6TH MEETING OF THE GERMAN NEUROSCIENCE SOCIETY
- 30TH GÖTTINGEN NEUROBIOLOGY CONFERENCE -

SATELLITE SYMPOSIUM V

"Joint Symposium of the DFG Neuroscience Graduate Schools"

FINAL PROGRAM

February 16, 2005, 13.00 – 19.30 h
Georg-August-Universität Göttingen
Geisteswissenschaftliches Zentrum, ZHG ("Blauer Turm")
Platz der Göttinger Sieben 5, Göttingen, Germany

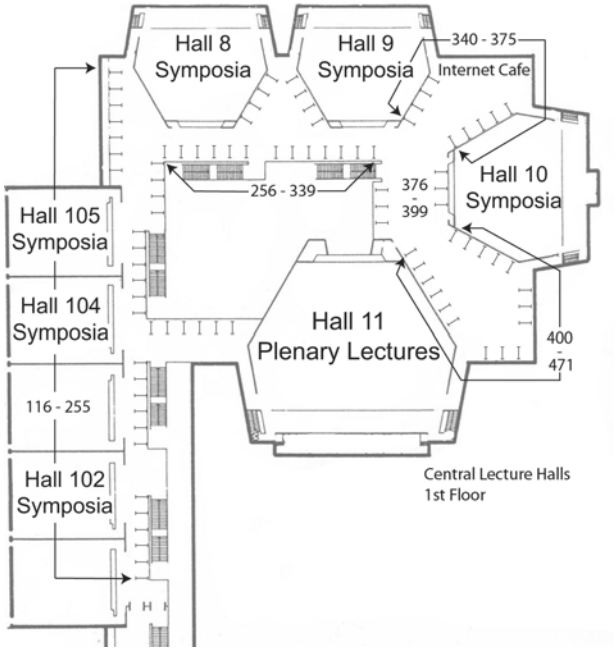
GENERAL INFORMATION

Organizers of the Satellite Symposium:
Walter Paulus, Göttingen
Guido Reifenberger, Düsseldorf

Venue of the Satellite Symposium:
Georg-August-Universität Göttingen
Geisteswissenschaftliches Zentrum
ZHG ("Blauer Turm")
Platz der Göttinger Sieben 5
Lecture Hall 105

Date and Time:
Wednesday, 16.02.05, 13.00-19.30 h

Symposium Language:
The symposium language is English.



Registration:
Participants of both Satellite Symposium V and the 6th Meeting of the German Neuroscience Society must officially register via the congress homepage at http://www.neuro.uni-goettingen.de. Online registration is possible until February 6, 2005. Doctoral students who participate only in Satellite Symposium V do not need to pay a registration fee.

Slide Presentation:
Speakers should provide a CD or a USB memory stick with a PowerPoint file of their presentations before the first session of the symposium. Windows format is preferred. Time for doctoral student lectures will be 15 min (10 min presentation plus 5 min discussion).

Poster Presentation:
Posters for Satellite Symposium V will be displayed for the entire afternoon on February 16, 2005. During the poster session (15.30-17.15 h), the poster authors should briefly present their results at the posters and should be available for answering questions. The best poster will be awarded by a poster prize. The poster exhibition area is in the ZHG close to Lecture Hall 105 The recommended poster size is 100 cm x 100 cm or DIN A0. Posters should be mounted on Wednesday, February 16, 12.00-13.00 h, and must be taken down on the same day after the end of the symposium (19.30-20.00 h). Please check your poster number in this program and stick your poster with pins (not tape) to the designated poster wall.

Coffee Break:
Coffee and cake will be provided during the coffee break and poster session.

Contact Information:
In case of questions concerning Satellite Symposium V, please contact the organizers at the following E-mail addresses: wpaulus@med.uni-goettingen.de or reifenberger@med.uni-duesseldorf.de.

PLATFORM PRESENTATIONS

13.00-13.15 h Welcome Address and Introduction to the Symposium

Prof. Dr. Walter Paulus, Göttingen,
Prof. Dr. Guido Reifenberger, Düsseldorf

13.15-14.00 h Invited Lecture I

Chairs: Guido Reifenberger (Düsseldorf) & Heiko Luhmann (Mainz)

GK-L1 **Recognition molecules and the rejuvenating nervous system**
Prof. Dr. Mellita Schachner
Zentrum fur Molekulare Neurobiologie, Universität Hamburg

14.00-15.30 h Doctoral Students' Lectures I

Chairs: Guido Reifenberger (Düsseldorf) & Heiko Luhmann (Mainz)

GK-L2 **Lipid raft mediated signalling events during early phases of myelination in the central nervous system**
Robin White, C. Klein, E.-M. Krämer, J. Trotter
Department of Biology, Unit of Molecular Cell Biology, University of Mainz
DFG Graduiertenkolleg 1044

GK-L3 **An essential role of disulfite bridges in myelin proteolipid protein (PLP): implications for protein misfolding in Pelizaeus-Merzbacher disease**
Ajit Dhaunchak, K.-A. Nave
Dept. of Neurogenetics, Max-Planck-Institute of Experimental Medicine, Göttingen
MSc/PhD/ MD-PhD Program Neurosciences Göttingen

GK-L4 **Human umbilical cord blood stem cells: analysis in vitro and after implantation into the intact adult rat brain**
Susanne Greschat, C. Rosenbaum, H. W. Müller
Molecular Neurobiology, Dept. of Neurology, Heinrich-Heine-University Düsseldorf
DFG Graduiertenkolleg 320

GK-L5 **The role of N-cadherin in synapse formation and function**
Kay Jüngling
Department Cell Physiology, Ruhr-University Bochum
DFG Graduiertenkolleg 736

GK-L6 **Characterization of the interaction between Drosophila X11L alpha/dMint1 and Rst and its possible role in the nervous system**
Smitha Vishnu, R. Braun, A. Hertenstein, G. H. de Couet, K. F. Fischbach
Institut für Biologie III, Albert-Ludwigs Universität Freiburg
DFG Graduiertenkolleg 843

GK-L7 **The mechanism of IL-8-like chemokine (GRO/CINC-1) release from rat astrocytes mediated by protease-activated receptor**
Yingfei Wang, W. Luo, R. Stricker, G. Reiser
Institut für Neurobiochemie, Medizinische Fakultät, Otto-von-Guericke-Universität Magdeburg
DFG Graduiertenkolleg 253

15.30-17.15 h Poster Session with Coffee and Cake

Chairs: Hans Werner Müller (Düsseldorf), Petra Wahle (Bochum),
Bernhard Keller (Göttingen)

17.15-19.00 h Doctoral Students' Lectures II

Chairs: Walter Paulus (Göttingen) & Georg Reiser (Magdeburg)

GK-L8 **Examination of the D-amino-acid oxidase locus as a genetic risk factor for schizophrenia**
Husam Suliman
Institute for Human Genetics, University of Bonn
DFG Graduiertenkolleg 246

GK-L9 **The state of the actin cytoskeleton determines its association with gephyrin**
Melanie Bausen, G. A. O´Sullivan, H. Betz
Dept. Neurochemistry, Max-Planck-Institute for Brain Research, Frankfurt
DFG Graduiertenkolleg 361

GK-L10 **Abnormal K^+ buffering in the blood-brain-barrier disrupted cortex**
Sebastian Ivens[1], E.Seiffert[1], Ingo Bechmann[2], U.Heinemann[1], A.Friedman[1,3]
[1]Institute for Physiology and [2]Anatomy, University Medicine Berlin,
[3]Zlotowski Center of Neuroscience, Ben Gurion University, Israel
DFG Graduiertenkolleg 238

GK-L11 **Preferred spatial frequency and spatial summation field size in upper layers of monkey V1**
Tobias Teichert, T. Wachtler, A. Gail, M. Wittenberg, F. Michler, R. Eckhorn
Philipps University, Department of Physics, NeuroPhysics Group, Marburg
DFG Graduiertenkolleg 885

GK-L12 **How are eye movements involved in landmark recognition?**
Yu Jin, S. Gillner, H. A. Mallot
Dept. of Cognitive Neuroscience, University of Tübingen
DFG Graduiertenkolleg 778

GK-L13 **Motion adaptation leads to energy efficient neuronal encoding of natural optic flow**
Jochen Heitwerth, R. Kern, M. Egelhaaf
Lehrstuhl für Neurobiologie, Universität Bielefeld
DFG Graduiertenkolleg 518

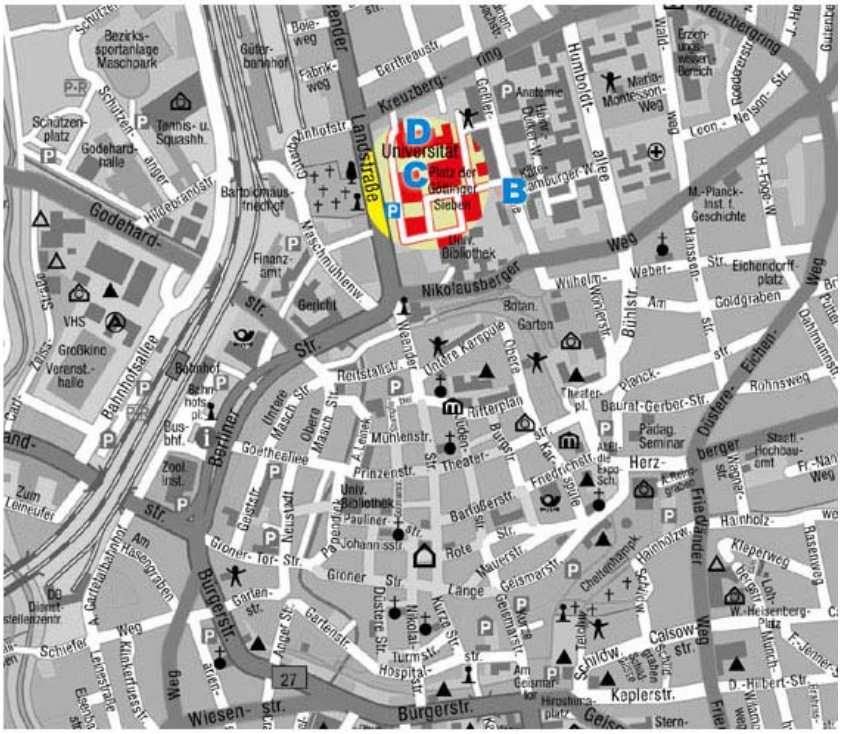
GK-L14 **Theta burst stimulation of the motor cortex: an interleaved TMS/fMRI study**
Elisabeth Rounis[1,2,3], J. Baudewig[2], Y. Huang[3], W. Paulus[1], J. Rothwell[3]
[1]Department of Clinical Neurophysiology, Univ. of Göttingen; [2]MRT Department of Clinical Neuroimaging, Univ. of Göttingen; [3]Sobell Department of Motor Neuroscience and Movement Disorders, UCL, London
DFG Graduiertenkolleg 632

19.00-19.30 h Invited Lecture II

Chairs: Walter Paulus (Göttingen) & Georg Reiser (Magdeburg)

GK-L15 **"New features of DFG research training groups"**
Dr. Priya Bondre-Beil
Deutsche Forschungsgemeinschaft Bonn

19.30 h End of the Symposium



POSTER PRESENTATIONS

GK-P1 **Synaptic vesicle proteins with four transmembrane domains are not essential for neuronal functions in *C. elegans***
Christian Abraham*, Erik Jorgensen[‡], Harald Hutter[†], Rudolf E. Leube*
**Department of Anatomy, Johannes Gutenberg University, Mainz; [‡]Department of Biology, University of Utah, Salt Lake City, Utah; [†]Max-Planck-Institute for Medical Research, Heidelberg*

GK-P2 **Hyperpolarization-activated cation channels in fast-spiking hippocampal interneurons**
Y. Aponte, C.C. Lien, P. Jonas
Physiologisches Institut, Universität Freiburg

GK-P3 **Stimulus induced plasticity of the olfactory receptor neuron**.
Jon Barbour*, Bettina Warscheid∞, Kai Stühler, Helmut Meyer[∞], Dirk Wolters[§], Hanns Hatt*, Eva Neuhaus*.
**Lst. Zellphysiologie, [∞]Medizinisches Proteom-Center, [§]Analytische Chemie, Ruhr-Universität Bochum*

GK-P4 **Frequency organization of the medial geniculate body in the Mongolian gerbil**
Peter Bäuerle, Manfred Kössl
Zoological Institute, J.W. Goethe University, Frankfurt Main

GK-P5 **Gene expression profiling during gliogenesis in the *Drosophila* embryo**
A. Becker[1], B. Altenhein[1,] B. Beckmann[2], J. Hoheisel[2], G. M. Technau[1]
[1]Institute for Genetics, University of Mainz; [2]DKFZ Heidelberg, Members of the Heidelberg FlyArray Consortium

GK-P6 **Identification and characterization of *Drosophila* genes involved in glia cell specification and differentiation**
Ruth Beckervordersandforth, Benjamin Altenhein, Gerd Technau
Institute of Genetics, University of Mainz

GK-P7 **Synaptic targeting and time course of secretion of neurotrophins from hippocampal neurons**
T. Brigadski, R. Kolarow, M. Hartmann, V. Lessmann
Institute of Physiology and Pathophysiology, University of Mainz

GK-P8 **Expression of constitutively activated Ras promotes enhancement of dopaminergic properties in differentiating neurospheres**
K.Chakrabarty, S.Mann, I. Dietzel, R. Heumann
Department of Molecular Neurobiochemistry, Faculty of Chemistry, Ruhr-University Bochum

GK-P9 **Analysis of tenascin-C isoform complexity in neural stem cells and their regulation by Pax6**
Ursula Egbers, Alexander von Holst, Andreas Faissner
Department of Cell Morphology and Molecular Neurobiology, Ruhr-University Bochum

GK-P10 **Broca's area and its language specific function**
Tanja Grewe
Graduate program "Neuronal representations and action control", Research Group Neurolinguistics, Philipps University Marburg

GK-P11 **Age-related changes of cerebral white and grey matter structures from childhood to adulthood using voxel-based morphometry**
Samuel Groeschel, Brigitte Vollmer, Alan Connelly
Radiology and Physics Unit, Institute of Child Health, University College London, UK

GK-P12 **Selective ER export of HCN4 channels**
Nadine Hardel, Gerd Zolles, Bernd Fakler, Nikolaj Klöcker
Department of Physiology II, University of Freiburg

GK-P13 **Developmental expression pattern of ionotropic glutamate receptors in differentiating embryonic stem cells**
Joshi I.*‡, Werner M.*, Smith A.°°, Grunwald T.°, Gottmann K.†‡, Hollmann M.*‡
**Department of Biochemistry, Receptor Biochemistry and ‡Graduate School Development and Plasticity of Nervous System, Ruhr University, Bochum; †Department of Neurophysiology, Heinrich-Heine-University, Düsseldorf; °°Institute of Stem Cell Research, University of Edinburgh, UK; °Department of Virology, Bochum*

GK-P14 **Remodelling of the damaged brain by fetal transplants?- Implication from studies in a rat model of Parkinson`s disease**
Klein A., *Metz G.A., Wessolleck J., Papazoglou A., **Knieling M., Timmer M., Nikkhah G.
*Laboratory of Molecular Neurosurgery, Dept. of Stereotactic Neurosurgery, University Hospital Freiburg; *CCBN, Univ. of Lethbridge, Lethbridge, Alberta, Canada; **University Hospital Jena, Dept. of Neurology, Jena*

GK-P15 **Contribution of somatostatin to long term potentiation in the mouse hippocampus**
Christian Kluge, Csaba Szinyei, Hans-Christian Pape
Institut für Physiologie, Medizinische Fakultät, Otto-von-Guericke-Universität Magdeburg

GK-P16 **Promoter hypermethylation and transcriptional downregulation of the carboxyl-terminal modulator protein (CTMP) gene in glioblastomas**
Knobbe C.B.[1], Reifenberger J.[2], Blaschke B.[1], Reifenberger G.[1]
[1]Department of Neuropathology and [2]Department of Dermatology, Heinrich-Heine-University, Düsseldorf

GK-P17 **Role of munc-18-1 as a docking factor for granules in chromaffin cells, revealed By evanescent wave microscopy**
O. Kochubey[1], R.F. Toonen[2], H. de Wit[2], M. Verhage[2], J. Klingauf[1]
[1]Department of Membrane Biophysics, Max-Planck Institute for Biophysical Chemistry, Göttingen; [2]Department of Functional Genomics, Free University of Amsterdam, The Netherlands

GK-P18 **Differential expression of the homeobox gene Pitx 3 in midbrain dopaminergic neurons**
Tatiana M. Korotkova, Olga A. Sergeeva, Alexei A. Ponomarenko, Helmut L. Haas
Institut für Neuro- und Sinnesphysiologie, Heinrich-Heine-Universität, Düsseldorf

GK-P19 **LTP and reinforcement of early-LTP by stimulation of ventral tegmental area in freely moving rats *in vivo*.**
Sergiy Kostenko, Julietta U. Frey, Sabine Frey
Dept. Neurophysiology, Leibniz-Institute for Neurobiology, Magdeburg

GK-P20 **Role of cannabinoids in excitotoxic neuronal damage, as assessed in organotypic hippocampal slice cultures**
S. Kreutz, M. Koch, H.-W.Korf, F. Dehghani
Institute of Anatomy II, Johann Wolfgang Goethe-University Frankfurt am Main

GK-P21 **Sustained asynchronous activity in random network of spiking neurons**
Arvind Kumar, Sven Schrader, Stefan Rotter, Ad Aertsen
Institute for Biology III, Albert-Ludwigs-Universität Freiburg

