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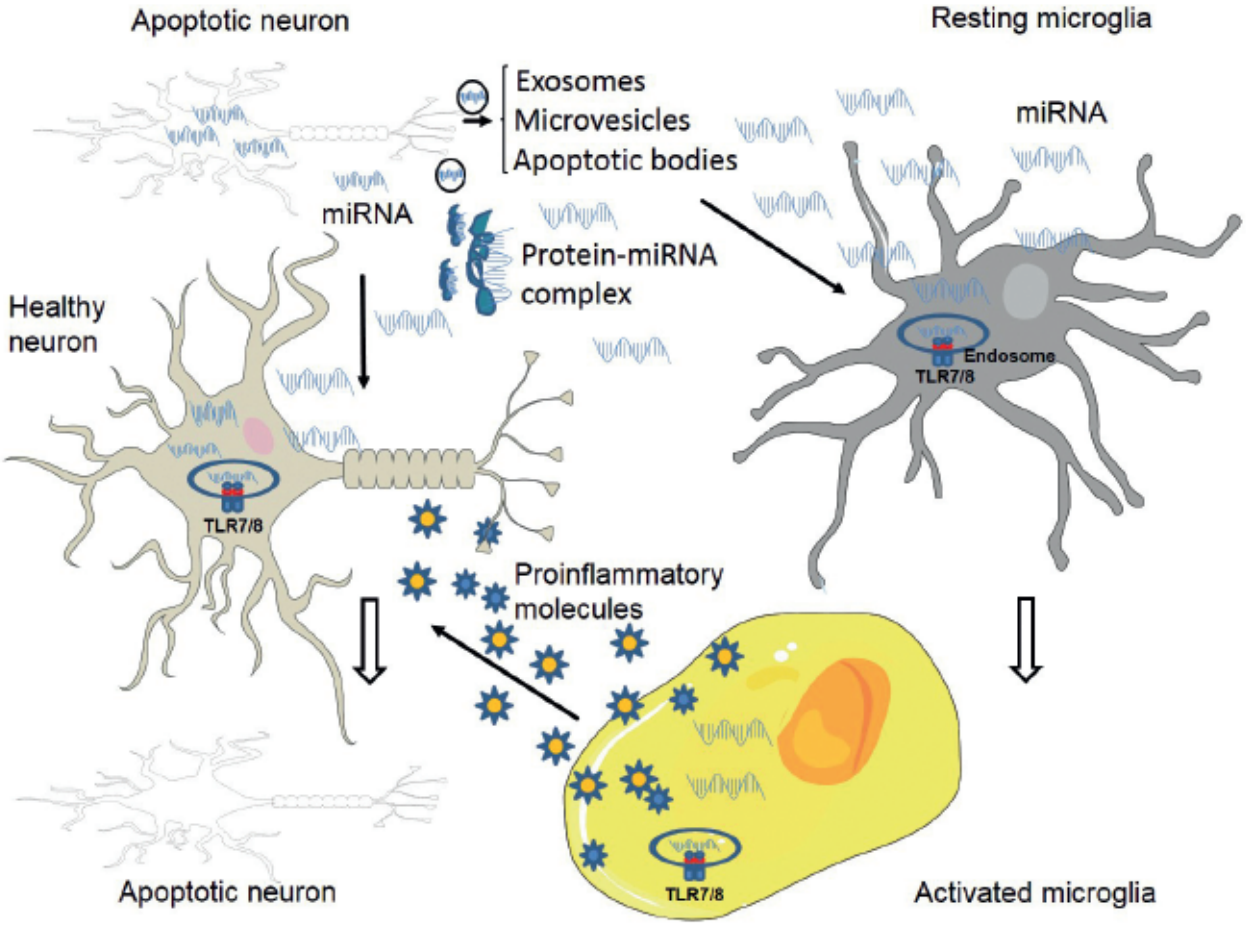

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CHEFREDAKTEURIN
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HERAUSGEBER Neurowissenschaftliche Gesellschaft e.V. (NWG), Kontakt: Meino Alexandra Gibson, Max-Delbrück-Centrum für Molekulare Medizin, Robert-Rössle-Straße 10, 13092 Berlin, Tel.: +49 (0)30 9406 3336, gibson@mdc-berlin.de, www.nwg-info.de

CHEFREDAKTEURIN Petra Wahle, AG Entwicklungsneurobiologie, Fakultät für Biologie & Biotechnologie, Ruhr-Universität, ND 6/72, 44780 Bochum, wahle@neurobiologie.ruhr-uni-bochum.de

REDAKTION Susanne Hannig, Max-Delbrück-Centrum für Molekulare Medizin, Robert-Rössle-Str. 10, 13092 Berlin (Germany), Tel.: +49 (0)30 9406 3336, susanne.hannig@mdc-berlin.de

JOURNAL MANAGER Torsten Krüger, De Gruyter, Genthiner Straße 13, 10785 Berlin, Germany. Tel.: +49 (0)30 260 05-173, Fax: +49 (0)30 260 05-250, E-Mail: Neuroforum.Editorial@degruyter.com

ANZEIGENVERANTWORTLICHE top-ad Bernd Beutel, Schlossergäßchen 10, 69469 Weinheim, Tel.: +49 (0)6201 290 92-0, Fax +49 (0)6201 290 92-20 20, disposition@top-ad-online.de

COVER ILLUSTRATION Role of miRNAs as signaling molecules for TLRs in CNS inflammation.Neurons suffer an initial insult and undergo cell death, thereby releasing ssRNAs, such as miRNAs, into the extracellular space. These host-derived molecules bind to TLR7/8 expressed in (i) microglia that in turn release numerous proinflammatory mediators (e. g. TNF, yellow stars; IL-6, blue stars), or (ii) in neighboring neurons. In the case of microglial activation, this neuroinflammatory response causes injury of neighboring neurons leading to the release of endogenous TLR ligands. In the case of TLR7/8 stimulation in neighboring neurons, apoptosis and cell-autonomous neurodegeneration are induced, also leading to the release of endogenous TLR ligands and thereby closing the vicious cycle of neuronal injury. Cover figure provided by Seija Lehnardt (nf-2018-0032, in this issue).

