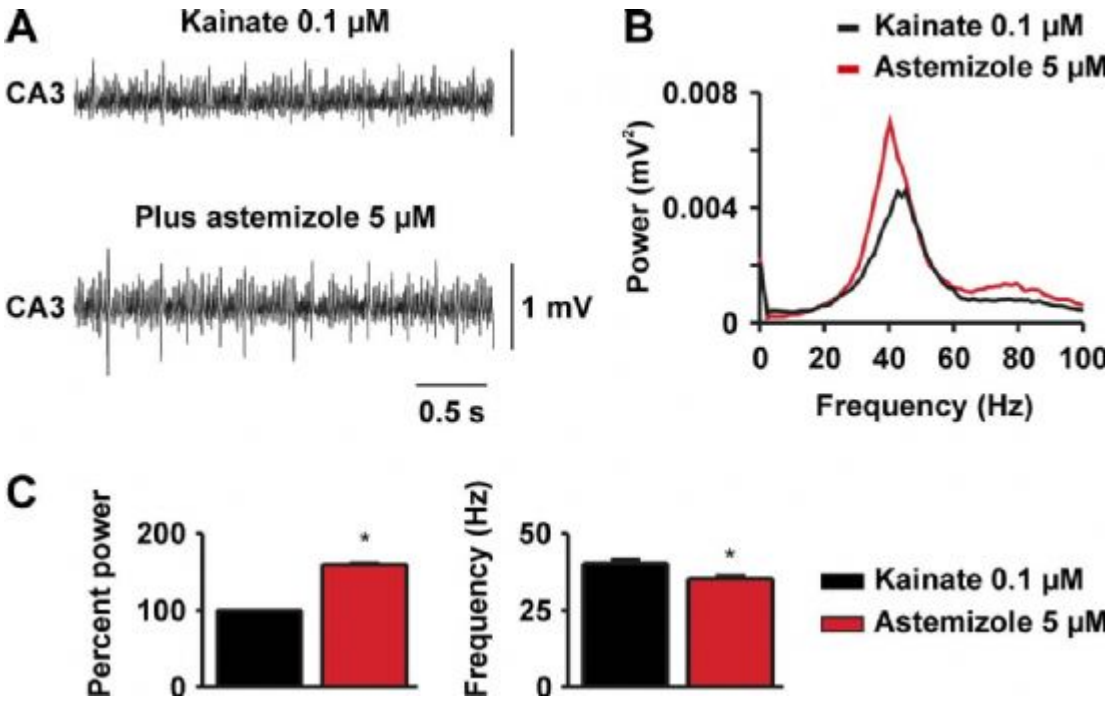
The mammalian hippocampus displays a variety of neuronal network oscillations, which are related to different functional states. During active wakefullness and spatial exploration theta (~5-15 Hz) and gamma (~30-100 Hz) rythms dominate. These network oscillations are necessary for retrieval and storage of information. In rat hippocampal horizontal slices kainic acid (100-150 nM) reliably induces gamma oscillations (30-100 Hz), while in coronal slices theta activity is promoted.

The aim of this work is to test the effect of Astemizole, an histamine H1-receptor antagonist and Kv 11.1 blocker on oscillatory activity at theta and gamma frequency. Experiments were performed on horizontal hippocampal slices obtained from brains of young adult Wistar rats (~ 160 g). Kainic acid, continuosly bath applied at a concentration of 100 nM was used to induce gamma oscillations. Gamma oscillations were extracellularly recorded from `stratum pyramidale´ of area CA1 and CA3b.

Preliminary results showed that Astemizole significantly increases the power by about 40% of gamma oscillations at the concentration of 5 µM. When the concentration of Astemizole was increased to 30 µM also the power was even more increased by 80%. By contrast, the application of Astemizole 1 µM didn´t affect gamma oscillations. We are now performing experiments concerning theta oscillations in coronal slices. It will be also tested the influence of Astemizole on the inducibility and incidence rate of sharp waves ripple complex, the fast network oscillations (200 Hz) involved in memory consolidation.



Dopamine modulated plasticity enables TD learning in a spiking actor-critic neural network model

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Synaptic plasticity is thought to underlie learning, but the exact relation between changes in synaptic efficacy and system-level learning remains unclear. One theory often considered in this context is reinforcement learning, in particular the variant known as temporal-difference (TD) learning. In a previous study [1] we showed that a spiking neural network model with biologically plausible plasticity rules is able to implement TD learning. However, the model does not assign a role to the dopaminergic neurons of the basal ganglia, although much of the neurophysiological evidence supporting the theory of TD learning in the brain is based on the dopamine system. Most notable in this context is the resemblance of dopaminergic activity to the TD error signal [2] and the finding that plasticity in the striatum is modulated by dopamine [3].

Here, we present a spiking neural network implementing actor-critic TD learning (see figure inset) that for the first time simultaneously describes the generation of a dopamine mediated TD error signal and the synaptic plasticity required to exploit the error information. The critic module is based on a simplified model of the basal ganglia [4]; the dopaminergic neurons respond to movements of the agent between states of different values with rate excursions of similar amplitudes to those observed in conditioning experiments [1]. The dopamine signal is in turn interpreted as the third factor in the plasticity of the synapses encoding the value function and the policy. Although the synaptic plasticity rules were postulated using a top-down approach, there is excellent agreement between the predictions of our synapse model and experimental findings on corticostriatal synapses [5].

We show that the network is capable of solving a non-trivial grid-world task with sparse rewards. It learns to evaluate the states with respect to reward proximity and adapts its policy accordingly. The learning performance is similar to that of a discrete-time TD learning algorithm (see figure; red: spiking network model, black: discrete time algorithm). Furthermore we analyse the learning behavior with respect to changes in the dopamine level and compare it to the learning behavior in Parkinson patients.

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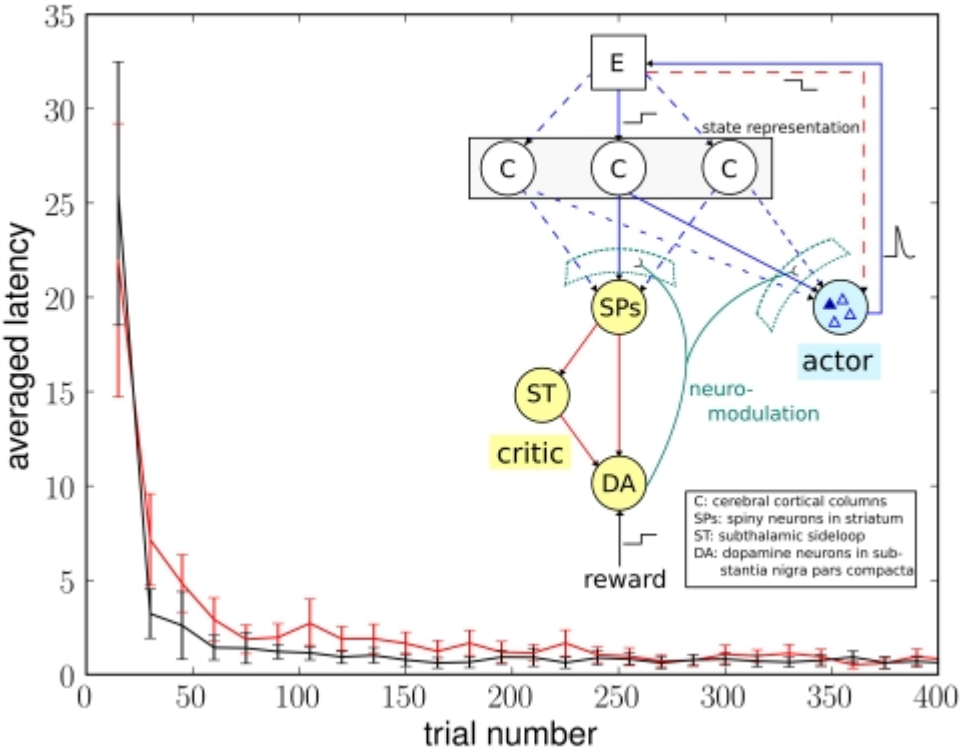
averaged latency
trial number
0
5
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15
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35
0
50
100
150
200
250
300
350
400
E
state representation
C
C
C
SPs
ST
critic
DA
reward
actor
neuro-
modulation
C: cerebral cortical columns
SPs: spiny neurons in striatum
ST: subthalamic sideloop
DA: dopamine neurons in sub-
stantia nigra pars compacta

The role of protein synthesis during LTP-reinforcement and memory formation under different learning paradigms

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The formation of a long-term memory and the maintenance of late-LTP requires protein-synthesis. In former studies we could show that specific behavioral stimulation (acute stress or spatial training) could reinforce a protein synthesis-independent early-LTP into a protein synthesis-dependent late-LTP in the dentate gyrus of freely moving rats, if the behavioral paradigm is temporarily related to the weak tetanic stimulation of the perforant path. We have now studied the effects of a transcription (actinomycin D) or protein synthesis inhibitors (anisomycin, emetine, rapamycin) on this transformation of early- into late-LTP. Acute stress-related LTP-reinforcement could be completely blocked by anisomycin, emetine or actinomycin D. In contrast, the effect of both classes of substances on spatial training-LTP-reinforcement was more complex and heavily dependent on the training paradigm as well as the used protocol. Holeboard training-related LTP-reinforcement, which is known to be less stressful, could not be blocked by translation inhibitors but not by actinomycin D. The transcription blocker did not affect memory formation, whereas the translation inhibitors prevented it. Water maze training-induced LTP-reinforcement showed more diverse results. LTP-reinforcement could be blocked without affecting the behavioral parameters of learning and memory formation in dependence of the training protocol. The data suggest that the involvement of transcription and translation during learning, memory formation and LTP-maintenance widely depends on how much stress is elicited by the testing paradigm.

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Hippocampal Activation of Immediate Early Genes Zenk and c-Fos in Zebra Finches (*Taeniopygia guttata*) During Learning and Recall of a Spatial Memory Task

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Zebra finches (*Taeniopygia guttata*) are able to learn the location of hidden food by orienting on spatial cues in a "dry water maze". In the course of spatial learning, the hippocampus shows high expression of the immediate early genes Zenk and c-Fos, indicating high activation of this area during learning. In contrast, the immediate early gene activity is nearly absent, if the birds do not have to rely on spatial cues (Bischof et al., 2006). In the present experiment it was examined whether hippocampal activation can also be observed if the learned task is recalled. For this purpose, the hippocampal Zenk and c-Fos activation of birds in an early learning stage was compared with others having reached their maximum performance. The results unequivocally show that the avian hippocampus is also active during recall of a learned spatial task, but the activation is significantly lower than in the learning animals. This suggests that the immediate early gene expression correlates with plastic changes that occur during memory formation. Hippocampal Zenk and c-Fos expression showed a large variation not only in the position of the active patches of neurons, but also in size and cell density.

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Associative learning is impaired upon lack of the presynaptic protein SAP47

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The synapse associated protein of 47 kDa (SAP47) is a member of a phylogenetically conserved, yet functionally diverse gene family. In *Drosophila*, SAP47 is encoded by a single gene and is expressed throughout all neuropil regions of the wild-type larval brain; specifically, electron microscopy reveals anti-SAP47 immuno-gold labeling within 30 nm of presynaptic vesicles. To analyze SAP47 function, we use the viable and fertile deletion mutant $Sap47^{156}$, which suffers from a 1.7 kb deletion in the regulatory region and first exon of the gene. SAP47 cannot be detected by either immunoblotting or immunohistochemistry in $Sap47^{156}$ mutants. $Sap47^{156}$ mutant larvaeexhibit normal sensory detection of odors and tastants as well as motor performance and basic neurotransmission at the neuromuscular junction. However, $Sap47^{156}$ mutant larvae show an approximately 50 % reduction in learning ability when compared to wild-type animals. A similar learning impairment is observed upon reduction of SAP47 expression using RNA-interference.
These data underscore the importance of presynaptic, “vesicular” mechanisms for associative plasticity and provide the first hint to the function of SAP47 in flies and its homologues in other species.

STRESS ACTIVATED PROTEIN KINASE IN LEARNING AND MEMORY OF HONEYBEE: IMPLICATIONS FOR A ROLE IN SLEEP

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Sleep like phenomena exist across the whole animal kingdom. The high variability in behavioral characteristics of sleep makes it difficult to identify general principles required to understand the evolutionary and functional implications of sleep. Despite this enormous variability it has been demonstrated that the cAMP/CREB system and epidermal growth factor signaling play conserved roles in mechanisms that regulate sleep. Sleep deprivation, on the other hand, can affect various physiological processes including synaptic plasticity and different aspects of learning. Since sleep deprivation activates cellular stress responses (e.g. heat shock response) in mammalian brain, we tested whether the highly conserved stress activated protein kinase /Jun N-terminal kinase (SAPK/JNK) contribute to mechanism underlying sensory processing, learning and memory formation after stimuli suited to interfere with sleep in honeybee.

Using the honeybee, we show that application of stimuli suited to interrupt sleep for over-night activates the SAPK/JNK in the honeybee brain and leads to an increased responsiveness to appetitive stimuli and specific impairments in memory formation. Inhibition of SAPK/JNK activity during overnight stimulus application reverses SAPK/JNK activity to normal level and rescues the changes in responsiveness, learning and memory formation. In addition, inhibition of transcription reveals that these changes are non-transcriptional. Taken together, our results confirm the critical role of the SAPK/JNK signaling cascade in mechanism underlying learning and memory formation in honeybee and provide first hints for a possible link between stress, SAPK/JNK signaling, and sleep deprivation.

MOLECULAR MECHANISM OF SYNAPSIN ACTION IN ASSOCIATIVE LEARNING OF LARVAL *DROSOPHILA*

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My main research interests are neuroethology, cellular neuroscience and synaptic plasticity, especially as they pertain to learning and memory. I use larval *Drosophila*, because of their cellular simplicity which obviously facilitates connecting analyses of learning in particular between neural circuits and behaviour. Specifically, I focus on the molecular function of the evolutionary conserved presynaptic phosphoprotein Synapsin. Synapsin knock-out flies (syn^{97CS}) are viable and show no obvious structural defects in brain anatomy, but are impaired in olfactory associative learning by approximately 50 %. As *Drosophila* Synapsin includes phosphorylation consensus motives for PKA, it may function as downstream target of the cAMP PKA cascade during memory formation. I currently investigate whether transgenic expression of Synapsin with non-phosphorylatable PKA consensus motive(s) can rescue the syn^{97CS} learning deficit. Furthermore, as one of the PKA consensus motives undergoes ADAR-mediated RNA editing (converting the protein motive from R-R-F-S into R-G-F-S), I ask whether transgenic expression of Synapsin carrying either of these motives can rescue the syn^{97CS} learning deficit.

The Effect of Ovarian Hormones on Strategy choice in the Morris Water Task and on Neurogenesis in the Hippocampus

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Independent memory systems, such as amygdala, striatum or hippocampus, can be affected by estradiol (E). In competitive memory systems, where not only the learning strategy of one, but also of several memory systems can be applied, the female sex hormone can cause a shift in strategy choice. For example, in a paradigm using the T-maze where either a striatum-dependent strategy or a hippocampus-dependent learning strategy could be used, high levels of E shifted the strategy choice towards using the hippocampus-dependent strategy. It is thought that E differentially affects the efficiency of the memory systems and thus leads to the shifting in strategy us.

One aspect, which might influence the efficiency of the hippocampus, is neurogenesis. Adult neurogenesis consists of at least two stages, cell proliferation (CP, birth of new cells) and cell survival (CS, new cells that become mature neurons). A recent study used male rats in a paradigm of the Morris Water Task (MWT) where rats could be distinguished by the strategy choice they used. Rats could either use the hippocampus-dependent learning strategy (place responder) or the striatum-dependent learning strategy (cue responder). It was found that the rate of CP is less in place responder that in cue responder. This indicates, perhaps paradoxically, that a lower rate of CP increases the efficiency of the hippocampus and shifts the strategy choice towards the hippocampus-dependent place strategy.

Adult neurogenesis is influenced by ovarian hormones. Generally E up-regulates CP acutely although this effect depends on many factors, such as the timing of estradiol administration, dose, sex, form of administration, and hormonal history of the animal.

In this study, we sought to determine whether E level shifted strategy choice in the MWT and whether the same relationship between strategy choice and CP in the hippocampus existed in female rats. In this study three groups of female rats were trained in a modified paradigm of the MWT. Intact rats (Sham+Oil), ovariectomized and estradiol-treated (OVX+E) and control (OVX+Oil) each received a bromodeoxyuridine (BrdU) injection i.p. on the day before training started. During the 9 training days, E/vehicle injections s.c. were given 2h before testing. The rescue platform stayed at the same location throughout acquisition trials. On d1 and d2 the platform was visible, on d3 it was exchanged by a hidden platform and this pattern was repeated three times. On d10 the visible platform was placed to the opposite quadrant. Rats swimming towards the new location were considered cue responders, while rats targeting the old location were considered place responder. Exogenous E did not affect strategy choice, but intact rats with very low levels of E were biased towards cue-strategy. The effect of E on neurogenesis (using BrdU and Ki67-IHC) supported the hypothesis, that a low rate of CP increases the efficiency of the hippocampus, as we found a positive correlation between the distance to find the visible platform and the CP-rate in cue responder.

Our findings suggest that ovarian hormones, as opposed to E alone, may shift strategy use and that both ovarian hormones and neurogenesis levels influence hippocampal and striatal efficiency.

Nutrition affects histone modifications and appetitive learning in honeybee

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Different studies show that nutrition influences physiological and developmental processes of an organism. One of the best known examples of a dietary impact is the *agouti* mouse experiment: changing the mother's nutrition before, during, and after pregnancy results in different phenotypes of the offspring. This effect is due to different gene expression caused by epigenetic mechanisms such as altered DNA methylation and histone modifications. Effects of nutrition on development and physiology also occur in invertebrates indicating basic and probably conserved processes. Feeding royal jelly to genetically similar larvae for different times during development leads either to fertile long-living queens or to sterile short-living worker bees. A recent study suggests an epigenetic contribution to this nutrition mediated effect on developmental processes.

This, and the fact that the honeybee queen is fed with royal jelly throughout its life prompted us to investigate whether nutrition also affects epigenetic mechanisms after development. We provide evidence that feeding royal jelly to adult honeybees affect histone modification, sensory processing, and learning, supporting the idea that nutritional effects on behaviour are modulated by epigenetic mechanisms.

Modification of olfactory learning and memory induced by RNA interference targeting a7 nicotinic acetylcholine subunit in the honeybee

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Acetylcholine is the major excitatory neurotransmitter in the central nervous system of insects and targets the numerous nicotinic acetylcholine receptors (nAChRs). The recent honeybee genome sequencing has described 11 a and non-a nicotinic subunits, but the molecular composition of the nAChRs remains unknown, in honeybees as in invertebrates. Many studies have already demonstrated the involvement of nAChRs in olfactory learning and memory using nicotinic antagonists injection into the honeybee brain. Behavioral pharmacological experiments suggested the existence of two nAChR subtypes: the a-Bungarotoxin (a-Bgt)-sensitive and the a-Bgt –insensitive nAChRs. The a-Bgt-sensitive nAChRs are necessary for long-term memory formation and the a-Bgt-insensitive nAChRs are needed for retrieval processes. In vertebrate, it is well known that a-Bgt-sensitive nAChRs are homomeric and made of a7 subunits which are phylogenetically conserved. We used the genetic tool of RNA interference in the honeybee to induce specific a7 subunit deletion to study its role in the formation of memory.

The role of a7 nicotinic subunits in olfactory learning and memory was studied using siRNA to block a7 subunit expression. Quantitative PCR analysis reveals that siRNA reduced a7 subunit expression from 3 h and to 18 h after injection. Honeybees injected with siRNA 18 h before multiple-trial olfactory conditioning had poor performance during acquisition and memory tests compared to control animals. siRNA injected 18 h before the 24 h-retrieval test had no effect on memory, this result excluding an effect on retrieval processes. This result also indicates that olfactory perception is not depending on the presence of a7 subunit.

In conclusion, deletion of a7 nicotinic subunit specifically impairs olfactory conditioning and this leads to low performances during retrieval test. As a consequence, nicotinic acetylcholine receptors including a7 subunit seem to be necessary for olfactory learning but not for retrieval processes. Participation of hypothetic a7 homomeric a-Bgt sensitive receptors in the formation of long-term memory has to be investigated by inducing a7 deletion during the consolidation phase of memory.

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STRESS ACTIVATED CASCADES IN HONEYBEES: ROLE IN LEARNING AND MEMORY FORMATION

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Learning and memory formation do not follow a strict pattern but strongly depend on a variety of internal and environmental conditions including satiation, motivation state, circadian rhythm, or stress. With regard to stress, *in vitro* studies show that distinct stress stimuli trigger different stress-activated cascades, including the MAPK kinases JNK and p38. Thus, depending on the physiological context, this network enables a fine-tuned response to stress. With regard to behavior, it is known that stress can either repress or improve learning and memory depending on parameters as strength, duration and type of stress as well as training conditions and learning context. However, the molecular processes that link specific environmental inputs to their appropriate behavioral response *in vivo* are unclear yet.

In our study we address the question which molecular pathways are responsible for changes in learning and memory in response to external stimuli *in vivo*. Using different behavioral paradigms in honeybees we demonstrate that in addition to other external factors, heat has consistent and specific effects on distinct aspects of the learning process. We selected the conserved stress-activated kinases JNK and p38 as potential pathways involved in the mediation of the behavioral response induced by heat. Immunohistochemical staining shows that JNK and p38 are located in different brain regions. Based on first evidence we presently test the hypothesis that the stress-activated kinases JNK and p38 are differently implicated in mediating the behavioral response to different stress stimuli.

Chromatin remodelling: Protein acetylation facilitates memory formation in honeybee

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Modifications of the chromatin structure are required for gene expression during development but are also involved in learning and memory formation. These modifications include DNA methylation as well as covalent modification of histone tails. Especially the acetylation and deacetylation catalyzed by histone acetyltransferases (HATs) and histone deacetylases (HDACs) play a key role in the regulation of gene expression in eukaryotic cells. Hyperacetylation of histones opens the chromatin structure, a process essential for the access of the transcription machinery. This suggests that these processes specifically contribute to the transcription-dependent formation of long-term memory (LTM), but not for short-term memory that is independent of transcription.

In the present study we use the olfactory associative learning paradigm to study the effect of Trichostatin A (TSA), a commonly used HDAC inhibitor, on memories induced by different training strength. As expected, TSA improves LTM formation induced by a strong training procedure but, unexpectedly, also improves memory induced by a weak training procedure that is independent of gene expression. Using the transcription inhibitor actinomycin D in addition to TSA we tested whether the “memories” improved by TSA depend on transcription processes. We show that actinomycin D totally abolishes the TSA effects after weak training, while the TSA effects after strong training are only partially reduced. Thus, depending on the strength of training, TSA affects different transcription-dependent and in addition yet unknown transcription-independent processes. The later finding suggests that in addition to histone acetylation, other targets not involved in transcription processes are modified by acetylation.

Stability and plasticity of transient hippocampal cell assemblies studied by self-organizing maps

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Information processing in the mammalian brain may be achieved by the transient generation of neuronal assemblies in which selected cells act coherently within neuronal networks. An example for information processing by spatio-temporal activity patterns is the formation and storage of declarative memories. Spatial memory formation in rodents (a homolog of declarative memory) is believed to follow a two-step process with transient storage of representations in the hippocampus and later transfer of information into neocortical networks for long-time storage. Storage and readout within the hippocampus are carried by specific type of network activity, the gamma oscillation and sharp wave-ripple complexes, respectively. This model suggests that several different neuronal assemblies are present in the hippocampus at the same time.

Based on this concept we hypothesized that spontaneously occurring sharp wave-ripple complexes can be sorted into different patterns, each of which corresponds to one memory representation. Using our in vitro model of sharp wave-ripple complexes in mouse hippocampal slices we applied mathematical sorting algorithms to field potentials of spontaneously occurring network events. As a first step we derived the characteristic signal parameters by principal component analysis. Subsequently, we sorted the data with self-organizing maps that provide a two-dimensional representation of similarities and differences between sharp wave-ripple complexes. In undisturbed recording conditions these maps (representing all activated assemblies) were stable over recording periods of more than 1 h. Single identified units were selectively activate on specific sub-types of sharp-wave ripple complexes. Changing synaptic weights in the network by long-term potentiation alters the maps in a defined manner, indicating that synaptic plasticity does induce changes in assembly formation during coherent network activity patterns which are involved in memory processing.

These data show that individual hippocampal neurons are members of defined assemblies which are selectively activated during sharp wave-ripple complexes. Moreover, the neuronal composition of assemblies can be altered by classical paradigms of synaptic plasticity.

Fluorescence microscopy used to visualize spontaneous high-frequency network oscillations in hippocampal slices from mouse and rat

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Synchronous action potential firing by which transient neuronal assemblies are formed is believed to play an important role in information processing in the mammalian brain. One important model system is the encoding of spatial memory by place cells and transient storage of these representations in the hippocampus. It has been shown that sequences of place cell firing encoding for the trajectory of the animal during exploration are re-played during awake immobility and sleep. This shows that memory traces are imprinted in the hippocampal formation and retrieval of stored memory from this region is reliably possible. However, the mechanisms underlying memory formation, consolidation and retrieval are poorly understood. This is mainly due to the lack of appropriate methods to monitor large neuronal networks: Tetrode recordings provide only a few identified units per tetrode and common scanning microscopy techniques lack a high temporal resolution which again prevents the recording of many neurons at the same time. In addition, it is demanding to monitor single action potentials with optical methods.

We have adapted a simple epi-fluorescence microscope to an interface recording chamber to visualize spontaneously occurring high-frequency network oscillations *in vitro*. We have previously shown that these oscillations have similar properties to *in vivo* sharp wave-ripple complexes that are implicated in memory consolidation during sleep (Maier et al., J. Physiol 2003, Both et al., Hippocampus 2008). To monitor neuronal activity we used different genetically encoded fluorescent Ca^{2+} indicator proteins by transfecting the cells with a recombinant adeno-associated virus. Transfection of cultured hippocampal slices yielded sufficient expression to detect spontaneous Ca^{2+} transients going along with single action potentials (Wallace et al,. Nat Methods 2008, Hasan et al., PLoS Biol 2004). With this approach we were able to monitor a field of view of 410 µm x 410µm with sub-cellular optical resolution (20x objective, 0.4 NA). Propagation of network activity could be monitored in a field of view of 2 mm x 2 mmwith single cell optical resolution (4x objective, 0.1 NA). With the high-end camera used (Hamamatsu Image EM C9100-13) the fluorescence signal is strong enough to identify calcium transients accompanying single action potentials in identified neurons with frame rates of up to 62 Hz.

Our data show that fast network dynamics in the millisecond rage can be recorded for large assemblies of neurons (>200 cells) allowing to study assembly formation and -stability in networks serving memory formation.

Circadian rhythmicity and olfactory learning in the honeybee (*Apis mellifera*)

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Circadian rhythmicity is a general feature among all groups of organisms, including animals, plants and bacteria (for an overview see Goldbeter, 2008). Circadian rhythms influence olfactory responses in *Drosophila melanogaster* (Tanoue et al. 2004, Krishnan et al. 2008) and pheromone reception of the moths (Merlin et al. 2007). Circadian rhythms also influence memory formation in *Drosophila* (Sakai et al. 2004) and in cockroaches (Decker et al. 2007).

Because the honeybee is an important model species for studying olfactory learning, we investigated whether honeybee learning is also modulated by circadian timing. Therefore we differentially conditioned harnessed honey bees at different circadian phases (first and last quarter of day as well as night) and tested their performance in an odour-discrimination test. We both tested short-term memory (30 min after training) and long-term memory (24 hours after training). To assess the influence of illumination we trained and tested the bees under different light regimes as well. We tested groups of bees taken from a controlled light regime of LD 12:12, and bees from the outside to unveil the impact of changing day length on the honeybees' learning ability and the transferability of a strictly controlled system to natural conditions.

Our results indicate that learning abilities of honeybees are modulated by the circadian system. This influence differs between outside bees and those from the controlled light regime. In addition, we found that light conditions during training also influence learning performance, in particular for those from the controlled environment. Further studies are needed to investigate the roles of cellular mechanisms at diverse levels of odour reception and memory formation.

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Characterization of a *c-fos* reporter mouse for *in vivo* imaging

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Experience and learning creates new memories which are stored as memory traces in the brain. To get insights into the organization of memory traces at the circuit level it would be helpful to identify distinct subsets of neurons being activated during memory formation, consolidation and recall. Thus, a mouse expressing a reporter in an activity dependent manner in combination with chronic *in vivo* imaging techniques could be a valuable tool for the investigation of these processes.

It has been shown that both memory formation and memory recall induce de novo protein synthesis and activate the expression of the immediate early gene *c-fos* (Radulovic *et al* 1998, J. Neuroscience). The reporter mouse created by Fleischman *et al* has a floxed *c-fos* gene and EGFP inserted downstream of the second loxP site. Upon crossing this mouse with a CRE line the *c-fos* gene is deleted and EGFP is expressed under the control of the endogenous *c-fos* promoter (Fleischmann *et al* 2003, J. Neuroscience).

The reporter mice used in this study are heterozygous with a wt *c-fos* allele and a deleted *c-fos* allele. Mice have also been crossbred with the tg-GFP-M line (Feng *et al* 2000, Neuron) and therefore contain very few neurons which constitutively express EGFP in high levels which serve as reference landmarks for unambiguous identification of the same subset of neurons in subsequent imaging sessions.

We characterized the properties of this reporter mouse and tested its suitability for *in vivo* imaging. We found that heterozygous reporter mice show no learning impairments in an auditory fear conditioning task compared to their wt littermates. After fear conditioning the mRNA levels of the immediate early genes *c-fos* and *Arc* are upregulated in the auditory cortex. Conditioning induces *c-fos* and EGFP expression in the auditory cortex and the expression is restricted to neurons. The EGFP signal is strong enough to be detected by 2-photon laser excitation microscopy *in vivo* and individual neurons can be followed over weeks.