GK-P22 **Two types of protease-activated receptors (PAR-1 and PAR-2) mediate calcium signaling in rat retinal ganglion (RGC-5) cells**
Weibo Luo, Yingfei Wang, Georg Reiser
Institut für Neurobiochemie, Medizinsche Fakultät, Otto-von-Guericke-Universität Magdeburg

GK-P23 **The scaffold protein harmonin (USH1C) also integrates Usher syndrome 2 proteins into synaptic Usher protein complexes in retinal photoreceptor cells**
Tina Märker, Jan Reiners, Karin Jürgens, Nora Overlack, Tobias Goldmann, Uwe Wolfrum
Institut für Zoologie, Johannes Gutenberg-Universität, Mainz

GK-P24 **Neuro-protection and neuro-regeneration using adeno-associated virus**
Malik I., Michel U., Shevtsova Z., Bähr M., Kügler S.
Department of Neurology, University Hospital Göttingen

GK-P25 **Long term neuroprotection in an optic nerve transection model**
Malik I. J. M., Shevtsova Z., Bähr M., Kügler S.
Department of Neurology, University Hospital Göttingen

GK-P26 **Role of GM-CSF for development and function of dendritic cells in the central nervous system**
Anne-Kathrin Mausberg[1], Sebastian Jander[2], Gaby Reichmann[1]
[1]Institute for Medical Microbiology and [2]Department of Neurology, Heinrich-Heine-University, Düsseldorf

GK-P27 **Developmental changes of GABAergic input synapse distribution in an identified insect motoneuron**
Maurice Meseke, Jan Felix Evers, Carsten Duch
Free University of Berlin, Institute of Neurobiology, 14195 Berlin

GK-P28 **Neurogenesis in the adult subventricular zone: a functional role for extracellular nucleotides**

S.K. Mishra[1*], N. Braun[1], C. Schomerus[2], H.-W. Korf[2], J. Sévigny[3], S.C. Robson[4], H. Zimmermann[1]

[1]Frankfurt University, Biocenter; [2]Frankfurt University, Medical School, Germany; [3]Sainte-Foy, Québec, Canada; [4]Harvard Medical School, Boston, USA

GK-P29 **Paired-pulse TMS effects on single unit activity in cat primary visual cortex**

Moliadze V.[1], Giannikopoulos D. [1], Kammer T. [2], Funke K. [1]

[1]Department of Neurophysiology, Ruhr-University Bochum, D-44780 Bochum

[2]Department of Psychiatry, University of Ulm, Leimgrubenweg 12-14, D-89075 Ulm

GK-P30 **Expression and function of the neuronal protein 25 (NP25) in the developing chicken embryo**

Manuela Pape[1], Markus Geißen[1,2], Chi Vinh Duong[1], Hermann Rohrer[1]

[1]Max-Planck-Institute for Brain Research, Department of Neurochemistry, Frankfurt am Main; [2]present address: Bundesforschungsanstalt fur Viruskrankheiten der Tiere, Insel Riems

GK-P31 **Schwann cells express Toll-like receptors**

Meike Ramacher, Wei Hu, Hans-Peter Hartung, Bernd C. Kieseier

Department of Neurology, Heinrich-Heine-University, Düsseldorf

GK-P32 **The transmembrane form of agrin reorganizes the cytoskeleton in neurons and non-neuronal cells**

Rene Ramseger[§], Maik Annies[§], Stefan Wöll[§] , Jürgen Löschinger[#], Stephan Kröger[§]

[§]Dept. of Physiological Chemistry, University of Mainz; [#]Max-Planck-Institute for Developmental Biology, Tübingen

GK-P33 **Shedding light on glutamate receptor dynamics during synapse formation of living *Drosophila***

Tobias Rasse

Universität Göttingen

GK-P34 **Electrical intracochlear stimulation induces c-Fos expression in specific neuronal populations of the auditory brainstem nuclei**

Adrian Reisch, Robert-Benjamin Illing

Neuropathological Research Laboratory, Dept. of Otorhinolaryngology, University of Freiburg

GK-P35 **Rats can navigate in virtual environments**

Alexander Schnee[1], Hansjürgen Dahmen[1], Christian Hölscher[2], Hanspeter A. Mallot[1]

[1]LS Kognitive Neurowissenschaft, Universität Tübingen; [2]School of Biomedical Sciences, University of Ulster, Coleraine

GK-P36 **Solution structure of KChIP4a and characterization of its interaction with the Kv4.3 N-terminus**

J. Schwenk[a], D. Bentrop [a] , M. Covarrubias[b], B. Fakler[a]

[a]Physiologisches Institut, Universität Freiburg; [b]Thomas Jefferson University, Philadelphia, USA

GK-P37 **Light-mediated regulation of Ras and Rho GTPases in the visual system of mice. Involvement of Ras in photoentrainement of the circadian clock**
Serchov T.[*], Thor F.[*], Jilg A.[#], Stehle J.[#], Heumann R.[*]
[*]*Department of Molecular Neurobiochemistry, Faculty of Chemistry, Ruhr-University Bochum;* [#]*Institute of Anatomy II, Dr. Senckenbergische Anatomy, Johann Wolfgang Goethe University Frankfurt*

GK-P38 **Association of the ecto-ATPase NTPDase2 with transient cell populations of the neurogenic pathway in the adult dentate gyrus**
V. Shukla[1*], N. Braun[1], J. Sévigny[2], S.C. Robson[3], S. Raab[4], H. Zimmermann[1]
[1]*Biocenter, Frankfurt University, Germany;* [2]*Sainte-Foy, Québec, Canada;* [3]*Harvard Medical School, Boston, USA;* [4]*Neurological Institute, Frankfurt University*

GK-P39 **Effects of contrast on smooth-pursuit eye movements**
Miriam Spering[1], Dirk Kerzel[2], Doris I. Braun[1], Michael J. Hawken[3], Karl R. Gegenfurtner[1]
[1]*Justus-Liebig-Universität, Gießen;* [2]*Faculté de Psychologie, Université de Genève, Switzerland;* [3]*Center for Neural Science, New York University, New York, USA*

GK-P40 **Plasticity of the somatosensory cortex in thalidomide-induced dysmelia**
Stoeckel M.C.[1], Pollok B.[1], Jörgens S.[1], Witte O.W.[2], Schnitzler A.[1], Seitz R.J.[1]
[1]*Department of Neurology, University Hospital Düsseldorf;* [2]*Department of Neurology, University Hospital Jena*

GK-P41 **Involvement of somatostatin in formation of contextual fear memory**
Christian Stoppel, Oliver Stork, Hans-Christian Pape
Institut für Physiologie, Medizinische Fakultät, Otto-von-Guericke Universität Magdeburg

GK-P42 **Glial cells in the respiratory network express functional transporters and receptors for glycine**
Katalin Szöke[1], Johannes Hirrlinger[2,4], Melanie Handschuh[3], Clemens Neusch[3], Frank Kirchoff[2,4], Swen Hülsmann[1,4]
[1]*University of Göttingen, Department of Neuro- and Sensory Physiology;* [2]*Max Planck Institute for Experimental Medicine, Department of Neurogenetics;* [3]*University of Göttingen, Department of Neurology;* [4]*DFG Research Center Molecular Physiology of the Brain, Göttingen*

GK-P43 **Calcium dependence of BK channels in auditory inner hair cells**
Henrike Thurm, Bernd Fakler, Dominik Oliver
Physiologisches Institut II, Universität Freiburg

GK-P44 **Malignant progression of astrocytomas is associated with decreased expression Of *AMOG, DRR1* and *APOD***
Jörg van den Boom, Britta Blaschke, Guido Reifenberger
Department of Neuropathology, Heinrich-Heine-University, Düsseldorf

GK-P45 **The mechanism of IL-8-like chemokine (GRO/CINC-1) release from rat astrocytes mediated by protease-activated receptor-1**
Yingfei Wang, Weibo Luo, Rolf Stricker, Georg Reiser
Institut für Neurobiochemie, Medizinische Fakultät, Otto-von-Guericke-Universität Magdeburg

GK-P46 ***In vitro* analysis of the neuroprotective potencies of natural compound derivatives**
Wendt W.[1], Sontag B.[2], Lübbert H.[1, 2, 3], Stichel C. C.[3]
[1]Department of Animal Physiology, Ruhr-University of Bochum; [2]Biofrontera Discovery GmbH, Heidelberg; [3]Biofrontera Pharmaceuticals GmbH, Leverkusen

GK-P47 **Trafficking of proteins and lipids in myelinating oligodendrocytes**
Christine Winterstein, Jacqueline Trotter, Eva-Maria Krämer
Department of Biology, Molecular Cell Biology, University of Mainz

GK-P48 **Decreased dopamine D2/D3 receptor binding in temporal lobe epilepsy: an ^{18}F-fallypride PET study**
I. Yakushev[2], K. Werhahn[1], H.G. Buchholz[2], S. Klimpe[1], C. Landvogt[2], W. Müller-Forell[3], S. Höhnemann[4], M. Schreckenberger[2], F. Rösch[4], and P. Bartenstein[2]
[1]Department of Neurology, [2]Nuclear Medicine, [3]Institute of Neuroradiology and [4]Institute for Nuclear Chemistry, Johannes Gutenberg-University Mainz

ABSTRACTS

GK-L2 **Lipid raft mediated signalling events during early phases of myelination in the central nervous system**

Robin White, Corinna Klein, Eva-Maria Krämer & Jacqueline Trotter
Department of Biology, Unit of Molecular Cell Biology, University of Mainz

Efficient saltatory propagation of action potentials on neuronal axons in the Central Nervous System is mediated by oligodendrocytes. These glial cells extend membrane processes towards an axonal contact site which is followed by an ensheathment and finally results in a compacted multilamellar myelin sheath. It has been shown previously that the Src family non receptor tyrosine kinase Fyn is a key player in early myelination events. Fyn co-precipitates with the IgG superfamily membrane proteins F3/ Contactin and N-CAM. All three proteins have been shown to localise to distinct membrane subdomains referred to as "lipid rafts". Antibody crosslinking of F3 results in activation of the Fyn kinase and Fyn has also been shown to bind to cytoskeletal microtubules and the microtubule associated protein Tau. Therefore, it has been proposed that an unknown neuronal interaction partner could bind to oligodendroglial F3 and trigger a signalling cascade that involves the activation of Fyn and the recruitment of the cytoskeleton towards the axon-glial contact site which leads to a polarisation of the cells and initiates the myelination process. Further biochemical investigations will shed more light on proteins that are involved in these signalling events and may reveal neuronal interacting partners that may trigger the recruitment of the oligodendroglial cytoskeleton. Furthermore, live cell imaging with fluorescently tagged cytoskeletal elements will help to visualise our biochemical data.

GK-L3 **An essential role of disulfide bridges in myelin proteolipid protein (PLP): implications for protein misfolding in Pelizaeus-Merzbacher disease**

A.-S. Dhaunchak, K.-A. Nave
Dept. Neurogenetics, Max-Planck-Institute of Experimental Medicine, Göttingen

In humans, point mutations of the X-linked PLP gene (Plp) cause a severe dysmyelinating phenotype and define Pelizaeus-Merzbacher Disease (PMD). The encoded Proteolipid Protein is highly abundant, comprising about approximately 50% of myelin protein in the central nervous system. At the molecular level, PLP is a 30kDa polytopic membrane protein with four transmembrane domains (tetraspan), and one intracellular and two extracellular loop regions.. Both N- and C-termini of PLP protrude into the cytosol. PLP point mutations that map into the second extracellular loop region (EC2) have a particular severe phenotype in vivo, but their effect on protein structure and function are poorly understood. PLP has two disulfide bridges, involving 4 cysteine residues in EC2. Here we asked whether the ability of PLP to form these disulfides is required for the normal tetraspan conformation and for the normal intracellular trafficking. Moreover, we investigated whether unpaired cysteines contribute to the retention of mutant PLP in the endoplasmic reticulum. For transfection with cDNA expression constructs, encoding PLP lacking 1-4 cysteine residues (Cys->Ser), we used an oligodendroglial cell line (Oli-neu; kindly provided by J. Trotter) and primary mouse oligodendrocytes. By laser confocal microscopy, we determined that bridge C1C4, but not C2C3, is required for intracellular sorting and surface expression of PLP. Moreover, some PMD point mutations that map into EC2, may act by exposing cysteines C1 and C4, that remain unpaired and are recognized by luminal chaperones.

GK-L4 **Human umbilical cord blood stem cells: analysis *in vitro* and after implantation into the intact adult rat brain**

Greschat S., Rosenbaum C., Müller H.W.
Molecular Neurobiology, Department of Neurology, Heinrich-Heine-University, Düsseldorf

Human umbilical cord blood represents an easily available source of unrestricted somatic stem cells (USSC) which can give rise to neural and other cell types. USSC represent the neonatal stage of multipotent human umbilical cord blood stem cells. These cells are defined and characterized as a CD34- and CD45-negative, non-haematopoietic, stem cell compartment, which can be easily expanded in cell culture up to at least 16 passages. We have developed a protocol to differentiate USSC into the neural direction followed by lineage selection of neuronal cells *in vitro*. The differentiation of USSC was monitored by immunocytochemical detection of neural antigens, different neurotransmitters and transmitter synthesizing enzymes. These findings enabled us to discriminate between neuronal subtypes, like GABAergic, and dopaminergic cells. Additional treatment with a dopamine containing medium led to a massive increase of tyrosine-hydroxylase immunoreactivity. In order to test whether immunocytochemical data correlate with functionality we currently analyse the cellular release of neurotransmitters by HPLC. To investigate the potential of the USSC with regard to cell replacement therapies, we carried out transplantation experiments. PKH26 pre-labelled and unlabelled USSC were stereotactically and unilaterally implanted into intact adult rat brains. In these experiments PKH26 labelling could be followed up for at least three months after implantation.

Currently, immunohistochemistry with a human specific nuclei antibody (Nuc) is carried out to confirm these data. Both methods, PKH26 labelling and Nuc-staining, are indicating high migratory activity of the implanted USSC. Further human Tau-protein positive cells could be found distributed in different regions of the rat brain three months after implantation, e.g., in cortex, hippocampus and the striatum giving hints for neuron-like differentiation in vivo.

GK-L5 **The role of N-cadherin in synapse formation and function**

Kay Jüngling
Department of Cell Physiology, Ruhr-Universität Bochum

Synaptic adhesion molecules of the cadherin superfamily, consisting of classical cadherins and protocadherins, have been proposed to control selective synapse formation. Classical cadherins, e.g. neural (N-) cadherin, mediate homophilic cell-cell adhesion in a Ca^{2+}-dependent manner and are found in the perisynaptic region, bordering the active zone and the postsynaptic density. We are analysing the role of N-cadherin in synapse formation, function and plasticity using embryonic stem (ES) cells from N-cadherin knock-out mice (Radice *et al*., 1997). Because of the early embryonic lethality of N-cadherin knock-out mice, we established *in vitro* differentiation and immunoisolation of ES cell-derived neurons (Jüngling *et al*., 2003) genetically null for N-cadherin. Glutamatergic synapses were studied in microisland cultures of these cells after 10-14 days *in vitro* by ultrastructural, immunocytochemical and electrophysiological methods. Previously we have shown that axon outgrowth, dendrite morphology and the number and ultrastructure of synapses are not altered in homogenous cultures of N-cadherin knock-out neurons. However, we found a clear-cut defect in the activity-induced recruitment of synaptic vesicles to the active zone (Jüngling et al., submitted).

GK-L6 **Characterization of the interaction between *Drosophila* X11Lα/dMint1 and Rst and its possible role in the nervous system**

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The *Drosophila* cell adhesion molecule Rst plays key roles during the development of the embryonic musculature, spacing of ommatidia in the compound eye and of sensory organs on the antenna, as well as in the neuronal wiring of the optic lobe. In rst^{CT} mutants lacking the cytoplasmic domain of the Rst protein cell sorting and apoptosis in the eye are affected, suggesting a requirement of this domain for Rst function. In order to study the role of the cytoplasmic portion of Rst, yeast two hybrid screens were performed using this domain and the intracellular domain of the paralogous protein Kirre as bait. Among several putative interactors, we identified a subset of overlapping clones in both screens, encoding two paralogous Drosophila PDZ motif proteins related to X11/Mint. Conserved homologues of this family are present in *C.elegans* (Lin-10) and in vertebrates and are believed to function as adaptor proteins and in the assembly of multi-subunit complexes at the synapse, where they link the vesicle cycle to cell adhesion. Using genetic, cell biological, and bioinformatic approaches, we show that the interaction of Rst with X11Lα/dMint1 is of biological significance. Both proteins co-localize during eye and optic lobe development and are concentrated in synaptic layers of the adult optic lobe. Reduced levels of X11Lα/dMint1 result in defective cell sorting and reduced apoptosis in the pupal eye disc, reminiscent of the rst^{CT} mutation. Retinula cells do not form proper rhabdomeres. The role of the X11/dMint1 - Rst interaction is discussed in the context of the proposed properties of this protein family in membrane vesicle transport and as scaffolding proteins for larger protein complexes.