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VORSTAND DER AMTSPERIODE 2019–2021

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Review Article

Presentation of Scientific Institutions

Nachrichten der Gesellschaft

Josef Priller and Marco Prinz*

Editorial

https://doi.org/10.1515/nf-2019-0019

Dear reader,

This special edition of the Neuroforum is dedicated "Neuroinflammation". What does inflammation mean? The definition of the term 'inflammation' has changed significantly throughout the years. Initially, Celsus defined inflammation based on the clinical cardinal signs *"tumor, rubor, calor et dolor"*, that was found later to be accompanied by histological signs of extravasation of blood cells such as granulocytes and lymphocytes in the tissues including the brain. Nowadays, the term "neuroinflammation" is used in many cases to describe totally different and diverse CNS diseases. While viral, bacterial and autoimmune diseases as well as the acute ischemic stroke are histopathologically characterized by extravasation of hematopoietic cells, the concept of neuroinflammation has also expanded to neurodegenerative diseases, e. g. Alzheimer's disease, Parkinson's disease, and Huntington's disease. From a neuropathological perspective though, these diseases are rather neurodegenerative instead of inflammatory pathologies because one can´t find immune cells of the adaptive (that blood-derived) rather than of the innate immune system (that is yolk sac-derived) in the CNS. Consequently, in classic neuroinflammatory diseases the blood-brain barrier is destroyed and in this case transmigration of hematopietic cells takes place. In general, all these inflammatory diseases show an activation of the innate immune system, especially of myeloid cells, for instance in microglia.

This special edition aims to address some of the currently most important questions in the field of neuroinflammation and to pose central questions about the pathogenesis and treatment of neuroinflammatory diseases. It includes contributions from members of the Collaborative Research Centre/Transregio (CRC/TRR) 167 ("Development, function and potential of myeloid cells in the central nervous system", NeuroMac). Research within this CRC/TRR is supposed to contribute significantly to a better understanding of inflammatory CNS diseases.

The review article by Chotima Böttcher, Roman Sankowski, Josef Priller and Marco Prinz deals with the characterization of myeloid cells in CNS diseases on single-cell level. By the recent development of new methods such as single-cell PCR and antibody-based single-cell flow cytometry (CyTOF) it is now possible to define new subpopulations of myeloid cells during homeostasis as well as during inflammatory CNS diseases. Henceforth, these new techniques allow now a precise definition of new context-associated subpopulations of myeloid cells and lead us to expect a better targeted therapy of CNS diseases in the future.

Charlotte Mezö, Omar Mossad, Daniel Erny and Thomas Blank give an overview about the interaction of the microbiome with the innate immune system in the CNS. Their article is based on the idea that the human body contains trillions of microorganisms that play a vital role for human health: the host microbiota that are important for the development and function of the immune system inside and outside of the CNS. Recent studies of the gut-brain axis give reasons to hope that the interactions of both organs will be better understood in the future, thus, achieving a therapeutic intervention of CNS diseases such as Alzheimer's or Parkinson's through modification of the digestive system.

In their article, Maximilian Lenz and Andreas Vlachos focus on the neuro-immunological synapse in the CNS. The topic is based on several recent studies which have shown that microglia as tissue macrophages of the CNS, are paramount for the neuronal development and the homeostasis of neurons in the adult brain as well as for the modulation of the synapses. Moreover, it was be shown before that a neuro-immunological synapse exists in the CNS and that microglial factors such as the tumor necrosis factor alpha can significantly shape the plasticity of the neuronal synapses together with astrocytes.

Philipp Henneke and Seja Lehnardt give an overview about molecular mechanisms through which viruses, bacteria, fungi and parasites are sensed by CNS cells during infections of the central nervous system. So-called Toll-like Receptors (TLRs) play an important role in detecting

***Corresponding author: Prof. Dr. Marco Prinz**, Institute of Neuropathology, Faculty of Medicine, University of Freiburg, Freiburg, Germany; Signalling Research Centres BIOSS and CIBSS, University of Freiburg, Freiburg, Germany; Center for Basics in NeuroModulation (NeuroModulBasics), Faculty of Medicine, University of Freiburg, Freiburg, Germany, e-mail: marco.prinz@uniklinik-freiburg.de
Prof. Dr. Josef Priller, Department of Neuropsychiatry and Laboratory of Molecular Psychiatry, Charité – Universitätsmedizin Berlin, Berlin, Germany; DZNE and BIH, Berlin, Germany; University of Edinburgh and UK DRI, Edinburgh, UK, e-mail: josef.priller@charite.de

those pathogens that can be found on different CNS cells. Aside from pathogens also destroyed neurons can secrete RNAs which can activate also endosomal Toll-like receptors though endosomes. These processes can contribute to neuroinflammation, too, and these possibilities are discussed in their review article.

The final review article by Daniel Berchthold, Luise Weitbrecht, Christian Meisel and Andreas Meisel focuses on another hematopoietic cell within the diseased CNS: the B cell. The authors discuss in depth the role of B cells for the acute ischemic stroke. For a long time, only certain cells of the adaptive immune response, the T cells, were the focus of most research activities. Just recently, it was shown that also B cells are important in acute and chronic phases of ischemic stroke. In their review article the authors describe the role of B cells in brain ischemia and possible antibody-dependent and independent mechanisms for the development of a cognitive impairment after a stroke.

We hope that these contributions will not only increase the knowledge about mechanisms during neuroinflammation of neuroscientists, but will hopefully convince the reader that a deeper understanding of the underlying pathomechanisms of CNS inflammation is urgently needed in order to help us to successfully treat these diseases in the future.

We sincerely thank the Society for Neuroscience and the editorial team of the Neuroforum for selecting the above-mentioned scientifically valuable and clinically relevant topics and, of course, all authors for their willingness to participate.

Yours faithfully,
Josef Priller and Marco Prinz

Josef Priller

Marco Prinz

Maximilian Lenz and Andreas Vlachos*

The neuroimmunological synapse: from synaptic homeostasis to brain disease

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Abstract: Microglia are the resident immune cells of the central nervous system (CNS). They play fundamental roles in active immune defense and neuroinflammatory responses. Historically, it has been assumed that microglia exist in a resting state until pathological stimuli trigger their activation. However, a series of recent landmark studies revealed important physiological functions of microglia in neural development, synaptic remodeling and homeostasis. Likewise, accumulating evidence suggests that immune mediators and inflammatory cytokines may assert physiological roles in synaptic transmission and plasticity. Hence, the concept of a *neuroimmunological synapse* has started to emerge based on the observation that microglial factors, such as tumor necrosis factor alpha (TNFα) modulate plasticity at tripartite synapses. In pathological conditions, in which microglia are activated by non-physiological stimuli (and/or circulating immune mediators and immune cells enter the CNS), homeostasis between microglia, astrocytes and neurons at synaptic sites will be altered, which may initiate, promote or sustain pathological brain states.