Together, these results indicate that this reporter mouse could be used in the future for *in vivo* studies because it allows monitoring the activation state of distinct neuronal populations over long periods of time.

Conditioned memory of complex sounds depends on the auditory cortex

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Auditory fear conditioning (AFC) is a simple and often used associative learning paradigm: Animals learn to associate a neutral stimulus (CS, conditioned stimulus) - usually a pure tone - with an aversive stimulus (US, unconditioned stimulus), e.g. a foot shock. Both auditory and sensory information converge onto cells in the amygdala. Projections from the amygdala to output brain areas, e.g. the brainstem, support fear behaviour, e.g. freezing. Such an absence of all non-respiratory movement serves as a behavioural indicator of successful learning.

We report the results of AFC to complex sounds in mice which are strikingly differ from findings derived form pure tone AFC:

Memory testing one day after AFC to complex sounds revealed significant freezing levels during CS presentation when compared to baseline freezing, so far resembling the results of pure tone AFC and proving that AFC to complex sounds is possible.

But what role does the auditory cortex (AC) play in AFC with complex sounds?

There are two auditory input pathways to the amygdala. On the one hand the direct thalamic route and on the other hand the thalamo-cortical route, the latter traversing both the auditory thalamus and the AC. It has been previously demonstrated that pure tone AFC can occur even in the absence of the AC because it can be sufficiently supported by the thalamic pathway alone (Romanski & LeDoux, 1992). With lesion studies we demonstrate that this is only true as long as pure tones are used as CS:

When bilateral lesions of the entire AC were set after AFC to complex sounds, freezing levels during CS presentation decreased to baseline levels. This indicates that the AC is both involved in AFC to complex sounds in the intact brain and necessary for memory recall.

Moreover, when bilateral ablation of the AC was done in naïve animals and AFC to complex sounds was performed *afterwards*, no significant freezing was observed compared to baseline freezing. The learning deficit could not be mastered by an extended AFC protocol with several conditioning sessions.

Thus we conclude that the AC is necessary for AFC to complex sounds.

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Development of avoidance behavior: Role of the functional activity of the medial and lateral septum.

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Recently, we showed that young rats (P17-21) failed to learn an active avoidance task in the shuttle box. However, as adults, these animals showed improved learning in this task (higher number of avoidance reactions throughout all training days meaning a higher cumulative number of avoidance reactions; Schable et al., 2007, Neurobiol Learn Mem). Using the non-invasive 14C-fluoro-desoxyglcose autoradiography, we now investigated the functional activity of the medial (MS) and lateral (LS) septum in freely behaving animals during different ages and avoidance training stages. The MS which prominently projects to the hippocampus and entire neocortex should be preferentially activated during learning irrespective of the emotional burden. In contrast, the LS which receives strong hippocampal and cortical input but also has reciprocal connections with the amygdala, nucleus accumbens and a variety of lower brain regions was expected to show high metabolic activity preferentially or additionally depending on the stress the rats had to face during the training. In a first series of experiments, female Wistar rats (P21=young and P42=adolescent) were alloted to four different training groups: 1) acquisition (A): functional activity was analyzed during one series of 50 learning trials in the shuttle box; 2) novel environment (control A): activity was analyzed during a single exposure to the shuttle box for 40 min meaning pure exploration without presentation of CS and UCS; 3) retrieval (R): activity was analyzed during the fifth day of a series of five consecutive learning days (50 trials each day); 4) familiar environment (control R): activity was analyzed after five consecutive days of shuttle box exposure without CS and UCS. Surprisingly, we found no age difference in the metabolic activity at the level of of the septum. The functional activity of the MS was higher than that of the LS in all four groups. In the MS, the highest activity was found in the acquisition group with a significant difference compared to the familiarity group. In the LS, activity was significantly higher in the acquision group compared to all other groups, however, the retrieval group was least different from the acquisition group. Correlation of functional activity and behavioral parameters revealed a heterogeneous pattern. Taken together, the results reflect some of the initially described morpho-functional differences of the MS and LS but do not explain the behavioral differences in avoidance learning in young (P21) and adolescent rats (P42).

Smells like home: olfactory landmarks in desert ants *Cataglyphis*

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Cataglyphis fortis forages individually for dead arthropods that are killed by heat stress in the inhospitable saltpans of Tunisia. Both high surface temperatures and a non-patched food source distribution result in the absence of any trail-laying behaviour. Instead, the fundamental system of long-distance navigation is path integration which constantly informs an ant about its position relative to the nest. Additionally, the ants rely on visual landmarks as geocentric navigational aids in the vicinity of the nest. In the present study we found place-specific differences in the composition of environmental odours, i.e. potential landmarks that could be used by the ants as olfactory cues for navigation. We show that these vision-guided insects make use of this olfactory information for homing. We designed an experimental paradigm in which the ants had to associate a visually inconspicuous nest entrance with a given odour. We trained ants to forage within an open aluminium channel. The exit from the channel to the nest was marked by 5µl of a single odour dropped on the channel ground. When being tested in a second channel, ants focused their nest search at the position of the training odour. When being tested with an odour different from the training one, the ants did not respond to the odour. The ants did not only learn to associate the training odour with the nest, but were also able to discriminate this odour against a set of non-trained odours. Thus, *Cataglyphis* ants are able to memorize an environmental odour in the vicinity of the nest entrance and use this odour as a landmark in order to pinpoint the nest during homing.

Heat responses measured by BOLD fMRI to study initial processes of chronic pain

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The experience of acute pain is an elementary sensation necessary for maintaining individual integrity and well-being in interaction with the environment. However, repetitive noxious input can induce chronic pain states without any biological advantage. Long-term changes in the excitability of neurons have been shown at the peripheral and spinal cord levels. Maladaptive supraspinal reorganization is thought to play an important role in central sensitization, reconfiguring the central pain-processing matrix through learning, extinction, and memory processes. But it is a major challenge to investigate cerebral mechanisms and structures that contribute to sensitization of pain.
Traditional behavioural pain examinations in animals are highly stressful and subjective. Non invasive imaging approaches like fMRI in anesthetized animals would significantly reduce the stress for animals and simultaneously refine and improve objective measurements of chronic pain. Moreover, a model which would allow the investigation of chronic pain processes would open a new avenue in pain research.
Having established a model for acute heat pain in a rat model we now seek to identify brain areas that may be involved in initial processes of chronic pain using an experimental model of repetitive pain exposure in healthy rats which results in reliable and quantifiable BOLD responses in pain related brain areas.
fMRI data were acquired with mild noxious heat stimulation (max. 48 °C, 12 repetitions over 1h) every second day over 6 days. Highly specific activity of the pain pathway was found (e. g. thalamus, primary and secondary somatosensory cortex, cingulate cortex, insular cortex, frontal cortex and parietal cortex). The comparison of fMRI data of the first versus the last stimulation indicated increased activation in terms of increased stimulus coupling in cingulate cortex, entorhinal cortex and hippocampus. This finding nicely compares to a human study (Valet et al., 2006) suggesting that in these structures first processes of pain chronification take place. Interestingly, no significant increases for response amplitudes in these structures could be found. Structures reported to be involved in pronounced chronic pain like parietal cortex and structures of the medial prefrontal cortex (Baliki et al., 2006) also showed increased stimulus coupling between the first versus last session. These results could neither be found comparing the first versus earlier days nor during innocuous heat stimulation.

In conclusion, our computer controlled topical repetitive painful heat stimulation of the rat hind paw is a robust stimulation paradigm leading to reliable BOLD activation of sensory and especially pain related pathways. It is well suited for repetitive stimulations hence for investigations of chronic pain. The results can be quantified with respect to brain area, size, and intensity in relation to the temperature applied. Therefore, this non invasive animal pain model is highly objective and well qualified for studying chronification of pain responses.

Single-trial phase precession in the hippocampus

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During the crossing of a place field in the rat hippocampus, the firing phase of a place cell decreases with respect to the local theta rhythm. This phase precession is usually studied on the basis of trial averages, in which data from many place field traversals are pooled together. Here, we study properties of phase precession in single trials and compare them to the properties of trial-averaged phase precession. We find that single-trial and trial-averaged phase precession are different with respect to three fundamental properties: phase-position correlation, phase-time correlation, and phase range. Comparison with surrogate trials indicates that single trials are not randomly drawn samples from the trial-averages. Thus, an important source of variability of phase precession pooled over trials is the large trial-to-trial variability. Part of this trial-to-trial variability may be explained by running speed and firing rate differences across trials but the larger part of the variability remains to be explained.

Modulation of extracellular monoamine transmitter concentrations in the rat hippocampus after weak or strong tetanization of the perforant path

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Depending on the stimulation pattern, an electrical weak tetanization of the medial perforanth path results in a short-lasting (up to 4 h), protein-synthesis independent early hippocampal long-term potentiation (early-LTP) within the dentate gyrus, whereas a stronger tetanization leads to late-LTP (> 4 h), which is protein synthesis-dependent and requires heterosynaptic activation during its induction. During memory formation in the behaving animal these heterosynaptic events can be provided by afferents from cortical brain regions or subcortical nuclei. Little is known about the concentrations and temporal dynamics of neuromodulators required for LTP, although they are essential to induce the maintenance and thus late-LTP. In particular, noradrenaline is required for late-LTP in the dentate gyrus whereas dopamine for late-LTP in the apical CA1-dendrites. We now implemented the microdialysis method to study this topic in more detail after stimulating the perforant pathway, the main input to the dentate gyrus. A weak tetanus of the perforanth path, which normally leads to early-LTP, transiently but significantly decreases the concentration of noradrenaline and increases the concentration of serotonin und dopamine. In contrast a strong tetanus, normally resulting in late-LTP, increases significantly and long-lasting the concentration of noradrenaline and dopamine, and leads to a delayed short-lasting increase of serotonin. These preliminary data suggest that different stimuli result in different release patterns of neuromodulators into the hippocampus. Since the microdialysis probe is not yet optimized for the dentate gyrus of the rat, further studies are required to determine region-specific action of the neuromodulators in more details.

Estimation of homing distance in desert ants remains unaffected by severe disturbances of walking behaviour

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Desert ants, *Cataglyphis fortis*, use a stride integrator as distance gauge in their well-studied path integration system (Wittlinger et al. (2007) J Exp Biol 210: 198-207; while a skylight compass provides the direction gauge; e.g. Wehner R (2003) J Comp Physiol A 189:579–588). To further scrutinize the mechanisms of the ant odometer, we tried to disturb the stride integrator by interfering with normal walking behaviour.

First, a pair of legs that contributes to one of the two leg tripods alternately used in normal walking was selectively amputated. Removal of the left middle and right hind leg indeed prevented the normal tripod gait and should have interfered with both, the normal walking program controlled by the central nervous system, and normal sensory feedback from the legs.

Second, manipulation of the walking substrate in the form of regular corrugations was observed to interfere with normal walking behaviour, at least for corrugation wavelengths in the range of normal stride lengths (12 – 25 mm). The animals fell and stumbled (**figure**), or footfall patters were entrained to the corrugation wavelength. The relationship between stride length and stride frequency was altered in several experimental situations.

Surprisingly, distance estimation and homing performance remained virtually unaffected even by the most severe interferences with walking behaviour. This demonstrates a remarkable robustness of walking behaviour and homing, in agreement with anecdotal field observations (e.g. *Cataglyphis* finds the nest even after it has lost one or more legs in a predator attack).

There are two major, though not mutually exclusive, possibilities of how a stride integrator may work. First, stride length–stride frequency relationships in normal walking are relatively stable, such that stride length might be deduced from stride frequency without the need for sensory measurements. A copy of the central motor command for walking could thus be fed directly into a stride integrator. Second, sensory feedback from the legs monitors several aspects of leg movement and walking behaviour. It is well conceivable that an accurate measure of stride length and stride number may be derived from this sensory input. This would allow purely proprioceptive measurement of stride length.

In view of the above results, a purely central nervous implementation of a stride integrator is unlikely. Altered stride frequency-stride length relationships are the most important argument here. Rather, stride integration would appear to rely on robust signals from leg sense organs, for instance, load signals.

Figure. Cataglyphis *ants walking on substrates with 15mm (left) or 25mm (middle & right) corrugation period frequently fall (left, right) or stumble (middle).*

Appetitive and Aversive Reinforcement Integration and their Nature of Interaction During Auditory Learning.

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Conditioning involves the association of neutral stimuli with appetitive or aversive reinforcers. Animals direct their behaviors, in both natural and laboratory situations (e.g. instrumental conditioning experiments), in such a way as to obtain appetitive reinforcement ("rewards") and avoid aversive reinforcement ("punishments"). However, it is not clear how both types of reinforcement interact. The main question in this study was whether effective learning could be derived by combining both reinforcers and how they exert their effect on behavior. By reinforcing the identical behavioral response (tone-conditioned hurdle-crossing) in shuttle-box auditory learning, we studied the interaction of appetitive and aversive reinforcement using footshock as aversive reinforcer and electrical stimulation of the ventral tegmental area as appetitive reinforcer. Three experiments were conducted: (1) acquisition and extinction using separate and combined reinforcers, (2) omission of one reinforcer after a phase of training with the combination of both reinforcers, later followed by omission of the remaining reinforcer (extinction), (3) analogous omission experiments after partial reinforcement procedures. We found that the combination of both reinforcements potentiated speed of acquisition and asymptotic level of performance as compared to single type of reinforcement. While the aversive reinforcer appeared to be more effective for initial acquisition of the conditioned behavior, the appetitive reinforcer was more effective for maintaining the high levels of conditioned response rates. The extinction was slower in the group trained with combination of both types of reinforcements compared to the groups trained with just one type. In summary, our learning paradigm allowed us to study the appetitive reinforcement ("the carrot") and aversive reinforcement ("the stick") in the same training session allowing direct comparison of the motivational states elicited by both types of reinforcers.

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PROTEASOME ACTIVITY RESTRICTS LONG-TERM MEMORY FORMATION IN HONEYBEES (*APIS MELLIFERA*)

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Proteasomes are multi-protein complexes that are degrading proteins. Their target proteins are tagged with ubiquitin by a ubiquitin ligase before degradation, to be recognized by the proteasomes. The ubiquitin-proteasome system plays a crucial role in a number of neuronal processes including axon guidance, synaptic development, synaptic function and synaptic plasticity. Moreover, a role of the ubiqitin-proteasome in long-term memory formation has been demonstrated. But these results are contradictory. In the crab *Chasmagnathus* the ubiquitin-proteasome system is necessary for the formation of long-term memory, whereas activity of the ubiquitin-proteasome system in the amygdala of rats restricts formation of a long-term memory in a fear conditioning paradigm. In this study we examine the role of the ubiquitin-proteasome system in the honeybee (*Apis mellifera*), an invertebrate model system for learning and memory. We examined an appetitive pavlovian learning paradigm, the olfactory conditioning of the proboscis extension response (PER). We systemically injected two proteasome inhibitors at different time points before and after acquisition and test their effects on learning and memory formation. We found out that only a long-term memory is affected by the application of these inhibitors after acquisition, whereas acquisition itself and middle-term memory remain unaffected regardless when the inhibitors were applied. Our results support data from the rat showing that the ubiquitin-proteasome system restricts long-term memory formation. Thus it seems that the ubiquitin-proteasome system is important to prevent inadequate long-term memory formation.

Operant learning in larval Drosophila?

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In operant conditioning, an animal learns that its behaviour has a particular effect. Despite the obvious importance of such learning for the organization of behaviour, its underlying mechanisms are only beginning to be understood (e.g. Lorenzetti *et al.* 2008; Brembs & Pendl 2008).

We use the Drosophila larva as a model system (Gerber & Stocker 2007). We follow individual larvae crawling on an agarose-filled Petri dish and determine on-line whether the larva turns left, turns right, or shows forward locomotion. Operant training consists in systematically punishing the larva whenever it turns to a particular side (e.g. its "left"). After such treatment, a change in turning behaviour (e.g. the larva turns more often to the previously non-punished side) would indicate operant learning. Using high-intensity light flashes or mechanical disturbance of the larvae, we will report our preliminary experiments of such operant learning. This, we hope, may open the door to unravel the neurogenetics of operant learning in this relatively simple animal.

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How to Resume an Approach after a Detour - A Spatial Orientation Memory in *Drosophila melanogaster*

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Walking *Drosophila melanogaster* flies are frequently approaching visual targets. In our test they are lured out of their path by presenting a distracter target lateral to their original goal, while at the same time this goal disappears. Wild-type flies are able to resume the approach of their initial target, in case the distracter disappears as well so that none of the targets is visible any more (Neuser et al. 2008 Nature). The underlying spatial orientation memory is goal-driven, stable over prolonged distraction times of up to 4s and the percentage of positive choices does not change during the ten consecutive trials per fly. Here we present further analyses of the spatial orientation memory basically concentrating on three questions.
Firstly, which neurons are necessary and/or sufficient for the memory? We used the GAL4/UAS (Brand and Perrimon, 1993 Development) system to silence specific subsets of neurons by expressing the neurotoxin Tetanus. Time of toxin expression has been controlled via the temperature sensitive GAL80 construct (McGuire et.al. 2003 Science). We found that subsets of the ellipsoid body ring neurons are necessary for a functional spatial orientation memory. Studies on other neuronal subsets are on the way.
Secondly, which proteins and cascades are involved in the memory? We found that the protein kinase S6KII (*ignorant*; Putz et.al. 2004 J. Neuroscience) is required, and its expression in R2 and R4 ring neurons in the ignorant mutant background sufficient to display the memory. The S6KII kinase belongs to the ribosomal serine kinase (RSK) family, which interacts with mitogen-activated protein (MAP) kinase signalling in *Drosophila*. Therefore, we investigated flies mutant for other proteins involved in the MAP kinase pathway. Moreover, we rescued ignorant protein levels in other subsets of neurons to unravel putative parallel pathways and we studied other known proteins involved in learning and memory cascades. We are currently looking into the neurotransmitters involved.
Thirdly, what strategy are the flies using to actually solve the orientation task? In principle, orientation can be either idiothetic, that is the position information is based only on information about the own movements, or allothetic, that is orientation is based on external reference systems. To address this question, we modified the detour paradigm. At the end of the presentation of the distracter the LEDs in the test arena turn all off instead of all bright by that excluding any possible visual information. Flies are being automatically tracked using dark red light that they cannot see. Regardless of this change, wild-type flies can nevertheless perform the task with the same high rate of positive choices for the previous target as in the original setup with ambient light, suggesting the orientation to be idiothetic.

Interaction of long-term plasticity and structural plasticiy for cortical map formation

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Learning of cognitive and motor tasks is well known to induce cortical remapping. Trained tasks enlarge their cortical representations at the expense of less used representations (Kleim et al., 2004). The mechanisms that contribute to lasting changes in cortical connectivity are not fully understood. Long-term plasticity in terms of LTP and LTD certainly plays a role in cortical map formation and remapping. However, it is not clear 1) what makes cortical representations persistent not changing with every input. Moreover, learning of a novel pattern by LTP goes along with increasing neuronal activities in the potentiated networks. 2) How does the brain find a connectivity which represents the memory content equally well but is energetically more efficient? We propose that structural plasticity is suitable to solve both problems in sofar as it 1) leads to stable clusters of connected neurons and 2) contributes to network homeostasis because neurons regulate their synaptic input and output spectrum in an activity-dependent manner. To show this, we implement our algorithm for activity-dependent structural plasticity (Butz & Wörgötter, 2009) onto a Kohonen-network (Kohonen, 1981). The main novel idea of this approach is to replace a predefined kernel of a Kohonen-network by a developing connectivity arising from our algorithm for structural plasticity. Each neuron expresses axonal and dendritic elements that can merge to form synapses. Structurally connected neurons will develop similar input specifities by means of long-term plasticity. Axons and dendrites grow on slower time scales than fluctuations in neuronal activities so that only lasting alterations in neuronal activities leads to changes in structural connectivity. Summarizing our modelling results, we postulate that learning first of all leads to a strengthening of exisiting synapses and a rising in neuronal activities in the trained representations. At a later stage, axonal growth and synaptogenesis establish an increasing structural connectivity into wider cortical areas that are recruited for a particular representation by long-term plasticity, subsequently. LTD and synaptic pruning rebalance network activities. As an emergent effect, synaptic pruning constitutes sharp reprentational borders in a competitive process. Our modelling data is in line with experimental data showing that synaptogenesis accompanies late but not early phases of LTP (Kleim et al., 2004).

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Glomerular plasticity related to olfactory long-term memory in the antennal lobe of honeybees

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The storage of stable memories is generally considered to rely on changes in the functional properties and/or the synaptic connectivity of neural networks. However these changes are not easily tractable given the complexity of most brain circuits, thus little is known about how memory traces of different stimuli are stored in the same neural structure. Such a search can be facilitated by studying memories involving neural networks with a modular and relatively simple organization. We have therefore focused on associative memories of individual odors and the possible related changes in the honeybee primary olfactory center, the antennal lobe. As this brain structure, like its vertebrate counterpart the olfactory bulb, is organized in well-identified units called glomeruli, we looked for evidence of structural and functional plasticity in these units in relation with the bees' ability to store stable memories of specific odors associated with food. Restrained bees were trained to form an odor-specific long-term memory in an appetitive Pavlovian conditioning protocol. The stability and specificity of this memory was tested behaviorally three days after conditioning. At that time, we studied possible changes in the structure and function of glomeruli within these individuals, compared with controls which had not formed any memory. We thus measured i) glomerular volume and ii) odor-induced activity, using *in vivo* calcium imaging, of a subset of 17 glomeruli easily identified across individuals. This experiment was carried out with two different odors, in order to relate possible specific changes to the glomeruli coding these odors in the antennal lobe. We show that long-term olfactory memory for a given odor is associated with volume increases in a defined subset of glomeruli which depends on the learned odor. The subset of plastic glomeruli differed from the pattern of glomeruli activated during conditioning. Besides, long-term memory formation did not modify glomerular odor-induced activity. Taken together, these results point towards a class of synapses made by a specific population of neurons in the network (the local interneurons), as the primary sites of memory-related plasticity. Our experimental data, in accordance with previous computational models, thus indicate that the olfactory network undergoes local plastic changes as memories of specific odor-food associations are formed, and that such changes can last over days, thus potentially serving as memory traces allowing long-term retention of previous experience.

Associative Learning Modulates the Total Amount of AmCREB in the Honeybee

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The transcription factor CREB (cAMP response element binding protein) plays an essential role in the formation of long term memory (LTM). CREB is required for the induction of a consolidated LTM by inducing gene expression after getting phosphorylated (pCREB) by several kinases. A huge body of work analyzed this CREB phosphorylation after learning but little is known about learning induced changes of it's total amount. To investigate this topic we are using the honeybee (Apis mellifera), a well known invertbrate model system for learning and memory.
We aim towards analyzing the role of *Apis melifera* CREB (AmCREB) in the formation of LTM in the honeybee. The AmCREB gene has been thourouly analyzed and several splice variants have been characterized. An antibody against vertebrate CREB detects a 33kDa AmCREB splice variant. The amount of this variant differs between forward and backward conditioned animals in the dorsal part of the honeybee brain 3h, but not 1h, 6h or 24h after acquisition. This suggests that the amount of CREB depends on the sequence of stimuli presented during acquisition.

Effects of hippocampus-dependent passive avoidance learning on freezing behaviour and NCAM180 expression in the domestic fowl (*Gallus gallus domesticus*)

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Domestic chickens are an established model organism for studies on learning and memory. Here, we tested in adult domestic chickens (1) whether the hippocampus is involved in a one trial passive avoidance learning task, and (2) the relationship between the learning performance in this task, the gene expression of NCAM180 in the hippocampus, and freezing behaviour. As learning task we used a step down avoidance test (SDA) which is frequently used in rodents.
In part 1 a total of 76 subjects was used and received an intra-hippocampal injection of D-AP5 before training in order to inhibit or not. We hypothesised that subjects of group A (D-AP5, foot shock) will not learn the SDA. The sham injected subjects in group B (sham injection with ACF, foot shock) and C (sham injection, no foot shock) served as a control for effects of anesthesia, surgery and injection procedure. Subjects of group D (no injection, foot shock) were expected to learn the SDA task, and subjects of group E (no injection, no foot shock) should not learn the SDA. Our results revealed that subjects treated with D-AP5 showed an impaired learning performance compared to sham-injected control subjects (see Krause et al., 2008 – Acta Neurobiol Exp 68).
In part 2 we compared the gene expression of NCAM180 in the hippocampus of good and poor learners of the SDA. Expression of NCAM180 on RNA level was verified by real time PCR. Subjects that successfully had learned (N_1=12) the SDA had a significantly higher gene expression of NCAM180 in the hippocampus compared to non-learners (N_2=11; $p<0.001$). In addition, learners showed significantly more freezing behaviour in the retest than non-learners.
Our results give strong evidence that the hippocampus is involved in passive avoidance learning (SDA), and that NCAM180 gene expression is affected by this learning task in adult domestic chickens.

Place learning vs. route learning in rodents – an operational approach

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Foraging animals are capable to remember the locations at which they have previously received food reward. This condition is used extensively for behavioral analysis of learning and has lead to a great variety of spatial tasks for animals. However, the question, how locations and routes are represented in an animals brain, still remains. Here we present a novel experimental paradigm using an elevated maze setup with automatic feeders that allows comparing place learning and route learning in rats. In the place learning condition, rats are trained to distinguish two places according to the amount of food reward they obtain at these places. In the route learning condition, rats can reach a single rewarded place along two different routes. Food reward depends on which route they take to get to the feeder. This paradigm allows answering the question, whether the learning rates in a place learning task and a route learning task are different. We hypothesize that the mental representations of places and routes are different resulting in different learning rates when route complexity is constant in both tasks.

Self-Organizing Control in Autonomous Robots and Intelligent Prostheses

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We consider a biologically inspired algorithm for adaptive control of an autonomous robotic agent in a complex environment which is intended as a model for the generation of play behavior, self-exploration and low-level learning in higher animals. The algorithm aims at a compromise between two antagonistic objectives, namely sensitivity to environmentally mediated feedback and predictability of future sensory inputs. Both the controller and the internal predictive model are realized by neural networks. The learning dynamics of the system causes an on-going modification of the network weights which results in an active and flexible interaction of the robot with its environment. By learning to connect actions and their perceptual effects the sensorimotor loop is established, which, as an additional benefit, turns out to generate sensorimotor coordination between different degrees of freedom via the environment. The emerging behaviors are well-adapted to both the robotic hardware and the environment and may be further shaped by intrinsically available rewards such as measures of the learning progress, contextual sensory information or by externally specified constraints. The algorithm is applied to a number of life-like simulated robots and to the control of a myoelectric hand prosthesis with the goal of an automated, user-specific interaction between patient and prosthetic device.

Electrical Stimulation of Lateral Habenula vs. Ventral Tegmental Area Produces Opposite Effects on Avoidance Learning

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The interaction between lateral habenula and dopaminergic neurons may play a role in opponent processes such as aversion and reward. Previous studies found that aversive stimuli or electrical stimulation of the lateral habenula inhibited dopaminergic neurons. Recently, in reward-dependent learning, it was found that lateral habenula neurons teach information about reward loss to dopaminergic neurons. We hypothesized that artificial activation of the lateral habenula coincident with avoidance responding would suppress a dopamine signal associated with successful avoidance and thereby impair avoidance learning. In a two-way active-avoidance task, we delivered electrical stimulation to either the lateral habenula or the ventral tegmental area (VTA) at the moment of negative reinforcement, when the avoidance response results in the omission of an expected aversive stimulus. Under this contingency, VTA stimulation enhanced avoidance learning while habenula stimulation impaired it. Subsequent omission of VTA stimulation caused animals to regress from brisk performance to normal performance while omission of habenula stimulation had no effect. Also, habenula stimulation after successful learning had no effect, indicating a specific impairment of learning and not the ability or motivation to perform the avoidance response. Overall, the results obtained from VTA and habenula stimulation during avoidance learning are consistent with their opponent roles in learning.

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Complex Associations in Pigeons: The Ability to Combine Two Visual Dimensions

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Most research on the avian brain is related to the visual system, its asymmetrical organization and its high visual skills. Birds are able to accomplish various complex cognitive processes within the visual domain like categorization, transitive inference and object permanence. Recent studies indicate that pigeons not only possess the ability to discriminate visual stimuli on the basis of one feature but are also able to differentiate complex stimuli on the basis of more than one dimension. However, it is less clear in how far pigeons are able to combine different stimulus dimensions.

The aim of the present study was to explore if pigeons are capable of integrating multiple dimensions of artificial stimuli to find a combination of positive visual features. The combination of multiple dimensions involves several processes which exceed simple perceptive skills. To solve these tasks, animals have to discriminate, compare and recognize relevant features and set them in relation to each other. Therefore, a paradigm was created which enables to investigate combinatorial skills of pigeons.

Pigeons were trained in a forced choice paradigm to discriminate pairs of visual stimuli that differed either in colour or form. Each pair consisted of one rewarded and one non rewarded stimulus (Figure 1a and b). After reaching learning criterion, the experimental design was adopted to more challenging conditions by presenting four new stimuli. The form and colour stimuli were combined with each other to generate four two-dimensional stimuli. Two of them were composed of the rewarded form and the non rewarded colour and vice versa. One stimulus consisted of a combination of the rewarded features in both discrimination conditions and the last one just out of the non rewarded features (Figure 1c). Hence there was only one combination with all correct features learned in the discrimination task, two that contained one correct feature and one that only combined incorrect features. These four stimuli were simultaneously presented on four pecking keys and the pigeons had to decide for one of them. Pigeons decided significantly more often for the stimulus which integrated all rewarded features. These data suggest that the animals had learned to combine different dimensions of previously learnt stimuli. This result confirms the ability of pigeons to learn complex associations and demonstrates an interesting and convenience skill via the combination paradigm.