GK-L7 **The mechanism of IL-8-like chemokine (GRO/CINC-1) release from rat astrocytes mediated by protease-activated receptor-1**

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Protease-activated receptors (PARs), a unique class of G protein-coupled receptors, are widely expressed in the central nervous system (CNS), such as neurons, microglial cells, astrocytes and oligodendrocytes. Now, it is clear that PARs are involved in multiple physiological processes, such as platelet aggregation, inflammation, apoptosis, cell proliferation, immune response, pain, morphological changes and calcium mobilization. The inflammatory roles of PARs are well understood in some systems, but not in the CNS. Till now, only very limited direct evidence has shown that PARs play a role in inflammation in the CNS. But the inflammatory mechanisms of PARs remain largely unknown. Rat chemokine growth-regulated oncongene/cytokine-induced neutrophil chemoattractant-1 (GRO/CINC-1), a counterpart of the human growth-regulated oncogene product (GRO), has been suggested to play critical roles as a mediator of inflammatory reactions with neutrophil infiltration in rats. In the present study, we investigated by RT-PCR and ELISA whether PAR-1 activation could increase chemokine GRO/CINC-1 in rat astrocytes. We found that thrombin and TRag time- and concentration-dependently upregulates GRO/CINC-1 at both mRNA level and protein level. Then we further investigated the mechanism of PAR-1-mediated GRO/CINC-1 production by rat astrocytes in vitro. ELISA results suggested that inhibitors of protein kinase C (PKC), mitogen-activated protein kinase kinase 1/2 (MEK1/2), phosphatidylinositol 3-kinase (PI3K), p38 MAPK, c-Jun N-terminal

kinase (JNK), nuclear transcription factor-kappa B (NF-κB) and Janus kinase 2 (JAK2) significantly reduced PAR-1-induced GRO/CINC-1 production. Signaling cascades of GRO/CINC-1 production were further studied by western blot. Our observations for the first time indicate that JNK is very sensitive to thrombin and TRag stimulations in rat astrocytes. Both thrombin and TRag can time- and concentration-dependently phosphorylate JNK, but not p38 MAPK. JNK activation was mediated by PKC, PI3K and MEK1/2. In addition, three interesting nuclear transcription factors, which are supposed to play important roles in regulating GRO/CINC-1 release, were also studied. Our observations indicate that phosphorylation and activation of c-Jun was involved in upregulation of GRO/CINC-1 and c-Jun gene expression. NF-κB, which could be activated by thrombin independent of PAR-1 activation, maybe have indirect effects on GRO/CINC-1 release. However, signal transducer and activator of transcription (STAT)3 functions as a negative regulator of GRO/CINC-1 release.

GK-L8 **Examination of the D-amino-acid oxidase locus as genetic risk factor for schizophrenia**

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D-amino-acid oxidase (DAO) catalyzes the oxidative deamination of D-amino-acids. Particulary the neurotransmitter D-serine, acting on the glycine binding site of NMDA-receptors (NMDA-R) is a specific substrate of DAO and several lines of evidence suggest an involvement of D-serine as well as NMDA-R in the pathogenesis of schizophrenia.Most interestingly, genetic studies point to an association between variants at the DAO locus and schizophrenia. Genotyping of 3 SNP markers within the DAO gene in a German sample with schizophrenia confirmed a French Canadian study, in which 4 SNP markers were analyzed and gave significant association results. To detect additional genetic variants, we re-sequenced all 11 exons of the DAO gene and putative regulatory regions in patients with schizophrenia and controls. We identified 22 variants that were not known so far and validated 17 SNPs already described in public databases. Based on our sequencing results we are currently genotyping 18 SNP markers in a case-control sample comprising 530 patients with schizophrenia. Our results strengthen the evidence for association between psychoses and the DAO gene locus.

GK-L9 **The state of the actin cytoskeleton determines its association with gephyrin**

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The clustering of glycine receptors and major subtypes of $GABA_A$ receptors at inhibitory synapses is mediated by the anchoring protein gephyrin. Although a direct interaction between gephyrin and the microtubule network has been shown, the association of gephyrin with the actin cytoskeleton is not well understood. However several studies have shown that gephyrin interacts with actin binding proteins such as profilin I, neuronal profilin IIa, and with members of the VASP family. In order to determine how gephyrin is linked to the cytoskeleton, full-length and mutated constructs of gephyrin were transfected into HEK 293 cells. Over-expressed gephyrin forms large intracytoplasmic aggregates (blobs) in HEK293 cells, which enrich gephyrin binding proteins whether endogeneous or coexpressed. Here, the actin cytoskeleton was subsequently either stabilised or disrupted with compounds, such as latrunculin A, jasplacinolide or cytochalasin D, in order to distinguish between F- or G-actin mediated interaction. The site of interaction of each of these proteins was further determined by the generation of gephyrin deletion mutants, used in the ´blob´ colocalisation assay and in immunoprecipitation experiments. Based on these results we propose a model how gephyrin may interact with the actin cytoskeleton.

GK-L10 **Abnormal K^+ buffering in the blood brain-barrier disrupted cortex**

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The blood brain-barrier (BBB) is a complex structure designed to maintain a unique neuronal environment and limit the penetrance of serum components to the brain. We have recently demonstrated that opening of the BBB leads to a delayed (~4 days) appearance of a longlasting focus of epileptiform activity. This was associated with early (24 hrs) activation of astrocytes. In the present study we investigated K^+ buffering in neocortical slices maintained *in vitro*. Extracellular recordings using ion-sensitive microelectrodes were done in treated and sham-operated cortices 1-30 days after surgery. K^+ buffering was studied following tetanic stimulation or K^+ ionophoresis. By both methods buffering was found to be compromised, strongest 1 day after BBB-treatment and fully recovered within 4 weeks. To differentiate between different buffering mechanisms we applied low concentrations of BaCL (100µM) to the bath to block inward rectifier K^+ channels (K_{IR}) . This was significantly less effective in augmenting K^+ signals in the 24 hrs BBB-disrupted cortex. In contrast, further increase in BaCl (2mM) to block K^+ leak currents was equally effective in control and treated cortex. Quabain in concentrations of 9µM which predominantly blocks the α2/3 subunits of the Na^+/K^+-ATPase had a smaller effect on the clearance of extracellular K^+ in treated slices one day after treatment, indicating a smaller activity of the enzyme. This compromised buffering showed functional significance as slow repetetive stimulation (0.33-0.67 Hz) evoked abnormal afterpotentials after several stimuli of a

train only in treated cortex which were never seen after the first stimulus. Our results show that development of epileptiform activity in BBB disrupted cortex is preceeded by activation of astrocytes and impaired K^+ buffering. This could contribute to neuronal hyperexcitability and later on to the development of chronic cortical dysfunction.

GK-L11 **Preferred spatial frequency and spatial summation field size in upper layers of monkey V1**

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Striate cortex is commonly believed to perform a localized two-dimensional spatial frequency (SF) decomposition of the visual scene. Models of early visual processing often use a set of localized SF-filters, typically based on oriented Gabor-wavelets, that are scaled in size proportional to the wavelength of their preferred SF. While this relationship has been examined extensively for V1 simple cells, little effort has been made so far to examine this relationship for V1 complex cells. We recorded multi-unit activity in the upper layers of macaque V1, thereby recording mainly from complex cells. While the monkey performed a passive fixation task, we presented Gabor-wavelets of different sizes and spatial frequencies. Consistent with our previous observations we found no correlation between the wavelength of the preferred SF and the size of the classical receptive field. We did however find a significant positive correlation ($p < .001$) between the wavelength of the preferred SF and the size of the spatial summation field. Further analysis revealed however, that this relationship is not linear as would be predicted by a Wavelet analysis. In conclusion our data suggest, that by modeling V1 as a localized set of wavelet filters one does not do justice to V1 complex cells, which are known to form a big part of the efferents from V1.

GK-L12 **How are eye movements involved in landmark recognition?**

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To better understand the concept of landmarks, it is necessary to understand not only how agents use them in navigation, but also how they are identified in the scene. Landmarks are often noticed or memorized because they are capable of acttracting attention. Landmark saliency may accrue by a unique visual property of an object, but also depends on the functional content of this object: how an agent uses it in a navigation task. In our experiment we used landmarks and distractors with matched visual properties. Unlike landmarks, distractors are identical in the whole environment and contain no spatial information. Eye gaze patterns were analyzed while subjects performed a navigation task in a virtual reality environment. Subjects were trained to learn a route in the virtual environment and perform tests using landmarks. We examined the gaze fixation with respect to landmarks and distractors in the environment. Our results showed that there are more gaze fixations on landmarks than on distractors. Also, the fixation duration on landmarks is longer than on distractors. Interestingly, an object elicits longer fixation when it acts as landmark in environment than as distractor. This suggests that gaze fixation selectively focuses on landmarks, rather than random free-viewing. Additionally, the distribution of eye-gaze is strongly relative to the focus of expansion, but biased by the position of landmarks in the scene. Our experiment demonstrates the highly task-specific nature of our vision system: information is actively extracted from the fixation point for certain task demands. Here, subjects actively search for landmark information for the purpose of performing navigation task.

Figure 1: Bird-eye view of virtual reality environment with landmarks and distractors

GK-L13 **Motion adaptation leads to energy efficient neuronal encoding of natural optic flow**

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When animals move around in their environment their retinal images are continually displaced. This so-called optic flow provides much information about the animal's self-motion as well as about the spatial layout of the surroundings. Blowflies have served as excellent model systems to analyse the neuronal mechanisms underlying optic flow processing: On the one hand because they rely heavily on optic flow information in orientation behaviour. On the other hand, recordings in the visual motion pathway of the blowfly are easily performed and its neurons can be identified individually. Neurons sensitive to visual motion change their response properties during prolonged motion stimulation. These changes have been interpreted as adaptive and were concluded, for instance, to adjust the sensitivity to velocity changes or to increase the reliability of encoding of motion information. These conclusions are based on experiments with experimenter-designed motion stimuli that differ much from the optic flow an animal experiences during normal behaviour. We analyse for the first time motion adaptation under natural stimulus conditions. The experiments are done an identified neuron in the blowfly visual motion pathway. We reconstructed the optic flow perceived by a blowfly in free flight. This optic flow was used to study motion adaptation. A variety of measures suggests that the coding quality does not improve with prolonged stimulation. However, the number of spikes decreases much during stimulation with natural optic flow. But interestingly the amount of information that is conveyed stays nearly constant. Thus the information per spike increases. Since spikes are energetically costly, motion adaptation serves as a mechanism to ensure parsimonious coding. Thus under natural stimulus conditions motion adaptation saves energy without sacrificing the reliability with which behaviourally relevant information is encoded.

GK-L14 **Theta burst stimulation of the motor cortex: an interleaved TMS/fMRI study**

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It has been 30 years since the discovery that repeated electrical stimulation of neural pathways can lead to long-term potentiation in hippocampal slices. To date, the most promising method for transferring these methods to human is repetitive transcranial magnetic stimulation (rTMS), a non-invasive method of stimulating neural pathways in the brain of conscious subjects through the intact scalp. However, effects on synaptic plasticity reported are often weak and variable between individuals despite the long periods of stimulation required to produce a lasting effect. A novel method of conditioning the human motor cortex using very rapid rTMS that produces a controllable, consistent, long-lasting and powerful effect on motor cortex physiology and behaviour after an application period of only 40 seconds has been developed. This novel method of stimulating the brain has been named theta burst stimulation (TBS). Here we present a study in which combined TMS/fMRI was used in order to characterise the effect of TBS on motor cortex excitability and motor network connectivity. The TMS/fMRI approach promises a direct representation of neurophysiological alterations induced by TMS which otherwise are only measured indirectly from motor evoked potential measurements or reaction time recordings in behavioural studies. We used a 2X3 experimental design in order to study the effects of TBS on BOLD response within the motor network. 12 right-handed healthy volunteers underwent 2 fMRI scanning sessions in which they either received TBS or no TBS. Each scanning session involved 3 conditions in a block design. The conditions involved subjects receiving suprathreshold and subthreshold interleaved TMS/fMRI to their left 'motor hot-spot'. A control condition was added, in which they had to voluntarily move their right thumb timed with the TMS pulse using the same timing as in the interleaved conditions but at an intensity that could not produce any stimulation of the brain. The order of the conditions and of the sessions was counterbalanced across subjects to avoid any session effects. We hypothesized that the TBS protocol we used, known as cTBS, would decrease the activity of the stimulated area, i.e., the left primary motor cortex. This is based on neurophysiological evidence that this paradigm depresses motor cortical excitability for a period of 1 hour following the stimulation. We were also interested in the activity of nonprimary motor areas such as the dorsal premotor cortex and the supplementary motor area. The former is known to play a key role in recovery from motor cortical strokes both in patients and in animal models. The latter (SMA) is known to be involved in the performance of internally generated finger movements in tasks such as our voluntary thumb movement task. We hypothesized that this area would be differentially affected in the voluntary versus externally triggered (suprathreshold TMS) thumb movements. Moreover, we were interested in looking at changes in connectivity between these regions such that the balance of activity might shift towards areas of increased excitability. Preliminary results of the comparison between the TBS and the no TBS session suggest that there is a a global decrease in BOLD response in the whole brain and more specifically within the stimulated area, the left primary motor cortex. BOLD activity in the left dorsal premotor and supplementary motor areas was increased. These results and any changes in motor network connectivity will be discussed in further detail at the meeting.

GK-P1 **Synaptic vesicle proteins with four transmembrane domains are not essential for neuronal functions in *C. elegans***

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Neurotransmitter vesicles contain an abundance of integral membrane proteins with four transmembrane domains that belong to three different polypeptide families, the physins, gyrins and SCAMPs (secretory carrier associated membrane protein). Different combinations of members of each family are expressed in various mammalian neurons. Despite their abundance knockout experiments in mice have provided little functional insights. Arguing that this may be due to molecular redundancy we have turned our attention to the genetic model organism *C. elegans* that presents only a single ancestral polypeptide for each family. We prepared functional null mutant for each polypeptide and bred double as well as triple mutant worms. Detailed analysis revealed, however, that neuronal functions are mostly intact including all aspects of motility and most sensory functions except for slight deficiencies in osmotic avoidance behaviour. Even though some of these nematode polypeptides are also synthesized in non-neuronal tissues as detected by fluorescent-tagged gene constructs we do not find any other obvious disturbances. We conclude that these polypeptides fulfil non-essential functions in neurotransmission and are only needed for specialized aspects of neuronal plasticity.

GK-P2 **Hyperpolarization-activated cation channels in fast-spiking hippocampal interneurons**

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Hyperpolarization-activated channels (I_h or HCN channels) are widely expressed in principal neurons in the CNS. However, I_h in inhibitory interneurons is poorly characterized. We examined the functional properties of I_h in fast-spiking basket cells of the dentate gyrus, using acute hippocampal slices from 17- to 20-day-old rats. To examine the possible presence of I_h channels in the somatodendritic domain, we tested the effects of the I_h channel blocker ZD7288. Bath application of 30 µM ZD7288 induced a hyperpolarization of 7.7 ± 1.4 mV (n=7). Furthermore, ZD7288 blocked hyperpolarization-activated current in a concentration- dependent manner (IC50= 13µM, Hill coefficient n_H= 0.87). To test whether I_h channels were also present in the axon of basket cells, we examined the antidromic propagation of action potentials from an axonal stimulation site to the soma. ZD7288 decreased the current threshold for evoking antidromic action potentials, consistent with the axonal expression of I_h. To examine whether Ih channels were present near presynaptic terminals, we recorded miniature IPSCs in hippocampal granule cells in the presence of 1 µM tetrodotoxin. 30 µM ZD7288 reduced the frequenzy of mIPSCs 1.85 ± 0.13-fold ($P < 0.01$), whereas the amplitude was unchanged ($P > 0.1$). In conclusion, our results suggest that I_h channels are expressed in the perisomatic region, axon, and presynaptic elemnts of GABAergic interneurons.

GK-P3 **Stimulus induced plasticity of the olfactory receptor neuron**

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Olfactory receptors (OR) are G-protein-coupled membrane receptors that encode the largest vertebrate multigene family ([a]1,000 ORs in the mouse and rat, [a]500–750 in human); they are expressed individually in the sensory neurons of the nose and have also been identified in human testis and sperm. OR signal transduction is facilitated via adenylyl cyclase upregulation of cAMP culminating in the opening of cyclic nucleotide-gated cation channels at the cell surface which elicits a graded receptor potential. We examined short-term and long-term olfactory receptor plasticity using established biochemical techniques in conjunction with a relatively novel proteomic strategy. Firstly, the question of short-term plasticity (e.g. receptor desensitization) was addressed by identifying novel OR receptor-protein-protein interactions using tagged fusion peptides. Tagged peptides from the intracellular loop 3 (IC3) and carboxyl termini of various mouse OR were used as bait to pull-out binding partners from mouse olfactory epithelium (OE). Interaction partners were identified using LC-MS/MS. In order to determine how OR mediate odour perception and how they influence long-term neuronal responses a differential proteomic strategy was employed. Test mice were exposed to odorants and the olfactory epithelium was compared to the OE from control mice using Fluorescence 2-D Difference Gel Electrophoresis (DIGE). Statistically significant differences in protein expression and peptide fingerprinting were performed using DIGE and MALDI-TOF respectively. Both proteomic strategies afford a powerful means whereby novel protein-protein interactions can be elucidated and thereby provide greater insight into olfactory receptor plasticity.

GK-P4 **Frequency organization of the medial geniculate body in the Mongolian gerbil**

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A common organization pattern for the primary auditory pathway is the systematic variation of different frequencies along a spatial arrangement (tonotopic map). In the central part of the auditory pathway information is relayed from the inferior colliculus (IC) in the midbrain via the medial geniculate body (MGB) of the thalamus to the auditory cortex (AC). Based on cytoarchitectonics the MGB is classically divided in three main parts: the ventral, dorsal and medial part, while only the ventral part belongs to the primary auditory pathway. The aim of this study was to investigate the parcellation of auditory information processing and the appearance of a putative tonotopic organization in the vMGB in the Mongolian gerbil. We used extracellular single and multi-unit recordings techniques with white noise or randomly presented pure tones stimuli in varying intensities to map basic neuronal response properties. The recording sites were histologically verified. We found according to the literature that neurons in the ventral part of the MGB are organized in a tonotopic fashion with a gradient in all three spatial directions. In medio-lateral direction low frequencies are located dorso-lateral, while high frequencies located ventro-medial. Additional we found a gradient in rostro-caudal direction with low frequencies represented dorso-rostral and high frequencies ventro-caudal. In this direction we could also observe a gradient in latencies with the occurrence of shorter latencies rostral and longer latencies caudal.