Keywords: Neuroinflammation, Synaptic Plasticity, Plasticity, TNFα, Synaptopodin, Microglia

Zusammenfassung: Mikroglia sind Zellen des angeborenen Immunsystems im zentralen Nervensystem, die eine bedeutende Rolle bei entzündlichen Veränderungen im Nervengewebe spielen. Ursprünglich galt die Annahme, dass Mikrogliazellen ihre Funktion erst nach Aktivierung durch pathologische Stimuli aufnehmen. Neuere Studien deuten darauf hin, dass Mikroglia physiologische Funktionen bei neuronalen Entwicklungsprozessen oder synaptischen Anpassungsreaktionen hat. Basierend auf dem Konzept, dass die Freisetzung mikroglialer Faktoren synaptische Eigenschaften tripartiter Synapsen (Präsynapse, Postsynapse, Astrozyt) beeinflussen kann, wurde der Begriff der *neuroimmunologischen Synapse* geprägt. Unter Bedingungen, bei denen der Aktivitätszustand der Mikroglia durch endogene oder exogene pathologische Stimuli verändert wird, kann dadurch das physiologische Zusammenspiel von Mikroglia, Astrozyten und Nervenzellen an Synapsen gestört sein, wodurch krankhafte Prozesse im zentralen Nervensystem angestoßen, befördert oder erhalten werden können.

Schlüsselwörter: Neuroinflammation, Synaptische Plastizität, TNFα, Synaptopodin, Mikroglia

Introduction

The characterization of structure-function interrelations in the central nervous system (CNS) was significantly advanced in the end of the 19th century when Franz Nissl developed a new staining method which allowed for the visualization of the CNS cytoarchitecture. Early neuropathological investigations pointed to a non-neuronal, i. e., glial cell type (Virchow, 1846), which showed intriguing similarities to macrophages of the immune system. These intricate glial cells were further characterized based on modified Golgi staining protocols (Robertson, 1899) which revealed their ramified appearance and the comparatively small cell bodies (c.f., Fig. 1*A*). Eventually, Pío del Río Hortega named this class of glial cells 'microglia' (Cajal, 1920).

In the 1960 s, the first transmission electron microscopy images of microglia were published [(Schultz et al., 1957) c.f., Fig. 1*B*]. These ultrastructural studies provided direct experimental evidence for the earlier proposed phagocytic properties of microglia (Penfield, 1925). It was soon suggested that microglia could be involved in the removal of dysfunctional neuronal synapses [(Gray, 1959); c.f., Fig. 1*C* and Table 1], a phenomenon termed 'synaptic stripping' (Blinzinger and Kreutzberg, 1968; Kettenmann et al., 2013). Several years later, the role of microglia as resident immune cells of the CNS was firmly established (Giulian and Baker, 1986), also pointing towards the relevance of dynamic properties of microglia – long before *in*

***Corresponding author: Prof. Dr. Andreas Vlachos**, Department of Neuroanatomy, Institute of Anatomy and Cell Biology, Faculty of Medicine, University of Freiburg, Germany; Center for Basics in Neuromodulation, University of Freiburg, Germany, e-mail: Andreas.Vlachos@anat.uni-freiburg.de

Dr. Maximilian Lenz, Department of Neuroanatomy, Institute of Anatomy and Cell Biology, Faculty of Medicine, University of Freiburg, Germany, e-mail: Maximilian.Lenz@anat.uni-freiburg.de

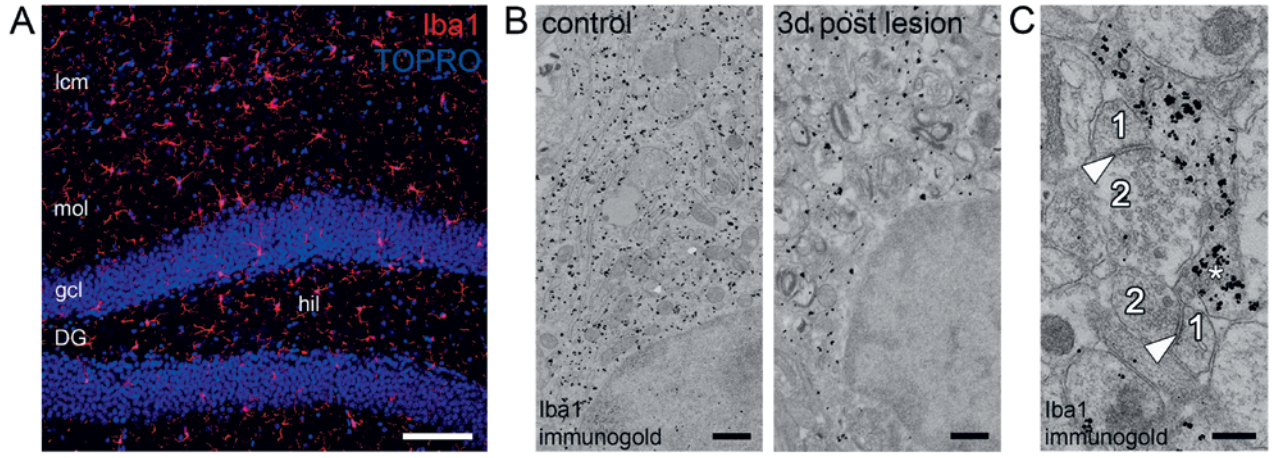

Figure 1: Microglia are small ramified cells that interact with synapses (A) Iba1 immunostaining of the mouse dorsal hippocampus reveals the morphology and distribution of microglia under physiological conditions. TOPRO nuclear staining was used to visualize cyto-architecture. (DG, dentate gyrus; gcl; granule cell layer; mol, stratum moleculare; lcm, stratum lacunosum; hil, hilar region). Scale bar, 100 µm. (B) Transmission electron micrograph of Iba1 immunogold labeled microglia in the molecular layer of a three-week-old hippocampal tissue culture. Numerous inclusion bodies are detected 3 days after lesioning the entorhino-hippocampal fiber tract *in vitro*. Scale bars, 500 nm. (C) Microglial processes (asterisk) in close proximity to neuronal synapses. Postsynaptic compartments indicated by '1', arrow heads point to synaptic clefts, and '2' indicates presynaptic compartments. Scale bar, 250 nm.