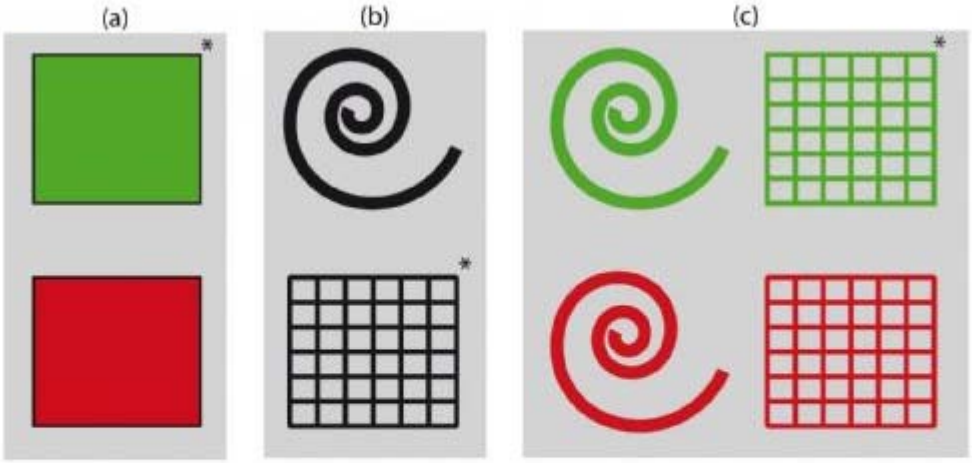

Figure 1: In a forced choice task, pigeons were trained to discriminate colours (a) or figures (b) and then they were tested in finding the combination of the correct form and colour(c). Stars represent correct stimuli.

fMRI of a Macaque Monkey Performing an Object Working Memory Task

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The active maintenance and manipulation of visual information after its disappearance for a short time period is an important features of working memory (WM). This ability has been extensively studied in humans and non-human primates with delayed-match-to-sample (DMS) tasks. To complete such a task successfully an initially presented sample needs to be held in memory and be compared with a subsequently shown probe in order to detect a matching item. Accordingly, the different periods of a DMS task (i.e. encoding, maintenance, and match) demand different information processing. This predicts a dynamic change of the underlying activity in the network of involved brain regions. To investigate the corresponding patterns of brain regions recruited during a DMS task we used event related primate fMRI.
Macaque monkeys were trained to perform a DMS task inside a 3T Siemens Allegra scanner. Following an initial fixation period of 1-2.5 s a sample shape was presented for 0.8 s in the lower left or right hemi field. After a variable delay period (1.5-13.0 s) another shape was shown. The monkey had to indicate a matching shape by releasing a bar, or continue holding the bar if it did not match. After a correct response the monkey had to maintain fixation for another 0.5-6.5 s in order to receive a reward. Trials were separated by 2-8 s. Dimming trials, where the monkey had to respond to a slight contrast change of the fixation spot, were interspersed as a baseline condition. Functional whole brain images were acquired with a BOLD weighted EPI sequence (TR 1.83 s, TE 25 ms) and analyzed with FSL.
We identified brain regions involved in the DMS task using GLM analysis. Prominent foci of activity were found in visual (V1, V2, V4, TEO, FST, LIP, TEa), and prefrontal areas (A10, A12, A45, vlPFC, anterior cingulate cortex). In addition, we detected reliable activation in subcortical structures such as the caudate nucleus, Putamen and thalamic nuclei including pulvinar and Medial dorsal nucleus. A comparison of activity maps for matching and non-matching shapes showed increased activity in vlPFC, A10, A30, LIP, caudate nucleus and the thalamic nuclei during the matching condition, whereas higher activity in the posterior cingulate cortex and A12 occurred during the non-match.
Extensive single subject trial averaging of the BOLD signal for anatomically defined regions of interest (ROI) allowed for estimation of response profiles for the task. Already for short delay periods (> 3.8 s) distinct peaks for the sample and the probe occurred in ROIs for prefrontal areas (A45, vlPFC) as well as for visual areas (V4, TEO, LIP). Especially prefrontal regions (A45, vlPFC) showed increased activity during the delay period followed by a response peak for the matching or non-matching probe that is higher than for the sample. This response profile is well in line with existing monkey electrophysiological and human fMRI data, demonstrating the capability of primate fMRI to link results obtained from both species.

The ratio of reinforced and non-reinforced conditioned stimuli influences odour responses in honeybees (*Apis mellifera*)

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Bees have an exquisite olfactory memory. Using differential conditioning, they will quickly learn which odour is associated with an appetitive reward, and which odour is not rewarded. In a natural environment, this behavior corresponds to a foraging situation: hovering over a flower-rich meadow, bees quickly find the rewarding flowers using the flowers' visual and olfactory displays. No two flowers are exactly alike, and therefore bees also generalize to somewhat similar flowers. In addition, bees will occasionally sample new flowers, allowing the hive to move to better crops when they start blossoming. When a bee comes to a new flower, therefore, it has to decide whether to explore it or not to explore it. Exploration will occur either because the flower's display is within the generalization boundaries of the bee's perceptual system, or because the bee is appetent for new flowers. Here, we analyze whether bees` choices to explore or not to explore a new flower depends on the ratio of rewarded and non-rewarded flowers encountered by a bee during previous foraging. We transported this question into a controlled laboratory environment using harnessed bees.

We differentially trained fixed bees to the odours 1-hexanol or 2-octanol as conditioned stimuli (CS). One of the odours (CS+) was paired with a sucrose reward (which elicits the proboscis extension reflex, PER) while the other odour (CS-) was presented without reward (and does not elicit any behavioural response after training). We tested three groups which differed in how often CS+ and CS- were given during training. The CS+/CS- ratios where: 75/25, 50/50 and 25/75. Great care was taken to keep the quantity of the total food intake equal across groups, in order to avoid differences in satiation. After 30 minutes and again after 24 hours each group of bees was tested with the two training odours in order to quantify learning performance. We also assessed their choice behavior towards a third, unknown odour. This was either 2-nonanol, or a binary mixture of 1-hexanol and 2-octanol. By recording odour-evoked calcium activity patterns in the antennal lobes of naive bees, we determined that the binary mixture was similar and somewhat intermediate to both training odours, while 2-nonanol was dissimilar to both training odours, with a higher dissimilarity towards 1-hexanol. We found that the bees' probability to respond to a new odour with a proboscis extension reflex depended on the CS+/CS- ratio during training: Bees of the 25/75 group responded significantly more to the new test odour than bees of the 75/25 or 50/50 groups. That increased response probability was observed for both test odours, 1-nonanol and the mixture, in the 30 minute test. In the 24 hours-test, however, there was no difference in the response rate to the new odours across the CS+/CS- ratio-groups.

We conclude that the generalization landscape of an odour is affected by the previous exposure and reward history of that odour. In particular, an odour that is presented more often will lead to a more restricted neural template, and therefore generalization to other odours is reduced. Alternatively – or additionally - it is also possible that the CS+/CS- ratio alters the behavioural state of bees, in that bees that are exposed to fewer rewards are more apt to perform explorative behavior. Interestingly, either one or both effects are short-term and no longer visible after 24h.

Odour avoidance after conditioning of the sting extension response in honeybees

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In Pavlovian conditioning, an originally neutral sensory stimulus (conditioned stimulus – CS) gains control over an animal's reflex response after its association with an unconditioned stimulus (US). In such a situation, the subject is not freely making decisions but exhibits a reflex response, so that whether the CS thereby acquires a positive or a negative value for the animal is difficult to assess. In honeybees *Apis mellifera*, an odour CS can be associated to a sucrose solution US in the appetitive conditioning of the proboscis extension response (PER) or to an electric shock US in the conditioning of the sting extension response (SER). To evaluate the positive or negative value acquired by the CS in both types of conditioning, we have compared the orientation behaviour of freely-walking honeybees in an olfactory-cued Y-maze after bees had either learned an odour-sucrose association in a PER paradigm, or an odour-electric shock association in a SER paradigm. We show that the same odours can acquire either a positive value when associated to sucrose or a negative value when associated to an electric shock, as bees respectively approach or avoid the CS in the Y maze. Importantly, these results establish for the first time the true aversive nature of SER conditioning in honeybees.

Correlations and Synchrony in Threshold Neuron Models

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Neurons in the CNS exhibit temporally correlated activity that can reflect specific features of sensory stimuli. In order to understand the origin, temporal properties and strength of interneuronal spike correlations it is essential to analyse how neurons subject to correlated synaptic inputs coordinate output spiking activity.

We used a simple statistical framework for the analysis of spike correlations between neurons driven by correlated inputs [1] to examine the quantitative determinants of the synchronization acuity of a pair of neurons subject to a variable percentage of common fluctuating input of different correlation times. First, we calculated the autoconditional firing rate of an individual neuron and analyzed its short and long time asymptotics. For large time lags, we find a substantial influence of the second derivative of the voltage correlation function. In the limit of small times, we find an algebraic rise out of period of intrinsic silence after each spike. We then evaluated the cross conditional firing rate of a pair of neurons for low and high common input fraction and with firing rate heterogeneity. In the low correlation regime, we identified a rate dependence of the rate of synchronous firing corroborating previous observations [2] and predict that spike correlations in this regime reflect detailed properties of input correlations and are typically temporally more precise than input correlations. In the high correlation regime, however, the synchronous rate ceases to depend on the stationary firing rate of individual neurons and the structure of spike correlations is governed by the input correlation time and the coupling strength but is insensitive to firing rate and the detailed form of input correlations. For all strengths of correlations the model predicts the appearance of a systematic delay of firing of the lower rate neuron relative to the higher rate neuron. We tested these theoretical predictions in in vitro experiments in slices of rat visual cortex and injected in pyramidal neurons fluctuating currents with a varying degree of common input. Cross and autoconditional firing rates computed from these recordings, confirmed all basic theoretical predictions of our formalism. [1] S. Treue, Soc. Neur. Abst. 2007 [2] Rocha J. et al., Nature, 448:802-806, 2007

Sensory Space Representations based on Motor Capabilities

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Recent studies demonstrate that early sensory representations optimize statistical properties over the set of natural stimuli (Olshausen & Field, 1996). Such a type of coding makes no direct reference to the behavioural repertoire of the agent and, hence, it is unclear whether the supposedly optimal representation is suited to generate behaviour. Here we present an optimization principle directly referring to the predictability of sensory state transitions resulting from motor actions and investigate its relation to a purely sensory coding scheme optimizing the temporal stability of representations (Wyss et al., 2006). Wyss et al. found that optimizing a stability objective leads to sensory representations that resemble hippocampal place-fields (see also Franzius et al. (2007)).

We use a number of simulated agents with varying motor capabilities in different two-dimensional mazes. Our optimization algorithm continuously adapts the agent's sensory space representation to increase the predictability of transition between sensory states caused by an action as well as to decrease representational redundancy. This process is guided by rule-based heuristics instead of the gradient of the objective function. After optimization, we compare the resulting sensory representation to the stability-optimized representations reported by Wyss et al (2006).

We found that the proposed algorithm successfully creates sensory space representations with high predictability values. Optimized states correspond to spatially compact regions of the agent's 2D maze environment (see figure). These regions resemble the place-fields found in the hippocampus. The emergence of this place-field like structure throughout the optimization process is illustrated in the figure below. Here, the sequence from a) to d) indicates progression of the optimization process. Maze positions assigned to the same representational state bear the same color.

The compactness of optimized states is hardly influenced by a change in motor parameters. In contrast, the size of optimized states is strongly dependent on motor parameters (data not shown). The spatial distribution of states depends on motor parameters as well as on maze type.

We compared the place-fields obtained from the optimization of predictability to those obtained from the optimization of temporal stability (Wyss et al, 2006). The former possessed a mean stability of 0.902 and a mean predictability of 0.584. The latter had a mean stability of 0.899 and a mean predictability of 0.216. Here, a temporal stability value close to zero corresponds to the stability of a randomly scrambled place-field distribution, a value of one denotes perfect stability. Zero and one predictability correspond to complete uncertainty and complete certainty of state transition, respectively. We see that both optimization procedures produce representations of similar temporal stability. In contrast, stability-optimized representations possess markedly less predictable state transitions.

Thus, we conclude that predictability is a more general coding principle than temporal stability. Our results also suggest that the motor apparatus could play a profound role in the formation of hippocampal place-fields in the actively behaving animal.

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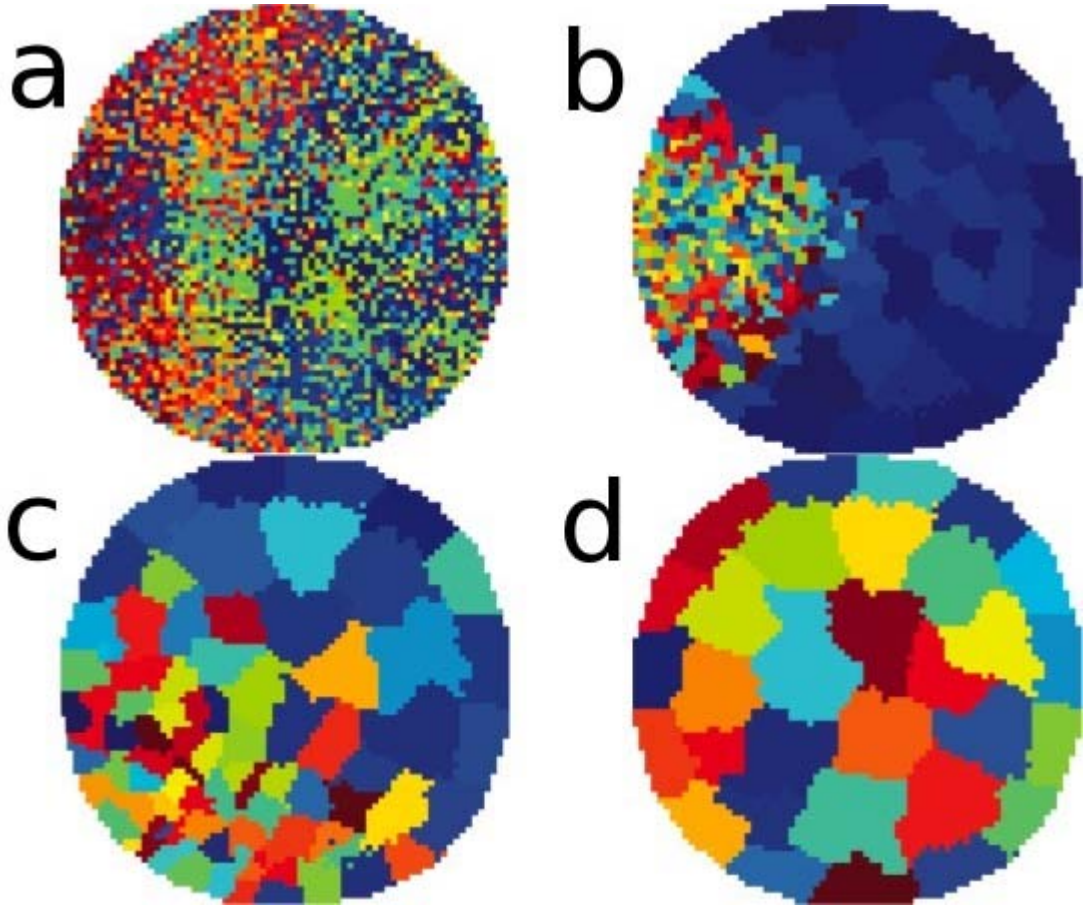
a
b
c
d

A model of the neuronal circuit of a Figure Detection cell in the visual system of the fly

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Detecting objects is an important task when moving through a natural environment. Flies, for example, may land on salient objects or may avoid collisions with them. The neuronal ensemble of Figure Detection cells (FD-cells) in the visual system of the fly is likely to be involved in controlling these behaviours, as these cells are more sensitive to objects than to extended background structures.
Until now the computations in the presynaptic neuronal network of FD-cells is not entirely understood. The FD1-cell, one member of the FD-cell ensemble, is inhibited by another cell from the same optic lobe, the so called vCH-cell [1] which receives input via electrical synapses from HS cells. Previous model simulations suggest that this inhibition is performed in a spatially distributed way and indirectly via the retinotopic input elements of the FD1-cell [2].
The FD1-cell model used in these simulations focussed on the interaction between the vCH-cell and the FD1-cell and was tested with experimenter designed stimuli. Currently the model is being elaborated stepwise by also taking the input of the vCH into account: its ipsilateral motion sensitivity is, amongst others, mediated by the HSE cell via dendro-dendritic electrical synapses; moreover, it receives motion input from the contralateral eye through, amongst others, the H1 and H2 cell. The model performance is assessed with naturalistic visual stimuli (see figure, only the mentioned input elements of the vCH-cell are shown).

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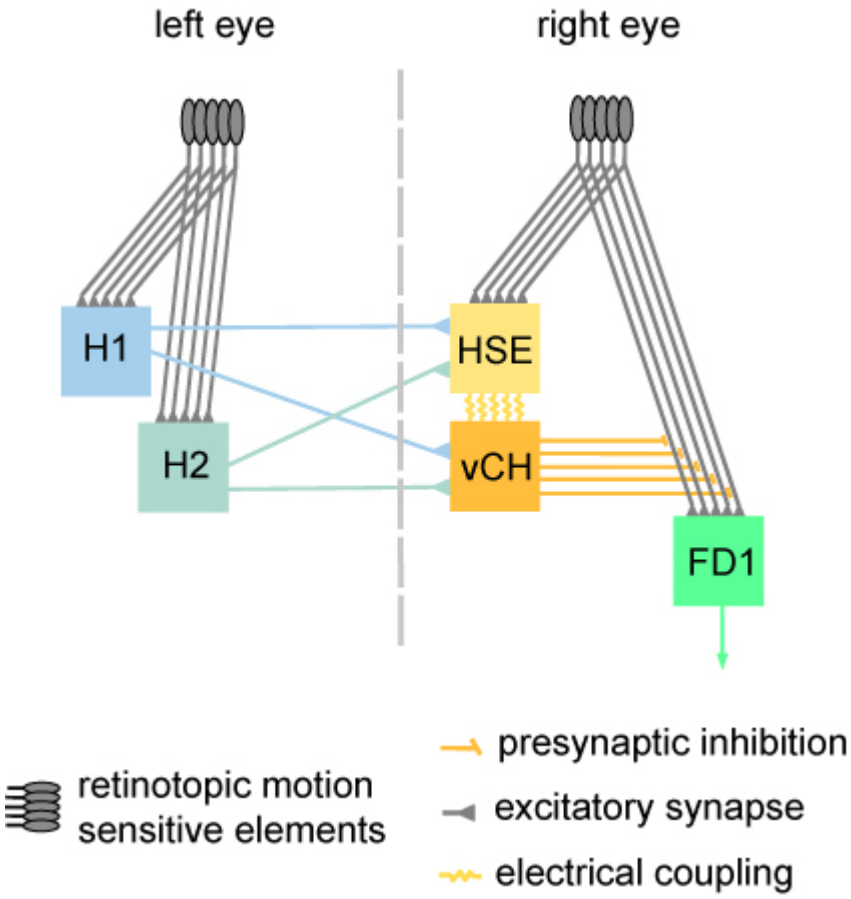
left eye
right eye
H1
H2
HSE
vCH
FD1
retinotopic motion
sensitive elements
presynaptic inhibition
excitatory synapse
electrical coupling

Connectivities and structures of the rat central nervous system

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Generating atlases of brains are done in many projects. Most use single ***modalities*** (structural/functional MRI/DTI or histological structures, connectivities, terminologies, ontologies, chemoarchitectures) and a specific ***type*** of a brain (human, rat, mouse, macaque brains). These atlases are restricted to certain terminologies (e.g. BAMS), however, brain terminologies develop very quickly and are limited in most cases to the brain and do not cover the CNS and PNS (***levels***) to map whole nervous systems of different species. Furthermore many structural and functional mapping problems are addressed to comparsions of genetically modified animals, changes in development and experimental conditions (***states***) which are not considered in atlas projects.

Therefore, we developed a system that can cope with different nervous systems (***types***), ***modalities***, ***states*** and ***levels*** with regard to 2D and 3D multimodality structural and connectitional information. Here, we focus on the rat central nervous system based on a complete series of isotropic (5 µm³) histological sections that were affine and elastically registered.

Additional, the connectivity of the amygdaloid complex has been integrated into the system and analysed by standard graph analysis procedures (adjacency, distance matrix, joint distribution, simple complexity measures).

Because lateralization should be analyzed also, 4160 structures of the right and the left central nervous systems of the rat have been organized in a hierarchical hypertree that enables us to manage concurrent terminologies (Paxinos and Swanson atlas terminologies) and fit in new functional and structural terminologies in emerging publications. 12600 nodes of the hypertree are linked, meaning that 12600 structures have directed connections. These connections are characterized by strength and density information obtained from 400 publications.

Visualization of three dimensional structures was realized by integrating the VTK package into our Java based mapping system whereby different import and export interfaces were realized to use many graph formats in this open system and to visualize with optimized graph visualization tools complex neural network connectivity (CGV,Cytoscape,Protege,SVG).

In this contribution we will provide an overview of the capabilities of the mapping managing system ***BrainSuite*** and present first results of connectivity analysis of the amygdaloid complex.

Huge Neural Nets, Biomorph, based on Engrams and Fractal Connectivity

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Introduction: Problems remain to explain the overwhelming ability of brains to learn, memorize and process a plenitude of patterns. In this study a huge neural net will be presented, which uses engrams as base of data-processing. The net consists of neurons, which belong to neural chains of 8 neurons. Each neuron will activate its subsequent neuron. Thus, in the neural chain will spread a sequence of activation, specific for the starting pattern.

The last neuron of the neural chain will produce engrams by recording the sequence of degrees of activity, in the computer model in form of values, in the hypothetical biological model in form of a molecular chain, for instance ribonucleic acids, oligonucleotides, each base triplet encoding a different degree of neural activity.
These molecular chains-memory-strings- could thus be used to store, compare, and, by retrograde activation of the net, to reconstruct a pattern within each neural chain(1).

New patterns will cause each storage cell to produce an according memory-string. This will be compared (in the computer model by regression) to the memory-strings, learned earlier. The memory-string with the most homologous sequence will be chosen to activate the according neural chain in reversed direction. The rate of activation of the neurons, belonging to each neural column will determine the degree of the resulting activity of the neural column.

Several neurons belong to one neural column. After each cycle of pattern-processing, the neural columns will decide, depending of the rate of activated neurons, to which degree they will be active at the beginning of the next cycle. Thus, the neural chains will influence others by their reaction.

The connectivity between the neurons could be determined by randomization, but a very interesting and more promising way is to use fractal algorithms to decide, which subsequent neuron should be activated by any neuron. The result are biomorph and fractal spatial structures with some morphological and functional features of biological nervous systems.

Methods: The net consists of 459 257 neural columns, which contain 3 674 056 neurons, which will form 459 257 neural chains, each with 8 neurons. Each pattern, which will be learned will be stored in 459 257 (one for each neural chain)different memory-strings, the engrams.
The connectivity between the neurons will be determined by a mapping algorithm. The coordinates of each neurons(x,y,z) will be interpreted as a triple of numbers which could be squared, by interpreting them as quaternions(x,y,z,w-with w set to be zero)(Described in detail in (2)). By this, we get a conformal mapping of each neuron to another neuron of the net, resulting in functional neural chains, which will finally form the neural net.

Three patterns of activation are learned by the net. Then, an incomplete pattern will be presented to the net, the reaction of the net will be recorded.

Results: The presented net is able to learn, store, compare and reconstruct patterns quite satisfyingly. It will perform all tasks, which may be done by neural nets, using changes of synaptical weights to store patterns.
Despite the great number of involved neurons, the net is quite fast, each pattern will be learned by one activation cycle. As result of the certain fractal connectivity, the net will present sructural and functional

aspects, which we may find in biological structures(rich topographic(“somatotopic”) connectionism, gyri and sulci, hemispheres, lobes).
The results of this first little study confirm former studies, demonstrating very conclusively, that even very large neural nets with remarkable biomorphism, using engrams as base of pattern-processing, will be able to perform all tasks of storing, comparing and reproducing patterns very effectively and that they should be worth to be further studied.

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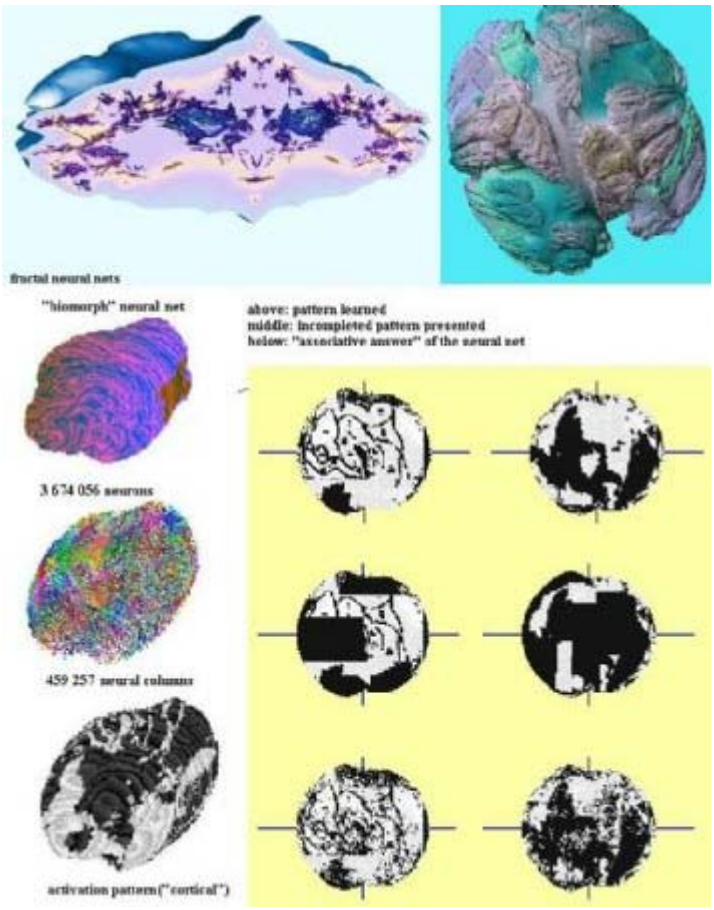

Compensating for Temporal Variation in Event-Related Potential Analysis

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We propose an averaging method for segments of electrophysiological data (Event-Related Potentials, ERPs) that has been designed to compensate for processing speed variability across trials in psychophysical experiments. The algorithm is based on the estimation of single-trial ERPs by wavelet-denoising combined with a parameterized dynamic time-warping algorithm to guide selective averaging. The averaging scheme is hierarchical based on dendrograms generated by agglomerative cluster-analysis.

A cross-validation approach is taken in order to estimate optimal parameter settings for the restricted time-warping procedure. As the main parameter goes to zero, our method converges against the method in [1] or a pointwise average, depending on the choice of external time-markers. This allows for a direct comparison of the methods in terms of the estimated prediction error. We define the average temporal distortion as the measure obtained by averaging warping functions computed on all pairs of trials independently. We find that electrodes are well divisible into distinct clusters of physically neighbouring electrodes that correspond to brain regions (frontal, occipital; lateralization) by applying cluster analysis with this measure (see Figure). This indicates that the process of activating neural assemblies and pathways might be well captured in the timing information thus obtained.

We apply our methods to artificial data and data obtained in a psychophysical experiment. We contrast the results with those obtained by using other relevant averaging techniques and show that our method leads to clear quantitative improvements, which are due to the following facts: (i) the questionable assumption of an identical ERP signal in different realizations is dropped and different processing speeds are considered explicitly, (ii) the choice of the warping function is estimated from the data and does not require an a-priori specification as in [1] and (iii) External information about the temporal alignment of the processing steps is incorporated by using arbitrarily many time-markers of externally observable events (such as saccades and response-markers).

A software library written in the C programming language (with MatLab-support for some of the functions) that implements the described algorithms is available from http://www.bccn-goettingen.de/projects/libeegtools.

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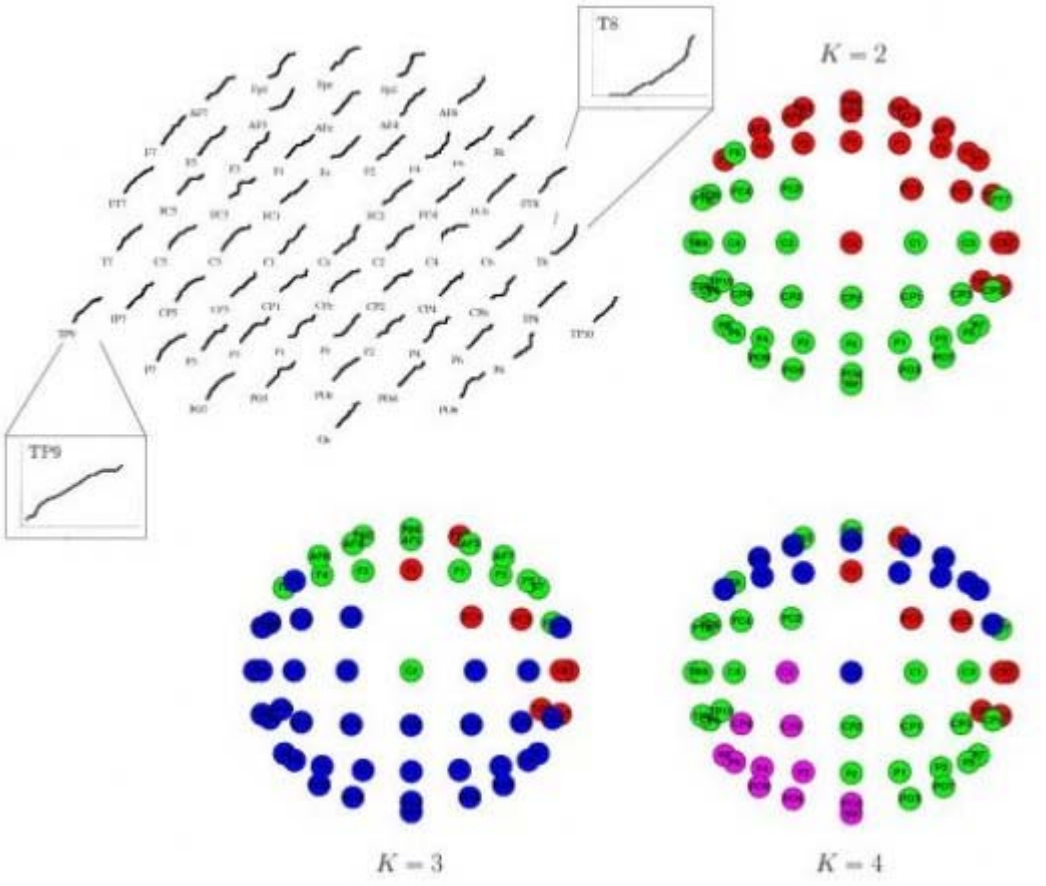
T8
TP9
$K = 2$
$K = 3$
$K = 4$

Learning as a Cause for Aging Impairments

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The performance in psychological tests of fluid intelligence such as Raven's Advanced Progressive Matrices, tends to decrease with age [1]. These results are in obvious contrast to performance improvements in everyday situations [2]. This suggests the hypothesis that the observed aging deficits are partly caused by the optimization of cognitive functions by learning.