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GK-P5 **Gene expression profiling during gliogenesis in the *Drosophila* embryo**

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The central nervous system of *Drosophila melanogaster* comprises two basic celltypes, neurons and glial cells, which derive both from common precursor cells. These so-called neuroblasts can either generate only neurons (neuroblast), only glia (glioblast) or neurons and glial cells (neuroglioblast). The glial cell fate is determined by the master regulator gene *glial cells missing* (*gcm*), a transcription factor, which promotes gliogenesis on the one hand and supresses the neuronal fate on the other. Whereas the function of *gcm* in glial determination is well described, little is known about genes that maintain and regulate glial differentiation. To date only a few genes acting downstream of *gcm* are known and chraracterized, like *reverse polarity* (*repo*), *pointed* (*pnt*) and *tramtrack* (*ttk*). The aim of our screen is the identification of further *gcm* target genes to explore the molecular processes underlying gliogenesis in *Drosophila*. In order to achieve as high an accuracy as possible in our microarray data, we designed two antagonistic screens: first an ectopic expression of *gcm* in the CNS to transform all presumptive neurons into glial cells, and second the *gcm* loss-of-function, which shows a lack of all lateral glial cells. Both mutant backgroundswere compared to wild-type on a whole-genome microarray („Flyarray", Heidelberg*)* comprising about 21.400 predicted genes. We performed both screens as time course experiments throughout embryogenesis, thus obtaining expression profiles for every single *Drosophila* gene between developmental stages 9 to 16. These complemetary approaches enabled us to identify differentially regulated candidate genes either from different developmental stages and/or from different genetic backgrounds. Here we present the outcome of our combined screens and show that carefully selected combinations of filters for expression intensity, fold regulation and reproducibility as well as expression profiling allow the reliable detection of target genes in microarray experiments.

GK-P6 **Identification and characterization of *Drosophila* genes involved in glia cell specification and differentiation**

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Glial cells are crucial players in the development of a complex nervous system. For example, they play a role in the correct migration and proliferation of neurons in providing guidance cues for growing axons, in providing electrical insulation by the ensheatment of neurons and their axons controlling neural proliferation, and in the maintenance of the CNS structure. The determination of the glial cell fate is controlled by the master regulatory gene *glial cells missing* (*gcm*). Only few downstream targets are known so far. There exist several subtypes of glial cells categorized according to their morphology and position. With the aim to find new genes involved in glial differentiation and specification we are working on two different approaches. A large-scaled EMS (Ethyle-methane-sulfonate) mutagenesis-screen was performed in order to find genes involved in glial cell migration and differentiation. More than 2000 EMS-mutants were generated and screened for their phenotyps. Here we present the phenotypical characterization and the mapping of stocks that show a severe reduction in the number of glial cells in the CNS. The second approach for candidate genes was build up on the characterization of enhancer-trap lines selected for their glial specific reporter gene expression. The chosen strains show an expression not in all glial cells, but in a subset of glia, which might suggest a functional difference between the CNS glia. With this approach we try to demonstrate a

molecular classification of the glial subtypes. I will present enhancer-trap strains with reporter gene expression in different subgroups of glia, such as the longitudinal glia, a subgroup which is neuropile-associated and ensheats the longitudinal tracts, and the cell body glial, a subgroup that is cortex-associated and surrounds the neuronal cell bodies in the cortex.

GK-P7 **Synaptic targeting and time course of secretion of neurotrophins from hippocampal neurons**

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The neurotrophins (BDNF, NT-3, NT-4/5 and NGF) modulate the survival, differentiation and synaptic plasticity in the central nervous system (CNS). These homodimeric proteins are secreted from innervated target tissue and neurons, or from the axon terminals of projecting neurons and mediate their biological effects via activation of specific tyrosine kinase receptors (Trks) and the p75 neurotrophin receptor, respectively (Patapoutian and Reichardt, 2001). In spite of the wealth of knowledge regarding the biological downstream targets of neurotrophin action there is relatively little information about the sites and mechanisms of neurotrophin secretion (Lessmann et al., 2003). BDNF is secreted upon intense synaptic stimulation of CNS neurons (Hartmann et al., 2001), thus mediating changes in synaptic efficacy (Lu, 2003). However, the signaling mechanisms regulating synaptic release of neurotrophins are far from being understood. In order to enable a direct comparison, we overexpressed GFP-tagged versions of NGF, BDNF, NT-3 and NT-4 in hippocampal neurons, and explored quantitatively the vesicular targeting and the synaptic release of these NTs. Hippocampal neurons were chosen, because all NTs have been described to be expressed endogenously in this brain area (Murer et al., 2001). Cultured rat hippocampal neurons were transfected at 8 DIV with the GFP fusion constructs, and investigated at 9-11 DIV. NT secretion was monitored by time lapse video microscopy as a decrease in intracellular fluorescence intensity. BDNF and NT-3 were targeted more efficiently to secretogranin II positive, dendritic secretory granules (BDNF: in 98% of cells; NT-3: 85%) than NGF (45%) and NT-4 (25%). Fusing the BDNF pre-pro sequence to NT-4 redirected NT-4 to the regulated pathway of secretion, suggesting the existence of targeting signals in the pre-pro domain of BDNF. All neurotrophins were detected near synapsin I positive presynaptic terminals and colocalized with PSD95-DsRed, suggesting postsynaptic targeting of the neurotrophins to glutamatergic synapses. Depolarization induced (50 mM K+ or high frequency electrical stimulation) release of all neurotrophins from synaptic secretory granules was slow (delay in onset: 10-30 s, : 120-307 s) compared to transmitter release kinetics monitored with FM 4-64 destaining (onset: <5 s, : 13 ± 2 s). Among the neurotrophins, NT-4 secretion was most rapid but still proceeded seven times more slowly than transmitter secretion. Preincubation of neurons with monensin (neutralising intragranular pH thus solubilising the peptide core) increased the speed of secretion of BDNF, NGF and NT-3 to the value of NT-4. These data suggest that synaptic secretion of the four mammalian NTs proceeds substantially more slowly than release of conventional transmitters, and is determined by the speed of peptide core dissolution in secretory granules.

GK-P8 **Expression of constitutively activated Ras promotes enhancement of dopaminergic properties in differentiating neurospheres**

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Parkinson's disease is a neurodegenrative disorder and affects 2% of the aged population. Its primary pathology involves degeneration and loss of dopaminergic neurons. In order to replace the lost dopaminergic neurons, the concept of neural transplantation / replacement has been developed. However therapeutical transplantation of dopaminergic neurons generated from neural/precursor cells suffers from a dramatic loss in number after implantation by as yet unknown mechanisms. A number of extracellular signals such as neurotrophic factors and extracellular matrix proteins are able to promote neuronal survival and differentiation. These effects may be mediated at least in part through the intracellular plasma membrane bound protein Ras leading to a downstream activation of MAPkinases (ERK1,2, and Erk5). Using a synRas mouse model expressing permanently activated Val 12-Ha-ras (ras oncogene) under the control of the neuronal synapsin 1 promoter we could demonstrate that the toxic in vivo treatment with MPTP resulted in a strongly reduced loss of dopamine (DA) (Heumann, R. et al. 2000, J. Cell Biol. 151, 1537-1548). Using synRas mice embryo derived ventral midbrain neurospheres we investigate the possibilities to produce transplants with an increased number of stabilized and functionally active dopaminergic neurons.

GK-P9 **Analysis of Tenascin-C Isoform Complexity in Neural Stem Cells and their Regulation by Pax6**

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We are interested in the regulation and function(s) of the modular extracellular matrix glycoprotein Tenascin-C (Tn-C) in the central nervous system. Tn-C occurs in the adult neural stem cell niche and in the ventricular zone of the developing brain, where it is thought to control neural stem cell (NSC) development. Structurally, Tn-C 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 Tn-C isoforms could be generated, and in cerebellum 27 different Tn-C isoform have been detected. In this study on the complexity of Tn-C isoform expression in NSCs, which were grown as free-floating neurospheres, we found 20 different Tn-C isoforms to be present. The pattern of Tn-C isoforms in NSCs was comparable to the one in P6 cerebellum. However, one novel Tn-C isoform was discovered that, thus, might be NSC-specific. In order to study the regulation of Tn-C and its isoforms, overexpression of Pax6 in NSCs was chosen, as Tn-C expression is altered in Pax6-deficient small eye (*sey*) mouse mutants during embryonic forebrain development. Therefore, neurospheres were generated from the medial ganglionic eminence and co-transfected with Pax6 and EGFP expression plasmids. Transfected neurospheres clearly overexpressed Pax6 on mRNA and protein level as shown by RT-PCRs and immunocytochemistry, respectively. The Pax6 overexpressing neurospheres were analysed for their expression of tenascin-C isoforms by RT-PCR in comparison to EGFP- or non-transfected sibling cultures. Pax6 did not alter the total Tn-C mRNA expression level but showed a pronounced regulative effect on different tenascin-C isoforms. When Pax6 was overexpressed the largeTn-C isoforms containing four, five and six additional alternatively spliced FNIII domains were upregulated whereas the small isoforms without any or with one additional domain were downregulated. We also analysed the Tn-C isoform complexity after Pax6 overexpression, but to our surprise we did not observe any significant change in the combinatorial code of Tn-C isoform expression after the analysis of several hundred clones. Not surprisingly, Pax6 overexpression lead to an increase in the number of neurons formed in neurosphere differentiation assays as shown by others. Taken together, Pax6 selectively regulates Tn-C expression but the functional significance remains to be investigated.

GK-P10 **Broca's area and its language specific function**

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Within the field of cognitive neuroscience there is a debate on the nature of language focussing extensively on the role of "Broca's area". Several neuroimaging studies show that Broca's area - and particularly the pars opercularis of the left inferior frontal gyrus (IFG) - is involved in the processing of complex sentences. There are different functional interpretations for this activation increase. On the one hand a number of researchers argue that the activation in this cortical region is grounded in general cognitive operations because the processing of complex permuted sentence structures entails higher working memory demands, and factors like task difficulty and acceptability may lead to increased processing costs. On the other hand Broca's area has been selectively associated with properties deemed to be particular to language since highly specific syntactic operations have to be resolved within complex sentences (e.g. transformations). A dissociation between these potential influences is often impossible because the processing of complex permuted sentence structures involves general cognitive as well as specific syntactic processes. We used functional magnetic resonance imaging to explore the precise nature of the pars opercularis activation in the processing of permuted sentences by examining the permutation of pronouns in German. Whereas deviations from a subject-before-object order in sentences only including non-pronominal arguments are associated with increased processing costs there is a special rule in German that licenses an object-before-subject order when the object is a pronoun. In this case, both the subject- and the object-initial order are judged to be equally acceptable although the latter clearly involves a permutation. The results of our experiment show that, in contrast to non-pronominal permutations, sentences with a permuted pronoun do not engender enhanced pars opercularis activation. Thus, the predictions of working memory-based and transformation-based accounts of Broca's area are not borne out. Our findings therefore speak against both of these theories of this region's role in sentence comprehension. Rather, we argue that the pars opercularis of the left IFG is selectively sensitive to language-specific rules concerning the linearization of hierarchical linguistic dependencies.

GK-P11 **Age-related changes of cerebral white and grey matter structures from childhood to adulthood using voxel-based morphometry**

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Introduction: Voxel-based morphometry (VBM) is an objective technique for characterising regional cerebral volume and tissue concentration differences in structural magnetic resonance images of the brain [1]. VBM was used to characterise the development of brain structures. Volumes of grey matter (GM), white matter (WM), and their substructures were studied in childhood, adolescence and young adulthood, in order to identify age- and sex-related differences.

Methods: 3D-images were obtained from 115 normal subjects (49 males, 66 females) from 0.5 to 30 years of age using the FLASH sequence. VBM was carried out using Statistical Parametric Mapping 2 (SPM2) software (Wellcome Department of Imaging Neuroscience, UCL, London, UK). Age-group-specific templates were created. Images were spatially normalised to the appropriate age-related template, segmented into GM, WM and cerebro-spinal fluid (CSF) segments using age-group-specific prior maps of GM, WM and CSF for automated segmentation. In order to preserve the volume of a particular tissue within a voxel, the voxel values in the segmented images were then modulated by the Jacobian determinants derived from the spatial normalisation. An analysis of modulated images tests for changes in the total volume of the tissue. The segmented images were finally smoothed using an 12mm isotropic Gaussian kernel. This procedure is described in detail elsewhere [2]. Raw volumes of the segments were measured over the whole age range and compared for evaluation of the method with manually measured intracranial volume. The subjects were then divided into 4 different age groups for statistical comparison employing the framework of the general linear model. The total volume of GM (for GM analysis) or WM (for WM analysis) was modelled as confounding effect to study regional specific differences. For the age-related changes gender was modelled as a nuisance variable (and age for the sex differences).

Results: Relative to brain size, overall grey matter volume decreases non-linearly over this age range, whereas overall relative white matter volume increases. Most rapid changes occur in early childhood. The total volumes of both grey and white matter increase non-linearly over this age range, with clearly stronger increase of white matter than grey matter, resulting in an increase of the white-to-grey-matter ratio. In addition to these overall changes, regionally specific changes are also observed. Sex differences are found to be more prominent after puberty with females having more cortical GM volume and males having bigger subcortical GM structures. Age-related regional changes of GM and WM volume are observed in various areas and some have consistently been described in the literature [3,4].

Discussion: Our results show that age-related changes of overall grey and white matter volumes occur most rapidly in early childhood but continue into adulthood. In addition to these overall changes, regionally specific changes are also observed. It appears likely that these changes reflect the functional development of the human brain, including both progressive phenomena (cell proliferation, arborisation, myelination) and regressive phenomena (cell death, synaptic and axonal pruning, atrophic processes). While these data are from a cross-sectional sample and need to be replicated in a longitudinal study, the reported regionally dependent developmental and gender differences may be important to take into account when functional or structural changes are being investigated in healthy persons as well as in patients.

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GK-P12 **Selective ER export of HCN4 channels**

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Pacemaking activity in spontaneously active neurons and heart cells is believed to rely on a hyperpolarization-activated, cyclic nucleotide-gated cation current carried by members of the HCN channel family. Four HCN channel subunits have been identified, with HCN4 being the predominant HCN transcript in the heart. Mice lacking the HCN4 channel subunit do not develop a mature sinoatrial node-like pacemaker potential and mutations in HCN4 have been found in patients with sinus node dysfunction. Here, we investigated the intracellular protein processing of HCN4 channel in more detail. Unlike other HCN channel subunits, HCN4 did not show only efficient surface transport but also significant accumulation in the Golgi complex upon heterologous expression in epithelial cells. Co-expression of dominant-negative mutants of Sar1 GTPase, which is essential for the recruitment of the COPII complex mediating selective protein export from the endoplasmic reticulum (ER), prevented Golgi accumulation of HCN4 and retained the channel within the ER. By designing chimeric constructs between HCN4 and other HCN channel subunits, we identified a C-terminal domain in HCN4 that is both necessary and sufficient to drive ER

export of HCN channels. Currently, we are addressing the question whether preferential ER export of HCN4 affects surface transport of other HCN subunits known to form heteromers with HCN4 in heart and brain.

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GK-P13 **Developmental expression pattern of ionotropic glutamate receptors in differentiating embryonic stem cells**

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Ionotropic glutamate receptors play an essential role in neuronal development and are expressed in neural precursor cells. Some studies have demonstrated the critical role of glutamate in early development of the ventral telencephalon, particularly in promoting the proliferation of cortical progenitors via a NMDA receptor-dependent mechanism while the proliferation of cortical progenitors derived from dorsal telencephalon is regulated by activation of AMPA/KA receptors. The developing mammalian CNS has an extremely wide array of progenitor cells that are heterogeneous in their lineage specification and proliferation potential even within the same brain regions. Therefore we study the developmental expression pattern of ionotropic glutamate receptors in well defined neural precursors derived from embryonic stem cells. We analyse a wide range of ionotropic glutamate receptor subunits and their splice variants at the cellular level. Techniques used are RT-PCR, real time PCR, and immunocytochemistry. For analysis, the 46C mouse embryonic stem cell line is used. 46C ES cells were generated by gene targeting in E14Tg2a.IV ES cells (Ying et. Al.; 2003). The open reading frame of the Sox1 gene was replaced with GFPiresPac. The transcription factor Sox1 is the earliest and most specific marker for mammalian neural progenitors. We obtained neural stem cells from ES cells by adherent monoculture method (Ying et. Al.; 2003). We were able to get around 40 % Sox1-positive cells which are retrieved by FACS sorting. We are looking at three cellular stages for expression of glutamate receptor subunits: undifferentiated ES cells, neural stem cells and terminally differentiated neurons. Our preliminary results are based on real time RT-PCR analysis where the specificity of the band amplified is determined by size analysis. So far we showed that GluR7, KA1 and KA2 are present in undifferentiated embryonic stem cells. Receptor subunits present in neural stem cells are GluR3, GluR4flip, GluR5, GluR6, GluR7, KA1 and KA2. Detailed analysis of other subunits at these two cell stages and analysis of terminally differentiated neurons and glial cells is on-going.