Table 1: Cell-to-cell contact sites

Name	Description	Reference (example)
Neuronal (electrochemical) Synapses	Presynaptic specialization + synaptic cleft + postsynaptic specialization (c.f., Fig 1C)	(Gray, 1959)
Tripartite Synapses	Neuronal Synapses + astrocytic endfeet	(Panatier et al., 2014)
Quadpartite Neuroimmunological Synapses	Tripartite Synapse + Microglia	(Schafer et al., 2013)
Immune Synapses	Leukocyte/Leukocyte-Interactions	(Llodra, 2017)
Enteroendocrine-Vagal-Synapses	Enteroendocrine/Vagal Nerve-Interactions	(Kaelberer et al., 2018)

vivo multiphoton microscopy discovered the considerably high motility of microglial processes [(Nimmerjahn et al., 2005); for detailed information on the historical context see (Tremblay et al., 2011)]. Meanwhile, high throughput gene expression analyses have started to decipher the origin and progeny of microglia (Prinz and Priller, 2014; Prinz et al., 2011) and their relevance in various physiological and pathological brain conditions (Butovsky and Weiner, 2018).

While the myriad roles of microglia in health and disease have been comprehensively reviewed by leading experts in the field [e. g., (Butovsky and Weiner, 2018; Kettenmann et al., 2013; Prinz and Priller, 2014)], this concise review article focuses on recent experimental evidence which suggests a fundamental role of microglia in modulating the ability of excitatory tripartite synapses to express plasticity (Figure 2). We will describe and discuss the emerging concept of the (quadpartite) neuroimmunological synapse and its implications in synaptic plasticity at the interface between health and disease.

Bidirectional interactions between microglia and neurons under physiological conditions

Based on structural and functional similarities between microglia and macrophages it was initially assumed that microglia exist in a resting state until pathological stimuli trigger their activation, e. g., proliferation, ameboid migration, phagocytosis and the release of inflammatory

cytokines. Meanwhile, a series of landmark studies has shown that microglia continuously survey the healthy CNS, with their processes getting close to pre- and post-synaptic compartments [Fig. 1*C*; (Nimmerjahn et al., 2005; Tremblay et al., 2011)] including axon initial segments (Baalman et al., 2015), i. e., all major structural and functional microdomains of neurons which generate, propagate or transmit signals.

These interactions are activity-dependent as a reduction in neural activity also reduces microglia dynamics (Li et al., 2012; Tremblay et al., 2010; Wake et al., 2009). Consistent with these observations, various neurotransmitter receptors, such as adrenergic, purinergic, glutamatergic and GABAergic receptors, are found on the surface of microglia which enables them to detect and respond to neurotransmitter release and changes in neural activity (Biber et al., 2007; Fontainhas et al., 2011; Pocock and Kettenmann, 2007).

In turn, microglia are known to mediate synapse formation and synaptic pruning during development (Paolicelli et al., 2011; Parkhurst et al., 2013; Wu et al., 2015), and they have been implicated in the modulation of excitatory and inhibitory synaptic transmission and plasticity [e. g., (Cantaut-Belarif et al., 2017; Pascual et al., 2012; Schafer et al., 2013)]. Interestingly, these physiological effects of microglia depend on signaling pathways traditionally studied in the context of neuroinflammation, e. g., complement and fractalkine systems (Bertollini et al., 2006), pro- and anti-inflammatory cytokines (Habbas et al., 2015), or partial phagocytosis (Weinhard et al., 2018). While the precise signals which recruit these neuroimmunological pathways under physiological conditions remain not well-understood, an indisputable activity-dependent interaction between microglia and neurons seems to exist, which is expected to play fundamental roles in complex brain function.

Tumor necrosis factor alpha (TNFα) mediates homeostatic synaptic plasticity and modulates the ability of neurons to express Hebbian plasticity

Among the best studied microglial factors that influence synaptic plasticity is the pro-inflammatory cytokine TNFα (Cahoy et al., 2008; Zhang et al., 2014). TNFα acts through two canonical receptors: TNF-receptor 1 (TNFR1)

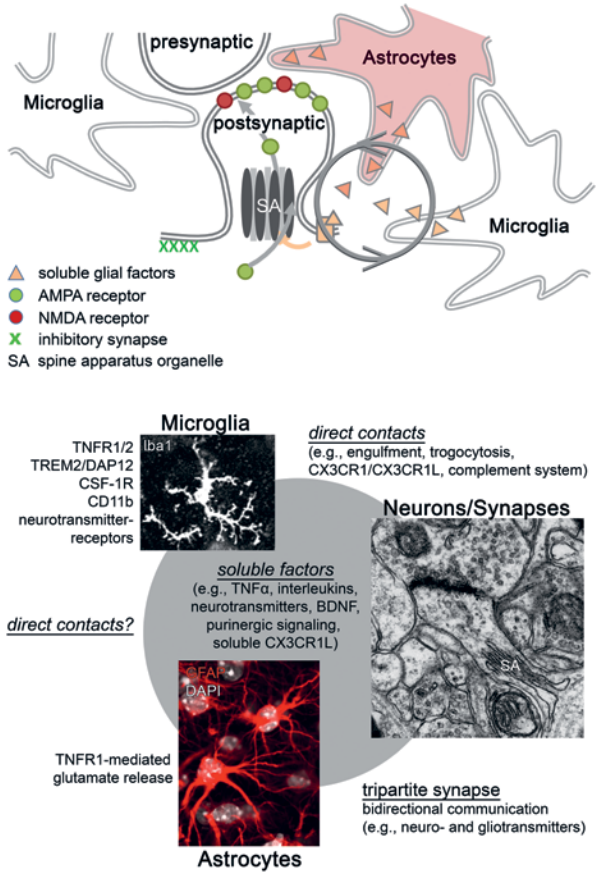

Figure 2: The (quadpartite) neuroimmunological synapse
Schematic illustration of structural and functional interactions between microglia, astrocytes and neuronal presynaptic and postsynaptic compartments (SA, spine apparatus organelle). Details provided in the text.

and TNF-receptor 2 (TNFR2). TNFR1 is activated by membrane-bound and soluble TNFα while TNFR2 predominantly binds to membrane-bound TNFα (Dopp et al., 1997; Probert, 2015). In the CNS both receptors are detected on neurons and glial cells. Hence, TNFα-signaling may account for both, microglia mediated secretion of TNFα (via TNFR1) and cell-cell interactions (via TNFR1/TNFR2) at synaptic sites (Figure 2).