In order to provide evidence for this hypothesis we consider a computational model consisting of integrate-and-fire neurons with dynamical synapses. Critical behavior being a generic phenomenon in such networks [3] might provide a suitable basis for tasks like Raven's test where the exploration of a large set of combinations of feature is required. The model comprises also the life-long improvement in 'crystallized' intelligence by an adaptation of the interconnectivity in the course of the learning of a number of neural-activity patterns. Learning is performed in graded fashion by reducing the learning rate such that reoccurring patterns can still be learned but relatively harder than early pattern avoiding in this way a memory overflow. The synaptic adaptation is shown to cause a breakdown of the critical state which can be explained by the formation of densely connected clusters within the network. The avalanche-like activity waves in the network will then tend to remain inside the cluster thus reducing the exploratory effects of the network dynamics while retrieval of patterns stored in the early phase of learning is still possible. Mimicking the Raven's test we presented the model with new combinations of previously learned subpatterns during various states of learning. Networks with comparatively lower memory load achieve more stable activations of the new feature combinations than the 'old' networks. This corresponds well to the results of the free-association mode in either network type where only the 'young' networks are close to a self-organized critical state. Where on the one hand laerning leads to impaired performance in unusual situations it may on the other hand also be the way out of the decline in fluid intelligence when the situations to be learned are carefully chosen.

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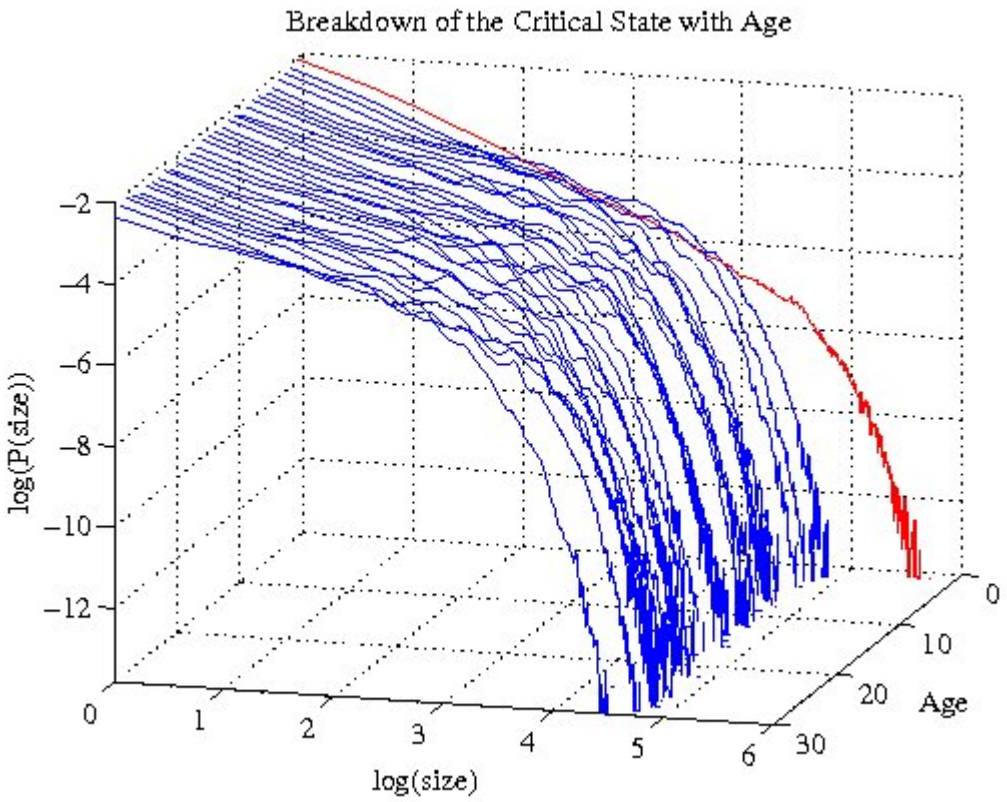
Breakdown of the Critical State with Age
log(P(size))
−2
−4
−6
−8
−10
−12
0
1
2
3
4
5
6
log(size)
0
10
20
30
Age

Stability analysis of pulse-coupled oscillators with delay

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About 20 years ago Mirollo and Strogatz have shown mathematically that two excitatory pulse-coupled oscillators will synchronize if the state variables or amplitudes are described by a smooth, monotonically increasing, concave function. While these early works included instantaneous couplings, more realistic models account for the finite propagation time of signals. This asks for a discussion of excitatory (phase advance) versus inhibitory (phase delay) synaptic connections. Ernst and coworkers (1995) reported that a finite propagation time causes temporal delays that yield an out-of-phase relation between the two excitatory oscillators. They also showed that the finite delay between two inhibitory oscillators can lead to anti-phase and in-phase synchronization. However, all these models were developed for the case of symmetric coupling (equally strong for both neurons) although the connectivity of biological neurons is hardly symmetric. E.g., the feed-forward projections of a neuronal population to another one are much stronger than their feed-back counterparts. Furthermore, synaptic strengths may change dynamically due to plasticity and learning, rendering full symmetry rather unlikely. Here, we analyze the dynamics of two asymmetric pulse-coupled neuronal oscillators with delays. We realize this for both purely excitatory and purely inhibitory coupling, as well as for a mixture of excitatory and inhibitory coupling, similar to the PING-models of Whittington and co-workers. We find analytical expressions for the stable states of the dynamical system of two interacting (populations of) neurons. Next to the stable states we show the corresponding bifurcation diagrams and Arnold tongues as a function of the differential coupling strength and of the delay time. Interestingly, the in-phase behavior is found only for two symmetric pulse-coupled inhibitory neuronal oscillators.

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Contralateral eye dominance induces pinwheel crystallization in models of visual cortical development

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The formation of orientation preference maps during the development of the visual cortex is sensitive to visual experience and impulse activity[1]. In models for the activity dependent development of these maps orientation pinwheels initially form in large numbers but subsequently decay during continued refinement of the spatial pattern of cortical selectivities [2]. One attractive hypothesis for the developmental stabilization of orientation pinwheels states that the geometric relationships between different maps, such as the tendency of iso-orientation domains to intersect ocular dominance borders at right angles can prevent extensive orientation map rearrangement and pinwheel decay[2]. Here we present a analytically tractable model for the coupled development of orientation and ocular dominance maps in the visual cortex. Stationary solutions of this model and their dynamical stability are examined by weakly nonlinear analysis. We find three different basic solutions, pinwheel free orientation stripes, and rhombic and hexagonal pinwheel crystals locked to a hexagonal pattern of ipsilateral eye domains. Using amplitude equations for these patterns, we calculate the complete stability diagram of the model. In addition, we study the kinetics of pinwheel annihilation or preservation using direct numerical simulations of the model in model cortical areas encompassing several hundred orientation hypercolumns. When left and right eye representations are symmetrical, inter-map coupling per se is not capable of stabilizing pinwheels, in this model. However, when the overrepresentation of the contralateral eye exceeds a critical value intermap coupling can stabilize hexagonal or rhombic arrays of orientation pinwheels. In this regime, we find a transition from a dominance of low pinwheel density states (4/cos(60deg)) to high density states (6/cos(60deg)) with increasing strength of inter-map coupling. We find that pinwheel stabilization by inter-map coupling and contralateral eye dominance leads to the formation of perfectly repetitive crystalline geometrical arrangements of pinwheel centers. These results suggest that while inter-map coupling can prevent pinwheel annihilation it is not sufficient to explain the spatially aperiodic arrangement of pinwheel centers in the visual cortex.

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How do Axonal Initiation and Backpropagation Shape AP Waveforms in Cortical Neurons?

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Cortical neurons typically fire at low rate, however they can encode fast changing stimuli by the firing pattern of a population. Theoretical studies suggested that the population response speed depends on the rapidness of single neuron AP generation. Furthermore, such fast onset has been observed in whole cell recordings of cortical neurons, which is unexpected from many Hodgkin-Huxley type models. It was speculated that APs initiated at axon and back propagating to the soma may account for the fast onset dynamics. Here we comprehensively characterize the somatic AP waveforms in a multi-compartment Hodgkin-Huxley-type neuron with a `ball-and-stick' geometry such that APs are initiated in the axon and back propagate through soma and dendrite. AP onset dynamics is quantified in simulations varying the distance between soma and the initiation site, the \Na channel density and the soma geometry. Our results suggest that the geometry of soma and axon initial segment and AP back propagating *per se* can not explain the fast AP onset observed in cortical neurons.

Supervised spike-timing dependent plasticity - A new neuronal learning rule for decisions and function approximation using spatio-temporal input

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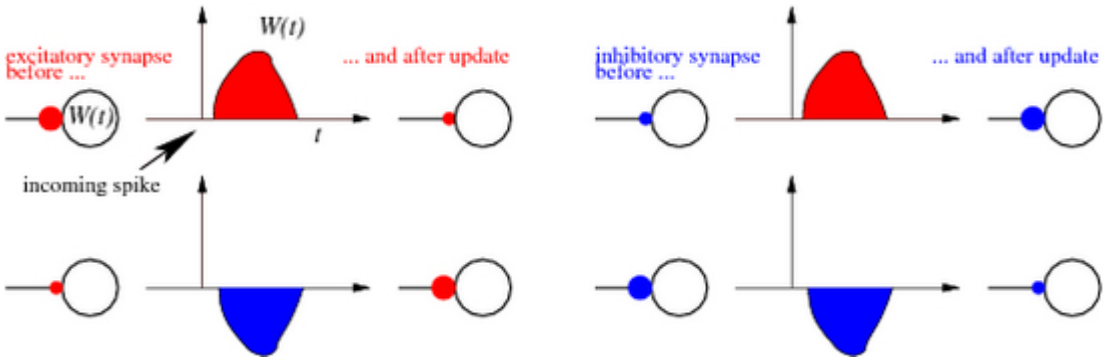
How can an animal learn from experience? How can it train sensors, such as the auditory or tactile system, based on other sensory input like the visual system? ``Supervised spike-timing dependent plasticity" (supervised STDP) trains one modality using input from another one as ``supervisor". One can prove that, under very general conditions, supervised STDP converges to stable synaptic weights leading to a reconstruction of primary sensory input. Example problems can be learned successfully.



Dynamic Action potential encoding in spatially extended neurons from an analytical tractable model

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In the cerebral cortex, the results of all neuronal operations performed at the single cell level are coded into sequences of action potentials (APs). In the living brain, cortical neurons are subject to an immense synaptic bombardment, resulting in large fluctuations of their membrane potential and in temporally irregular AP firing. Recently, the AP encoding under conditions of such synaptic bombardment has received much attention and has been analyzed extensively in single compartment neuronal models. Real neurons, however, are spatially extended systems and physiological studies indicate that the site of action potential initiation of cortical neurons is located in a relatively small neuronal process, the proximal part of the axon. The impact of this geometry on AP wave form and AP encoding is a matter of ongoing controversy. To elucidate the impact of axonal AP initiation, we here take the axon as a semi-infinite cable and calculate the transfer of voltage fluctuation in the axon using the Green's function method. In the framework of Gaussian neuron model we obtain the spike-triggered averaging voltage and variance at the soma when a spike is triggered at axon. We find that the spike-triggered variance is very small compared with the experimentally observed variability of thresholds at the soma. We also study the linear response function for the dynamical firing rate when action potentials are elicited in the axon and a small sinusoidal current is injected at soma.

Phase differences in local field potentials from macaque monkey area V4 predict attentional state in single trials with 99.6% accuracy

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Coherent oscillations and synchronous activity are suspected to play an important role in selective processing and routing information across the primary visual areas. In this contribution we show that phase coherency between distant recording sites allows to distinguish almost perfectly between two attentional states in a behavioural task, thus giving strong quantitative support for a functional role of oscillatory neural dynamics.

A macaque monkey was trained to perform a delayed-match-to-sample task, in which the animal had to direct attention to one of two sequences of morphing shapes (1 deg. diameter) presented on a computer screen and separated by a gap of less than one degree of visual angle. The task was to signal the reoccurrence of the initial shape of the attended morphing sequence. Recordings of local field potentials (LFPs) were performed with an array of chronically implanted intracortical microelectrodes, covering parts of areas V1 and V4.

The LFPs were split into their frequency components by applying a Morlet-wavelet transform. From the transformed signals, we computed the phase coherency (i.e. a complex-valued scalar with amplitude <=1 and a phase difference) averaged over a time interval of 2500 ms, for every electrode pair. We then used a support vector machine (SVM) to classify the attended state (attention directed either to one or to the other sequence) from the phase differences. Strikingly, nearly perfect state identification (up to 99.6% correct) was possible from several pairs of electrodes in V4, mainly in the frequency bands of 48 Hz and 61 Hz. From V1-V4 electrode pairs, classification with up to 76% correct was possible.

Our results show that phase differences between signals from V4 can accurately inform about the direction of attention to different locations in visual space in every single trial. This effect is both, stable over time and robust against continuous changes of the shapes at the attended location. Our results give strong quantitative support for the hypothesis that coherent oscillations are a key mechanism underlying information processing under attention.

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Influence of facilitation and connectivity pattern on the criticality in neural networks

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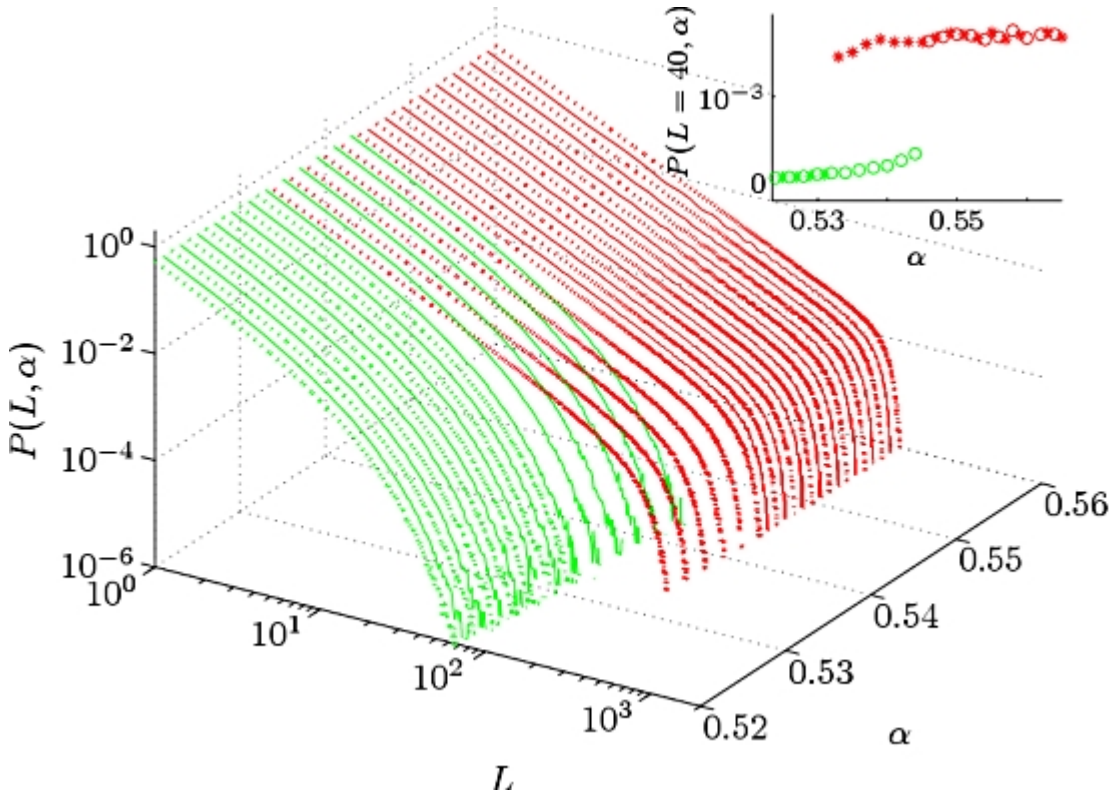
The concept of self-organized criticality (SOC) describes a variety of natural phenomena ranging from plate tectonics, the dynamics of granular media, and stick-slip motion to neural avalanches. In all these cases the dynamics are marginally stable and event sizes obey a characteristic power-law distribution.

Biological evidence of power-law distributions suggests that it is possible for particular feedback systems to actively and robustly maintain the critical behavior. This would allow the system to benefit from optimal computational capabilities, optimal transmission and storage of information, and sensitivity to sensory stimuli.

Here we present a neural network equipped with facilitating synapses, such that the feedback mechanisms are able to facilitate or attenuate the interaction among the elements. We show analytically that the adaptive model attains criticality in an extended region of the parameter space that is bounded by phase transitions.
The system exhibits a rich dynamical behavior including a hysteresis between critical and non-critical dynamics, switching of the dynamics in dependence of external input, and first- and second-order phase transition that form a cusp bifurcation.
Since the critical regime exhibits a bistable dynamic it may be related to wake-sleep cycles or to a partial suppression of activity in the absence of external input such as assumed to cause up and down states in the prefrontal cortex.

We also discuss the impact of the specific connectivity graph on the stability of critical region. We consider simple ring network with stochastic or small-world connectivity as well as the more elaborate model of neural tissue with distance dependent probability of connections.

Figure caption:
Distributions of avalanche sizes in dependence on the interaction parameter a for a network of size N=300.
At $a<a_c=0.534$ the distributions are subcritical (green), while between a_c and $a^c=0.547$ a critical phase emerges (red), which survives beyond a^c.
Finite networks also have a supercritical phase that is present for $a>0.57$ (not shown), but disappears in the limit of large system sizes.
The inset illustrates the hysteretic behavior of the network by showing a section of the main plot perpendicular to the L-axis at L=40.
Circles are obtained by incrementing a and stars by decrementing a.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Decoding of motions with multielectrode data acquired from a retinal ganglion cell population

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One of the properties of any visual scene is motion, which is coded by the retina and transmitted in form of spike responses from the retinal ganglion cells (RGCs) to the brain through the optical nerve. This work presents a method to decode motions using the temporal information of spikes inside short time windows. The isolated retina of a turtle is stimulated by a one-dimensional moving light pattern and the spike responses are recorded from the RGCs with a multielectrode array. The initial unicellular analysis is restricted to evaluate the reliability of each unit of the RGC population and to construct a couple of new populations with the most reliable units. So, the analyses were developed on three RGC populations of different sizes, whose spike responses were condensed in single spike trains. Having these data we measure the timings of the first three spikes fired during the first milliseconds of the spikes responses. Subsequently, a Bayesian classifier contained in the function classify of Matlab ® was utilized with these measures to decode the motions, obtaining values of classification efficiency greater than the random guess for this case.

The results indicate that including the timing of the first spike does not improve the classification efficiency. Therefore, the classification efficiency is better using only the timings of the second and the third spikes of the responses. Nevertheless, it is probable that the performance of the classifier improves including other properties of the retinal code in wider time windows. However, higher efficiency of classification is a trade-off between gained efficiency and extended classification time, so collecting the temporal information of two or three spikes is done faster than counting spikes in longer time windows of the responses. We found also that the classification improves when the coding strategies are evaluated over populations composed of reliable cells.

Stable information processing in spiking neural networks by synchronization

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Generally information processing in biological nervous systems is done by means of numerous parallel working neurons. These neurons interact by means of spikes on a high dynamical level. However, this interaction has to be organized to provide structured information processing of specific pattern components. Such binding to a specific pattern is based on the assumption that a selection of cues activates a population of neurons, a cell assembly coincidentally. This could be achieved by choosing a recurrent neural net of the Hopfield type which allows for extracting a specific target pattern stored in a correlation matrix. It has been analysed here whether spike synchronisation in a recurrent neural net leads to a pattern specific activation of a cell assembly. These modelling consideration have been extended in two ways. Firstly, it could be shown that a sequence of patterns emerge by choosing adaptive synaptic weights. Secondly, a stability analysis of specific activation states of a cell assembly could be performed by applying noise to the spike generation process.

For simulation of a spiking network physiologically plausible neurons of the Wilson type were chosen. This neuron type is a simplification of the well known Hodgkin-Huxley-Model and provides the physiological key property of voltage dependent activation of ion channels. Postsynaptic potentials was modelled on the basis of Rall's alpha-function. To produce adequate random spike noise an extra layer was introduced. The recurrent neural network applied resembled the connection schema of the CA3 region of the hippocampus. The synaptic connection weights of a pattern were stored in an auto-associative weight matrix. A pattern sequence was built in terms of cross-associations additionally stored in the weight matrix. To keep global activity in a reasonable operational range an additional inhibitory layer was applied.

In a recurrent neural net synchronisation may provide fault-tolerant and stable information processing. During the simulation without noise a pattern sequence could be reproduced. Even during the application of an intense of noise the neural net was able to separate patterns and recalled the pattern sequence stored correctly. Besides synchronous assembly activation of specific patterns, the simulated network showed high error tolerance and functional spike rate adaptation by inhibition.

Chaotic Dynamics in Balanced Neural Networks

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Mammalian neurons embedded in operational cerebral circuits fire action potentials in temporally irregular, seemingly random fashion [Softky1993]. The irregular firing is thought to arise from high temporal fluctuations of the synaptic inputs [Mainen1995], resulting from a balance between excitatory and inhibitory inputs [Bell1994]. This balanced state emerges robustly in large sparse neural networks [Vreeswijk1998,Amit1997] and its existence is widely accepted. The dynamical nature of the balanced state however, is controversially discussed.

To clarify the dynamical nature of the balanced state we here analyze the dynamics of networks of theta-neurons [Gutkin1998]--a standard form of type I neurons with active spike generation--using standard methods of nonlinear dynamics. In numerical simulations we calculate all Lyapunov exponents, which quantify the exponential divergence of small perturbations. In our study of non-delayed, pulse coupled networks of purely inhibitory neurons as well as mixed networks of excitatory and inhibitory neurons, we find positive finite largest Lyapunov exponents, hence conventional deterministic chaos. This is in contrast to dynamics of networks of binary neurons [Vreeswijk1998], which exhibit a kind of hyperchaos with positive but infinite largest Lyapunov exponents, and contrasts recent results of networks of leaky integrate and fire neurons [Zillmer2006,Jahnke2008], that exhibit stable chaos, characterized by negative Lyapunov exponents but irregular firing.

Beyond the largest Lyapunov exponent, the full Lyapunov spectrum allows us to characterize the dynamics of the balanced state in more detail. The attractor dimension and entropy production rate, derived from the Lyapunov spectrum, appear both very high in the balanced state. They also scale linearly with the network size, thus the chaos is spatially extensive. The mean of the Lyapunov exponents is negative, thus the system is dissipative. In a random matrix approximation we derive an analytic expression of the mean Lyapunov exponent, which is in very good agreement with the simulation results. Analyzing the statistics of the Lyapunov vector that corresponds to the largest Lyapunov exponent we find spatio-temporal chaos with neurons in the intermediate firing regime contributing the most to the chaotic dynamics.

The impact of target type selection on the stability of layered cortical network dynamics

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The local cortical network consists of distinctively interconnected layers. A feed-forward pattern of connections (layer 4 (L4) to L2/3 to L5 to L6) has been hypothesized on the basis of axonal branching properties as well as the tuning properties of cells in primary visual cortex (see [1] for review). Recently, evidence has been accumulated that this pattern is accompanied by "feedback" connections inverse to the feed-forward connections; strikingly these connections specifically select interneuronal targets (see e.g. [2]). This lead to the hypothesis that the resulting lack of excitatory feedback increases the sensitivity for time-dependent signaling and decreases the susceptibility to "over-excitation and epileptiform activity" [2]. However, the impact of target type selection on the activity dynamics of the local network remains unstudied - largely due to the incompleteness and shortcomings of even the most comprehensive data sets on cortical connectivity.

We overcome this problem with the compilation of an integrated data set on layer-specific connectivity based on anatomical and physiological data [3]. In addition, we incorporate information on target type selection from laser-scanning photostimulation [4] and electron microscopy studies [5]. On this basis, we present an algorithmic procedure to construct self-consistent data sets strictly fulfilling target type selection constraints.

We investigate the dynamical implications of target type selection in large-scale simulations. Our model consists of 80,000 I&F neurons and explains around 90% of the synapses that constitute the local cortical microcircuit. We find that networks exhibiting specific target type selection (target specificity) show superior stability compared to control networks lacking this feature. The figure shows the population rates (left, separate for excitatory, mean of inhibitory populations) and Fano Factor (right, mean of excitatory, inhibitory populations) for different values of target specificity (+/-1 for only selecting excitatory/inhibitory neurons; 0 for non-specific targeting). Note, that we solely alter the specific selection of targets of the feedback connections shown in the inset (arrow thickness represents connection probabilities, target specificity fixed to -0.4). With otherwise identical parameters, these minor changes in the connectivity drive the network non-linearly from a low-rate asynchronous irregular state to global over-excitation and epileptiform activity. Thus, we identify specific target type selection as a potential structural tipping element for network stability, establishing a link between microcircuitry and activity dynamics.

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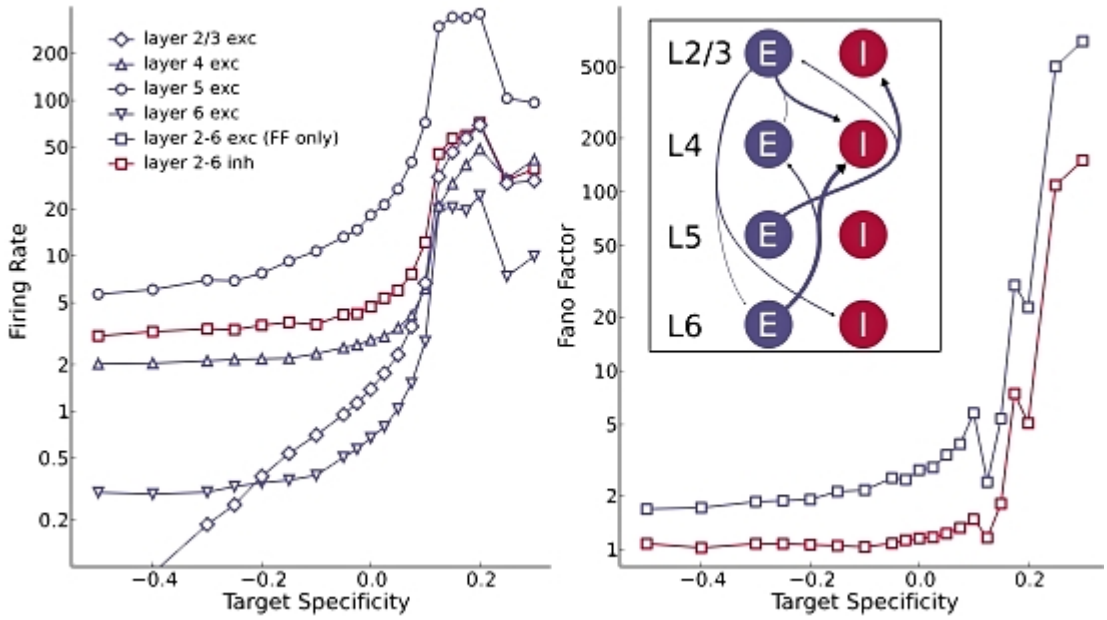
Acknowledgements
Partially supported by EU Grant 15879 (FACETS), Next-Generation Supercomputer Project of MEXT, Japan, and the Helmholtz Alliance on Systems Biology.


layer 2/3 exc
layer 4 exc
layer 5 exc
layer 6 exc
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Target Specificity
Fano Factor
Target Specificity
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Dual measures for assembly activation based on the LFP and spike coincidences

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A common hypothesis concerning the strategies of information coding employed by cortical networks involves the propagation of activity through synchronously firing groups of neurons, termed assemblies. Despite recent advances in increasing the number of recorded neurons from cortical networks, the inherent undersampling of the system still prevents us to directly verify the existence of assembly activity in the living brain. However, a growing body of experimental studies indirectly substantiates the assembly idea with findings of significant synchronous spiking activity that relates to behavior (e.g., [1]). Independently thereof, a signal measuring directly on the population level, like the local field potential (LFP), typically exhibits temporally structured oscillations commonly interpreted as correlated network activity. Recently, we demonstrated a direct link between coincident spike events and their phase relationship to LFP beta oscillations in motor cortex of the awake behaving monkey [2]. In particular, we showed that Unitary Events (UEs, significant coincidences, cf. [3]) exhibit an exceptionally strong locking to the LFP that cannot be explained by the locking of the individual neurons.