GK-P14 **Remodelling of the damaged brain by fetal transplants?- Implication from studies in a rat model of Parkinson`s disease**

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Unilateral injections of 6-OHDA into the medial forebrain bundle lead to permanent impairments of skilled limb movements (SLM), especially in forelimb reaching tasks and gait performance. Fetal dopaminergic (DAergic) grafts promote an improvement of sensorimotor behaviour, however, the mechanisms and the qualitative aspects of graft-induced recovery are still unclear. The aim of this study was to determine whether transplantation of fetal DAergic neurons mediates recovery in SLM and gait performance by rewiring neural motor patterns for reaching movements and limb coordination, or whether transplants support compensation of motor deficits. The rats were trained in skilled reaching tasks (single pellet grasping task for qualitative and quantitative evaluation, staircase test for quantitative evaluation). Gait analysis (rung walking task and footprint analysis) was performed to test skilled walking and forelimb/hindlimb coordination. After 6-OHDA lesion and transplantation of E14 VM neurons rotational behaviour was assessed. After transplantation grafted rats showed overcompensation under amphetamine administration ans a reduction of apomorphine-induced rotation. This indicates a significant graft survival and graft effect on simple motor behaviour. Morphological and stereological analyses demonstrated substantial graft survival and reinnervation. For gait performance an incomplete but significant improvement after transplantation could be observed. The quantitative evaluation of SLM demonstrated significant enhancement whereas the qualitative analysis revealed no improving graft effect. We conclude that in hemiparkinsonian rats graft-induced recovery in SLM is due to graft-induced compensatory mechanisms.

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GK-P15 **Contribution of somatostatin to long term potentiation in the mouse hippocampus**

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The neuropeptide somatostatin (SST) is released from hippocampal interneurons during high frequency discharges. Although a number of effects of SST have been described at the cellular and subcellular level (for review see: Møller LN et al., 2002), little is known about its effects on the properties of the hippocampal synaptic network. Here we show a contribution of SST to synaptic long term potentiation (LTP) in the CA1 region of the rat hippocampus elicited by high frequency stimulation of the Schaffer-collateral – pathway in slice preparations *in vitro*. Recordings were obtained as field potentials in the *stratum radiatum*. Mice with a knock-out of the SST gene (SST-/-) showed a significant reduction of LTP (as compared to SST+/+ litter mates) with respect to the immediate post-tetanic potentiation and the overall magnitude up to one hour following tetanic stimulation. Baseline synaptic transmission as measured by an input/output relationship and paired-pulse ratio as determined by varying the interpulse interval stepwise from 50 to 300ms were not different in wild type compared with knock-out animals. Furthermore, the effect of the SST gene knock-out on hippocampal LTP could be mimicked by somatostatin depletion in wild type animals, achieved by intraperitoneal injection of cysteamine (50 mg/kg) four hours prior to slice preparation. The time course and magnitude of LTP after cysteamine injection closely resembled that in SST-/- mice, whereas a saline injected control group showed no alterations. To control for effects of cysteamine other than depletion of SST, we injected SST-/- mice with the compound. An effect of cysteamine on LTP was not observed. In conclusion, our results show that SST contributes to synaptic plasticity in the hippocampus and thereby provides one possible explanation of the effects of SST depletion on fear-learning and memory formation (Stoppel C et al., 2005, German Neuroscience Society meeting - Abstract).

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GK-P16 **Promoter hypermethylation and transcriptional downregulation of the carboxyl-terminal modulator protein (CTMP) gene in glioblastomas**

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Introduction: The carboxyl-terminal modulator protein (CTMP) has been identified as a negative regulator of protein kinase B/Akt [1]. Aberrant Akt signaling is frequently observed in glioblastomas, the most common and most malignant glial brain tumors. Because loss of CTMP function and/or expression may remove the inhibitory effects on Akt and promote tumorigenesis, we investigated 93 primary glioblastomas and 9 glioblastoma cell lines for genetic alteration, promoter hypermethylation and expression of CTMP [2].
Methods: A total of 93 primary glioblastomas and 9 established glioma cell lines were investigated. All tumors and cell lines were analyzed for CTMP mutations using single-strand conformation polymorphism analysis and DNA sequencing, and for homozygous deletion of CTMP using duplex-PCR analysis. The CTMP mRNA levels in tumors and cell lines were assessed by real-time reverse transcription-PCR and compared with non-neoplastic brain tissue. The methylation status of the CTMP promoter was determined by sequencing of sodium bisulfite-treated DNA. In addition, we used a methylation-specific PCR assay based on HpaII restriction digestion. To assess whether hypermethylation was responsible for the decreased mRNA expression, glioma cell lines were treated with the demethylating agent 5-aza-2'-deoxycytidine and the histone deacetylase inhibitor trichostatin A. Akt activation was determined by Westen blotting using phospho-Akt specific antibodies.
Results: Constitutive activation (phosphorylation) of Akt was found in all investigated glioblastomas but not in non-neoplastic brain tissue. None of the tumors or cell lines demonstrated CTMP homozygous deletions or coding sequence mutations. However, CTMP mRNA expression was lower by at least 50% relative to non-neoplastic brain tissue in 37 (40%) glioblastomas and six (67%) glioma cell lines. Reduced CTMP mRNA levels were closely associated with aberrant hypermethylation of the CTMP promoter. Furthermore, treatment of CTMP hypermethylated A172 glioma cells with the demethylating agent 5-aza-2'-deoxycytidine and the histone deacetylase inhibitor trichostatin A resulted in partial demethylation of the CTMP promoter and increased CTMP mRNA expression. CTMP promoter hypermethylation and reduced mRNA expression did not show any correlation with PTEN mutations or EGFR amplification in our tumor series.
Conclusions: Taken together, our results demonstrate that epigenetic downregulation of CTMP transcription is a common aberration in glioblastomas that likely contributes to aberrant Akt signaling in these tumors. Thus, CTMP is a novel tumor suppressor gene candidate that seems to be involved in the pathogenesis of a substantial fraction of glioblastomas.

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GK-P17 **Role of munc-18-1 as a docking factor for granules in chromaffin cells, revealed by evanescent wave microscopy**

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Munc-18-1, a syntaxin-1A interacting protein, is an important upstream regulator of exocytosis. Its null mutation in mice eliminates synaptic transmission at central nervous synapses and neuromuscular junction and impairs docking of large dense-core vesicles (LDCVs) in adrenal chromaffin cells. Its exact function however is not known. Here we applied evanescent wave microscopy (TIRFM) for real-time imaging of fluorescently labeled single LDCVs beneath the plasma membrane in intact chromaffin cells from munc-18-1 null mutant (KO) and wild-type (WT) mice. The average density of fluorescently labeled LDCVs at the membrane footprint of KO cells was two times smaller than in WT cells or cells rescued by viral expression of munc-18-1. Also, detected LDCVs in KO cells were localized more distantly from the plasma membrane in line with electron microscopic data. By three-dimensional tracking of docked LDCV in x, y, and z we found parameters of simple diffusion to be similar for KO and WT cells. However, the velocity autocorrelation function (VACF) calculated for LDCV movement perpendicular to the membrane revealed a distinct negative component at small correlation times . ~0.5-1 s for WT cells, absent in KO cells. This feature was rescued in KO cells by munc-18-1 overexpression and partially recovered after introduction of a mutated munc-18-1 with low affinity for syntaxin-1A. Also, phorbol ester (PMA) application rescued KO cells completely. The negative component of the VACF indicates a deviation from simple free diffusion and can be interpreted as repetitive changes in direction of movements along the z axis. Monte Carlo simulations show that it can be attributed to either restricted diffusion (when the LDCV is 'encaged' near the membrane) or tethering forces acting on the LDCV. Since the 'cage' could be provided by the cytoskeleton, we applied latrunculin-A to disrupt the cortical actin network. This treatment did not significantly change the VACF in both WT and KO. Our data indicate that munc-18-1 is an essential positive regulator during docking by strengthening tethering forces probably requiring interaction with the t-SNARE syntaxin.

GK-P18 **Differential expression of the homeobox gene Pitx 3 in midbrain dopaminergic neurons**

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In Parkinson's disease and its models loss of dopaminergic (DA) neurons in substantia nigra (SN) is higher than in the ventral tegmental area (VTA). This variation could be related to developmental gene regulation and expression of neuroprotective markers such as calcium-binding protein calbindin (CB). Pitx3 is a novel transcription factor that is expressed selectively in the midbrain and regulates the differentiation and survival of DA neurons. Here we studied the pattern and level of expression of Pitx3 in isolated midbrain cells. Single-cell RT-PCR revealed the presence of Pitx3 in 72 % of VTA and in 78% of SN neurons in young adult Wistar rats. The level of Pitx3 mRNA expression, determined by semi-quantitative RT-PCR, was 5.8 fold higher in VTA than in SN single neurons. In the VTA but not in SN the level of Pitx3 expression correlated with CB presence: in CB-positive neurons the expression of Pitx3 mRNA was 3.6 times higher then in CB-negative cells. In the VTA CB was expressed exclusively in Pitx3-positive neurons (50%). A higher Pitx3 expression level and higher coexpression of Pitx3 and CB in VTA in comparison to SN neurons may contribute to the different vulnerability of these dopaminergic nuclei to neurodegeneration.

GK-P19 **LTP and reinforcement of early-LTP by stimulation of ventral tegmental area in freely moving rats *in vivo***

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It has been shown that the prolonged maintenance of hippocampal long-term potentiation (LTP) requires heterosynaptic events during its induction. In recent studies was shown that stimulation of modulating brain structures within a distinct time window can reinforce a transient early-LTP into a long-lasting late-LTP in the dentate gyrus in freely moving rats. This reinforcement was dependent on β-adrenergic and/or muscarinergic receptor activation and protein synthesis. The question arose as to whether also similar mechanisms can be described for forms of LTP in the hippocampal CA1 area. Experiments in vitro revealed that here, other modulatory inputs such as the dopaminergic system maybe required than those important for the dentate gyrus. The ventral tegmental area (VTA) is a heterogeneous group of dopaminergic cells and a major component of the mesolimbic dopamine system. Neurons of the VTA are involved in the regulation of motor and motivational aspects of behaviour and reveal high neuronal plasticity. The VTA is the major dopaminergic input to the CA1 region of the hippocampus. Thus, we have studied the effect of VTA-stimulation of CA1-LTP in freely moving animals. First, we have solved technical problems with respect to reliable recordings of the population spike and field-EPSPs in freely moving animals. Thus, we stimulate the contralateral CA3 and record ipsilaterally from CA1 principal cells. This allowed us to induce different forms of LTP in the CA1-region in vivo in a very reliably manner. We now present

first data studying the effect of VTA-stimulation on early-LTP in CA1. Stimulation of the mesolimbic input to the hippocampus resulted in the reinforcement of this early-LTP into the long-lasting late-form of LTP.

GK-P20 **Role of cannabinoids in excitotoxic neuronal damage, as assessed in organotypic hippocampal slice cultures**

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Acute CNS pathologies are characterized by loss of neurons, activation of microglial cells and the presence of reactive astrocytes. Microglial cells are responsible for the exacerbation of the initial injury (secondary damage) by releasing neurotoxic substances such as proinflammatory cytokines and free radicals. It is also known that a massive proliferation of microglia and astrocytes occurs. There is recent evidence that endogenous Cannabinoids in the CNS as well as the exogenous plant derived substance Δ^9- Tetrahydrocannabinol protect granule cells from excitotoxicity. We therefore intended to investigate whether pharmacological treatment with Δ^9- Tetrahydrocannabinol or the endogenous Cannabinoids Anandamide (AEA), 2-Arachidonylglycerol (2-AG) and Palmitoylethanolamide (PEA) inhibits secondary neuronal damage after excitotoxic injury by suppression of microglial and astrocytic activation. Organotypic hippocampal slice cultures (OHSC) derived from 8 day old (p8) Wistar rats were lesioned by the application of N-methyl-D-aspartate (NMDA) after 6 days in vitro (div) and treated with THC (0,03-15µM) or AEA, 2-AG or PEA (0,1-0,001µM, at four different time points) up to 9 div. Propidium iodide (PI) was used to visualize the extent of neuronal damage, microglial cells were stained with FITC-conjugated *Griffonia simplicifolia* isolectin B_4 (IB_4), and OHSCs were analysed by confocal laser scanning microscopy. The dentate gyrus of control OHSCs contained almost no PI^+ neurons and few, ramified microglial cells, whereas NMDA-treated cultures displayed a massive increase in PI^+ neurons and large numbers of amoeboid microglial cells accumulating at sites of neuronal injury. However, treatment of lesioned OHSCs with THC (3µM) resulted in a 48.6% decrease in the number of microglial cells, compared to OHSCs only lesioned with NMDA. Treatment of lesioned OHSC with certain concentrations of the three used endocannabinoids led to a strong increase of microglial cells which was partly correlated to a change in microglial morphology.
Our findings show that THC as well as the endocannabinoids do not protect granule cells from excitotoxic injury. THC leads to a significant decrease, endocannabinoids to an increase in the number of activated microglial cells on the one hand and changes in microglial morphology on the other hand. Further experiments are needed to elucidate this discrepancy between the effects of THC and endocannabinoids on microglial cell numbers and morphology.

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GK-P21 **Sustained asynchronous activity in random network of spiking neurons**

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We investigate the dynamics of a large sparsely connected random network of spiking neurons, connected with conductance based synapses. Global population activity, single neuron firing rate and irregularity of firing is studied as a function of external input (V_{ext}) and relative strength of inhibition (g). The network is able to show asynchronous population activity while individual neurons spike in an irregular manner at low rates (AI state) for low V_{ext} in inhibition dominated regime. Increasing V_{ext} or excessive inhibition leads to synchronous activity and individual neurons spike regularly. We propose a numerical solution of the mean field approximation using a single neuron model of large sparsely connected network of spiking neurons [1] . We find that the single neuron model is a good approximation of network in the AI state. The deviations in predicted rate using single neuron approximation and network rates are presumably because of correlation in network due to shared connectivity. Using the single-neuron approximation, we predict that the random network of spiking neurons can show a sustained asynchronous activity at low and irregular firing rates, in absence of any external input. We test this prediction in large network simulation of size 10^5 . Indeed the network can show sustained AI type of activity lasting up to a second, in absence of any external drive. However there is a small deviation in sustained activity frequency predicted by single neuron and as is observed in network simulation. We systematically investigate this deviation and find that the deviation are a collective result of random fluctuations and slope of transfer function at the fixed point. This result contradicts previous study where it was shown that a network needs external input to show low asynchronous irregular activity [2].

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GK-P22 **Two types of protease-activated receptors (PAR-1 and PAR-2) mediate calcium signaling in rat retinal ganglion (RGC-5) cells**

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Protease-activated receptors (PARs), the unique G protein-coupled receptors, are widely expressed in various tissues, where they participate in many physiological and pathological processes, such as hemostasis, proliferation, tissue repair and inflammation. There are four members of this family: PAR-1, PAR-3, and PAR-4, which are activated by thrombin; and PAR-2, which is activated by trypsin. PARs activation could result in calcium mobilization. Calcium, an important second messenger, plays an important role in neurons. Increasing calcium activates various signal pathways that lead to the expression of genes that are essential for dendritic development, neuronal survival and synaptic plasticity. In the present study, we used RGC-5 cells, a rat transformed retinal ganglion cell line, as a cell model *in vitro* to study PAR-mediated calcium signaling. Using reverse transcription-polymerase chain reaction (RT-PCR), we demonstrate that RGC-5 cells mainly express PAR-1 and PAR-2, which was confirmed by indirect immunofluorescence. Short-term stimulation of RGC-5 cells with thrombin (0.001-1 U/ml) and trypsin (1-100 nM) dose-dependently induced a transient increase in intracellular calcium concentration ($[Ca^{2+}]_i$). An increase in $[Ca^{2+}]_i$ was also induced by both TRag (PAR-1 activating peptide, Ala-pFluoro-Phe-Arg-Cha-HomoArg-Tyr-NH_2) and PAR-2 activating peptide (PAR-2 AP; SLIGRL, Ser-Leu-Ile-Gly-Arg-Leu). The EC_{50} values were 0.3 nM for thrombin, 12.0 nM for trypsin, 1.3 M for TRag and 1.6 M for PAR-2 AP, respectively. Desensitization studies showed that a second addition of thrombin after pre-pulse challenge with thrombin or TRag significantly reduced the calcium response, but the preceding stimulation with PAR-2 AP did not decrease the amplitude caused by thrombin as a second challenge. A second addition of trypsin after a pre-pulse challenge with trypsin, TRag or PAR-2 AP also significantly reduced the calcium response. These results suggest that thrombin could activate PAR-1, but not PAR-2. Trypsin, however, could activate PAR-1, as well as PAR-2 in RGC-5 cells. Calcium source studies showed that PAR-induced calcium mobilization mainly comes from intracellular calcium stores in RGC-5 cells. This result was quite different from that of astrocytes and OLN-93 oligodendrocytes, where PAR-mediated calcium mobilization comes from both intracellular stores and extracellular space. Intracellular calcium overload via calcium influx could lead to neuronal degeneration. The role of calcium release from intracellular stores in neurons, however, is not clear till now. Some studies showed that PARs were involved in neuronal degeneration. Therefore, it will be interesting to investigate the effect of calcium from intracellular stores induced by PAR on neuronal degeneration in RGC-5 cells.