Consistent with the observation that microglia assert physiological functions, TNFα has been linked to homeostatic synaptic plasticity (Stellwagen and Malenka, 2006), which is a form of plasticity that plays a fundamental role in maintaining physiological brain function. It was shown that a reduction of network activity – which reduces microglia motility (Wong et al., 2011) – leads to glial TNFα release (Barnes et al., 2017; Habbas et al., 2015; Stellwagen and Malenka, 2006). In turn, TNFα induces a compensatory increase in excitatory synaptic strength (Stellwagen and Malenka, 2006) which brings neurons back to their former activity state (Beattie et al., 2002; Stellwagen et al., 2005). While evidence exists that TNFα also downregulates inhibitory neurotransmission (Pribiag and Stellwagen, 2013), it may be important to note that in a recent study we were not able to detect homeostatic changes in inhibitory neurotransmission in a lesion model that is known to trigger glial activation and increased TNFα levels (Lenz et al., 2019). Thus, the precise role of microglia and TNFα in coordinating homeostatic plasticity of excitatory and inhibitory neurotransmission remains a matter of future investigations.

Meanwhile, it has been also suggested that TNFα may act as a permissive factor in the context of synaptic plasticity (Becker et al., 2013; Maggio and Vlachos, 2014; Steinmetz and Turrigiano, 2010). Hence, microglia may assert their effects on plasticity not by inducing changes in synaptic transmission and strength *per se*, but may rather act as neuromodulators: Through the release of TNFα microglia modulate the ability of neurons to express plasticity without necessarily affecting baseline synaptic transmission. Indeed, a recent study demonstrated that low concentrations of exogenously applied TNFα improve the ability of neurons to express excitatory synaptic plasticity, i. e., long-term potentiation (LTP) of Schaffer collateral-CA1 synapses, without affecting synaptic strength or previously established LTP in the same set of hippocampal slices (Maggio and Vlachos, 2018). Interestingly, high doses of TNFα had an opposite effect and impaired LTP – again not affecting baseline synaptic transmission and previously established LTP (Maggio and Vlachos, 2018). These results demonstrate that TNFα can act as a mediator of metaplasticity, i. e., it modulates the ability of neurons to express LTP in response to the exact same stimulus. Hence, it is conceivable that microglia surveille synaptic transmission and upon changes in neural activity (or yet unknown neuronal or astrocytic co-stimulatory factors) they can modulate the ability of synapses to express further plasticity depending on the concentrations of membrane-bound or locally secreted TNFα.

Microglia-mediated modulation of the tripartite synapse

What are the cellular and molecular targets through which microglial TNFα affects synaptic transmission and plasticity? A solid line of experimental evidence exists which suggests that TNFα can act on astrocytes, leading to an increase in glutamate-release by astrocytes (Habbas et al.,

2015; Santello et al., 2011). In turn, presynaptic NMDA-receptors will be activated which modulate presynaptic release properties. Indeed, evidence has been provided that astrocytic TNFR1 mediates this phenomenon, which could be relevant in various physiological and pathological conditions (Habbas et al., 2015).

With respect to postsynaptic mechanisms, our recent work identified the actin-binding molecule synaptopodin as a target of microglial TNFα (Maggio and Vlachos, 2018; Strehl et al., 2014). Synaptopodin is an actin-modulating protein enriched in a subset of dendritic spines [and in the axon initial segments; (Schluter et al., 2017)] of cortical principal neurons (Mundel et al., 1997; Deller et al., 2000). It is a marker and essential component of the spine apparatus organelle, an enigmatic cellular organelle composed of stacked smooth endoplasmic reticulum [(Deller et al., 2003); c.f., Figure 2], which regulates homeostatic plasticity and LTP via intracellular calcium stores [(Vlachos et al., 2013; Vlachos et al., 2009); for a recent review see Jedlicka and Deller, 2017]. Indeed, in absence of synaptopodin low concentrations of TNFα do not improve synaptic plasticity in our experimental setting (Maggio and Vlachos, 2018). Consistent with this observation, low concentrations of TNFα increase synaptopodin expression and the sizes of spine apparatus organelles [c.f., (Vlachos et al., 2013)], while high concentrations of TNFα are expected to reduce synaptopodin expression and impair hippocampal plasticity (Strehl et al., 2014). Although it remains to be shown whether these effects of TNFα are mediated by TNFRs on neurons (and not through an astrocytic mechanism), they support the notion that microglia affect plasticity by modulating structural and functional properties of tripartite excitatory synapses (Figure 2).

The term tripartite synapse refers to the functional interactions and structural proximity of neuronal (1) presynaptic, (2) postsynaptic membranes and (3) the surrounding astrocytic endfeet (Figure 2). Work from recent years has started addressing the functional significance of tripartite synapses in synaptic transmission/plasticity and complex behavior [e. g., (Chever et al., 2016; Dallerac and Rouach, 2016)]. Also considering the well-established role of inflammatory cytokines and other immune mediators in modulating synaptic plasticity, it has been proposed that microglial processes, which interact with tripartite synapses (Fig 1C), may constitute the forth compartment of a *quadpartite synapse* (Schafer et al., 2013). Because microglia assert their effects on synaptic plasticity via signaling pathways traditionally studied in the immune system the term (quadpartite) '*neuroimmunological synapse*' (c.f., Table 1) seems applicable in this context.