In order to understand how the observed levels of synchrony and phase locking quantitatively translate to the assembly hypothesis we formulate a simplified model. It assumes that part of the spiking activity is involved in assembly activations, whereas the other part is not. In the model, UEs express observed assembly activity. Combined with the results in [2], we conclude that assembly spikes are more strongly entrained by the LFP than non-assembly spikes. In this framework, we demonstrate how to compute the minimal relative contribution of assembly spikes following two conceptually different approaches. First, we show how to estimate the fraction of spikes involved in assembly activations by comparing the phase distributions between time periods that exhibit UEs and those that do not. Second, we estimate this fraction as a function of the UE significance level given a model of injected spike synchrony into otherwise independent firing (cf. [4]), exploiting estimates of the expected and empirical coincidence distributions. Both methods are calibrated using simulated data before they are applied to the biological data. Finally, extending the former approach enables us to infer an estimate of the percentage of spikes a neuron contributes to assemblies. The consistency of the results of both approaches provides encouraging support for the assembly hypothesis.

Partially funded by the Helmholtz Alliance on Systems Biology, the French National Research Agency (ANR-05-NEUR-045-01), and EU grant 15879 (FACETS).

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Spike Frequency Adaption Reduces Noise in Neural Ensemble Activity

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A spontanoeus spiking pattern of negatively serially correlated intervals has been reported for a range of different neuron types and in various systems - in the periphery as well as in central brain regions, both in invertebrtates and vertebrates (for review see [1]). This phenomenon has been linked to neuron-intrinsic physiological mechanisms of spike-frequency adaptation (SFA) which exist in many different types of spiking neurons.

Here, we studied the causal relation between SFA and serial interval correlation (SIC) in different computational models. We show that (1) the negative serial correlation of ISIs is a direct consequence of SFA, (2) negative correlation reduces the noise level in single neurons as well as (3) in the merged activity of a neural ensemble which is representative of the integrated input to post-synaptic neurons.

We studied three different models. First, a biophysical model with slow AHP currents. In the presence of white noise current injection this model produces negative SICs which are most pronounced for neghbouring intervals but generally extend over several lags. This result is robust throughout the physiological spike frequency range. Second, we investigated the generality of this effect in two different phenomenological Integrate-and-Fire (I&F) models with SFA that had been proposed by Brette & Gerstner (2005) [2], and Muller et. al. (2007) [3], respectively. Both models captured the short-lived negative SIC. In both models we describe the relationship of the input statistics with SIC in the output spike train. In particular we confirm the expectation that the strength of the observed correlation is rate dependent. As a control, we compare our results to the leaky I&F model without SFA which produces a renewal output.

Independent of the detailed model we find that the observed negative serial correlation lead to a reduced single neuron variability by up to 50%, as measured by the Fano factor of the spike count. We have previously shown that this phenomenon also exists in in vivo recordings from single cortical neurons where the noise was reduced by abot 30% [4].

Interestingly, this reduction of the count variance carries over to the ensemble activity of SFA neurons. This activity shows the very same amount of reduction when observed on the same time scale. We may interpret this as a reduced noise in the fluctuating ensemble rate signal. The read-out of this signal in a receiving cell will be more accurate than in the case of serially uncorrelated single neuron activity of the presynaptic ensemble.

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Self-sustained cell assemblies in structurally plastic networks

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The connectivity structure of cortical networks was recently found to exhibit plastic changes even in adult animals [1]: there is a considerable continuous turnover of synapses. It is still unclear, how networks maintain their functionality despite this plasticity and what are the mechanisms ensuring network stability.

Here we investigate random and structured networks subject to structural reorganization. We exploit the idea of correlation based sorting, where new synapses are constantly created at a fixed rate between randomly chosen neurons and synaptic pruning is controlled by a correlation based selective elimination rule [2].
We show that this plasticity induces a homeostasis of firing rates in random recurrent networks. At realistically low firing rates as observed in neocortex, this structural plasticity effectively regulates the number of incoming connections per neuron.
To address the question how functionally important connections are maintained despite the synaptic turnover, we embed cell assemblies of G_e neurons into the network (see Figure A) and investigate the stability of their characteristic connectivity. Earlier theoretical work [3] showed that cell assemblies are a viable substrate of associative memory and that structural plasticity improves the memory capacity. Our own prior work [2] demonstrated cooperation among synapses that are activated in a correlated way, resulting in a stabilization of these connections. The neurons of an assembly in our model exhibit such correlation which, by cooperation, effectively stabilizes its connectivity. Furthermore, a critical minimal connectivity $f>f_c$ is required for this self-sustained maintenance.
However, the embedded cell assemblies continuously recruit more cells and hence expand over time. Therefore this work prompts for further constraints of structural plasticity that prevent cell assemblies from unbounded growth.
In parallel to direct numerical simulations, we derive analytical results that enable us to predict the point of homeostasis, which corresponds to a stable fixed point (see Figure B) as well as the critical connectivity needed for self-sustained cell assemblies which is an unstable fixed point.
Partially funded by DIP F1.2, BMBF Grant 01GQ0420 to BCCN Freiburg, EU Grant 15879 (FACETS), and
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[2] Helias, Rotter, Gewaltig, Diesmann, submitted
[3] Knoblauch (2007) BMC Neuroscience **8**(Suppl 2), S14.

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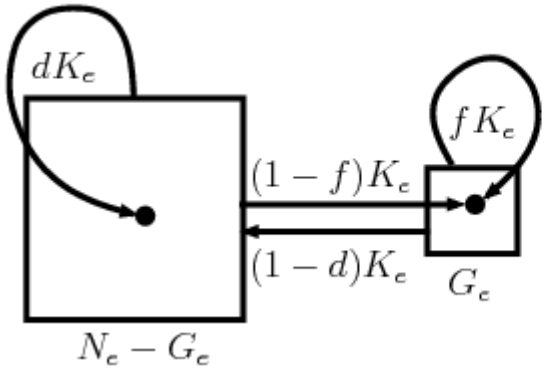
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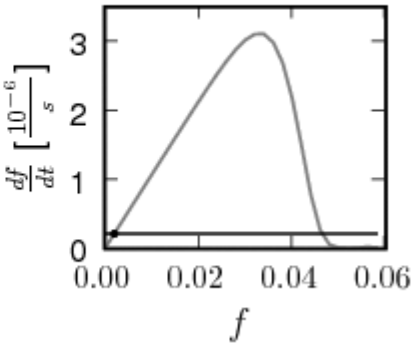
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Functional consequences of correlated excitation and inhibition on single neuron integration and signal propagation through synfire chains.

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[2]Neurobiology and Biophysics, University of Freiburg, Freiburg, Germany
[3]Bernstein Center for Computational Neuroscience, Freiburg, Germany

Neurons receive a large number of excitatory and inhibitory synaptic inputs whose temporal interplay determines their spiking behavior. On average, excitation (Gexc) and inhibition (Ginh) balance each other, such that spikes are elicited by fluctuations [1]. In addition, it has been shown in vivo that Gexc and Ginh are correlated, with Ginh lagging Gexc only by few milliseconds (6ms), creating a small temporal integration window [2,3]. This correlation structure could be induced by feed-forward inhibition (FFI), which has been shown to be present at many sites in the central nervous system.

To characterize the functional consequences of the FFI, we first modeled a simple circuit using spiking neurons with conductance based synapses and studied the effect on the single neuron integration. We then coupled many of such circuits to construct a feed-forward network (synfire chain [4,5]) and investigated the effect of FFI on signal propagation along such feed-forward network.

We found that the small temporal integration window, induced by the FFI, changes the integrative properties of the neuron. Only transient stimuli could produce a response when the FFI was active whereas without FFI the neuron responded to both steady and transient stimuli. Due to the increase in selectivity to transient inputs, the conditions of signal propagation through the feed-forward network changed as well. Whereas synchronous inputs could reliable propagate, high asynchronous input rates, which are known to induce synfire activity [6], failed to do so. In summary, the FFI increased the stability of the synfire chain.

Supported by DFG SFB 780, EU-15879-FACETS, BMBF 01GQ0420 to BCCN Freiburg

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Time-driven simulation as an efficient approach to detecting threshold crossings in precisely spiking neuronal network models

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In order to avoid artificial synchronization in neuronal network simulations, there is considerable interest in being able to compute spike times precisely. Recently, research has developed along two lines, which both enable the calculation of spikes with arbitrary precision: extending the set of neuron models that can be simulated in an event-driven scheme by developing appropriate spike-prediction algorithms [1,2], and detecting spikes between the sampling points of a time-driven scheme [3].

One potential advantage of the time-driven scheme is that a threshold crossing needs only be detected retrospectively, which is a simpler problem than the prediction required for the event-driven approach. We compare the simulation times of networks of neurons implementing spike-prediction algorithms as suggested by Brette [1] (Polynomial) and D'Haene et al. [2] (Envelope) and spike-detection algorithms as suggested by Morrison et al. [3] (Interpolation) and the extension of this framework to use iterative root-finding methods (Newton/Raphson), see figure. For orientation, the figure also shows the simulation times of a traditional grid-constrained neuron implementation as a function of the error in spike times of a single neuron simulation. All simulations were performed in NEST [4]. These results demonstrate that given a sufficiently high computational resolution (1 ms), sampling the membrane potential at every incoming spike provides an effectively fail-safe method of detecting recent threshold crossings that is more efficient than the elegant methods used for predicting a future spike proposed by Brette [1] and D'Haene et al. [2]. Moreover, the time-driven scheme can be applied to all linear and non-linear neuron models.
>
By analyzing the dependence of simulation time on the computational resolution and the input and output rates of a medium size neuronal network (12,000 neurons), we conclude that the only regimes in which an event-driven scheme could be more efficient than a time-driven scheme are small networks at very low rates, and applications where the research domain forces a high resolution for the time-driven scheme, e.g. due to very short synaptic delays.

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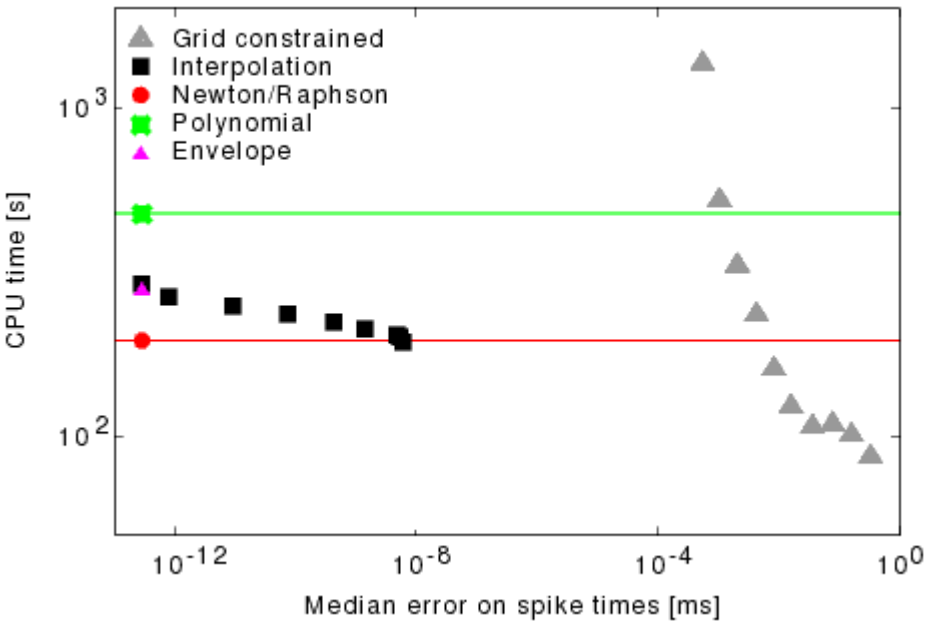
Grid constrained
Interpolation
Newton/Raphson
Polynomial
Envelope
CPU time [s]
10^3
10^2
10^{-12}
10^{-8}
10^{-4}
10^0
Median error on spike times [ms]

Spatially organized higher-order spike synchrony in cat area 17

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It is still an open question, how the concept of maps is related to temporal coding. Therefore we analyzed parallel spike recordings from cat visual cortex (10 x 10 grid, 3.6 x 3.6 mm) for spike correlation [2]. Application of pairwise cross-correlation analysis onto all (100) recorded multi-unit activities (MUA) allowed us to extract all significantly correlated MUA pairs. Graph theoretical analysis of all correlated pairs revealed a decomposition into a small number (4) of distinct clusters of inter-correlated MUAs, which also correspond to segregated clusters in cortical space. Their spatial scale is in agreement with the scale of orientation tuning maps. However, due to the limitation of the applied pairwise analysis, cell assemblies composed of larger groups of neurons could not be conclusively identified. Therefore, a test which extracts higher-order synchrony (HOS) needs to be used.

Therefore, we developed a new method for the detection of HOS, which combines the accretion method [3] with frequent itemset mining (FIM). Spike synchrony is detected by the accretion approach: pairs of spike trains are tested for significant correlation and then reduced to new point processes containing only synchronized events. These processes are in turn correlated with further, single neuron spike trains and so on, until the maximal order of correlation is found. Ideas from FIM algorithms help to search the space of all neuron subsets efficiently. However, such algorithms usually rely on a minimum support criterion to prune the search, as this guarantees soundness. However, HOS does not necessarily imply frequent occurrence of spike patterns, and we are rather interested to extract spike patterns that occur significantly more often than expected given by the firing rates. Therefore we designed a FIM related algorithm (AFIM), that processes large sets of data efficiently [4].

Using this approach we re-analyzed the above mentioned data. To account for non-stationarity in time we segmented the data into quasi-stationary segments, and analyzed these separately. In different time segments different sets of MUAs exhibit higher-order spike synchrony in groups of up to order 7. Within a time segment, the groups exhibiting HOS are highly overlapping, and are therefore combined into supersets. Most interestingly, the supersets of the different time segments do not overlap, but reveal the same, separate clusters of MUAs that were identified by the pairwise analysis. We verified our results by shuffling the trials of the individual MUAs, which did not reveal any HOS in any of the time segments. Thus, our results show strong evidence, that similar to the dynamic occurrence of activity patterns found in optical imaging, spatially segregated groups of higher-order correlated neurons are alternatingly active.

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Identification of neurons participating in cell assemblies

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Chances to detect assembly activity are expected to increase if the spiking activities of large number of neurons are recorded simultaneously. Although such massively parallel recordings are now becoming available, methods able to analyze such data for spike correlation are very rare. Extensions of methods developed for data sets of a small number of neurons is not feasible due to combinatorial explosion. We have taken new approaches [1,2,3] that can analyze massively parallel data for higher-order synchrony, but they do not identify neurons that participate in the correlation. Methods (e.g. [4]) that are able to identify assemblies and their participating neurons require considerably computing power, and would profit if the data set entering the analysis could be reduced. Thus, the goal of the present study is to directly identify neurons that are part in an assembly as expressed by coincident firing.

For a solution we present an approach that follows the idea to detect if an individual neuron is involved more often in coincident activity than expected by chance. Different variants of the approach differ in their test statistic. Background rate estimation (BRE) computes the statistic based on a comparison of estimates of the background firing rate and the total coincidence rate. Conditional pattern complexities (CPC) takes into account how many other neurons fire together with the tested neuron. Conditional spike frequencies (CSF) tests on the basis of how often other neurons fire together with the tested neuron. Since there are certain obstacles to find the distributions of these test statistics under the null hypothesis that the considered neuron is not part of an assembly, we rely on a spike shuffling procedure instead.

The sensitivity of the approach is tested by analyzing simulated massively parallel spike data which contained one, or two (overlapping) assemblies (cf. [2]). Data are generated by a stochastic model, which realizes coincident spiking activity of the neurons involved in an assembly in otherwise independent background. Control data are independently firing neurons, which may differ in their firing rates. BRE proved to be a fairly weak method in our experiments due to a high variance of the estimate of the background rate. However, including at least second order measures improved the results considerably. Since CSF and CPC include these naturally, they perform very well. However, CSF requires an estimate of the expected number of spikes based on the assumption of stationarity, which is a severe drawback for the case of realistic spike trains. In contrast, CPC provides the advantage that it leaves the spike trains of the other neurons and thus their correlation structure intact, and therefore does not interfere with additional features of the spike trains that may confound the analysis.

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The nonlinear response of a spiking neuron to a transient stimulus

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The propagation of synchronous firing activity in feed-forward neural networks has been proposed as a model ("synfire chain") to explain precise spatio-temporal spike patterns in the cortex [1]. Numerical studies have revealed that such propagation is possible in physiologically realistic parameter ranges and that two variables are sufficient to describe the dynamics of the travelling activity pulse [2]. To gain a general understanding we aim at a full analytical description of the activity dynamics. This inevitably requires the knowledge of the response of a single neuron to transient stimuli in the presence of background noise. An analytical solution of even this single neuron problem, which takes basic membrane properties into account, is still lacking, although progress has been made recently [3]. The most likely reason for this, are general technical difficulties to treat time-inhomogeneous random processes.

Here, we present an explicit formula for the time-dependent firing rate of a leaky integrate-and-fire neuron in response to an arbitrarily strong, transient stimulus. With this formula, we are finally equipped with a theory that accurately predicts the expected response of a population of neurons to an incoming activity pulse. In other words, we are able to construct a transmission operator that iteratively maps the firing response of one neuron group to the firing response of the next group. This allows us to study the pulse propagation along the feedforward network analytically. The key idea to solve the time-dependent problem is based on an exact series representation for the first-passage-time density of differentiable random processes and its approximations [4-6]. Already the first term of the series excellently accounts for the transient firing rate (see figure). For strong or fast stimuli, this approximation recovers previous results [3]. Furthermore, the mean membrane potential excursion and its temporal derivative naturally appear in the formula, which justifies assumptions of previously used phenomenological models.

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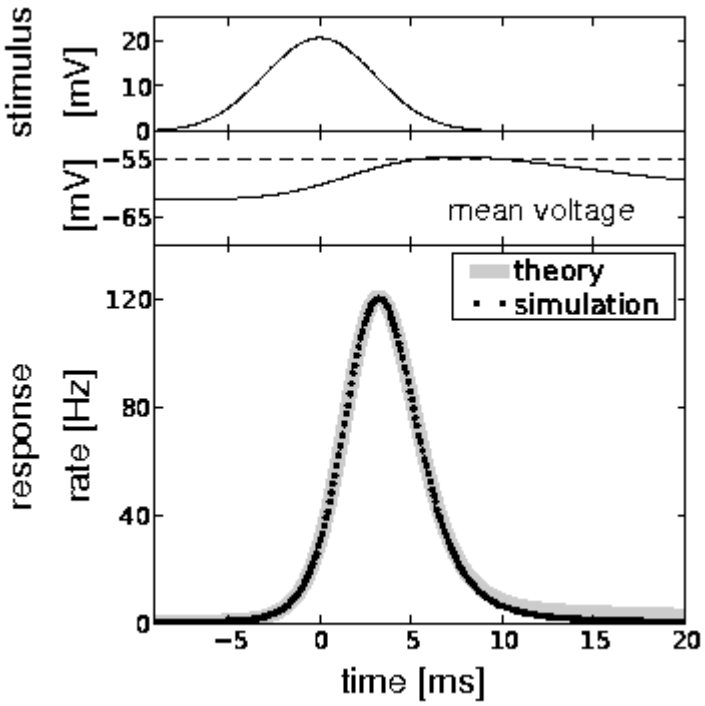
stimulus
[mV]
20
10
0
−55
−65
[mV]
mean voltage
theory
simulation
response
rate [Hz]
120
80
40
0
−5
0
5
10
15
20
time [ms]

Reduction of Scattered Light by Müller Cells in the Human Retina

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It is a long standing question why in the mammalian eye photo-receptors are positioned at the back of the retina, such that photons have to travel through various neuronal layers of the retina before the light-sensitive rods and cones can detect them. Recent studies have shown that Müller cells can act like optical fibers [1]. Their collective parallel arrangement is reminiscent of highly efficient fiber-optic plates, which are technologically used to precisely transfer images between spatially separated planes. If light conduction by Müller cells in fact improve optical precision, it may appear puzzling that in the fovea, the center of highest acuity of vision, no Müller cells are found.

To understand the potential optical impact of light conduction of Müller cells, we theoretically analyzed the reflection and transmission of incident light from Müller cells surrounded by tissues of the neuronal retina. We showed that Müller cells reduce the scattered light in the surrounding area of the fovea and help to increase the signal-to-noise ratio on the entire retina.

The wave properties of light allows to map the electro-magnetic Helmholtz equation to the quantum-mechanical Schrödinger equation, where the refraction indices are represented by negative potentials. Transmission and reflection measurements have revealed the refraction indices of Müller cells and of their surrounding tissue for different species [1-4].

Using the Fisher-Lee relation we obtained transmission and reflection values from numerically calculated scattering wave functions. Taking the geometry of the eyeball into account, we calculated the angle-dependent amount of back-scattered light which is reflected by the surface of the retina. We find that the signal-to-noise ratio of incident light at a point on the retina to back-scattered light from the entire surrounding retina is enhanced in the presence of Müller cells. The specific characteristic of Müller cells responsible for this is the horn-like shape of the head of the Müller cell, as in horn-shaped quantum point contacts [5].

Calculations were done for the human retina, but can be applied to species with a similar visual system.
In summary, Müller cells are responsible for a reduction of scattered light in the entire retina, most effectively in the center highest acuity of vision, the fovea. The increase of the signal-to-noise ratio up to a factor of 2 is predicted to lead to sharper vision.

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Clustered network topology and noise directly influence quasi-stable bursting behaviour

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Disassociated cortical cultures grown on multi-electrode arrays (MEA) have been established as a useful biological model in the analysis of network dynamics. Present in their dynamics are periods of strongly synchronized spiking by the network, termed 'bursting', whose purpose is not understood but may saturate network dynamics. It has been demonstrated that bursts have different motifs and contain structure, refuting the possibility that they are merely chaotic activity. However, in order to minimize bursting and promote closed-loop communication with the disassociated culture, it is of interest to understand what conditions are necessary for bursting to arise. Descriptive models have been promoted in order to understand their bimodal activity; however, a functional description might be of more use in investigating characteristics of bursting networks.

A type of recurrent neural network model termed Echo State Networks was proposed as a functional model of the disassociated cultures. We have previously demonstrated the effect of altering parameters such as connectivity and temporal resolution of individual units and reported that of all possible parameter changes, altering the structure of the reservoir most drastically changed network dynamics. Of specific interest were subreservoir configurations that resulted in a marked tendency for quasi-stable activity dynamics, which appears analogous to bursting. Furthermore, we established that temporal recall is related to the ratio between cluster size and number of clusters. Here, we further analyze this finding and examine network topology against dynamics by considering an extended collection of network configurations.

The robustness of the bursting-like model to noise was previously established by perturbing the system using small uniformally distributed noise and found to be lower for non-homogeneous reservoir architectures, supporting the hypothesis that these networks operate in a quasi-stable or 'critical' range as proposed elsewhere for reservoir networks. In addition, noise was also found to be responsible for determining the amount of variability observed in artificial bursts. Where previously only small amounts of noise were considered, we now examine the effect of larger noise values in order to identify an acceptable range of noise values and distributions that result in dynamics that are both quasi-stable and biologically plausible.

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Structural plasticity in recurrent cortical networks

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We study recurrent neural networks exhibiting structural plasticity by formation and elimination of synapses: synaptic death is controlled by a biologically realistic correlation dependent learning rule [1] and synapse formation with a fixed rate takes place in a random manner.

The interplay between network dynamics and correlation dependent evolution of network structure exhibits an interesting feature: We observe the emergence of cell assemblies in initially random cortical networks upon correlated stimulation of a subgroup of neurons.

To gain a quantitative understanding, we reduce the detailed spike based learning dynamics to effective rate equations where the network dynamics follows the structure adiabatically. We show that this description captures the essential features of the interplay between structural plasticity and network dynamics. Comparison to direct numerical simulations proves the approximate procedures applied on many levels adequate.

With the theoretical treatment of network evolution ready, we can inquire into modes of stimulation that facilitate the induction of cell assemblies, and into the conditions that must be fulfilled to stabilize them for longer periods.

Extensions of our setup to include correlation dependent rather than random formation of synapses and sequential stimulation of several network subgroups will be treated as this project progresses.

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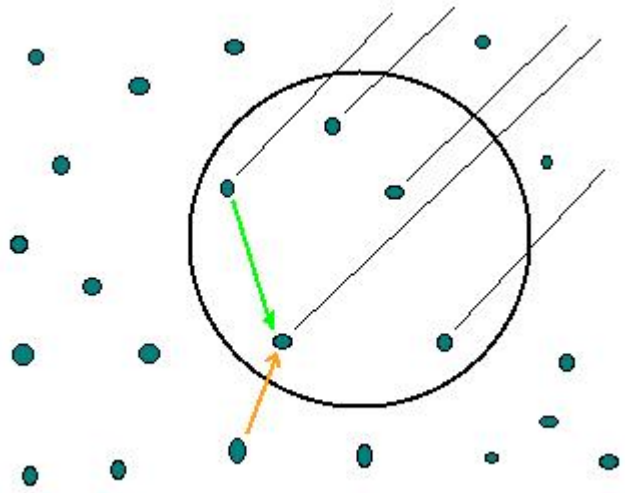

An evaluation of different copula models for the short-term noise dependence of spike counts

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Correlations between spike counts are often used to analyze neural coding. Traditionally, multivariate Gaussian distributions are frequently used to model the correlation structure of these spike-counts [1]. However, this approximation is not realistic for short time intervals.
In this study, as an alternative approach we introduce dependencies by means of copulas of several families. Copulas are functions that can be used to couple marginal cumulative distribution functions to form a joint distribution function with the same margins [2]. We can thus use arbitrary marginal distributions such as Poisson or negative binomial that are better suited for modeling noise distributions of spike counts. Furthermore, copulas place a wide range of dependence structures at our disposal and can be used to analyze higher order interactions.
We develop a framework to analyze spike count data by means of such copulas. Methods for parameter inference based on maximum likelihood estimates and for computation of Shannon entropy are provided. The methods are evaluated on a data set of simultaneously measured spike-counts on 100 msintervals of up to three neurons in macaque MT responding to stochastic dot stimuli [3] and of up to six neurons recorded from macaque prefrontal cortex. Parameters are estimated by the inference-for margins method: first the margin likelihoods are separately maximized and then the coupling parameters are estimated given the parameterized margins. Resulting parameters are close to the maximum likelihood estimation with the advantage that the approach is also tractable for moderate dimensions. Goodness-of-fit is evaluated by cross-validation for the likelihoods.
The data analysis leads to three significant findings: (1) copula-based distributions provide better fits than discretized multivariate normal distributions; (2) negative binomial margins fit the data better than Poisson margins; and (3) a dependence model that includes only pairwise interactions overestimates the information entropy by at least 19% compared to the model with higher order interactions.

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Anaesthesia monitoring by recurrence quantification analysis of EEG data

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In today's clinical practice, routine monitoring of general anaesthesia is mainly based on cardiovascular parameters and motor responses. However, during neuromuscular blockade, in the presence of beta-blockers, or in patients who only tolerate "light levels" of anaesthesia, these clinical parameters may fail to reliably monitor the depth of anaesthesia. As a consequence, intra-surgical awareness can occur, possibly leading to explicit memory of words spoken in the operating room, discomfort, or pain. Possible sequels of intra-operative awareness include nightmares, or even symptoms of a posttraumatic stress disorder (Schneider et al., 2005). These adversities make the development of safe anaesthesia monitoring devices, which directly analyze electrical activity from the brain as the main target organ of general anaesthetics, a major challenge.

We used a novel approach for anaesthesia monitoring from EEG data, namely recurrence quantification analysis (RQA). RQA is a highly sensitive technique of nonlinear time series analysis developed by J. P. Zbilut and C. Webber (1992). To test the applicability of RQA for anaesthesia monitoring, we applied RQA to EEG data from two clinical studies comprising 40 surgical patients each.

We found that RQA predicts the occurrence of intra-operative awareness with a prediction probability p_K of > 0.85, and a temporal delay of maximal 30 s. So far, comparable results had only been obtained if both spontaneous activity (EEG) and auditory evoked activity (auditory evoked potentials, AEP) were combined (Schneider et al., 2005). The analyzed data sets were also fed into different commercial anaesthesia monitors presently used in clinical practice. These monitors separated consciousness from unconsciousness with considerably lower p_K values (0.5 - 0.8) (Schneider et al. 2003, Schneider et al. 2004, Pilge et al. 2006), while mostly requiring longer time intervals before reflecting a change in the level of anaesthesia (Pilge et al. 2006). We therefore guess that our results open up a new avenue for the development of improved future anaesthesia monitoring devices.

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Efficient probabilistic wiring of spatial neuronal network using Walker's alias method

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Models of sensory and cortical neuronal networks are often composed of spatially organized neuronal layers. Connections between neurons in different layers (as well as within a layer) are commonly described by probabilistic kernels as follows: Each neuron is assigned a location **x**, which can either be the physical location of a neuron within the layer, or, e.g., the center of the receptive field of the neuron in visual space. The probability for a neuron at position **x** in layer A to make projection to (or receive a projection from) a neuron at position **y** in layer B is then given as a function of the distance **d** of the neurons, $p \sim \mathbf{d}=\mathbf{x}-\mathbf{y}$. A typical example would be a Gaussian kernel, where connection probability is maximal for aligned neurons and dropping exponentially with distance.

A common way to create network connections from kernel-based descriptions is to visit each pair of neurons, compute their distance and thus connection probability, and then create a connection with that probability. For exponentially decaying kernels, this is quite inefficient, since no connections will be created for most possible pairings of neurons, even if some cut-off is set at some large distance. The scheme becomes highly inefficient when a prescribed number of connections is to be created, since that will require revisiting neurons until the quorum is achieved.

We present here a far more efficient approach based on Walker's alias method (D.E.Knuth, The Art of Computer Programming, vol 2, 1998). The task at hand is to distribute a given number of connections from a presynaptic neuron in layer A across the postsynaptic neurons in layer B (within a cut-off radius). We begin by tabulating the connection probalities for all eligible postsynaptic neurons, thus obtaining the relative probability for connections to be made to each postsynaptic neuron. We then draw one postsynaptic neuron at a time according to these relative probabilities. Using Walker's alias method makes this selection process efficient even if probabilities vary by orders of magnitude. Optimal efficiency is achieved if pre- and postsynaptic layers are commensurable regular grids, since probabilities need not be re-tabulated for each presynaptic neuron. The algorithm oviously applies if presynaptic neurons are chosen probabilistically for a given postsynaptic neuron.