GK-P23 **The scaffold protein harmonin (USH1C) also integrates Usher syndrome 2 proteins into synaptic Usher protein complexes in retinal photoreceptor cells**

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The Usher syndrome (USH) is the most common form of combined deaf-blindness. USH is divided in 3 clinical distinct types USH1 to USH3. USH1, the most severe form, is characterized by profound congenital deafness, constant vestibular dysfunction and prepubertal onset of retinitis pigmentosa. Whereas, USH2 is a mild form of USH with moderate to severe sensorineural hearing impairment at birth, normal vestibular responses and progressive retinitis pigmentosa. USH1C encodes for the protein harmonin, which contains PDZ-motifs known to organize protein complexes via protein-protein interaction. In previous studies, we and others have demonstrated that harmonin binds all 5 known USH1-proteins via its 3 PDZ domains. Their colocalization in the photoreceptor synapses suggests that these interactions occur in the synaptic compartiment of the specialized neurons, where they may constitute the basis for a supramolecular USH1-complex. The aim of our present study was to validate the presence of the three USH2-proteins namely Usherin (USH2A), NBC3 (Na-Bicarbonate Co-transporter 3, USH2B) and the 7-transmembrane receptor VLGR1 (Very Large G-protein coupled Receptor 1, USH2C) in USH-protein-complexes. *In vitro* (GST-pull downs) and *in vivo* (yeast two hybrid system) assays of protein-protein interactions reveal that all three USH2-proteins also bind to the PDZ-domains of harmonin. Immunocytochemical analysis of cryosections of rat and mouse retinas further demonstrates additional colocalization of USH2-proteins with USH1-proteins in photoreceptor synapses. Our findings strongly suggest that ribbon synapses of photoreceptors bear supramolecular protein complexes composed of USH1- and USH2-proteins. Mutations of one of the components may cause dysfunction of the entire complex which probably leads to synaptic defects and in turn to retinal degeneration (retinitis pigmentosa), the phenotype observed in USH-patients.

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GK-P24 **Neuro-protection and neuro-regeneration using adeno-associated virus**

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Neuro-degeneration is a commonly used word which has a global impact. Neuro-degeneration corresponds to any pathological condition primarily affecting neurons.. A closer look at the pathogenesis of neuro-degeneration almost always reveals an apoptotic component that either contributes to disease progression or accounts for it. This raises the possibilities in the treatment of CNS diseases by potential interruption of the programmed cell death, apoptosis. In this study, we used Adeno Associated Virus-2 (AAV) to express various proteins in the retina and compared retinal ganglion cell (RGC) survival after optic nerve transection. Overexpression of Bcl-xl promoted high percentage of RGC survival after optic nerve lesion. Other transduced proteins (CalbindinD28K, GDNF) did not have a profound effect on cell survival. The combined effect of Bcl-xl and GDNF shows higher rescue effect of RGCs than Bcl-xl or GDNF alone. Having sorted the problem of survival, we proceeded with the next logical question. Can these RGCs regenerate? Currently, we are studying regeneration, in a microcrush lesion model of the optic nerve. Although Bcl-xl overexpression played a key role in long term RGC survival, it does not promote axonal outgrowth after microcrush lesion. Therefore we propose to use RNAi along with overexpression of Bcl-xl to promote axon regeneration. So far, we have shown that when two AAVs were injected simultaneously, they could be co-localized in nearly 90% of the RGCs. Also we have achieved a nearly complete knockdown of GFP transduced RGCs using shRNA carrying AAV-vectors.

GK-P25 **Long term neuroprotection in an optic nerve transection model**

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Programmed cell death plays a critical role in a wide range of physiological processes. Regulation of apoptotic cell death contributes to various neuro-degenerative diseases. The potential of gene delivery to the CNS to repair and treat neuro-degenerative diseases has received much recent attention.. Many studies have shown that gene therapy, with anti- apototic proteins, helps to prevent cell death. Until now, long-term survival studies have shown that cell death is only delayed, not prevented. Adeno associated virus has been developed as an attractive gene delivery system. They mediate long-term expression that can persist for upto an year and are not associated with any known human diseases. Bcl-XL prevents neuronal apoptosis during development and neuro-degenerative disease. It blocks the apoptotic cascade upstream of many caspases and the mitochondrial dysfunction. To study if long term survival can be achieved using Bcl-XL, we injected Adeno Associated Virus (AAV) expressing Bcl-XL into the intravitreal space of the eye of the wistar rat. We show that Bcl-XL prevents neuronal death in a retinal ganglion cell model of axotomy-induced apoptosis and also helps survival of 93%, 73%, 67% and 47% of retinal ganglion cells (RGC's) for 2, 4, 6 and 8 weeks *in vivo* respectively. Thus, Bcl-XL prevents neuronal death and it aids in the long-term survival of neurons.

GK-P26 **Role of GM-CSF for development and function of Dendritic Cells in the central nervous system**

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Granulocyte-macrophage colony-stimulating factor (GM-CSF) is an inflammatory cytokine and a hematopoietic growth factor which has profound effects on microglial cells. In vitro, GM-CSF promotes the differentiation of neonatal and resting adult microglia into cells which are phenotypically and functionally similar to dendritic cells (DC). In vivo, GM-CSF is expressed in the central nervous system (CNS) under inflammatory conditions; however, its precise role is still unclear. To address the role of GM-CSF in the CNS, mice were given GM-CSF intracerebrally over a period of 6 days using a microosmotic pump. Intracerebral leukocytes (ICL) were isolated at day 0, 4 and 6 of treatment for phenotypical and functional analysis. Similar numbers of ICL ($3x10^6$) were obtained from untreated and PBS-treated animals, while significantly more ICL could be isolated after GM-CSF treatment (d4: $4.5x10^6$, d6: $5.3x10^6$). This was due to an increase in leukocytes expressing high levels of CD45 as determined by FACS analysis. Within the $CD45^{high}$ population, the majority of the cells (~ 90 %) were $CD11b^+$ myeloid cells and approximately 50 % co-expressed the DC marker CD11c. In addition, an enhanced expression of MHC II and costimulatory ligands (CD80, CD86) was detected while expression of CD40 was not increased. When tested as stimulator cells for naïve T cells in a mixed leukocyte reaction, $CD11c^+$ ICL from GM-CSF-treated mice had a reduced stimulatory capacity compared to splenic DC or DC from the brains of mice with Toxoplasmic encephalitis. Taken together, intracerebral GM-CSF treatment induces the presence of DC in the brain which seem to be functionally immature. The origin and function of these CNS-DC will be further investigated.

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GK-P27 **Developmental changes of GABAergic input synapse distribution in an identified insect motoneuron**

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During insect metamorphosis, changes of motor behavior require the respecification of the neuronal circuits underlying the production of these behaviors. This is achieved by specific postembryonic alterations in neuronal structure, membrane properties and synaptic connectivity. During metamorphosis of the tobacco hornworm, *Manduca sexta*, the identified motoneuron, MN5, develops from a slow motoneuron that participates in larval crawling, into a fast adult flight motoneuron. This study aims to investigate the impact of changing dendritic architecture and altered distribution of GABAergic input synapses onto MN5 for its different behavioral roles. Dendritic architecture is assessed by novel methods for highly precise 3-D reconstructions from confocal image stacks. By combining these precise geometric reconstructions with immunolabeling for Synapsin I and GABA, the distribution patterns of putative GABAergic input synapses through entire dendritic trees of different developmental stages can be addressed by high resolution confocal laser scanning microscopy. In all stages investigated (larval crawling motoneuron, regressed pupal motoneuron without behavioral function, adult flight motoneuron), specific construction principles for the distribution of putative GABAergic inputs are found. For instance with regard to the distribution of GABAergic inputs along all individual dendritic segments, in low branch orders the larval crawling motoneuron seems to receive inhibitory inputs predominantly at branch points. This indicates that entire dendritic subtrees may be controlled by few GABAergic on-off switches. This construction principle is lost during the loss of the larval motor function. We currently test whether similar or different construction principles become implemented during the gain of the new adult function. With regard to the analysis of the tree topology, putative GABAergic inputs are lost to different extends in different branch orders, indicating a differential regulation of inhibitory synapse elimination within a single dendritic tree. Multi-compartment models will now be used to elucidate possible functions of the rules for the distributions of putative GABAergic input synapses with regard to synaptic input integration and the different behavioral tasks during various developmental stages.

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GK-P28 **Neurogenesis in the adult subventricular zone: a functional role for extracellular nucleotides**

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Studies in particular of the last decade showed that active neurogenesis continuously takes place in the subventricular zone (SVZ) of the lateral ventricles of the adult rodent brain. Neurogenesis in the SVZ leads to migration of neuroblasts within the rostral migratory stream (RMS) and neuron formation mainly in the olfactory bulb (OB). According to present understanding, glial cells with astrocytic properties represent the actual adult neural stem cells. The cell types representing the various cellular transition states leading to the formation of mature neurons as well as the mechanisms controlling adult neurogenesis and neuroblast migration are poorly understood. We show that the ATP-hydrolyzing enzyme nucleoside triphosphate diphosphohydrolase 2 (NTPDase2) is associated with type B cells, the presumptive neural stem cells. NTPDase2 is a protein of the plasma membrane with its catalytic site facing the extracellular space. It hydrolyzes extracellular nucleoside triphosphates to their respective nucleoside diphosphates. This raises the possibility that the signaling pathway via extracellular nucleotides is involved in the control of adult neurogenesis. Neurons as well as glial cells express several subtypes of receptors that are responsive to the nucleotides ATP, ADP, UTP, or UDP (P2 receptors). P2X receptors are ion channels, P2Y receptors are coupled to trimeric G-proteins. In order to probe for a functional role nucleotides we referred to an *in vitro* system for analyzing stem cell properties. Neurospheres produced from isolates of the mouse SVZ and cultured in the presence of EGF and FGF-2 express NTPDase2 as well as tissue non-specific alkaline phosphatase. Accordingly, neurospheres hydrolyze extracellular ATP to adenosine. Since these ecto-nucleotidases control the availability of extracellular nucleotide agonists we studied the potential expression and functional role of nucleotide receptors in isolated neurospheres. Neurospheres respond to extracellular nucleotides with a rise in Ca^{2+}_i (ATP = ADP > UTP). The $P2Y_1$ antagonist MRS 2179 strongly reduces the ATP- or ADP-induced increase in Ca^{2+}_i, suggesting the involvement of a $P2Y_1$ receptor. The agonistic activity of UTP and the lack of response to UDP imply the additional presence of a $P2Y_2$ and/or a $P2Y_4$ receptor. The $P2Y_1$ antagonist MRS 2179 also reduces neurosphere proliferation. Taken together with previous observations of a synergistic effect on cell proliferation of ATP and growth factors, our results suggest that P2Y-mediated nucleotidergic signaling is involved in neurosphere function and possibly also in adult neurogenesis *in situ*.

GK-P29 **Paired-pulse TMS effects on single unit activity in cat primary visual cortex**

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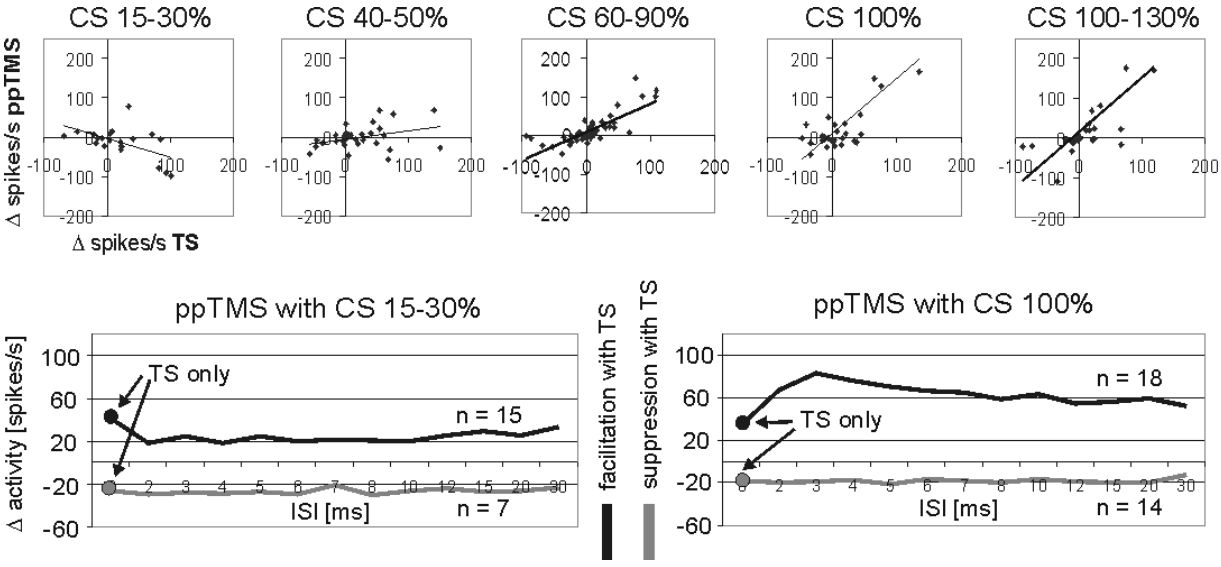
Paired-pulse transcranial magnetic stimulation (ppTMS) has become a tool for exploring the excitability of human cortical networks, especially in the motor system. Applying a subthreshold conditioning stimulus (CS) 2-5 ms prior to a suprathreshold test stimulus (TS) to human motor cortex has been found to weaken the motor evoked potential (MEP) triggered by the test stimulus, while an inter-stimulus interval (ISI) of 7-30 ms leads to a potentiation of the MEP (1,2). The effect observed with short ISIs has been interpreted as a strengthening of intracortical inhibition (ICI) while long ISIs favour intracortical facilitation (ICF). However, ICI evoked by short ISIs is only found with subthreshold CS. Increasing CS strength to that of TS, or a suprathreshold CS followed by a subthreshold TS leads to ICF also at short ISI (1,3,4). In the present study, we tested the paired TMS paradigm for visually evoked single and multi-unit activity in cat primary visual cortex. Paired pulses were generated by 2 MagStim 200 units connected via a BiStim module (Micromed) and were applied via a 2x 70 mm figure-of-8 coil to area 17/18 of the anesthetized (N_2O/O_2 [70/30%] + halothane [0,8%]) and paralyzed cat. Here, we defined the TS as being suprathreshold (100%) when it caused a significant increase or decrease in the visually evoked activity. By systematically varying the ISI between 2 and 30 ms and the strength of the CS within a range of 15-130% of TS, we found no dependence of ICI and ICF on ISI but a clear dependence of ppTMS effect on CS strength. By plotting the additional changes in visual activity achieved by ppTMS (ppTMS effect minus TS effect) versus the change evoked only with the single pulse TS (see upper row diagrams), we found a clear correlation between ppTMS and TS effect, but the correlation varied with CS strength. A significant negative correlation was found with weak CS strength in the range of 15-30%. Increasing the CS strength above 50% caused a flip to a clear positive correlation: within a CS range of 60-130% ppTMS resulted in a stronger facilitation if the TS caused facilitation of visual activity by itself and more suppression if the TS itself was suppressive. This correlation also indicates that the ppTMS effect scales with TS effect. Correlations shown are for an ISI of 3 ms between CS and TS. Correlations obtained with other ISI between 2 and 30 ms did not show significant differences.



The 2 lower diagrams of the figure show mean changes in activity achieved with different ISI between CS and TS in comparison with the change in activity resulting from TS alone (large dots at ISI 0). Cases in which TS had either a facilatory (black curve) or a suppressive effect (gray curve) are averaged separately. The data with 15-30% CS strength show a reduced facilitation by TS, while the data with 100% CS strength show potentiation of the TS effect. Qualitatively identical effects were found for all tested ISI, but the strongest effects were found for ISI of 2 to 4 ms and declined for longer ISIs. Our results show that ppTMS effects on sensory activity evoked in the visual cortex (of cat) differ from ppTMS effects on artificially evoked efferent activity in human motor cortex.

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GK-P30 **Expression and function of the neuronal protein 25 (NP25) in the developing chicken embryo**

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The neuronal protein 25 (NP25) has been initially identified as a gene of unknown function, expressed exclusivly in neurons of the adult rat brain. NP25 belongs to the calponin (CaP) family of actin binding proteins that are characterized by containing one calponin homology (CH) domain. Here we show that the expression of NP25 in the chicken embryo starts at embryonic day 2 (E2) stage 11 (st11) in the closed neural tube prior to the onset of expression of the neuron differentiation marker gene Superior Cervical Ganglion Clone 10 (SCG10, E3, st18). We also demonstrated that the expression is limited to postmitotic neuronal progenitor cells (BrdU labelling). This excludes a role of NP25 in the proliferation of neuronal progenitor cells and makes NP25 to one of the earliest marker for postmitotic neurons. These findings in the spinal cord were confirmed for the paravertebral dorsal root ganglia (DRG) and sympathetic ganglia (SG). To elucidate the so far unknown function of NP25, we performed overexpression experiments in Phaeochromocytoma (PC12) cells, a model cell line for peripheral autonomic neurons. NP25 overexpression was able to enhance neurite growth, resulting in increased length of longest neurite (159 % +/- 24%, $p < 0,05$) as well as in an increased number of neurites per cell (151% +/- 12%, $p < 0,01$).Our experimental data, together with the structural properties of NP25 suggest an important role of NP25 in the remodeling of the actin cytoskeleton, controlling neurite outgrowth in postmitotic neuronal progenitors.