Relevance of microglia-mediated neuromodulation in the context of brain disease

Alterations in cognitive function and behavior are often observed in the context of neurological diseases associated with neuroinflammatory responses and/or infection of the central nervous system [e. g., (Heneka et al., 2018)]. As pointed out, immune mediators have been identified that affect synaptic plasticity (Werneburg et al., 2017). This is of considerable relevance in the context of neurological and psychiatric diseases associated with increased brain levels of pro-inflammatory cytokines (Heneka et al., 2018). Hence, microglia activation by endogenous or exogenous non-physiological stimuli are expected to disturb physiological interactions and homeostasis between microglia, astrocytes and neurons at *neuroimmunological synapses* eventually leading to alterations in synaptic plasticity.

The biological consequences of alterations in synaptic plasticity are not well-understood. Apparently, a microglia-mediated impairment of synaptic plasticity – as seen for example under conditions of high TNFα levels – cannot be simply interpreted as detrimental, since it is possible that a reduction in the ability of neurons to express synaptic plasticity protects neural networks from maladaptive changes. However, microglia-mediated alterations in synaptic plasticity may hamper functional recovery at a later stage of the disease. Considering the emerging concept of the *neuroimmunological synapse* and the well-established bidirectional interactions between neural activity and microglia function, a vicious cycle between pathological microglia activation and neural network alterations may arise, which could initiate, promote or sustain pathological brain states. It is tempting to speculate that exogenous (therapeutic) modulation of neural activity and plasticity could affect and potentially counteract the detrimental effects of neuroinflammation on *quadpartite* synapses, since microglia are known to respond to changes in neural activity.

In this context, repetitive transcranial magnetic stimulation (rTMS) may represent an interesting approach (Lefaucheur et al., 2014). Based on the physical principle of electromagnetic induction, TMS allows for the non-invasive stimulation of distinct cortical regions in awake and non-anesthetized human subjects and has been shown to modulate cortical excitability beyond stimulation [for review see (Lenz and Vlachos, 2016)]. Using an *in vitro* model of r(T)MS we recently demonstrated that repetitive magnetic stimulation induces plasticity of excitatory and inhibitory synapses (Lenz et al., 2015; Vlachos et al., 2012). The role of microglia in rTMS-induced plasticity has not been tested so far. Yet, it is conceivable that rTMS may provide an efficient approach to modulate structural and functional properties of *neuroimmunological synapses*, which may influence and even restore physiological microglia function under certain experimental conditions. It is tempting to speculate in this context that rTMS may also act on synaptopodin-associated calcium stores in dendritic spines and the axon initial segment. Regardless of these considerations, it is clear that a comprehensive understanding of the role of microglia in modulating synaptic plasticity will be important to identify new strategies for the treatment of brain diseases associated with microglia activation and neuroinflammatory responses.

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Bionotes

Dr. med. Maximilian Lenz
Institut für Anatomie und Zellbiologie
Abteilung für Neuroanatomie
Albertstraße 23, D – 79104 Freiburg,
Germany
e-mail: Maximilian.Lenz@anat.uni-freiburg.de

Dr. Maximilian Lenz studied medicine at the Goethe-University in Frankfurt. After a research visit at the Chaim Sheba Medical Center (Tel Hashomer, Israel) in 2014 and 2015, he received his doctoral degree in 2017 for the investigation of neuronal mechanisms underlying synaptic adaptations following non-invasive brain stimulation. In 2016, he was awarded the 'Nachwuchspreis' of the Anatomical Society. After a Post-Doc at the Heinrich-Heine-University in Düsseldorf, he is now holding a Post-Doc position at the Department of Neuroanatomy in the Institute of Anatomy and Cell Biology at the University of Freiburg.

Prof. Dr. Andreas Vlachos
Institut für Anatomie und Zellbiologie
Abteilung für Neuroanatomie
Albertstraße 17, D – 79104 Freiburg,
Germany
e-mail: Andreas.Vlachos@anat.uni-freiburg.de

Prof. Dr. Andreas Vlachos studied medicine at the Goethe-University in Frankfurt and received his doctoral degree in 2010 after a research visit at the Weizmann Institute of Science (Rehovot, Israel). In 2011, he was awarded the Wolfgang-Bargmann Award of the Anatomical Society and in 2014 he received the Habilitation and Venia Legendi in Anatomy. In 2016, he was appointed W2-Professor of Anatomy at Heinrich-Heine-University Düsseldorf. In Mai 2017, he moved to Albert-Ludwigs-University Freiburg as full professor and director of the Department of Neuroanatomy at the Institute of Anatomy and Cell Biology.

Review Article

Daniel Berchtold, Luis Weitbrecht, Christian Meisel and Andreas Meisel*

Friend or foe? – B cells in stroke

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Abstract: Stroke is one of the leading causes of mortality and morbidity worldwide. Upon cerebral ischemia, an inflammatory reaction takes place in the brain. Infiltration of different immune cell subsets as well as activation of resident microglia cells have been shown to have both beneficial and detrimental effects on stroke outcome. For a long time, research in the field of adaptive immunity after stroke has mostly focused on T lymphocytes and only recently, several publications shed light on the importance of B lymphocytes in the acute and chronic phases of ischemic stroke. In this review, we will focus on the role of B cells in the ischemic brain and describe possible antibody-dependent and antibody-independent mechanisms in the development of post-stroke cognitive deficits.

Keywords: autoantibody; autoreactivity; ectopic lymphoid structure; cognitive decline; neuroinflammation

Zusammenfassung: Schlaganfall gehört nicht nur zu den häufigsten Todesursachen, sondern ist die wichtigste Ursache für lebenslange Behinderung weltweit. Unmittelbar nach einer zerebralen Ischämie kommt es zu einer lokalen entzündlichen Antwort, bei der neben den ortsständigen myeloiden Immunzellen früh aus der Peripherie in das ischämische Gehirn infiltrierende myeloide Zellen und später auch Lymphozyten eine Rolle spielen. Dabei werden neben schädlichen zunehmend auch für den Krankheitsverlauf positive Entzündungsreaktionen vermutet. Während bisher vor allem die Rolle von myeloiden Zellen und T-Lymphozyten im Vordergrund des Interesses standen, sind mit der Entdeckung der verzögerten Einwanderung von B-Lymphozyten in der subakuten und chronischen Phase des ischämischen Schlaganfalls diese zunehmend in den Fokus gerückt. In dieser Übersichtsarbeit beschreiben wir mögliche antikörperabhängige und –unabhängige B-Zellfunktionen für den Langzeitverlauf nach Schlaganfall.