We have implemented our algorithm as part of the Topology Library for the NEST neuronal network simulator. Benchmark tests indicate that our approach is up to ten times faster than the naive algorithm for realistic network architectures.

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Validating System Identification Models of the Locusts Hind Leg Reflex Control Loop Using Walking and Sinusoidal Inputs

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The established approach for investigating the mechanical, neural and muscular elements of invertebrate limb control systems is to optimise the design of experiments to investigate the control of a particular movement. By contrast, the ultimate goal of the system identification approach is to produce a quantitative model which can accurately predict the response of the control system to any input.

Previous work [1] used the system identification approach to model the response of motor neurons in the locusts hind leg reflex control loop. The parameters of Wiener/Volterra system identification models in [1] were estimated from the Gaussian White Noise (GWN) input used to mechanically excite the system, and the synaptic response of the extensor motor neurons was taken as output. A GWN input signal was chosen because it excites the system at all frequencies within its operating range and should therefore produce a model which can predict the response to arbitrary inputs. GWN is readily generated, facilitates the experimental procedures, and allows for relatively rapid measurements. So far, however, little work appears to have been carried out to test whether the models based on GWN can predict the responses to biologically relevant 'natural' inputs, such as those occurring during walking.

We have therefore extended previous work [1] by investigating the ability of GWN Volterra models to predict the response of the system to walking, as well as to sinusoidal inputs. Our results show that GWN Volterra models of the Fast Extensor Tibia (FETi) motor neuron can accurately predict the neurons response to further GWN noise stimulation, as residual errors are similar to background neural and measurement noise. These models, however, were found to be poor at predicting the response of the FETi motor neuron to natural walking and to sinusoidal inputs. Investigation into this failure has allowed a more general and accurate model to be proposed, which gives smaller errors in predicting the response of the FETi motor neuron to GWN and to sinusoidal inputs, and significantly improves the performance with walking inputs.

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Dynamics of cortical networks including long-range patchy connections

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Most studies of cortical network dynamics are either based on purely random wiring or neighborhood couplings [1], focussing on a rather local scale. Neuronal connections in the cortex, however, show a more complex spatial pattern composed of local and long-range patchy connections [2,3] as shown in the figure: It represents a tracer injection (gray areas) in the GM of a flattened cortex (top view): Black dots indicate neuron positions, blue lines their patchy axonal ramifications, and red lines represent the local connections. Moreover, to include distant synapses, one has to enlarge the spatial scale from the typically assumed 1mm to 5mm side length.

As it is our aim to analyze more realistic network models of the cortex we assume a distance dependent connectivity that reflects the geometry of dendritesand axons [3]. Here, we ask to what extent the assumption of specific geometric traits influences the resulting dynamical behavior of these networks. Analyzing various characteristic measures that describe spiking neurons (e.g., coefficient of variation, correlation coefficient), we compare the dynamical state spaces of different connectivity types: purely random or purely local couplings, a combination of local and distant synapses, and connectivity structures with patchy projections.

On top of biologically realistic background states, a stimulus is applied
in order to analyze their stabilities. As previous studies [1], we also find different dynamical states
depending on the external input rate and the numerical relation between excitatory and inhibitory synaptic weights. Preliminary results indicate, however, that transitions between these states are much sharper in case of local or patchy couplings.

This work is supported by EU Grant 15879 (FACETS).
Thanks to Stefan Rotter who supervised the PhD project [3] this work is based on.
Network dynamics are simulated with NEST/PyNN [4].

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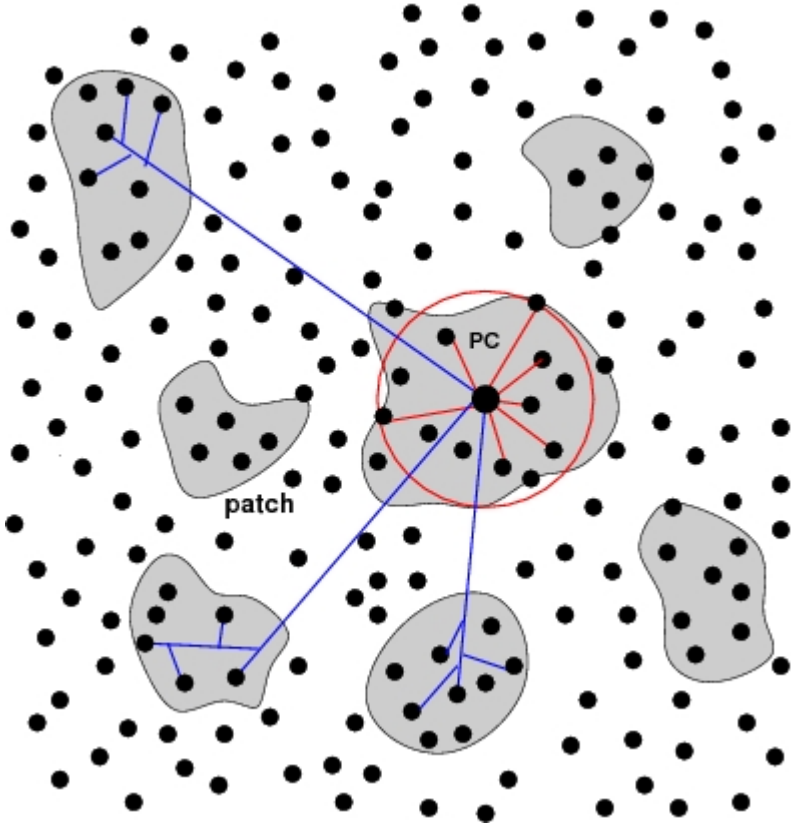
PC
patch

NeuralEnsemble: Towards a meta-environment for network modeling and data analysis

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NeuralEnsemble (http://neuralensemble.org) is a multilateral effort to coordinate and organise neuroscience software development efforts based around the Python programming language into a larger, meta-simulator software system.
To this end, NeuralEnsemble hosts services for source code management and bug tracking (Subversion/Trac) for a number of open-source neuroscience tools, organizes an annual workshop devoted to collaborative software development in neuroscience, and manages a google-group discussion forum. Here, we present two NeuralEnsemble hosted projects:

PyNN (http://neuralensemble.org/PyNN) is a package for simulator-independent specification of neuronal network models. You can write the code for a model once, using the PyNN API, and then run it without modification on any simulator that PyNN supports. Currently NEURON, NEST, PCSIM and a VLSI hardware implementation are fully supported.
NeuroTools (http://neuralensemble.org/NeuroTools) is a set of tools to manage, store and analyse computational neuroscience simulations. It has been designed around PyNN, but can also be used for data from other simulation environments or even electrophysiological measurements.

We will illustrate how the use of PyNN and NeuroTools ease the developmental process of models in computational neuroscience, enhancing collaboration between different groups and increasing the confidence in correctness of results.
NeuralEnsemble efforts are supported by the European FACETS project (EU-IST-2005-15879)

Reorganization of neuronal circuits in growing visual cortex

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The contribution of plasticity to normal development remains unsolved. Cortical growth may involve substantial modications of neural circuitry, thus enabling to study cortical plasticity under natural rearing conditions. Here, we present experimental evidence for reorganization of ocular dominance (OD) column layouts during postnatal cortical growth. Furthermore, we show that the observed mode of reorganization is predicted by a large class of models for activity dependent development of neural circuitry. We used an image analysis method based on wavelets to analyze the two-dimensional spatial organization of ocular dominance columns during postnatal cortical growth, both in flat mount sections of 2-DG labeled OD columns as well as in chronic optical recordings of intrinsic signals. Despite the fact that the size of area 17 increased by 50% between postnatal week 3 and 10, the mean spacing between adjacent ocular dominance columns increased only slightly. Consequently, the number of hypercolumns increased considerably during this expansion period. Furthermore, this process was paralleled by a strong tendency of columns to change appearance from stripe-like to disordered patterns. Theoretically, this process resembles a behavior known as the zigzag instability in dynamical systems. This is a generic behavior in expanding self-organizing systems dominated by Mexican-hat like interactions and is therefore predicted by a large class of models for activity dependent visual cortical development. We analyzed numerically the effect of cortical expansion on columnar layouts in the elastic net model and compared it to the degree of reorganization observed experimentally. We find that the observed reorganization indeed exhibits signatures of this type of instability. Thus, models for activity dependent self-organization of neural circuitry indicate that the specific mode of columnar reorganization observed in this study results from cortical expansion during postnatal growth.

Interacting point processes and neuronal modeling

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Inhomogeneous Poisson processes are much employed for modeling and analyzing neuronal spike data: an inhomogeneous Poisson process is a Poisson process the rate of which changes over time. If the change of the rate depends on the realization of some other processes, then we speak of interacting point processes.
We investigate networks of interacting point processes that use the instantaneous firing rates of their neurons as state variables.
Generalizing Hawkes' [2],[3] linear model, our nonlinear description also admits inhibitory interactions. In particular, we present
(1) a mathematical model of interacting point processes where spikes trigger changes in the instantaneous firing rates of the postsynaptic neurons;
(2) an ordinary differential equation for the mean of the firing rates;
(3) a point process equivalent of the leaky integrate-and-fire neuron;
(4) a Fokker-Plank type equation for the distribution of the firing rates of the perfect integrator.

We analyze the dynamic behaviour of the firing rates in response to nonconstant inputs; this could shed some light on the observed precise response to input transients as found e.g. in [4] and exemplified in the plot of a simulation of our system in the attached figure.

Support by the German Federal Ministry of Education and Research (BMBF grant 01GQ0420)
is gratefully acknowledged.

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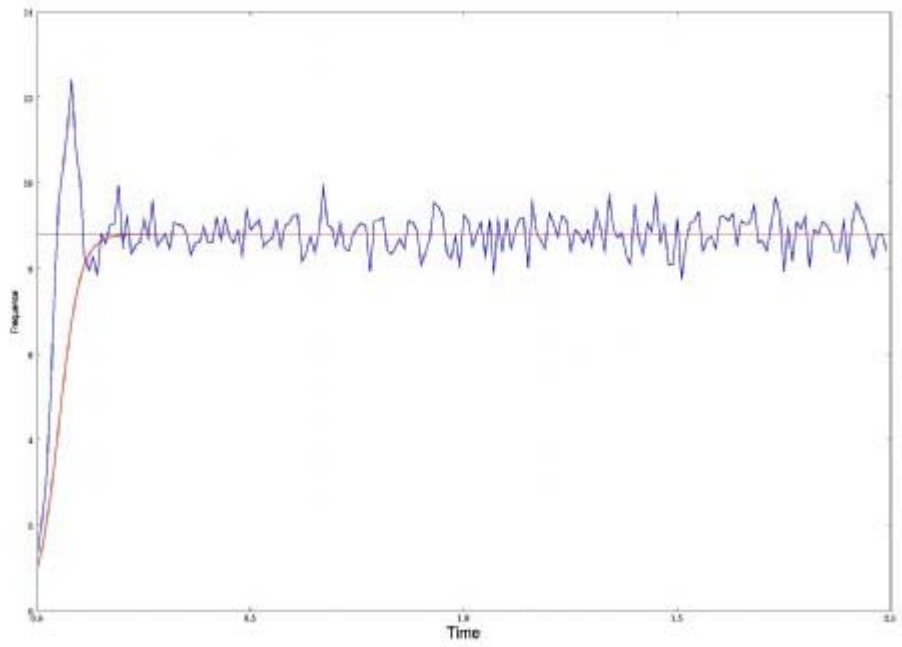
Frequency
Time

Modeling Free Monkey Scribbling by the Propagation of Synchronous Activity

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We present a model of free monkey scribbling where the propagation of synchronous activity represents unaccelerated movements in velocity space. Our architecture can be represented by a graph in velocity space: the edges of the graph represent synfire chains (SFCs) [1] which transform the velocity of the trajectory linearly between the start and end vertex. Thus, each neuron group has a velocity coding depending on its position along the edge. A trajectory is generated by integrating the population vector of all neurons. Activity in the final group of a SFC can stimulate the first group of any SFC whose start vertex corresponds with the end vertex of the previously active chain. Reliable switching to select one of several candidate SFCs with the same start vertex is feasible by a switching method based on cross inhibition of the SFCs. The network activity is sustained by a background network of highly recurrent backward and forward connected chains (BFoCs). Self-ignition within this background ignites synfire activity in the model. The BFoCs activities are suppressed while the model is active. This network architecture guarantees random ongoing parabola trajectories with smooth transitions and a drawing dynamics that obeys the 1/3 power law as observed in experiments [2,3]. An example of a 5 second trajectory generated by a graph of 10 SFCs simulated in NEST [4] is shown in the figure, where each color corresponds to propagating activity in a particular SFC.

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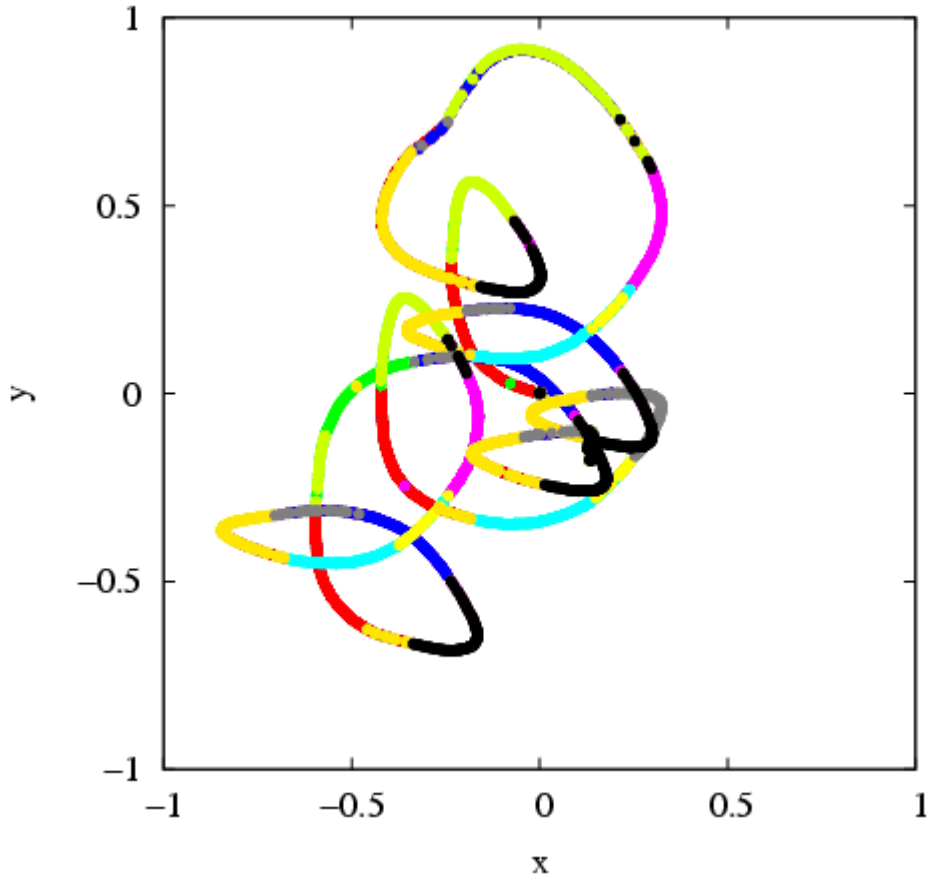
1
0.5
0
−0.5
−1
y
−1
−0.5
0
0.5
1
x

Count variability in doubly stochastic point processes

Oliver Muthmann[1], Stefano Cardanobile[1], Stefan Rotter[1,2]

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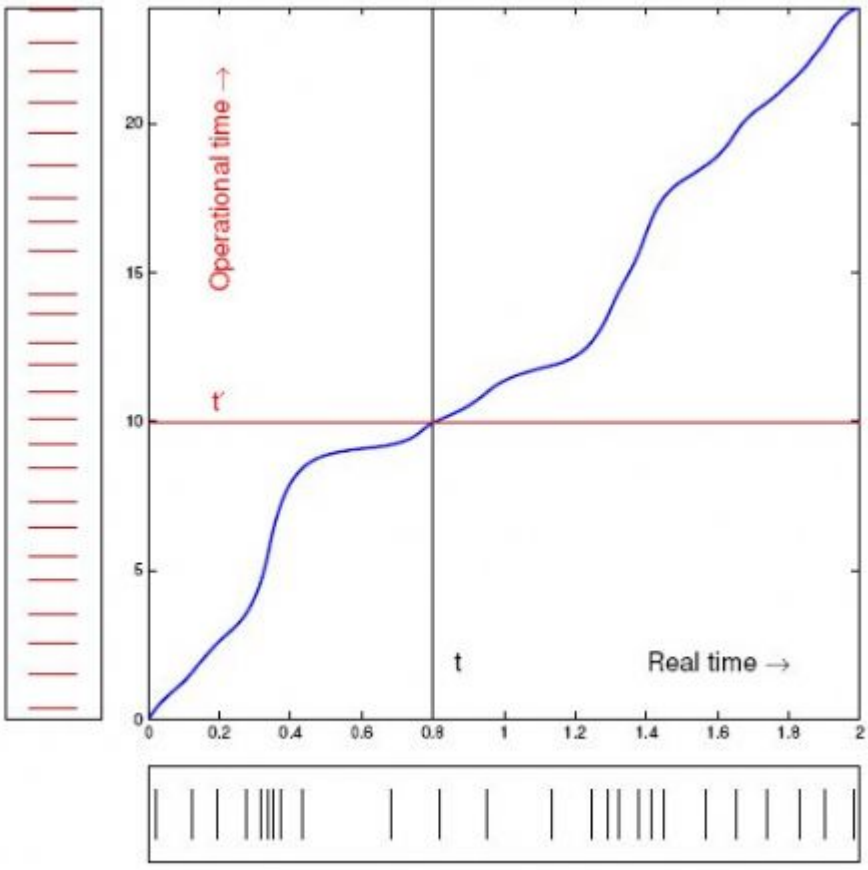
Analysis of neuronal spike trains often assumes stationarity. Slow rate transients can be accounted for by estimating the rate profile. However, it is not possible to separate fast changes in rate from the interspike interval fluctuations of the underlying point process. Here, we approach this problem by modeling the process as a doubly stochastic point process and analyzing the behaviour of the Fano factor.
The Fano factor $FF[N_T]$ is a measure of the variability of the number of events N_T (e.g. spike counts) in a fixed interval of time.
$FF(T)=Var[N_T]/E[N_T]$
It is normalized to one for a Poisson process and will be smaller if the process is more regular and larger if the process is more irregular.
In general, the Fano factor depends on the length of the observation interval and on the firing rate. We study this phenomenon in the case of doubly stochastic processes with a stationary rate process. For the simulation, this can be accomplished by generating a renewal process with a constant rate in operational time and then applying a nonlinear transformation on the time axis to adjust the rate (see Figure).
In doubly stochastic point processes, the spike count variability is increased by the rate variability and related to the autocovariance of the underlying rate.
We simulate such doubly stochastic point processes with different autocovariance functions. For the rate process, we exponentiate a gaussian process to ensure that the rate is always positive. We investigate how nonstationary firing rates affect the dependence of the Fano factor on the length of the observation. The insight gained from these experiments will supply us with information valuable for the analysis of neuronal variability.


Operational time →
t'
t
Real time →
0
5
10
15
20
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2

Frequency-invariant encoding of interaural time differences in the DNLL of gerbils

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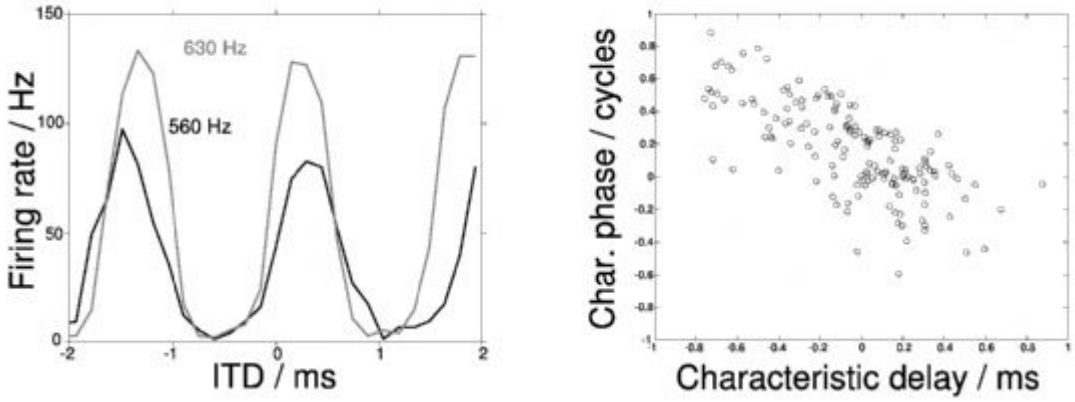
Whenever a sound originates from outside the sagittal plane, it produces a difference in time of arrival at the two ears. Many mammals, such as gerbils or humans utilize this interaural time difference (ITD) to localize the azimuth of the sound source. Neurons in the dorsal nucleus of the lateral lemniscus (DNLL) of gerbils vary their firing rates on ITD and the frequency of the sound stimulus. The firing rate as a function of ITD is called an ITD tuning curve (Fig. 1, left). The ITD that elicits the maximal response is called the best ITD. Its frequency dependence can be fitted by two parameters, the characteristic delay and the characteristic phase. We find a negative correlation between characteristic delays and phases of DNLL neurons (Fig. 1, right). In order to compute the distribution of characteristic delays and phases that maximizes the mutual information between firing rates and ITDs, we extract the noise distributions from the data. We find the noise to be suitably approximated by a Laplace distributions. The optimal distribution of characteristic delays and phases, as suggested by information theory, turns out to be qualitatively similar to the measured distribution.



Motion processing with wide-field neurons in the retino-tecto-rotundal pathway

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The retino-tecto-rotundal pathway is the main visual pathway in non-mammalian vertebrates and has been found to be highly involved in visual processing. Despite the extensive receptive fields of tectal and rotundal wide-field neurons, pattern discrimination tasks suggest a system with high spatial resolution. In this work, we address the problem of how global processing performed by motion-sensitive wide-field neurons can be brought into agreement with the concept of a local analysis of visual stimuli. As a solution of this problem, we propose a model of the retino-tecto-rotundal pathway based on a global Fourier theory of vision which offers an explanation of how spatiotemporal information is organized in the optic tectum and nucleus rotundus. The model incorporates anatomical and electrophysiological experimental data on tectal and rotundal neurons, and the basic response characteristics of tectal and rotundal neurons to moving stimuli are captured by the model cells. The model predicts that local velocity estimates may be derived from rotundal-cell responses via superposition in a subsequent processing step. Experimentally testable predictions which are both specific and characteristic to the model are provided. Thus, for the first time, a satisfactory explanation is given of how the retino-tecto-rotundal pathway enables the animal to detect and localize moving objects or to estimate its self-motion parameters.

Properties of Similarity Measures for Neural Spike Trains

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We explore three different possibilities of measuring similarity in spike trains:
A jitter measure introduced by I. Samengo, Pearson's correlation coefficient and a correlation-based measure introduced by S. Schreiber. The measures are tested on ensembles of surrogate data with varying similarity.
The Samengo measure computes the standard deviation of the spike-time jitter by means of a sliding window-technique with a fixed window size w. As the measure saturates with increasing jitter, only jitter values that are small compared to w are reliably detected.

Regarding spiketrains of high similarity, the Pearson and Schreiber measures result in similar values. However, in regimes of low similarity, the Schreiber measure is higher than the correlation coefficient; for independent poisson-distributed spike trains, where the Pearson coefficient yields zero, the Schreiber measure results in values greater than zero, dependent on the firing rate. We provide analytic estimates for the relation between Schreiber and Pearson measure for independent spiketrains at low rates.

We apply all three measures on recordings from grasshopper auditory receptor neurons that were stimulated with pulse trains with varying duty cycles and intensities.

Modelling thalamo-cortical network oscillations to study structures in real EEG data: The mathematical model as a bridge between microscopic and macroscopic dynamics

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The EEG gives an impression of the dynamic of cortical brain functions in humans and is used to investigate neurological or psychiatric diseases. Time-frequency methods are very helpful in disentangling the complexity of the EEG data and thereby contribute to a better understanding of neurobiological differences between diseases and their stages. The major aim of this work is to link certain EEG structures in the data to specific brain regions or neuronal circuits. Recent neurophysiological results on dynamics of single brain areas, the impact of inhibitory interneurons on synchronization, the dynamics on single neuron level including receptors, ion channels, and other cell dynamics enable the development of more realistic models of the underlying neuronal circuits. Usually, one models dynamics with equations of Hodgkin-Huxley type, which are very attractive when modelling a single neuron or a small number of neurons. But their complexity hinders the identification of those parameters that are most important for the dynamics. Therefore, as a first step we developed a mathematical phase oscillator model to describe dynamics of the thalamo-cortical circuit, where we concentrate on auditory sensory processing. We model macroscopic dynamics of brain areas visible in the EEG, but also take into account the neurophysiological basics. Every single area involved in the auditory processing is modelled by just one equation, which represents the mean dynamic of the local field potential of that area, which consists of postsynaptic potentials. In our model we just concentrate on the time course of the oscillation, the phase, and not on the amplitude. The hypothesis behind this is that the phase, i.e. the precise time when certain receptors are active and therefore produce post synaptic potentials, reflects time adjusted brain dynamics, which are disturbed in psychiatric diseases.

Results:

- The model allows us to simulate data, which are similar to real EEG data in humans.
- The mathematical description of the thalamo-cortical networks is used to mathematically analyze the influences of certain parameters, which is much more powerful than just doing simulations.
- Results from local field potentials of animal studies could easily be incorporated into the model.
- By using the simulations we got insights into the dynamical interactions of coupled brain areas of the thalamo-cortical circuit.
- The model mathematically specifies neurophysiological knowledge of single brain regions and their complex interactions, and finally links this on the one end to macroscopic EEG data and on the other end to microscopic measurements of local field potentials or single neurons.

In summary, the model functions like a bridge between the microscopic and the macroscopic neuronal world and the distinct ways of measuring those dynamics on different scales to understand dynamics of connected brain areas.

Detecting assembly-activity in massively parallel spike trains

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The cell assembly hypothesis [1] postulates dynamically interacting groups of neurons as building blocks of cortical information processing. Synchronized spiking across large neuronal groups was later suggested as a potential signature for active assemblies [2], resulting in specific higher-order correlations among assembly members. Recent advances in electrophysiological and optical imaging techniques allow to observe the spiking activity of large populations of neurons simultaneously, thereby increasing the chance to observe active cell assemblies in the working brain. The resulting multi-variate data sets, however, pose a major challenge to available analysis tools [3]. This holds in particular for approaches that transcend pure rate estimates or pairwise analysis, and aim for the analysis of higher-order correlations, as here the number of parameters grows exponentially with the number of recorded neurons [4]. The resulting requirements with respect to the size of the empirical sample limits the applicability of these approaches even for small populations of ~10 neurons.

We have recently presented novel procedures to detect higher-order interactions in massively parallel spike trains that are applicable to reasonable spike train samples of 10-100 seconds duration [5,6]. As a key ingredient, the procedures assume the compound Poisson process (CPP) as a parametric model to underly the superimposed and discretely sampled spiking activity of the neuronal population. This leads to a parsimoniously parametrized univariate estimation problem, circumventing the `curse of dimensionality' and greatly reducing the necessary sample size. The parametric nature of the approach yields unprecedented sensitivity, such that existing higher-order correlations are detected even in very weakly correlated populations (average correlation coefficient ~0.01) of >100 spike trains, as was revealed by numerical simulations. In this study, we systematically investigate the sensitivity of the novel procedures with respect to violations of the stationary compound Poisson assumption. Specifically, we analyze populations of correlated Gamma-processes with prescribed correlation structure, and populations with time varying rate profiles.

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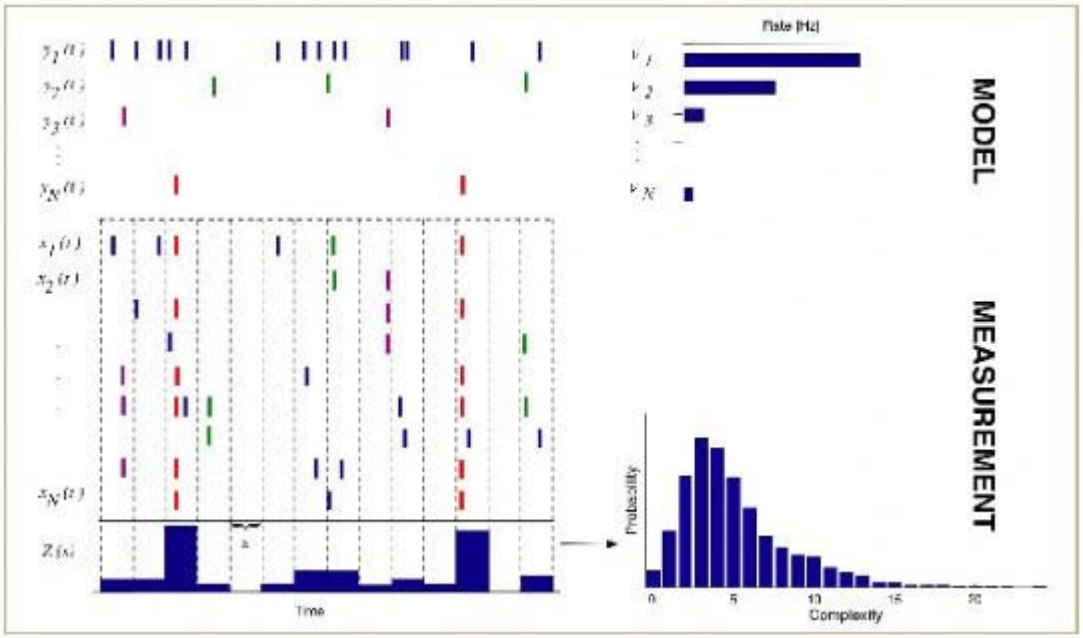
Rate (Hz)
MODEL
MEASUREMENT
Time
Probability
Complexity
0
5
10
15
20

Neural model for the visual tuning properties of action-selective neurons in premotor cortex

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The visual recognition of goal-directed movements is crucial for the learning of actions, and possibly for the understanding of the intentions and goals of others. The discovery of mirror neurons has stimulated a vast amount of research investigating possible links between action perception and action execution [1,2]. However, it remains largely unknown what the precise nature of this putative visuo-motor interaction is, and which relevant computational functions can be accomplished by purely visual processing.