GK-P31 **Schwann cells express Toll-like receptors**

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Schwann cells (SC), primarily responsible for the formation of myelin within the peripheral nervous system (PNS), might be involved in the local immune response in the PNS. Several lines of evidence indicate that SCs display immune-related functions, such as the expression of MHC class II antigens, suggesting that SCs can acquire the role of a facultative antigen-presenting cell. Toll-like receptors (TLRs) have emerged as key receptors responsible for recognizing specific conserved components of microbes, such as lipopolysaccharide (LPS), and have been implicated to play a critical role in various inflammatory disorders. LPS may be a relevant antigen in causing immune-mediated demyelination of the PNS; therefore we studied the expression of TLRs in these PNS resident cells. In SCs from rat various TLRs were detectable *in vitro*, out of which TLR-2 and -4 were found to be regulated by substrates, such as interferon-alpha, or phorbol 12-myristate 13-acetate (PMA) in a time- and dose-dependent fashion, as measured on the RNA-level by RT-PCR as well as on protein level by immunofluorescence. After stimulation with LPS the secretion of various inflammatory mediators was induced, as determined by a qualitative protein array and quantitated by ELISA. These mediators include chemokines, growth factors, and protease inhibitors. This induction was effectively blocked by specific anti-TLR-2 and anti-TLR-4 antibodies as well as inhibitors of nuclear factor-kappa B. Thus, our data indicate that SCs might act as a link between innate and acquired immunity via TLRs in the inflamed PNS.

GK-P32 **The transmembrane form of agrin reorganizes the cytoskeleton in neurons and non-neuronal cells**

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Agrin is a heparansulfate proteoglycan which is widely expressed in the central and peripheral nervous system as well as in non-neuronal tissues.. Agrin's function is best understood in skeletal muscle where it plays a key role during formation, maintenance and regeneration of the neuromuscular junction. Usage of alternative first exons generates two agrin isoforms: a soluble, secreted basement membrane-bound form responsible for synapse formation at the neuromuscular junction, and a type II transmembrane protein with a single membrane-spanning region. The transmembrane form of agrin is primarily expressed in the CNS on growing axons, and it was suggested that it might be a receptor with signal transducing activity. Since downstream signaling pathways of transmembrane receptors can be activated ligandindependently by antibody-mediated oligomerization, we clustered the transmembrane form of agrin using polyclonal anti-agrin antibodies. Clustering of TM-agrin induced the formation of numerous filopodia-like processes on growing axons from the CNS and PNS. The processes contained a complex cytoskeleton, required calcium and could be inhibited by cytochalasine D. Likewise, heterologous expression of TM-agrin in different cell lines induced the formation of numerous long, actin-containing processes extending from the transfected cells, indicating that TM-agrin alone is sufficient for reorganizing the cytoskeleton and that this activity of TM-agrin is not limited to neurons. While the intracellular and the transmembrane domains could be deleted without loss of the process-forming activity, the heparansulfate chains, and their close association with the plasma membrane were necessary and sufficient for the process inducing activity. These results are consistent with TM-agrin being a receptor or co-receptor involved in axonal growth of CNS neurons.

GK-P33 **Shedding light on glutamate receptor dynamics during synapse formation of living *Drosophila***
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Recent evidence suggests that the mobility of non-NMDA type glutamate receptors can control synapse efficacy over shorter time periods. However, so far glutamate receptors have not been imaged in vivo during naturally occurring synapse formation. We use confocal imaging on intact Drosophila larvae expressing fully functional GFP-labeled glutamate receptors marking individual postsynaptic densities. We find that new glutamate receptor fields form exclusively de novo, and reach their mature size in about 24 hrs The mobility of glutamate receptors is further analyzed with in vivo photo-bleaching and photo-activation. We find that glutamate receptor entry – mainly derived from diffuse pools of extrasynaptic glutamate receptors - controls the growth of new receptor fields.

GK-P34 **Electrical intracochlear stimulation induces c-Fos expression in specific neuronal populations of the auditory brainstem nuclei**
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Neuronal activity in sensory organs not only invokes fast electrical responses but may also trigger molecular changes via complex signalling cascades in central neurons. In the auditory system, unilateral changes of sensory activation trough electrical intracochlear stimulation (EIS) induces molecular and possibly morphological molecular changes in the adult auditory brainstem of the rat. Changes in the expression of the immediate early gene c-fos were observed in a tonotopically precise pattern in central auditory neurons of several brainstem nuclei after electrical stimulation for 2h with a cochlear implant under urethane anaesthesia. The stimulation-dependent modulation of the transcription factor c-Fos in the ascending auditory system suggests modifications in electrochemical und structural properties of the affected cells. We addressed the question if neurons are indiscriminately affected by EIS, or if only subsets of neurons respond to stimulation with the expression of c-Fos while others fail to do so. Fluorescence double-staining was performed to determine in which cell types c-Fos expression took place. Neurons were characterized by the neurotransmitter they contain and by the destination of their axon following injection of a fluorescence axonal tracer into the inferior colliculus I week before stimulation. The presence of c-Fos expression was simultaneously demonstrated with fluorescence immunohistochemistry. We found that about 50% of c-Fos positive nuclei belonged to glutamatergic neurons and about 50% to glycinergic neurons in both the anteroventral cochlear nucleus and in the lateral superior olive ipsilateral to the stimulation. GABAergic cells seem not to respond to the stimulation with upregulation of c-Fos. Remarkably, c-Fos is expressed in neurons of the AVCN projecting to the contralateral inferior colliculus, but not in cells of the LSO with the same target region. We therefore conclude that only distinet subpopulations of neurons responds to EIS with expression of the transcription factor c-Fos, suggesting that these cells preferentially undergo plastic changes upon changes of afferent activity. Interestingly, not only excitatory cells processing signals along the ascending auditory branch express c-Fos upon stimulation, but also inhibitory neurons mediating communication between ipsi- and contralateral cochlear nucleus.

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GK-P35 **Rats can navigate in virtual environments**
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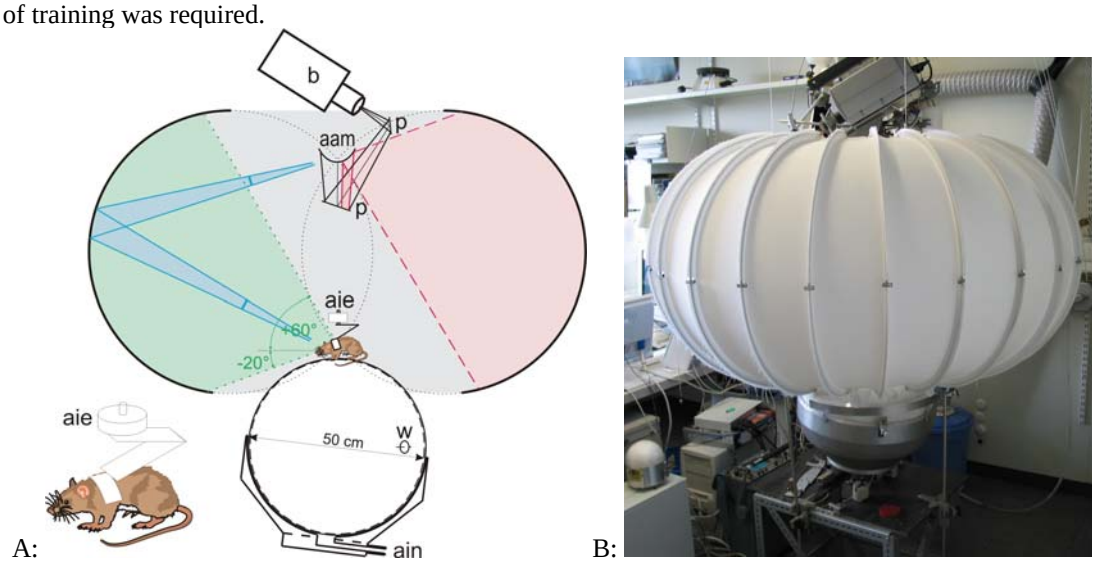
The use of virtual reality technology is meanwhile quite common in research on human spatial cognition. In rats, however, all previous attempts have failed to develop VR systems that are interpreted as spatial environments by the animals. We designed a VR system for rats that covers most of their visual field (360° of azimuth, -20 - +60 elevation). Our set-up consists of a projection system that displays a virtual environment via a mirror system onto a torus-shaped screen that completely surrounds the rat. The animal is stationarily running on top of an air cushioned polystyrene ball of 50 cm diameter. In its mount, the rat is free to rotate about the vertical axis, but its translational movements are transferred to the ball. Motion detectors register the ball´s movement and relay it to a computer system which produces the virtual environment and updates the projected view in relation to the current position of the rat. This novel set-up allows to present large environments with the advantage to quickly change and continuously control those environments. In a first experiment we presented an infinite environment with vertically striped, black and white cylinders of 50 cm diameter suspended from the ceiling (1m height). The cylinders where arranged on a squared grid of 2m side length and the animals were rewarded with sugar water when they approached the area below any cylinder. After 10 days of training the rats showed a significant increase of performance, reflected by the amounts of hits, walking distance and time interval between hits. In a continuously following experiment we enlarged the distance between these cylinders to 10 m and the animals still improved their performance to navigate towards these targets. In a second experiment the animals had to learn a discrimination task. For this purpose we introduced a virtual environment which also consisted of cylinders of 50 cm diameter

suspended from the ceiling. Twelve of these cylinders, with six cylinders colored in dark grey and six cylinders colored in light grey, were arranged alternatingly along a circle with a starting point in the centre. Only one type of those cylinders was rewarded. The animals where instantly able to discriminate between the two types of cylinders, but they also showed a strong preference to the light grey cylinders which were rewarded in the first part of the experiment. To retrain the animals to prefer the dark grey cylinders in the second part of the experiment, more than a month of training was required.



A: Schematic and, B: photo image of the set-up with the projection screen lowered onto the ball.

GK-P36 **Solution structure of KChIP4a and characterization of its interaction with the Kv4.3 N-terminus**

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K^+ channel-interacting proteins (KChIPs), members of the recoverin/NCS1 family of EF-hand calcium-binding proteins, specifically interact with Kv4 voltage-operated K^+ channels and modulate both channel gating and protein trafficking. Different from the other KChIPs, KChIP4a removes rapid inactivation of Kv4 channels by virtue of its KIS (K^+ channel inactivation supressor) domain, an approximately 40 amino acid domain in the N-terminus of the protein. At present, neither the molecular mechanism behind the inactivation removal, nor the structural properties of the Kv4-KChIP4a interaction are understood. Here, we use heteronuclear NMR spectroscopy on triple-labeled (13C, 15N, 2H) KChIP4a protein to characterize the solution structure of the protein and its interaction with Kv4.3 N-terminus. Three dimensional triple resonance experiments were performed on KchIP4a in the presence and absence of calcium and structure determination is in progress. The interaction between the N-terminus of Kv4.3 (amnio acids 1-40) and KChIP4a is investigated by intrinsic fluorescence and native polyacrylamide gel-electrophoresis (PAGE). Moreover one- and two-dimensional NMR studies are used to estimate the affinity of this interaction and to map the binding interface.

GK-P37 **Light-mediated regulation of Ras and Rho GTPases in the visual system of mice. Involvement of Ras in photoentrainement of the circadian clock**

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Introduction: Ras and Rho GTPases are intracellular signaling proteins, which cycle between inactive GDP bound and signaling competent GTP bound state. Ras integrates extracellular signals from multiple receptor types and mediates signal transduction to the cytosol and nucleus, as an upstream regulator of MAPK pathway (Marshall C.J. et al. *Curr. Opin. Cell Biol.* 8, 1996). To investigate its role in the central nervous system, constitutively activated V12-Ha-Ras was expressed selectively in neurons of transgenic synRas mice via a synapsin promoter (Heumann R. et al. *J Cell Biol.* **25,** 2000). The Rho family of GTPases act as molecular switches that transduce signals to the cytoskeleton, participating in the regulation of dendrite elaboration and plasticity (Cline H.T. et al. *Nature* **28**; 2002). We would like to investigate the light-induced regulation of Ras, cdc42 and Rac1 activity in the visual cortex (VC) of mice, as possible candidates for regulation of gene transcription and dendritic morphology. Retinal ganglion cells connect not only to the visual system but also to the suprachiasmatic nucleus (SCN) which is the main brain structure involved in the photoentranment of the circadian rhythm. In order to determine the possible role of Ras in the clock entrainment, we will investigate circadian and photic regulation of Ras activity in SCN and pineal gland

(PG). In addition we monitor the spontaneous locomotor activity of synRas mice expressing constitutively activated H-Ras also in SCN neurons at different dark/light conditions.
Results: Our results show that during duirnal rhythm Ras activity is not changed in the VC. Consistently, during night there is no short term photic regulation of Ras activity. In contrast, after keeping mice in the dark for 7 days there is a decrease of Ras, cdc42 and Rac1 activity in the VC. Direct exposure to light for 1h re-increases all GTPases activity, even exceeding basic levels. In order to investigate possible crosstalk between Ras and Rho GTPases, we used synRas transgenic mice. We found 2-fold increase of cdc42 activity in VC of synRas mice.
In contrast to visual coretx, we have found circadian regulation of Ras activity in SCN and PG. During day Ras activity in SCN is high and it decreases during night. Conversely in PG Ras activity is upregulated during night and decreases during day. In addition, our results show short term photic regulation of Ras activity in SCN during night. Our investigation on the spontaneous locomotor activity demonstrates that synRas mice show absence of light-induced phase advance during the subjective late night.
Conclusions: Taken together, these results indicate that Ras, cdc42 and Rac1 activity are regulated by light exposure in VC of dark treated animals. In contrast, in brain regions involved in photoentrainment of the circadian clock, Ras activity is regulated directly by light input during duirnal rhythm. In addition, the absence of light-induced phase advance during late night in synRas mice suggests that Ras is essential for photoentrainment of the circadian clock in the SCN.

GK-P38 **Association of the ecto-ATPase NTPDase2 with transient cell populations of the neurogenic pathway in the adult dentate gyrus**

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Active neurogenesis continuously takes place in the dentate gyrus of the adult mammalian brain. The dentate gyrus of the adult rodent hippocampus contains an astrocyte-like cell population that is regarded as residual radial glia. These cells reside with their cell bodies in the subgranular layer. Radial processes traverse the granule cell layer and form bushy ramifications in the inner molecular layer. The residual radial glial cells apparently represent neuronal progenitor cells that can give rise to functionally integrated granule cells. To date the cellular and molecular events driving a subpopulation of these cells into neurogenesis as well as the cellular transition states are poorly understood. We show that in the mouse dentate gyrus this cell type selectively expresses surface-located ATP-hydrolyzing activity and is immunopositive for nucleoside triphosphate diphosphohydrolase 2 (NTPDase2). NTPDase2 is a protein of the plasma membrane with its catalytic site facing the extracellular space. It hydrolyzes extracellular nucleoside triphosphates such as ATP or UTP to their respective nucleoside diphosphates. The enzyme becomes expressed in the hippocampus during late embryogenesis from E17 onwards, and is thus not involved in early brain development. Its embryonic pattern of expression mirrors dentate migration of neuroblasts and the formation of the primary and finally the tertiary dentate matrix. NTPDase2 is also expressed by a transient population of cortical radial glia from late embryonic development until postnatal day 5. NTPDase2 can be employed as a novel marker for defining cellular transition states along the neurogenic pathway. It is associated with subpopulations of GFAP- and nestin-positive cells. These intermediate filaments are typically expressed by the progenitor cells of the dentate gyrus. In addition there is a considerable overlap with doublecortin-positive cells. Expression of the microtubule-associated protein doublecortin is indicative of a transition of progenitors to a neural phenotype or an immature form of granule cell. NTPDase2 is no longer associated with mature granule cells as indicated by the lack of double-immunostaining for NeuN. Furthermore S100-positive astrocytes do not express NTPDase2. Experiments with the S-phase marker bromodeoxyuridine (BrdU) demonstrate that NTPDase2-positive cell proliferate. Postmitotic BrdU-labeled cells preferentially acquire an NTPDase-positive phenotype. Our results suggest that NTPDase2 is associated with cell types of varying maturation states but not with mature neurons. They suggest that the signaling pathway via extracellular nucleotides and nucleotide receptors may play a role in the control of hippocampal neurogenesis.

GK-P39 **Effects of contrast on smooth-pursuit eye movements**

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It is well known that moving stimuli can appear to move more slowly when contrast is reduced [Thompson, Vision Res., 22(3), 377–380 (1982)]. Here, we address the question whether changes in stimulus contrast lead to a similar change in the velocity of smooth pursuit eye movements. Subjects were asked to smoothly track a moving Gabor patch. Targets varied in velocity (1, 8, 15 deg/s), spatial frequency (0.1, 1, 4, 8 c/deg), and contrast, ranging from just below individual thresholds to maximum contrast. Results show that smooth pursuit eye velocity gain rose significantly with increasing contrast. Below a contrast level of two to three times threshold, pursuit gain, acceleration, latency, and positional accuracy were severely impaired. Therefore, the smooth pursuit motor response shows the same kind of slowing at low contrast that was demonstrated in previous studies on perception. These

results have implications for testing Bayesian models of perceived speed [Weiss, Simoncelli, & Adelson, Nat. Neurosci., 5(6), 598-604 (2002)].