Schlüsselwörter: Autoantikörper; Autoreaktivität; ektope lymphoidd Strukturen; kognitive Störung; Neuroinflammation

Immune mechanisms in stroke: does it matter?

According to the WHO, stroke is the second most common cause of mortality worldwide. In 2016 alone, 5.7 million people died after suffering from a stroke (WHO, Disease Burden and Mortality Estimates 2018). The clinical picture of acute traumatic or ischemic central nervous system (CNS) injury and stroke in particular is not only characterized by neurological deficits, but also by a high incidence of complications. Most prominent amongst these, is stroke-associated pneumonia (SAP) with a mortality of up to 37 % (Brommer et al., 2016; Chamorro et al., 2012; Hannawi et al., 2013; Meisel and Meisel, 2011). A key mechanism facilitating SAP is stroke-induced immune depression (Chamorro et al., 2012; Iadecola and Anrather, 2011a; Meisel et al., 2005; Westendorp et al., 2011). Lymphocytic apoptosis and dysfunction induced by an over-activation of the sympathetic nervous system (Prass et al., 2003) and anti-inflammatory effects on the innate immune system mediated by parasympathetic, cholinergic pathways (Engel et al., 2015) are involved in the pathogenesis of this temporary failure of host defense. Increased activity in the hypothalamus-pituitary axis results in release of corticosteroids further dampening immune responses (Chamorro et al., 2012; Meisel et al., 2005). The B cell compartment is equally affected by this lymphopenia. Especially splenic marginal zone (MZ) B cells are lost due to adrenergic over-activation. MZ B cells are innate-like immune cells rapidly producing immunoglubulin (Ig) M upon inflam-

***Corresponding author: Dr. Andreas Meisel**, Charité Universitaetsmedizin Berlin, Department of Neurology, Center for Stroke Research, NeuroCure Clinical Research Center, Charitéplatz 1, 10117 Berlin, Germany, e-mail: Andreas.Meisel@charite.de
Daniel Berchtold, Charite Universitaetsmedizin Berlin, Experimental Neurology, Chariteplatz 1, 10117 Berlin, Germany, e-mail: daniel.berchtold@charite.de
Luis Weitbrecht, Charite Universitaetsmedizin Berlin, Experimental Neurology, Charitéplatz 1, 10117 Berlin, Germany, e-mail: luis.weitbrecht@charite.de
Dr. Christian Meisel, Charité Universitaetsmedizin Berlin, Institute of Medical Immunology, Charitéplatz 1, 10117 Berlin, Germany, e-mail: chr.meisel@charite.de

matory challenge. Their loss due to adrenergic over-activation after stroke therefore increases the susceptibility to bacterial infections (McCulloch et al., 2017).

Opposed to peripheral immune depression, cerebral ischemia also induces a cascade of inflammatory processes in the CNS, which may result in autoreactivity as we will discuss in this review focusing on B cell responses. The release of damage-associated molecular patterns and reactive oxygen species leads to the activation of sensors of the innate immune system, such as Toll-like receptors and subsequent production of inflammatory mediators (Chamorro et al., 2012; Iadecola and Anrather, 2011b). This results in microglia activation within hours after stroke and recruits peripheral immune cells to the affected brain region. Neutrophils accumulate shortly after the ischemic insult and monocytes follow in the first days after stroke (Anrather and Iadecola, 2016; Chamorro et al., 2012; Otxoa-de-Amezaga et al., 2018). Cells of the adaptive branch of the immune system, namely T and B lymphocytes, infiltrate in a delayed manner. Before entering the damaged brain area, T cells appear to be mainly primed in cervical lymph nodes by recognition of their cognate CNS antigens that are drained via lymphatic vessels or leak over the damaged blood brain barrier (Gelderblom et al., 2009; Kuntz et al., 2014; Laman and Weller, 2013). Several studies report presentation of CNS-specific antigens such as myelin oligodendrocyte glycoprotein (MOG), myelin basic protein (MBP), and N-methyl-D-aspartate (NMDA)-receptor components in antigen-presenting cells (APCs) residing in cervical lymph nodes of patients and experimental animals (Planas et al., 2012; van Zwam et al., 2009).

Unpublished data from our group indicate that T cell infiltration into the ischemic brain is antigen-specific. By adoptive cell transfer of either naïve, MOG-specific or ovalbumin-specific T cells into immunodeficient mice before middle cerebral artery occlusion (MCAO), we found that only MOG-specific T cells accumulated in the ischemic region of the brain (Klehmet et al. unpublished data). Once in the brain, both detrimental and protective features have been attributed to infiltrating T lymphocytes (Chamorro et al., 2012; Gelderblom et al., 2009; Kleinschnitz et al., 2013; Liesz et al., 2009; Shichita et al., 2009). Stroke outcome depends on the immune cell composition in the CNS after stroke, as mice with a pro-inflammatory immune response show more severe dysfunction after stroke, compared to mice prone to anti-inflammatory responses (Kim et al., 2014). Recently, it has been shown, that anti-inflammatory immune phenotypes and protection from stroke can be promoted by hypoxic preconditioning and subsequent upregulation of chemokine ligand 2 (CCL2) (Monson et al., 2014; Stowe et al., 2012).

Studies from our group demonstrate that blocking peripheral immunodepression after stroke increases inflammatory T cell responses in the brain (Romer et al., 2015). This finding suggests that CNS inflammation is not an isolated process and might well be influenced by the ongoing immune challenges in the periphery. For instance, SAP increases the infiltration of CNS antigen-specific T cells into the brain. In contrast, prevention of SAP decreases the recruitment of inflammatory macrophages and T lymphocytes to the ischemic brain, but increases the influx of regulatory T lymphocytes, resulting in a significantly better neurological outcome. These findings are in line with human stroke studies showing increased autoreactive CNS antigen-specific immune responses in the blood of patients with post-stroke infections compared to stroke patients without bacterial infections. Since increased levels of autoreactivity correlate with poorer stroke outcome, preventing bacterial post-stroke infections is a crucial aim in post-stroke care (Becker et al., 2011).