Here, we present a neurophysiologically inspired model for the visual recognition of grasping movements from videos. The model shows that the recognition of functional actions can be accounted for to a substantial degree by the analysis of spatio-temporal visual features using well-established simple neural circuits. The model integrates a hierarchical neural architecture that extracts form information in a view-dependent way accomplishing partial position and scale invariance [3,4,5]. It includes physiologically plausible recurrent neural circuits that result in temporal sequence selectivity [6,7,8]. As a novel computational step, the model proposes a simple neural mechanism that accounts for the selective matching between the spatial properties of goal objects and the specific posture, position and orientation of the effector (hand). Opposed to other models that assume a complete reconstruction of the 3D effector and object shape our model is consistent with the fact that about 70% of mirror neurons in premotor cortex show view-tuning [9]. We demonstrate that the model is sufficiently powerful for recognizing goal-directed actions from real video sequences. In addition, it correctly predicts several key properties of the visual tuning of neurons in premotor cortex.

We conclude that the recognition of functional actions can be accomplished by simple physiologically plausible mechanisms, without the explicit reconstruction of the 3D structures of objects and effector. Instead, prediction over time can be accomplished by the learning of spatio-temporal visual pattern sequences. This 'bottom-up' view of action recognition complements existing models for the mirror neuron system [10] and motivates a more detailed analysis of the complementary contributions of visual pattern analysis and motor representations on the visual recognition of imitable actions.

Acknowledgments
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Computational modeling of the *Drosophila* neuromuscular junction

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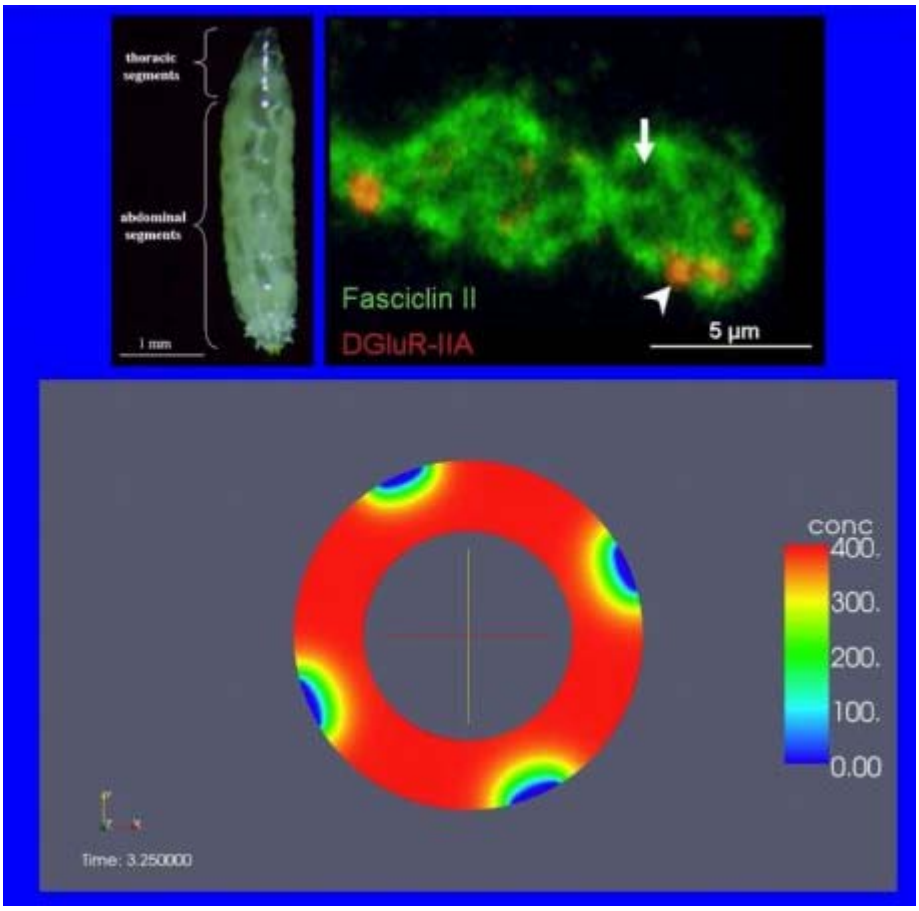
An important challenge in neuroscience today is understanding how networks of neurons go about processing information. Synapses are known to be important for this process however quantitative and mathematical models of the underlying physiologic processes which occur at synaptic active zones is lacking.
To counteract this problem, we are developing mathematical models of synaptic vesicle dynamics at a well characterized model synapse, the *Drosophila* larval neuromuscular junction. This synapses simplicity, accessibility to various electrophysiological recording and imaging techniques, and the genetic malleability which is intrinsic to the *Drosophila* system make it ideal for computational and mathematical studies.
We have employed a reductionist approach and started by modeling single presynaptic boutons. Synaptic vesicles can be divided into different "pools" however a quantitative understanding of their dynamics at the *Drosophila* neuromuscular junction is lacking.
We performed biologically realistic simulations of two bouton types characterized by partial differential equations - PDEs - taking into account not only the evolution in time but moreover also the spatial structure of two dimensions (the extension to the full three dimensions will be implemented soon). These PDEs are solved using UG.
UG is a program library for the calculation of multi-dimensional PDEs which are solved using a finite volume approach and implicit time stepping methods leading to extended linear equation systems which can be solved by using multi grid methods.
Numerical calculations are done on multi-processor computers allowing for fast calculations using different parameters in order to asses the biological feasibility of different models. In preliminary simulations, we modeled vesicle dynamics as a diffusion process describing exocytosis as Neumann streams at synaptic active zones.
The results obtained with these models are consistent with experimental results however this should be regarded as a work in progress.
Further refinements will be implemented such as models of glutamate diffusion and clearance from the synaptic cleft, post synaptic receptor kinetics, and physiologically realistic whole neuromuscular junction simulations.


thoracic segments
abdominal segments
1 mm
Fasciclin II
DGluR-IIA
5 µm
conc
400.
300.
200.
100.
0.00
Time: 3.250000

Temporal Processing in Perception and Action

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Time is a very important dimension in our life, both for perceiving stimuli changing in time, such as speech or music, and for precisely timed coordination of movements. Currently, it is debated to which extend these two domains rely on common neural mechanisms. Such a connection is believed to exist between time perception and discrete motor tasks like the production of intervals or finger tapping. However, no evidence has yet been presented to link time perception with smooth, continuous motion that is relevant in everyday tasks like reaching.

We investigate the influence of such a continuous motor task on a simultaneously performed time perception task in the hundred millisecond range. Our results show a twofold effect: On the one hand, there is a unidirectional interference of the motion task with the time perception task. On the other hand, the subjective duration of the presented intervals was systematically influenced by the state of motion.

Participants were required to follow an elliptic trajectory that was drawn on a screen, using the end effector of a robotic manipulandum. At specific segments of this guided arm motion, namely the apices of the ellipse, two short tones (standard duration 100 ms) were presented which the participants had to discriminate according to their duration. Both the discrimination performance and the perceived duration of the tones were assessed. We also performed control experiments which comprised only the time perception or the motion task, respectively, to test the performance effect of the dual task. We found that the duration of the tones were perceived as significantly shortened in the more curved segments of the ellipse, where the speed of motion is comparatively slower than near the short semi-axes. This is consistent with the notion that an internal clock rate is increased as the motion gets faster.

Furthermore, comparison of discrimination performance between the single and dual task experiments and the different states of motion revealed a rather unspecific interference of the motion task with the interval discrimination. Performance decreased in the dual task experiment compared to the single task, but there was no effect of speed or curvature. Moreover, the time task did not impair the ability to follow the target trajectory. Changes in this measure were in turn significantly affected by the amount of practice acquired during the experiment. Such an effect of unidirectional interference has so far only been observed for much longer intervals in the range of several seconds.

The present results suggest that there is a functional connection between the systems of time perception and timing of smooth motor actions.

Acknowledgement: This study was supported by a grant from the Bundesministerium fuer Bildung und Forschung (BMBF) in the framework of the Bernstein Center for Computational Neuroscience Goettingen, grant number 01GQ0432.

Vesicle tracking in neurones using an alkaloid of marine origin as marker

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Brominated pyrrole-imidazole alkaloids from sponges are known to show different properties as are the potency of some alkaloids to interact with voltage operated calcium channels (Bickmeyer et al. 2004). One pyrrole-imidazole alkaloid, Ageladine A, is known to be a metallo-proteinase inhibitor (Fujita et al. 2003), which shows additional properties: It reports pH values from pH 4 to 9 by fluorescence changes and -most probably because of its bromination- permeates cell membranes (Bickmeyer et al. 2008). The alkaloid can be used to measure pH values in individual cells as well as an indicator of acidic tissues in transparent animals. We applied Ageladine A in primary neuronal cultures from rat hippocampus. In the concentration range of 1-10 µM used for the imaging experiments, Ageladine A did not change the spontaneous synaptic activity of cultured hippocampal or cortical neurons. In video imaging experiments, single vesicles could be tracked during their directed movements in dendrites and axons. Estimated from the properties of Ageladine A, these vesicles should be very acidic. Which type of vesicles can be tracked remains to be elucidated.

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U. Bickmeyer, Achim Grube, Karl-Walter Klings, Matthias Köck, Ageladine A, a pyrrole–imidazole alkaloid from marine sponges, is a pH sensitive membrane permeable dye. BBRC 373 (2008) 419-422.

Preparations of the mouse spinal cord for in vivo imaging by 2-photon laser-scanning microscopy.

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2-Photon laser-scanning microscopy (2P-LSM) provides an excellent *in vivo* method to keep track of structural and functional changes within the spinal cord of transgenic mice with one or more cell types expressing fluorescent proteins (GFP, YFP, CFP). Together with transgenically induced chronic neurological diseases, this method may be used to follow not only structural changes, but also the outcome of therapeutical measures. Here, we describe two different experimental preparations of the mouse spinal cord for acute and chronic observations. Chronic experiments are designed for long-term survival of the animal, while for acute experiments the stability of image recording is optimized.

For chronic experiments, a volatile anaesthesia with isoflurane and N_2O was applied. The spontaneous respiration rate is used as an indicator for the depth of anaesthesia. The method is valid for access to the dorsal columns, dorsal roots, and the superficial layers of the dorsal horn. After removal of the muscle and tendon tissue, the cleft between two vertebral arches uncovers the structures of interest through the intact dura mater. If necessary, one vertebral arch may be removed (leaving the dura intact) for larger exposure of the spinal cord. With this preparation laser lesions can be applied through the intact dura to the spinal cord, or chemicals might be applied i.v., i.p. or intrathecally. Given a sufficiently sterile preparation, the animal can be repetitively sutured, awakened and re-inspected over several months.

For acute experiments with exposure of the dorsal or lateral spinal cord, we use pentobarbital-Na i.p. as initial anaesthesia, and after inserting a catheter into the jugular vein the anaesthesia is continued with methohexital-Na. A respiratory tube is inserted into the trachea for artificial respiration, which reduces respiratory movements, especially in preparations with large spinal cord exposure. Temperature and ECG are controlled throughout the experiment, and are used as indicator for the depth of anaesthesia. Two to four vertebral arches are removed to allow dorsal and/or lateral access to the spinal cord. For the lateral access, the arches have to be removed quite rigorously on one side. This procedure uncovers the dorsal root ganglia, too. For mechanical access to the roots, the dura is removed carefully. This enables to dislocate the dorsal roots for a better view to the lateral spinal cord and even the ventral roots. The vertebral column is rigidly fixed with clamps at the vertebrae rostrally or caudally to the exposed spinal cord. For the lateral approach, the clamps can be tilted by 90°. At the end of an acute experiment (with image recording for up to more than 10 h), the animals are sacrificed.

In conclusion, we are able to perform *in vivo* 2P-LSM of the mouse spinal cord in both chronic and acute experiments. These experiments may be performed over a period of several hours (acute) or weeks (chronic) even though quite severe surgery has been performed in the case of the acute experiments.

Identifying presynaptic circuitry of lobula plate tangential cells by Serial Block Face Scanning EM

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For visual course control, flies crucially rely on optic flow generated by their ego-motion. We study the neural mechanisms leading from moving retinal images to appropriate course control signals ultimately reaching the wings of the animals. Anatomically, the fly visual ganglia are divided into three layered structures: lamina, medulla and lobula complex (lobula, lobula plate). These structures are composed of repeated columns. Each column corresponds to the relative position of facets in the eye and consists of stereotyped, layer specific ensembles of neurons. Within the lobula plate, there exists a group of 60 individually identifiable motion sensitive visual interneurons, the lobula plate tangential cells (LPTCs). With their large dendrites, LPTCs spatially integrate the output signals of columnar neurons and respond to visual motion within large parts of the visual field in a directionally selective way. The computations leading from directionally unselective responses of the photoreceptors to the directionally selective response behavior of LPTCs is captured in remarkable detail by an algorithmic model which is called Reichardt-type elementary motion detector (EMD). Although some neurons of the medulla and the lobula are the main candidates, the precise neural implementation of the EMDs is still unclear.

To elucidate the anatomical correlate of EMDs we use the fruit fly *Drosophila melanogaster*. Electron dense contrast is introduced genetically by pan-neuronal expression of membrane bound HRP, visualized via DAB and Osmium staining. To obtain serial image stacks of the lobula plate and medulla columns we use the technique of Serial-Block-Face-Scanning-Electron-Microscopy (SBFSEM, Denk and Horstmann, PloS Biol 2, e329, 2004). It combines block-face imaging of backscattered electrons with serial ultra-thin sectioning (30 nm) inside the vacuum chamber of a scanning electron microscope. To image entire visual ganglia at sufficiently high lateral resolution (10-20 nm/pixel) the block-face is tiled. The ability to combine serial sectioning with EM-imaging avoids loss of sections as well as post-hoc image alignment and allows for 3D reconstructing all neuronal processes, including chemical synapses, within a given volume of tissue. Analysis of image stacks and 3D reconstruction of neural processes is performed using custom written software.

Construction of a custom-made multi-channel-electrode for investigations of frequency integration in the barn owl's midbrain.

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In the barn owl across-frequency integration takes place in the projection from the lateral shell of the inferior colliculus ('lateral shell') to the external nucleus of the inferior colliculus ('external nucleus'). The best frequency of the neurons increases from dorsal to ventral in the lateral shell. This arrangement allows the successive recording in one penetration of neurons having different best frequencies, if a single extracellular electrode is moved perpendicular to the iso-frequency slabs. It could be shown that all neurons in one penetration share one ITD (interaural time difference) and it was concluded that these neurons form a functional array that underlies the extraction of one interaural time differences from the phase equivalents present in the different frequency channels (Wagner et al. J Neurosci 7: 3105 (1987)). Strictly spoken this conclusion holds only, if responses are stationary over hours of recording time. The stationarity assumption can only be tested, if many neurons at located different depths could be recorded simultaneously. Since the nucleus lies about 15 mm deep in the brain, imaging techniques cannot be used. Instead, it would be most promising to have an electrode with multiple contact points. In principle such electrodes are available, but in fact none of these is optimally suited for recording from the arrays in lateral shell. Therefore, we aim at constructing a multi-channel-electrode which has a linear array of wires with spacing of contact points optimally adjusted to study across-frequency integration in the lateral shell.

Several prototypes were built, each having four to six insulated wires. Different wire diameters were tested. The single wires were embedded in an approximately four cm long Teflon tube, which served as a casting mould. At one end the tubing was cut into a half-pipe having a length of approximately 0.5 cm. In this region tiny holes were pricked into the Teflon tube at 0.1 mm distance. After this, one wire was mounted through each of the punctured holes. The wire was then bent at a right angle to the outer side of the tube to ensure stability in the following manufacturing process. Since the lateral shell is located at a considerable depth, the electrode has to be very stable and stiff when penetrating through the brain. To ensure this stiffness, a tungsten electrode (diameter 0.2 mm) was embedded in parallel to the wires lengthwise of the electrode. For bonding of the electrode wires and the tungsten electrode, the Teflon tube was carefully filled up with Epoxy resin. For curing, the electrode was brought to a furnace and heated at 160 °C for 2 hours. After cooling, the electrode had to be carefully removed from the casting mould and fine polished for later applications.

First tests showed that a diameter of 0.05 mm for the wires provided the best compromise of stability and stiffness of the electrode and a diameter below 0.3 mm for the electrode. Further properties like different materials (stainless steel or tungsten), resistance of the single channels, recording properties when used in experiments as well as the stationarity hypothesis will be tested in the near future.

Functional MRI on transgenic mice – genetic modification in the pain system

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Pain fulfils a protection and warning function for the living organism against noxious stimuli. In contrast pain arising from pathological dysfunctions offers no advantage and may be debilitating to patients afflicted.
The treatment of different pain states is often ineffective and efficient drugs against chronic pain are still an unmet medical need. Two cutting-edge technologies offer new ways gaining deeper insight in nociceptive processing thereby leading to the development of new compounds.
Firstly, non-invasive functional magnetic resonance imaging (fMRI) using the blood oxygenation level dependent (BOLD) signal is a standard method for studying (chronic) pain (in humans) and to discover pathways associated to the modulation and processing of pain. BOLD fMRI offers many advantages compared to conventional animal experiments, especially the non-invasiveness, the high quality of data, and the lower number of animals needed.
Secondly, genomic research has provided us with mouse strains showing highly specific genetic modifications along the nociceptive pathway.
For the first time, we were able to combine these two technologies, namely investigating non-invasive BOLD fMRI in (transgenic) mice. With this qualitative breakthrough we were able to examine central nociceptive processing in different mouse strains (hypo- and hyperalgesic) caused by peripheral heat pain (45-60 ºC, surface of hindpaws). We observed robust changes (specific for different mouse strains) in a highly specific manner (~ 64 brain areas, e.g. thalamus, somatosensory cortex, cingulate cortex, insula, hypothalamus). The distribution of these areas along the pain pathway is compatible with the knowledge of nociceptive processing in human and rat. Hence, we were able to successfully distinguish pathways conducting painful information and their modulation in (transgenic-) mice by BOLD fMRI.
We conclude that fMRI can be used as a reliable and valid method to monitor activity in the mouse brain. It is a useful tool in pain research especially in respect to genetically modified mouse strains for investigating new targets and compounds for a better pain relief.

Comparison of appetitive and aversive reinforcement in an auditory discrimination task in mice

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Behavior is influenced by both appetitive and aversive stimuli. We investigate the influence of the polarity of the reinforcement on learning-induced mechanisms. To this aim we had previously developed a paradigm that allows the application of reward (electrical stimulation of the ventral tegmental area) and punishment (footshock) in the same experimental design in the Mongolian gerbil (*Meriones unguiculatus*) (presented by Ilango *et al*. at the 7th Göttingen Meeting of the German Neuroscience Society). Here we report the transfer and adaptation of this paradigm to the species mouse (C57 BL/6). The animals were trained in a go/no-go paradigm (shuttle-box) to discriminate between two frequency modulated tones (4 to 8kHz versus 8 to 4kHz) using foot shock as aversive reinforcement and electrical stimulation of the medial forebrain bundle as appetitive reinforcement. The new experimental model permits proteomic analyses of distinct brain regions after appetitive or aversive reinforced learning or a combination of both. In addition, we plan to investigate the influence of the reinforcement polarity on learning correlates in the sensory processing using electrophysiological recording.

Novel Cre complementation indicates coincident activity of different genes *in vivo*

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Cre/LoxP recombination is the gold standard for conditional gene regulation in mice *in vivo*. The Cre DNA recombinase recognizes LoxP sites and catalyzes DNA excision between two LoxP sites. Despite the impressive success of cell-specific gene targeting, the expression pattern of a single promoter activity used to drive Cre expression is often insufficient to genetically define a distinct neural cell type that may be histologically or functionally easy to discern. To overcome this limitation of spatial precision of Cre/LoxP-mediated DNA-recombination, we added a second dimension of recombination control by designing a novel "split-Cre" system based on the complementation of Cre protein fragments. For that purpose, we constructed fusion proteins of a constitutive protein-protein interaction domain with either N- or C-terminal Cre fragments (designated NCre and CCre, respectively). Each of these split-Cre proteins did not catalyze DNA recombination alone. However, they readily assembled into a functional enzyme when co-expressed in the same cell *in vitro*. We generated transgenic mice which express NCre or CCre under the control of the *GFAP*- or *PLP*-promoter driving the expression of Cre fragments in astrocytes and oligodendrocytes, respectively. The transgenically expressed NCre and CCre fragments constitute DNA recombinase activity faithfully also *in vivo.* We found that during development, in double-transgenic split-Cre mice crossed to a recombination reporter mouse line cells were persistently labeled and were still visible in the adult. Furthermore, we have genetically defined by the simultaneous activity of the *GFAP*- and the *PLP*-promoter a subgroup of embryonic glial progenitor cells that give rise to both astrocytes and NG2-positive glia. The broad-range applicability of the split-Cre system was confirmed by using adeno-associated viral gene transfer to express NCre and CCre driven by the *Gad67*- and *Cck*1 promoter in interneurons *in vivo*. Similar to the transgenic approach, functional complementation of split-Cre was achieved, which allowed the identification of interneurons co-expressing Gad67 and CCK. Split-Cre will become a versatile tool to study transient cell populations during development and disease processes and to genetically target cell populations much more precisely, not only in the brain but also in other organs of the body.

Simultaneous behavioral and electrophysiological recording in freely moving awake rats by a novel method

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Various human neurological diseases, such as the epilepsies or Huntington's disease, have already good animal models based on behavioral, electrophysiological, neurochemical and other methods. By combination of two of the above approaches, a more powerful research tool can be achieved. Recording physiological and behavioral responses simultaneously may allow higher accuracy in obtaining correlation between these two kinds of data. Methods of investigating open field (OF) motility can be expediently combined with recording electrical activity from the cortex (but also from other parts of the nervous system or from peripheral organs).

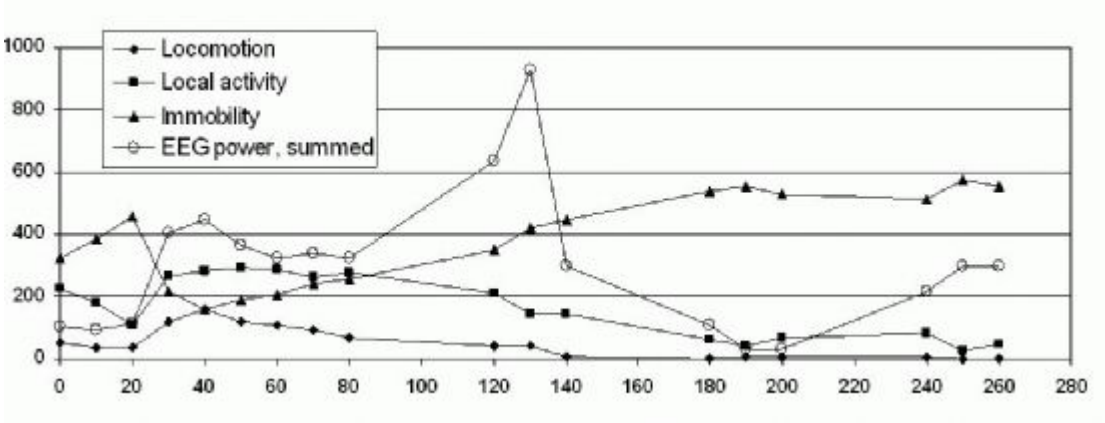
The core of the recording setup to be demonstrated is an automated (OF) box with a fine grid of infrared motion detectors. For the sake of electrical recording, it is placed in a Faraday cage. Beyond the infrared grid, a video camera is also used for recording the animal's behavior. The system was optimized for larger-size rats (ca. 300 g body weight). A connector crown is mounted on the rat's skull by means of screws and dental acrylic. The recording electrodes can be the screws, or silver wires placed epidurally. The crown allows for maximally 4 bipolar lead-offs, and connects during recording to a spring-suspended elastic cable containing the preamplifier. The recording and evaluation software enables synchronous recording of digital video image, OF motility, and electrophysiological signals; and displays the motions and the EEG spectrum in real time. The stored EEG records can be analysed for pre-defined wave bands, or over the full spectrum, at 0.5 Hz / 10 s resolution; and the OF records, for any defined behavioral element (such as ambulation, immobility, corner sitting etc.) with a resolution of 5 cm / 1 min. So, on playing back the records, it can be determined with high accuracy what kind of cortical activity corresponds to a given behavioral event recorded by the OF or the digital video.

The capacity of the system was tested, in repeated 3 x 600 s recording sessions, on the acute epileptic activity induced in rats by pentylenetetrazol (PTZ). As seen in the figure below, the rats showed increasing immobility in the first session as it habituated to the OF box. Then, 50 mg/kg b.w. PTZ was injected ip., immediately inducing increased locomotion and sharp increase of EEG power with epileptic bursts. A second injection (25 mg/kg) induced EEG power increase and bursts again, and a grand mal attack was seen on the video but no increased motility on the OF record. The changes in the EEG spectrum correlated well with onset and end of the ictal and onset of the post-ictal period.

This novel combination of methods can find applications in neurophysiological and behavioral research, but also in drug development and other applied fields.

Figure legend:

Abscissa: time. Ordinate: time (seconds) spent in the indicated form of OF behavior from the total 600 s of a session (filled symbols); or summed (1 to 50 Hz) EEG power in arbitrary units. Pentylenetetrazol was given ip. after the 20th (50 mg/kg b.w.) and another dose before the120th min (25 mg/kg).


Locomotion
Local activity
Immobility
EEG power, summed
1000
800
600
400
200
0
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280

High-resolution mapping of neuronal activity using the lipophilic thallium chelate complex TlDDC – comparison with the 2-deoxyglucose method

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Changes in neuronal activity are accompanied by changes in the metabolic rate of glucose. The patterns of glucose consumption can be mapped using Sokoloff´s 2-deoxyglucose (2-DG) method, which is the most widely used tracer technique for mapping neuronal activity in experimental animals.
The drawback of the 2-deoxyglucose method is the relatively low spatial resolution, which is due to the fact that the tracer cannot be chemically fixed and non-radioactively be detected within the tissue.
In order to overcome these problems we developed a novel tracer technique similar in rationale to the 2-DG method, but based on a tracer that can be chemically fixed and be detected non-radioactively at high spatial resolution.
This novel technique for mapping neuronal activity, thallium autometallography (TlAMG), was introduced in 2004 (Goldschmidt et al., NeuroImage 23:638-47). The technique is based on the strong coupling of neuronal activity and potassium (K^+) uptake. The K^+-analogue Tl^+, a heavy metal ion, is used as a tracer for mapping potassium uptake in a similar manner as 2-DG is used for mapping glucose phosphorylation. In contrast to 2-DG, however, Tl^+ can be chemically fixed and non-radioactively be detected in brain sections at very high spatial resolution by means of a modified histochemical method for the detection of heavy metals in the brain, a modified Timm-technique or autometallographic method.
In the initial version of TlAMG animals were injected i.p. with water-soluble thallium salts. While the changes in activity-dependent Tl^+-uptake could be mapped with high resolution at the cellular level, the uptake patterns at the regional level were influenced by regional differences in blood-brain barrier (BBB) potassium permeability. We therefore modified this approach. Instead of injecting the animals with water-soluble thallium salts we now inject the animals with the lipophilic thallium chelate complex thallium diethyldithiocarbamate (TlDDC).
After TlDDC injection regional differences in Tl^+-uptake, which are related to differences in BBB potassium permeability, are no longer present. At the cellular level, however, the Tl^+-uptake patterns remain the same as after injection of water-soluble thallium salts, indicating that Tl^+ is released from the lipophilic complex into the brain extracellular space, from which neurons and glial cells take up the ion in an activity-dependent manner.
With the regional differences in BBB K^+-permeability no longer influencing the Tl^+-distribution, the brain regional Tl^+-uptake patterns should be very similar to the patterns seen with the 2-DG method. We here tested this hypothesis. We intravenously injected animals with both tracers and analyzed the Tl^+ and the 2-DG distribution in neighboring serial sections through entire rodent brains. We find very similar uptake patterns with both tracers as expected from the fact that K^+/Tl^+-uptake is mediated to a large degree by the Na,K-ATPase, and is coupled, therefore, to the metabolic rate of glucose.

High-resolution mapping of neuronal activity using the lipophilic thallium chelate complex TlDDC – tracer kinetics

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The monovalent thallium ion Tl+ has been used for decades as a potassium (K+) probe in K+-uptake studies in vitro and in vivo. In neurons potassium permeability, Na,K-ATPase activity and, hence, the rate of K+-uptake increase upon depolarization or sodium influx, respectively. The activity-dependent increase in the rate of K+-uptake has been shown in squid axons in vitro already decades ago using radioactive isotopes of K+ or K+-analogues like thallium (Tl+).

Based on these in vitro findings we introduced in 2004 a novel method for mapping potassium uptake and neuronal activity in the rodent brain (Goldschmidt et al., NeuroImage 23:638-47) using the heavy metal ion Tl+ as tracer. We injected animals i.p. with the water-soluble thallium salt thallium(I)acetate, stimulated the animals for 15 min, and mapped the distribution of thallium in brain sections by means of a non-radioactive high-resolution histochemical technique, a modified Timm-technique or autometallographic method.