GK-P40 **Plasticity of the somatosensory cortex in thalidomide-induced dysmelia**

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Introduction: It is well established that functional representations in the cerebral cortex expand due to excessive use [1]. Less is known, however, about the reverse, e.g. the effect of restricted use due to acquired lesions. Lifelong effects due to congenital malformations have rarely been studied. In this study we investigated the relationship between congenitally missing fingers, compensatory foot use and the size of the somatosensory hand and foot representations in thalidomide embryopathy.
Methods: Twenty three subjects participated in our study, ten with normal (group C), and thirteen with upper extremity dysmelia due to intrauterine exposure to thalidomide (group D). The malformations varied from one missing finger on each hand to complete amelia of both arms in the dysmelic subjects. The compensatory foot use was documented using a questionnaire and varied from auxiliary functions such as lifting up objects (subgroup D1) to complete substitution of hand function (subgroup D2).
Localization capacities across digits were determined using von-Frey hairs. The somatosensory hand and foot representations were studied using electrical stimulation of the most distant digits of each hand and foot. Representations in Brodmann Areas (BA) 3b and 1 were localized using MEG and fMRT, respectively. To estimate the size of the somatosensory hand and foot area Euclidian distances between the representations of the most distant digits were determined. The rank-correlation between the size of the hand area and the number of developed fingers on the contralateral hand was calculated. The foot area was compared between group C and subgroups D1 and D2.
Results: Localisation capacities on the toes were significantly superior in D2 than in D1 and C subjects, while D1 and C subjects did not differ [2]. Toe representations were also enlarged in D2 subjects but not in D1 subjects as compared to the control group. The Euclidian distance between the most distant toes was larger in D2 subjects than in control subjects indicating an overall larger cortical foot area in this group.
The mean size of the hand area was significantly smaller in the dysmelic subjects (BA 3b: 5.67, BA 1: 8.6 mm) as compared to controls (BA 3b: 11.2, BA 1: 20.4 mm) [3,4]. There was a significant rank-correlation ($p < 0.05$) between the number of developed fingers and the Euclidian distance between the cortical representations of the most distant fingers (BA 3b: 0.62, BA 1: 0.69).
Conclusions: Our data show use-dependent cortical plasticity of the somatosensory foot and hand area in thalidomide-damaged subjects with malformed upper but normal lower extremities. While the enlargement of the foot area was most likely due to long-standing compensatory foot use, the shrinkage of the hand area could not fully be accounted for by the anatomical lesion but most likely also reflected a lifelong constraint of hand use caused by the thalidomide-induced malformation.

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GK-P41 **Involvement of somatostatin in formation of contextual fear memory**

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Somatostatin (SOM) has been shown to be involved in aversive learning and other memory tasks. However, the role of SOM in fear conditioning has not been elucidated so far. In this study we investigated the effects of genetically and pharmacological induced SOM deficiency onto cued and contextual fear conditioning in mice. After conditioning to an auditory cue (CS+) SOM null mutants (SOM -/- mice) were scored for freezing behaviour onto background context, the CS+ and an unconditioned tone (CS-; previously presented during adaptation). SOM -/- mice displayed selectively decreased freezing to the background context, while no differences in response to CS+ and CS- could be observed compared to their heterozygous and wild-type controls. To further investigate the involvement of SOM in different aspects of fear memory formation, foreground contextual fear conditioning was performed. As in the cued fear conditioning paradigm freezing behaviour to the context was reduced in SOM -/- mice. To test whether the observations in SOM -/- mice are due to an acute involvement of SOM in fear memory formation rather than to developmental deficits, pharmacological depletion of SOM was performed by intraperitoneal infusion of cysteamin (50 or 150 mg/kg). On one hand, application 4h pre-training led to a specific decrease in freezing to background context, thus phenocopying the null mutant phenotype. This cysteamin effect was not observed upon injection to SOM -/- mice. On the other hand, cysteamin infusion 10min post-training led to a more generalised fear-response to all stimuli presented (context, CS+, CS-). Together, our results indicate that

SOM plays a critical role in memorising contextual information interconnected with a threatening experience. This further suggests that hippocampal SOM may be relevant in acute contextual fear memory formation, as integration of complex contextual information crucially depends on processing in the hippocampal formation.

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GK-P42 **Glial cells in the respiratory network express functional transporters and receptors for glycine**

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Inhibitory synaptic transmission within the respiratory network plays a key role in the generation and modulation of the neuronal rhythm for breathing. To understand how astrocytes and oligodendrocytes are involved in glycinergic neurotransmission of the respiratory network, we used a combined approach of immunohistochemistry, electrophysiology and single-cell RT-PCR analysis to determine the expression of glycine transporter 1 (GlyT1) and glycine receptors (GlyR) in the ventral respiratory group of the medulla. Immunohistochemistry against GlyT1 and GlyR was performed in brainstem slices from transgenic mice, in which astrocytes (TgN(hGFAP-EGFP)) and oligodendrocytes (TgN(PLP-DsRed)) were fluorescently labeled. In the ventral respiratory group, GlyT1 was expressed preferentially by astrocytes with bright EGFP-fluorescence. In this type of astrocytes, GlyT1 expression overlapped with glycine receptor expression. To determine the functional expression of glycine transporters and –receptors, whole-cell currents were recorded in voltage-clamp experiments from identified astrocytes and oligodendrocytes using acutely isolated brainstem slices. Astrocytes with bright EGFP-fluorescence that showed linear steady-state IV-curves expressed both receptor- and transporter-mediated currents in response to glycine. In contrast, in an additional population of cells with weak EGFP-expression and outwardly rectifying IV relationship, we were not able to detect glycine-induced currents. Oligodendrocytes responded with both receptor- and transporter mediated glycine currents. As expected, mRNA of GlyT1 was detected by single-cell RT-PCR in astrocytes with linear IV relationship and in oligodendrocytes but also in a subset of outwardly rectifying astrocytes. These data suggests that in the respiratory network both astrocytes and oligodendrocytes may interfere with glycinergic synaptic transmission and thereby modulate respiratory rhythm generation. (supported by the DFG, SFB 406 TP C10)

GK-P43 **Calcium dependence of BK channels in auditory inner hair cells**

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Large conductance Ca^{2+} dependent potassium channels (BK or *slo* channels) are involved in the modulation of neurotransmitter release, smooth muscle contraction and in shaping the receptor potential in auditory inner hair cells. Being activated by voltage as well as by intracellular Ca^{2+}, BK channels operate as an integrator of these two cellular signals. BK channels in different tissues share a rather shallow voltage dependence (18 mV for an e-fold change in activation) but differ in their activation voltage range in the nominal (buffered) absence of intracellular Ca^{2+} (voltage of half-maximal activation (V_h) lies typically between +50 to +150 mV). This diversity may depend on the various local Ca^{2+} sources (i.e. extracellular Ca^{2+}, SR, ER), BK subunit composition and post-translational modification. Contrasting with this general pattern, BK currents measured in the intact IHC show a steep voltage dependence (8 mV) and a very negative V_h (-43 mV). To understand the unique BK channel activation properties, we determined (i) the Ca^{2+} dependence of BK gating, evaluated (ii) the role of voltage gated Ca^{2+} channels (Ca_v) as Ca^{2+} sources and (iii) the role of the BK subunit composition. Experiments were done in excised patch as well as whole-cell voltage-clamp conditions with pharmacologically isolated IHCs from acutely excised mouse and rat cochleae. (i) In excised patches, V_h was +10.8 (inside out) and -15.2 mV (outside-out) in the absence of $Ca^{2+}{}_i$ and was shifted to hyperpolarised potentials with micromolar $Ca^{2+}{}_i$. Similarly elevation of Ca^{2+} in the whole-cell mode by blocking the plasma membrane Ca^{2+}-ATPase led to hyperpolarisation of V_h. (i) The whole-cell BK activation curve was not changed by either withdrawal of extracellular Ca^{2+} or blockage of Ca^{2+}-channels by isradipine. Replacing extracellular Ca^{2+} with Sr^{2+}, which is equally permeable through the IHCs Ca_v-channels but less potent in activating BK, also did not substantially change V_h. (iii) In IHCs, the β_l-subunit is known to be the main BK β-subunit. However in IHCs of BK-β_l knockout mice. Ca^{2+}-sensitivity of BK in patches was only slightly reduced, but the BK whole cell characteristics were not affected. These results indicate that while BK channels in IHCs are perfectly Ca^{2+}-sensitive with an exceptional negative activation in Ca^{2+} free medium, their gating in the intact IHC does not rely on voltage-dependant Ca^{2+} influx, not on the channel's subunit composition. In general, these results suggest that BK channels may be gated exclusively by membrane potential in the physiological.

GK-P44 **Malignant progression of astrocytomas is associated with decreased expression of *AMOG, DRR1* and *APOD***

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We used suppression subtractive hybridization (SSH) combined with cDNA array analysis for the identification of genes involved in the spontaneous progression of primary astrocytic gliomas of World Health Organization (WHO) grade II to secondary glioblastomas of WHO grade IV. SSH products from 4 pairs of primary astrocytic gliomas and secondary glioblastomas were hybridized to high-density filter arrays of the RZPD UniGene set, which carry cDNA fragments representing approximately 12,000 genes. We identified 463 genes that were differentially expressed between primary and secondary tumors in at least one tumor pair. Eight genes were differentially expressed in more than 2 tumor pairs. Expression of these eight genes was further analysed by real-time reverse transcription-PCR analysis in 10 pairs of primary low-grade and recurrent high-grade astrocytic gliomas, as well as 43 primary astrocytic gliomas of different WHO grades. Thereby, we found that the genes for *apolipoprotein D* (*APOD,* 3q26.2-qter), *adhesion molecule on glia* (*AMOG*/*ATP1B2,* 17p13.1), and *downregulated in renal cell carcinoma 1* (*DRR1*/*TU3A*, 3p14.2), demonstrated significantly lower mRNA levels in high-grade as compared to low-grade astrocytic gliomas. Furthermore, treatment of glioma cell lines with the demethylating agent 5-aza-2'-deoxycytidine and the histone deacetylase inhibitor trichostatin A resulted in increased *AMOG* mRNA expression, indicating that transcriptional downregulation of this gene may be due to aberrant promoter methylation. Taken together, we identified three interesting novel tumor suppressor gene candidates that are likely to be important in astrocytoma progression.

GK-P45 **The mechanism of IL-8-like chemokine (GRO/CINC-1) release from rat astrocytes mediated by protease-activated receptor-1**

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Protease-activated receptors (PARs), a unique class of G protein-coupled receptors, are widely expressed in the central nervous system (CNS), such as neurons, microglial cells, astrocytes and oligodendrocytes. Now, it is clear that PARs are involved in multiple physiological processes, such as platelet aggregation, inflammation, apoptosis, cell proliferation, immune response, pain, morphological changes and calcium mobilization. The inflammatory roles of PARs are well understood in some systems, but not in the CNS. Till now, only very limited direct evidence has shown that PARs play a role in inflammation in the CNS. But the inflammatory mechanisms of PARs remain largely unknown. Rat chemokine growth-regulated oncongene/cytokine-induced neutrophil chemoattractant-1 (GRO/CINC-1), a counterpart of the human growth-regulated oncogene product (GRO), has been suggested to play critical roles as a mediator of inflammatory reactions with neutrophil infiltration in rats. In the present study, we investigated by RT-PCR and ELISA whether PAR-1 activation could increase chemokine GRO/CINC-1 in rat astrocytes. We found that thrombin and TRag time- and concentration-dependently upregulates GRO/CINC-1 at both mRNA level and protein level. Then we further investigated the mechanism of PAR-1-mediated GRO/CINC-1 production by rat astrocytes in vitro. ELISA results suggested that inhibitors of protein kinase C (PKC), mitogen-activated protein kinase kinase 1/2 (MEK1/2), phosphatidylinositol 3-kinase (PI3K), p38 MAPK, c-Jun N-terminal kinase (JNK), nuclear transcription factor-kappa B (NF- B) and Janus kinase 2 (JAK2) significantly reduced PAR-1-induced GRO/CINC-1 production. Signaling cascades of GRO/CINC-1 production were further studied by western blot. Our observations for the first time indicate that JNK is very sensitive to thrombin and TRag stimulations in rat astrocytes. Both thrombin and TRag can time- and concentration-dependently phosphorylate JNK, but not p38 MAPK. JNK activation was mediated by PKC, PI3K and MEK1/2. In addition, three interesting nuclear transcription factors, which are supposed to play important roles in regulating GRO/CINC-1 release, were also studied. Our observations indicate that phosphorylation and activation of c-Jun was involved in upregulation of GRO/CINC-1 and c-Jun gene expression. NF- B, which could be activated by thrombin independent of PAR-1 activation, maybe have indirect effects on GRO/CINC-1 release. However, signal transducer and activator of transcription (STAT)3 functions as a negative regulator of GRO/CINC-1 release.

GK-P46 ***In vitro* analysis of the neuroprotective potencies of natural compound derivatives**

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Natural products of microorganisms are a source of cure for many CNS diseases. Some of the products have been identified as neuroprotective and may be beneficial in the treatment of neurodegenerative diseases such as M. Parkinson and M. Alzheimer. We aimed to identify new chemical derivatives of natural compounds with improved neurotrophic efficacy.Therefore, we established several in vitro screening models that reflect major Pathological processes of neurodegeneration. In detail, we treated different neuronal cell lines with either (i) staurosporine, an unspecific protein kinase inhibitor, (ii) thapsigargin or (iii) tunicamycin, two endoplasmic reticulum-stress inducing

toxins. Moreover, we established a (iv) coculture model, composed of a neuronal cell line intoxicated with the supernatant of LPS-stimulated microglial cells. Cell death was analysed in the presence or absence of test compounds by MTS- and LDH-tests as well as immunohistochemical and histological stainings for degenerating cells. In the present study we concentrate on derivatives of cyclosporin A, which is produced by a wide range of fungal strains. Cyclosporin A possesses immunosuppressive and neurotrophic activities and has been shown to reduce neuronal cell death in neurodegenerative diseases. We will compare the neuroprotective activities of different derivatives and discuss their therapeutic potential.

GK-P47 **Trafficking of proteins and lipids in myelinating oligodendrocytes**

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During development of the vertebrate central nervous system (CNS), oligodendrocytes synthesise the myelin sheath by wrapping a lipid-rich multilamellar extension of the plasma membrane around the axon. This "myelination" is essential for the rapid conduction of the action potential along the axon and for the maintenance of axonal integrity. Membrane compaction results in exclusion of most of the cytoplasm from mature myelin, leading to membrane compartments with distinct lipid/protein composition, reminiscent of the segregated membrane domains of polarised cells. Development and maintenance of this sophisticated compartmentalised membrane system requires continual glial-neuron communication, coordination of protein and lipid synthesis and directed membrane trafficking towards the myelin sheath. Because lipid and protein trafficking in oligodendrocytes is a largely unresearched area, we are establishing assay systems to resolve vesicular transport pathways of myelin components. Future results will improve understanding of myelin formation and of the molecular mechanisms of pathology in myelin diseases, where trafficking of myelin components is impaired (e.g. Sphingolipidoses, Pelizaeus Merzbacher Disease).

GK-P48 **Decreased dopamine D2/D3 receptor binding in temporal lobe epilepsy: an ^{18}F-fallypride PET study**

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Objective: To quantify D2/D3 receptor availability in patients with mesial temporal lobe epilepsy (mTLE). **Background:** In animal models of TLE activation of D1-receptors has a pro- and of D2-receptors an anticonvulsant effect. Evidence for an alteration of extrastriatal dopamine in human focal epilepsy is missing.
Design/Methods: Six mTLE patients: 6 men, 1 women, aged 23 to 38 years, mean ($\pm$ SD) age 30.1 ($\pm$ 6.2) and 7 age matched control subjects were studied by PET using the high affinity dopamine D2/D3 receptor ligand ^{18}F-Fallypride, suitable for imaging extrastriatal binding. Mesial TLE was defined by interictal and ictal Video-EEG, MRI and ^{18}F-fluorodesoxyglucose (FDG-) PET and was due to hippocampus sclerosis in 6 patients. Anatomical regions of interest (ROIs) were drawn on MRIs. PET data were quantified using a simplified reference tissue model to assess binding potential (BP) values in each ROI, with cerebellum as reference. Data were stereotactically normalized using a ligand-specific template. For each patient, a normalized percentage BP change was calculated as the relative variation of BP in each ROI on the epileptogenic compared with the unaffected hemisphere. In addition, a voxel-based analysis was performed using statistical parametric mapping (SPM). Results were correlated with FDG-PET and MR-volumetric data.
Results: Compared to controls, all patients showed a significant decreased ^{18}F-Fallypride BP (SPM analysis corr. $p < 0.001$ at cluster level) restricted to the pole (by 36% in ROI analysis) and in lateral parts (35%) of the affected temporal lobe. This reduction was particularly evident in areas surrounding the seizure onset zone in the pole and lateral parts of the temporal lobe. In contrast, FDG hypometabolism apart from lateral structures included also mesial parts of the affected temporal lobe and extended to more posterior parts of the lateral temporal lobe. Although MR volumetry in patients revealed a mean reduction of the size of the hippocampus of 38 $\pm$ 6.3% on the epileptogenic side compared to the unaffected side there was no significant alteration of ^{18}F-Fallypride binding. Reduction of ^{18}F-Fallypride BP did not correlate with seizure or spike frequency, and hippocampal atrophy as measured by MR-volumetry.
Conclusions: There was a reduction in ^{18}F-Fallypride binding in anterior-lateral parts of the affected temporal lobe of patients with mTLE. This suggests a specific alteration of the dopaminergic system in an area attributable to the irrative zone. The observed changes might represent a D2/D3 receptor loss, decreased receptor affinity, or changes in receptor occupancy or a combination of these. D2/D3 binding in mesial structures, most affected by volume reduction, was not changed making partial volume effects an unlikely explanation.. Our findings are consistent with the hypothesis that a reduction of D2/D3 binding contributes to an increased excitability in areas surrounding the seizure onset zone. It remains to be determined if these changes are merely functional or related to the pathophysiology of chronic mesial temporal lobe epilepsy.