With this method we were able to reveal patterns of neuronal activity with cellular resolution, but the images were influenced by regional differences in blood-brain barrier potassium permeability.

We later found that, after injection of the lipophilic thallium chelate complex thallium diethyldithiocarbamate (TlDDC), regional differences in Tl+-uptake, which are related to differences in blood-brain barrier potassium permeability, were no longer present. At the cellular level, however, the Tl+-uptake patterns remain the same as after injection of water-soluble thallium salts, indicating that Tl+ is released from the lipophilic complex into the brain extracellular space, from which neurons and glial cells take up the ion in an activity-dependent manner.

We here provide further evidence that brain potassium metabolism and neuronal activity can be mapped with very high spatial resolution after intravenous injection of TlDDC. We reasoned that, if Tl+ is released from TlDDC into the brain extracellular space, the time course of Tl+-uptake in neurons in vivo - or in more general terms: the Tl+ tracer kinetics in vivo - should be the same or similar to the time course or tracer kinetics, respectively, of Tl+- or K+-uptake in neurons in vitro.

In contrast to 2-deoxyglucose, which is metabolized and trapped within cells by phosphorylation, Tl+ moves through the neuronal cell membrane in both directions. With increasing influx of the ion the efflux out of the neuron increases in return until influx and efflux equilibrate and no net flux across the neuronal cell membrane occurs. Neuronal activity can only be mapped as long as the influx dominates over the efflux. At equilibrium the Tl+-distribution in the brain is expected to be stable. As the turnover rates of K+ or Tl+ increase with increasing activity, neurons or brain regions with higher activity will approach the equilibrium faster than those with lower activity. We here show that this in fact the case, and that the time courses of approaching the equilibrium are similar to those of K+ or Tl+ in vitro as reported in the literature.

Relacs - a modular software platform for closed-loop and dynamic clamp experiments

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Relacs ("Relaxed ELectrophysiological data Acquisition, Control, and Stimulation") is a fully customizable software platform for data acquisition, online analysis, and stimulus generation specifically designed for electrophysiological recordings. Filters and spike detectors can be applied instantly on the recorded potentials. Freely programmable, hardware independent C++ plugins can access the preprocessed data for further online analysis and visualization. Therefore the experimental protocols can automatically adapt a stimulus (e.g. offset, variance, etc.) in a closed loop fashion and thus completely control the running experiment. For programming the experimental protocols, the Relacs package also provides a data analysis library containing algorithms for basic statistics (moments, quartiles, histogram), power spectra, coherence, transfer function, linear fits, non-linear fits (Simplex, Levenberg-Marquardt), firing rates (mean, PSTH binned/kernel, 1/ISI), CV, Fano factor, ISI correlations, vector strength, reliability, jitter, mutual information (lower and upper bound), etc. A simulation mode not only allows to test and improve the experimental protocols, but also to check the performance of models with the very same procedures that were used in the real electrophysiological experiments.

The dynamic clamp is a closed-loop experiment on a per sample basis where each sampled value of the cell's membrane potential is used to compute a current that is injected back into the cell. Relacs supports software dynamic clamp, i.e. no additional hardware is needed, that is implemented as an RTAI real time Linux kernel module. For using the full potential of discontinuous current-clamp amplifiers for the dynamic clamp we are synchronizing the switching cycle of an npi SEC amplifier with the software loop (in collaboration with H. R. Polder, npi electronic GmbH, Tamm, Germany).

Very important for enhancing the reusability of acquired data is their annotation with meta-data that specify the context of the experiment. Upon completion of a recording, Relacs immediately forces the experimenter to provide this important information through a freely configurable dialog. In addition, for each recording all configuration files, log files, and version numbers and settings of the experimental protocols are saved as well. All the meta-data can be automatically stored in LabLog (see Poster by Jan Grewe) for enhancing reusability of the recorded data.

Relacs is free and open software published under the GPL to foster development and exchange of innovative experimental protocols and analysis techniques. In particular, Relacs will be a test bed for the data analysis toolboxes and data format interfaces from the German Neuroinformatics Node (www.g node.org). For more information and downloads visit www.relacs.net.

Novel sensor proteins as tools for in vivo optical imaging of cAMP dynamics in *Drosophila* brain

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The ubiquitous second messenger cAMP is involved in numerous biological processes. However, technical limitations make it difficult to resolve the spatio-temporal dynamics of cAMP signalling using biochemical tools. Here we present an evaluation of multiple DNA-encoded fluorescent sensors, as candidates for real time monitoring and comprehensive studies of cAMP dynamics *in vivo*. Those sensors feature single cAMP binding domains, derived either from human and murine Epac protein or murine HCN2 channel, fused to YFP- and CFP-proteins (Nikolaev et al. 2004, Nikolaev et al. 2006). We express the different cAMP sensors in the mushroom body of the fly and assess their signal-to-noise-ratios and kinetics, as well as compare them to a tetrameric PKA based cAMP sensor. Our prospect is to elucidate changes in local cAMP levels in the context of learning and memory formation. First results will be presented.

Targeted-esterase-induced dye loading (TED): a new non-disruptive strategy to target calcium indicator dyes to the endoplasmic reticulum

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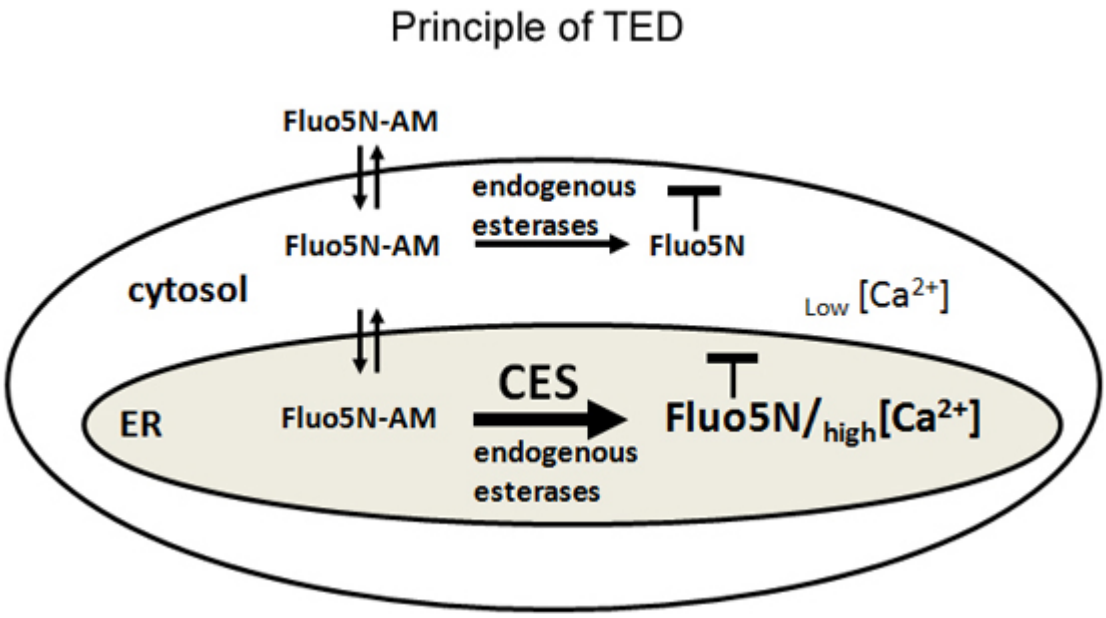
For the analysis of Ca^{2+}-dependent signaling, acetoxymethyl (AM)-derivatized ion indicators have become a popular tool. These indicators permeate membranes in an ion-insensitive form but, within cells, esterases hydrolyze these compounds to release ion-sensitive dyes. However, the properties of these indicators limit their targeting to subcellular structures such as the endoplasmic reticulum, the dominant intracellular Ca^{2+} store. Recently, we introduced a new strategy to target low-affinity, synthetic calcium indicator dyes to the endoplasmic reticulum (Cell Calcium: 44, 386-399, 2008). The method that we call Targeted-Esterase-induced Dye loading (TED) bases on the targeted, recombinant expression of a high Carboxylesterase activity in the lumen of the endoplasmic reticulum. After esterase-based dye loading, this additional esterase activity allows improved trapping of Ca^{2+}-sensitive forms of low-affinity Ca^{2+} indicators within the ER. Here we show an active soluble core element of the Carboxylesterase 2 (CES2) thatcan be used for the development of new targeting constructs. This allows the development of fusion proteins with an enzymatically active CES core. While this core-element showed excellent performance characteristics in the ER, it was inactive in the cytosol as well as in mitochondria. In addition, we will present the performance of the TED method in cortical astrocytes in vitro, where the method allows direct high-resolution imaging of intracellular calcium stores as well as the direct visualization of the ER-refilling process after agonist-induced store depletion.



The Laboratory Logbook - database approach for project documentation

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Laboratory-journals are used to document the progress of a project, to note results or to sketch ideas. Commonly these journals are handwritten notebooks or, in the worst case, a loose collection of notes. For personal use this might be sufficient but from a lab's perspective with a current flow of people this might not be optimal. In case a person leaves the department large parts of the knowledge leave as well. Furthermore handwritten journals, besides a sometimes dreadful scrawl, have no unique naming and sorting scheme which makes them extremely hard to search. The same applies for the data recorded in a laboratory. Lots of data is acquired and notes about the recording conditions may be written on protocol sheets or saved along with the data in some kind of log-files. Again, it is extremely hard to retrieve this information. Thus, lots of the knowledge gained throughout the years is basically not available to other members of the lab or project successors without huge efforts.

In order to facilitate finding information or data that has been acquired in the lab the Laboratory Logbook (LabLog) is a software that offers the framework to store project related documentation and meta-information about acquired data (including the file locations) in a more structured way. The LabLog includes tools to store information about the department, its subgroups and researchers as well as a journal for actual documentation and a data manager. Within the journal stimuli, experiments, analyses, and results are described. The journal also offers a personal diary to take short notes which also can be searched. As mentioned above, the database stores meta-information about the collected data which is managed in the data-manager, not the data itself. This part of the program offers the opportunity to search for certain kinds of data and to retrieve all stored information related to it as well as e.g. export retrieved data-file lists to text files e.g. for further processing with your preferred analysis-tool.

LabLog is written in Java and hence is platform independent. All information is stored in a relational database hosted by a mySQL server that provides the powerful searching capabilities of a database system. Furthermore, the LabLog can be linked to the **Relacs** recording environment (see Poster by Jan Benda; www.relacs.net) and then directly imports information about recorded data to the database.

An online algorithm for simultaneous spike detection and spike sorting based on matched filters and deconfusion

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To understand higher cortical brain functions, an analysis of the simultaneous activity of a large number of individual neurons is essential. One common way to acquire the necessary amount of neural activity data is to use simultaneous extracellular recordings, either with single electrodes or, more recently, with multi electrode arrays. However, the recorded data does not directly provide the isolated activity of single neurons, but a mixture of neural activity from many neurons additionally corrupted with noise. The task of so called "spike sorting" algorithms is to reconstruct the single neuron signals (i.e. spike trains) from these recordings.
In the past years many spike sorting algorithms have been developed. However there are only few algorithms which operate online and explicitly address the following needs: i) formulated for and making use of data from multi electrodes ii) high performance in detection and separation of overlapping spikes and iii) being able to adopt to non-stationarities of the data as caused by electrode drifts.

We present a combined real-time online spike detection and spike sorting algorithm which explicitly address these three issues. A spike sorting algorithm for multi electrode data is formulated, which detects and resolves overlapping spikes with the same computational cost as non-overlapping spikes and tracks variations of the data.

In an initial step, the number of neurons and their corresponding wave-form templates are estimated. From these templates a set of optimally matched filters is calculated. The filters constitute an approximation of an exact deconvolution (which is in general impossible for noisy data) and thus greatly improve the signal-to-noise ratio. Instead of thresholding directly the output of each individual filter in order to detect/cluster spikes, we apply an additional transformation called deconfusion. In particular, by considering the maximal responses of all filters to every template, an un-mixing matrix is obtained. Similar to the ICA technique, this matrix is applied stepwise to the filter output providing a source separation. This minimizes the energy of the filter output of every filter to non-matching templates. Finally, a well-defined threshold is applied which leads to simultaneous spike detection and spike sorting.

Our method needs an initialization phase, in which any supervised or unsupervised technique can be used to find the wave-form templates. Once the initial wave-forms are estimated, the algorithm can operate online and, because of the easy to implement linear operations, also in real-time. By incorporating a direct feedback from the newly found spikes to the existing templates, the matched filters are adapted constantly allowing for the detection of varying spikes shapes due to electrode drifts and non stationary noise characteristic.

We evaluate the method on simulated and experimental data, including simultaneous extra/intra-cellular recordings and data from prefrontal cortex of behaving macaque monkeys. We compare the results to existing spike detection as well as spike sorting methods including thresholding combined with principal component analysis. We conclude that our algorithm is indeed able to resolve successfully overlapping spikes and outperforms other methods under realistic signal to noise ratios.

Acknowledgments
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Peptidomics of single identified neurons of the Arcuate Nucleus of the Hypothalamus

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Neuropeptides are signalling molecules which show remarkable chemical and functional diversity in invertebrates as well as in vertebrates, and are involved in neuromodulation, neurotransmission, and hormonal signalling. The identification of neuropeptides from specific tissues or even single cells is an important step to understand physiological processes in an organism and to design novel experiments. Here, we describe a protocol for the isolation of identified peptidergic cells from a specific region of the mouse brain (pituitary gland) which contains POMC (proopiomelanocortin) expressing neurons. By using the Cre/IoxP recombination system to drive green fluorescent protein (GFP), we first identified individual pituitary cells in tissue slices. These neurons were isolated with the help of a fluorescence stereo microscope directly from the fresh untreated tissue and subjected to MALDI-TOF mass spectrometry. The profilings of these fluorescence labelled cells resulted in reliable mass spectra, containing at least 12 products of the POMC prohormone. Active post-mortem proteolytic processes are phenomenons which can be obtained during the preparation procedure of the brain. These protease activities may lead to the detection of protein/peptide degradation products. The combination of post-mortem microwave-irradiation with single cell dissection from brain slices (200µm) allowed the exclusive detection of endogenous neuropeptides. Thus, this method not only anabled the fast identification of neuropeptides on the single cell level but also the analysis of cell-specific precursor processing which is typical of neurons in living animals.

Identifying Electrically Active Cells in Neuronal Culture and Tissue using CMOS based Multi-Transistor Arrays (MTAs)

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A unique feature of CMOS based Multi-Transistor Arrays (MTAs) compared to Metal-Electrode Arrays (MEA) is the high density of the sensor pixels over a large sensor array [1-3]. Key parameters for MTAs are a spatial resolution of 7.8µm, a temporal resolution of 6 kHz (full frame readout) and a size of 1mm² (16384 sensors in total).
When using these chips for measuring the electrical activity of neurons in culture or tissue, usually the signal of one neuron is detected on several transistors.We make use of this feature to automatically identify action potentials and individual neurons in recorded data, even if the coupling area of neighboring cells overlap and therefore a sensor transistor records activity of different cells.
In a first step we detect statistically significant data by examining the combined deviation of the signal from its average on the considered transistor and its neighbors in space and time. This results in a map of data points in space and time for each action potential of all electrically active cells. By grouping signals that form cohesive neighborhoods in space and time we can identify action potentials. By examination of the cross correlation between pairs of action potentials it is possible to identify single cells, even if the coupling area of neighboring cells overlap and therefore a sensor transistor records activity of different cells. We show an application of this method to dissociated cultures of hippocampal rat neurons and rabbit retina.

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Quantitative dendritic organization of the rat deep cerebellar Nuclei: a MAP-2 immunostaining and laser scanning microscopic approach

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Dendrites together with axons constitute the majority of the brains neuropil. Previous attempts to describe the organization of dendrites within a brain region have relied on qualitative descriptive approaches, or single neurons staining. In this study we applied an alternative approach that in principle could allow to extract several important parameters that describe the dendrites characteristics and that is sufficiently fast to allow for large sample analysis. We have used previously developed algorithms to segment and trace dendrites that were stained with the specific dendritic marker MAP-2 a+b and that were subsequently imaged in 3D with a laser scanning confocal microscope. The main steps of the algorithm included segmentation and centreline thinning to produce a skeletonized representation of the dendrites with cylinders representing the dendrite thickness at the corresponding points. To validate the MAP-2 approach we compared our results to data obtained from intracellular neurobiotin filling of single rat DCN neurons and manual 3D tracing and reconstruction (Sultan et al., 455, pp 139-155, 2003). We found a good correspondence between the average dendritic thickness obtained by both methods (0.77um by single cell analysis and 0.84um by the group analysis approach). We also compared the dendritic volume fraction obtained from the group analysis to an indirect estimate utilizing neuron density, dendritic length and thickness obtained from previous studies. Here too we found a good correspondence between the two methods (indirect estimate: 12% vs. MAP-2 analysis 11%). The low dendritic volume fraction obtained by both our approaches (compared to 23-30% in Neocortex) may be due several factors:1) underestimation of the dendritic volume fraction due to an additional underestimation of the single neuron's dendrite length as obtained by our previous study, 2) extended extracellular space occupied by the well-developed extracellular matrix in the DCN, 3) or by the larger proportion of axon's in the DCN neuropil.

Thallium-Autometallography in chicken midbrain slices – a method for the study of neuroarchitecture?

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The architecture of neuronal circuits is a key factor for understanding their functionality. In principle there are two ways to visualize the neurons involved: a brute force approach by anterograde / retrograde labeling of all neurons in an area, or a one-by-one approach with single cell labeling. In both cases one does not know if neurons projecting to a similar area are indeed physiologically connected. Recent techniques using expression of fluorescent proteins ("brainbow" [1]) allow to draw conclusions on neuron-to-neuron connectivity and even networks, but are not feasible in all animal systems and in all labs.

Thallium autometallography seems to be an interesting alternative. Based on the fact that thallium is transported into cells by Na^+/K^+ pumps instead of potassium, neurons plus their postsynaptic targets should incorporate it upon electrical activity. Subsequently, it can be precipitated by sulphide and developed by silver staining [2]. This has been proved *in vivo* in gerbils[3] and pigeons[4].

Here, we present the results on thallium autometallography in slices of the chicken midbrain. The midbrain is involved in the processing of visual stimuli in vertebrates. It is related to spatial orientation by integrating all available sensory modalities and by generating a premotor signal. The principle architecture of neuronal circuits underlying in the signal processing in different layers of the optical tectum and the nuclei isthmi is mostly known. Here, neurons form exclusive feedback loops making this an ideal model for such a study.

Neurons were electrically stimulated to specifically activate Na^+/K^+ pumps, which resulted in an increased thallium uptake. We demonstrate the effects of different protocol parameters onto staining pattern and quality.

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2. Danscher, G., *Histochemical demonstration of heavy metals. A revised version of the sulphide silver method suitable for both light and electronmicroscopy.* Histochemistry, 1981. **71**(1): p. 1-16.
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Wireless raw data acquisition system for neuronal activities from freely moving animals

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Neurophysiological research aims at understanding the brain activity underlying behaviour. Many forms of complex behaviour in primates can only be investigated in natural environments. Recording neuronal activities in freely moving or socially interacting monkeys is still challenging due to the required bandwidth for signal transmission and recording stability, while at the same time energy supply, construction space, and acceptable weight for telemetric devices are very limited.
We designed a telemetric device to record neuronal activity without a need for constraining the animal's movements. In the current version we aim to monitor four input channels (four independent channels of a Thomas RECORDING tetrode), amplify and transmit the recorded data to a Lab PC for further analysis.
Different to most available systems the present system will be able to transmit the complete raw stream of all four channels (digitised at 25 ksmp/s) without the need for an online threshold pre-selection and thereby keeping the full information content of the signal.
Digital wireless transmission is very robust to electromagnetic noise sources which usually exist in a technical environment. Also, in contrast to widespread analogue systems our digital device is capable of event triggered actions like a wake-up in response to a change in signal intensity and pre-processing.
Importantly, we implemented a bi-directional wireless communication protocol. Together with the fully integrated micromanipulator, and different from other available systems, this allows to remotely re-adjust the microelectrode positions without the need to handle the animal. This new technology ensures a precise tracing of signals from individual neurons during free movement.
To allow the highest possible resolution a cascade of two programmable amplifiers fits the different amplitudes independently for each channel. In combination with the fixed pre-amplification a gain between 20 dB and 80 dB can be programmed remotely.
The platform is mounted on the animals head and contains the programmable microcontroller, the pre- and main amplifier, and the motor drive and controller. The system is powered by a LiPo-battery with a capacity of 380 mAh allowing a continuous operation for more than two hours.

Summary
A stand-alone digital wireless system for acquisition of neuronal activity on primates is described. It is capable of recording, processing and transmitting four independent neuronal signals. Bi-directional communication allows the integration of a micro-drive to feed the electrodes.

Photo-activation of neuronal tissue using a spatial light modulator (DMD)

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Photo-activation of excitable cells has proved to be a powerful tool to study connectivity patterns in neuronal slice tissue. Current experimental setups for the release of caged neurotransmitters or activation of transgenic cells with light sensitive ion channels employ a single light beam. In a conventional mapping experiment, an intracellular recording from a single cell in the slice is established, and the light beam is consecutively focused onto different locations within the surrounding tissue, eliciting spikes in the local cells. These spikes can be detected as postsynaptic events in the intracellular recording if a functional connection is present within the tissue, and a connectivity map can be generated from the location of light irradiation and the postsynaptic response measurement.

Single-beam based experimental systems impose certain limitations on spatial mapping experiments: the strictly sequential activation of cells limits the mapping speed, the spatial range of the maps depends on the optical properties of the microscope and objective. Moreover, the UV lasers usually used for photo-activation are expensive.

Here, we present a novel experimental setup for uncaging experiments that overcomes these limitations by employing a digital mirror device (DMD). It is used to project a spatial light pattern into the slice tissue, employing an ordinary arc lamp as light source. Our device can operate independently from the microscope optics and allows for an extremely flexible choice of stimulation parameters for each presynaptic location. In addition, the setup could also be used in dynamic stimulation paradigms and for parallel stimulation of multiple sites. Here, we explain the technical realization of the setup and demonstrate its use for the generation of connectivity maps in acute slices of neocortical tissue. Using spatial light modulators for photo-activation of neuronal tissue will open new possibilities for the investigation of connectivity and dynamic signal integration in neuronal tissue.

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Soundcards as Recording and Playback Device in Research and Education

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Modern soundcards are highly sophisticated hardware components, designed to record or playback sounds on a computer. Apart from its capability to produce or record sounds, a soundcard can also be regarded more generally as a digital-to-analog (DA) and analog-to-digital (AD) input/output (IO) interface. Modern soundcards support high sampling rates up to 192 kHz and high bit resolutions up to 24 Bit.Analog waveforms, e.g. extracellular recordings, muscle potentials or every other kind of biological potential, can be captured very precisely. The playback of auditory stimuli is also very precise with high signal-to-noise ratios of above 100dB. Thanks to high sampling rates also supersonic stimuli can be presented with a soundcard. Soundcards with eight input and output channels are available below 300 € whichcould make high quality multi-channel recording and playback extremely well-priced. If more than eight channels are required, it is possible to cascade soundcards to achieve even more channels.
Despite the fact that it is technically possible to use soundcards in research and education, the use of soundcards is still an exception. The lack of appropriate software is probably the main drawback. Although Software Development Kits for addressing soundcards are available, building a software application from the beginning can be very time consuming.
Here I present software tools that turn a soundcard into a high-class waveform generator and an oscilloscope. Recorded and generated waveforms can be analysed with respect to their frequency content and can be filtered if necessary. Additionally I provide a professional software environment for behavioural and electrophysiological experiments in education and research. Auditory stimuli can be designed easily with the integrated stimulus designer or applied as wav-files. Since these tools are built on the Windows sound architecture it is not restricted to a special kind of soundcard. Every soundcard with a Windows driver can be used. Most of the functionality of these tools is implemented in software. By this virtual instrument design, the user benefits both by software updates and by new and improved hardware components. This virtual instrument design makes it easier, cheaper and faster to adapt new trends in science and keeps the user always up-to-date.

Analysis of disulfide-bonds in neuropeptides by means of MALDI-TOF using the matrix 1,5-Diamino-Naphtalene

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Neuropeptides are neuroactive peptide messenger molecules which activate specific transmembrane receptor proteins (mostly G-protein-coupled receptors) to transduce signals to the inside of a neuron or other target cells.
Neuropeptides show a high degree of structural diversity and play vital roles both as neuromodulators within the nervous system and as hormones released from neurohemal sites or endocrine cells into the circulation. Thus, neuropeptides occupy a high hierarchic position in the coordination of physiological events, and are likely to be involved in the control of behaviour, reproduction, development, feeding and many other physiological processes.
During processing of inactive propeptides neuropeptides undergo posttranslational modifications such as N-terminal pyroglutamate-formation, amidation, phosphorylation, sulfation, O-methylation and disulfide-bond formation. The detection of such posttranslational modifications is an important step in the characterization of neuropeptides.
Here we describe a protocol for the suitability of MALDI-TOF (Matrix Assisted Laser Desorption Ionisation-Time of Flight-) mass spectrometry to detect disulfide-bonds in the secondary structure of amino acid sequences of synthetic and native neuropeptides, using the 955.4 Dalton CCAP (Crustacean Cardio Active Peptide). CCAP is a nonapeptide that contains one disulfide bond between the amino acids at the position three and nine. In our experiments we performed MALDI-TOF mass spectrometry with the matrix 1,5-Diamino-Naphtalene (1,5-DAN). We mapped the cysteine residues that are responsible for disulfide-bond formation and confirmed the disulfide-bond in native CCAP by analysing nervous tissues with of MALDI-TOF/TOF mass spectrometry.

My First Neuron: an Educational Tool for Teaching Neural Computation

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Although the type of parallel, distributed computation performed by neurons will probably be a prominent paradigm in future computing systems, there are virtually no accessible educational tools for teaching the basic principles of neural computation. We have developed a new educational tool called My First Neuron to address this need. It is comprised of a set of greatly enlarged toy "neurons", each spanning about 20 cm, which students physically connect together to form networks. By engaging in this construction process we aim to convey to students both the theoretical aspects of neural networks and an intuitive sense of the physical constraints under which neural networks are constructed and operate. Each battery-operated neuron has multiple user-selectable models, four adjustable model parameters and an integrated visual/auditory (LED bar graph and speaker) readout of internal states. The neuron models in My First Neuron are available as a neuromorphic VLSI silicon integrated-and-fire neuron chip, or as software running on a conventional microcontroller. The electrical connections between the neurons are implemented using standard plugs at the "axon" terminals and corresponding sockets at the "dendrites" or "soma".

My First Neuron has been tested in teaching on graduate neuroscience students. They were able connect the neurons and adjust the cell parameters to demonstrate simple computations such as winner-take-all circuits and central pattern generators. In addition, they were able to access the bare electronics inside the neurons to perform "intracellular recordings. Future students will be able to upload new software neuron models in the microcontroller-based embedded neuron system, and to connect elementary sensors (light detectors, whiskers) and effectors (artificial "muscle" units) to the system.

Quantitative measurements of cAMP concentration with a new Epac Based FRET-Sensor

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FRET-based biosensors are becoming more and more important for the analysis of intracellular signaling, including cyclic AMP. When employing a mono-molecular cAMP biosensor, the donor/acceptor emission ratio of the sensor, when excited at the donor excitation wavelength, can be used to follow relative changes in the cAMP concentration level. A quantitative concentration analysis under this conditions, however, is impossible, because the ratio varies not only with the changes in cAMP concentration, but also with the changes of the biosensors microenvironment, e.g. ion concentrations, pH, metabolic state, protein folding. Under hypoxic conditions, which for example are encountered during stroke, intracellular pH decreases significantly. To measure cAMP-dependent signaling pathways under these hypoxic conditions, we developed a spectral FRET analysis on the basis of two excitation wavelengths. Thus we obtained a reliable measure of the absolute cAMP concentrations with high temporal and spatial resolution. Additionally we improved a novel FRET-biosensor based on the cAMP binding protein Epac by exchanging the donor and acceptor with fluorescent proteins, with less sensitivity to changes in the microenvironment under pathological as well as physiological conditions.

A novel CreERT2 'knock in' mouse line to study gene functions in single projection neurons of the mouse neocortex

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To study gene functions in individual cortical projection neurons embedded in a complex neuronal network is a challenging task. Among several genetic engineering strategies that have been developed to study functions of a gene in vivo, 'conditional mutagenesis' permits the spatial control over genomic DNA manipulations. However, the standard versions of 'Cyclic recombinase' (Cre) do not allow for temporal control. Therefore, we have generated a novel mouse line that expresses a modified Cre recombinase (CreERT2) that is fused to a mutated human estrogen receptor (ER) ligand-binding domain. Addition of tamoxifen, a synthetic ligand of the ER, induces nuclear transfer of the CreERT2 variant and site-specific recombination of loxP-flanked genomic DNA.

To direct expression of CreERT2 to projection neurons of the telencephalon, we chose regulatory sequences from the Nex gene. Nex belongs to the NeuroD-family of neuronal basic helix-loop-helix (bHLH) proteins and is predominantly expressed in the dorsal telencephalon. To mimic the endogenous Nex expression pattern, the coding region of the Nex gene was replaced by a CreERT2 expression cassette using homologous recombination in ES cells. Resulting NEXCreERT2 'knock in' mice were breed to reporter mouse lines that express lacZ- and GFP-reporters upon Cre-mediated recombination. Application of tamoxifen to adult mice induced reporter gene expression in projection neurons of the neocortex and hippocampus, but was completely absent from interneurons, oligodendrocytes, and astrocytes. Since recombination occurred only in a subset of neocortical projection neurons, the NEXCreERT2 'knock in' mouse line will be a valuable tool to perform 'in vivo single cell genetics' in the murine cortex.

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