In experimental models, preventive antibiotic treatment succeeds in reducing the bacterial burden after stroke and effectively averts post-stroke infections, thus also improving the neurological outcome. However, two large randomized controlled phase III clinical trials failed to reduce bacterial pneumonia in stroke patients (Meisel and Smith, 2015). These findings warrant research into further treatment strategies to improve long-term outcome after stroke. One possible approach is to modulate immune responses by a pharmacological intervention in order to reverse stroke-induced immune depression in the periphery. However, temporal dampening of the immune system might be an adaptive mechanism to limit autoreactive CNS antigen-specific immune responses in the brain. Hence, any immune intervention after CNS injury must consider both, peripheral and central consequences. Another approach is to hinder peripheral immune cells from infiltrating into the brain after cerebral ischemia, restricting local inflammation and autoreactivity and therefore improving neurological function. Complete deficiency of T and B lymphocytes led to reduction of lesion size and inflammation in a mouse model (Hum et al., 2007), indicating that these cells contribute to secondary brain injury after stroke.

Most research in the field of stroke immunology has focused on innate immune cells or T lymphocytes, rather than B cells in the past. Nevertheless, it has been known for years that stroke patients can develop oligoclonal bands in their cerebrospinal fluid (CSF), which is an indication of local antibody production, and thus the presence of B lymphocytes, in the ischemic brain (Prüss et al., 2012; Tsementzis et al., 1986). Increasing evidence establishes the concept of B cells playing a pivotal role in CNS auto-

reactive processes (Ransohoff et al., 2015). Here we will review recent developments in investigating the role of B cells for stroke in comparison with findings in multiple sclerosis (MS) and spinal cord injury (SCI).

B cells in the ischemic brain

First experiments analyzing the effect of B cells on experimental stroke were carried out in $\mu MT^{-/-}$ mice. Due to a mutation in the μ chain of the B cell receptor, these mice do not develop mature B lymphocytes (Kitamura et al., 1991). When subjected to MCAO, they develop significantly larger lesions and suffer from higher mortality. Flow cytometric enumeration of infiltrating immune cells 48 hours after reperfusion demonstrated higher numbers of neutrophils, T cells, microglia and infiltrating macrophages in the ischemic hemisphere of the $\mu MT^{-/-}$ mice (Ren et al., 2011a). Intraperitoneal or intrastriatal transfer of B lymphocytes into $\mu MT^{-/-}$ mice rescued this phenotype which could be attributed to the production of interleukin (IL)-10 by the transferred lymphocytes since adoptive transfer of IL-10-deficient B lymphocytes did not affect the lesion size or the neurological outcome after MCAO (Chen et al., 2012; Ren et al., 2011a). Ren et al. found that IL-10-producing B lymphocytes decrease the infiltration of neutrophils (Ren et al., 2011a). This might partly explain the beneficial effect of early B cell infiltration on infarct growth, as several research groups could show that the recruitment of peripheral neutrophils contributes to the ischemic damage (Neumann et al., 2015, Neumann et al., 2018; Otxoa-de-Amezaga et al., 2018). Furthermore, in the case of lung infection, it was demonstrated that neutrophil infiltration is increased in IL-10-deficient animals, whereas it is decreased in a model of pulmonary IL-10 overexpression. This demonstrates that indeed IL-10 affects neutrophil recruitment and thus might play a similar role after cerebral ischemia (Peñaloza et al., 2015; Sun et al., 2009).

Moreover, transfer of IL-10 producing B lymphocytes into wild-type mice significantly reduced infarct lesion size which correlated with a reduced infiltration of pro-inflammatory T cell subsets into the ischemic brain while the infiltration of regulatory T cells was enhanced (Bodhankar et al., 2014, 2015a). In addition to protective effects via IL-10 secretion, B cells may also limit immune cell infiltration and infarct growth through the programmed death-1 (PD-1) coinhibitory pathway. PD-1 is an immunoreceptor expressed by activated T and B cells. Upon binding of PD-1 to its ligands PD-L1 or PD-L2, inhibitory signal pathways and peripheral T cell anergy are induced (Ren et al., 2011b). An additional line of research suggests that there are sex differences in B cell biology after MCAO. In brief, female mice develop smaller infarcts, the peripheral immune depression is less drastic and the regulatory B cell response is stronger (Bodhankar et al., 2015b; Seifert et al., 2017). However, a recent study found no major influence of B cells on infarct size and functional outcome for up to 3 days after experimental stroke (Schuhmann et al., 2017). Thus, further research is required to better understand the role of B cells in the acute phase of stroke.

The above-mentioned experimental studies investigated the role of B cells within the acute or sub-acute phase after experimental stroke and were focused on their cytokine-producing function, neglecting their capability to produce auto-antibodies specific to CNS antigens. It has been known for a long time that local antibody production can occur in the ischemic brain and oligoclonal bands can be found in the CSF of stroke patients (Prüss et al., 2012; Tsementzis et al., 1986). In an experimental model, B cells have been demonstrated to infiltrate into the ischemic hemisphere in a delayed manner starting after 7 days and at least over the following 12 weeks (Doyle et al., 2015). Findings from our group support these results. In our hands, B lymphocyte infiltration starts between day 7 and day 14 after MCAO. Thereafter, the number of B lymphocytes keeps increasing at least until 10 weeks after MCAO. Doyle et al. hypothesized that this accumulation of B cells is responsible for a delayed post-stroke cognitive decline. In fact, clinical data suggest that the risk of developing dementia after stroke is increased and a subgroup of patients shows a remarkable cognitive decline (Levine et al., 2015; Pendlebury and Rothwell, 2009). In experimental stroke, mice developed delayed cognitive deficits 7 weeks after stroke onset. This phenotype was verified in different experimental models as well as with different cognitive tests and ultimately linked to the production of MBP-specific antibodies (Becker et al., 2016). In contrast, cognitive deficits after stroke were prevented in B cell-deficient $\mu MT^{-/-}$ mice or after B cell depletion. On a functional level, this cognitive decline was mirrored in a deficient long-term potentiation in the hippocampus developing between week 1 and week 7 after stroke (Doyle et al., 2015). These findings are mirrored by data from human brains obtained from deceased patients demonstrating that the frequency of B cells is significantly higher in the group with post-stroke dementia compared to the stroke group without dementia (Doyle et al., 2015).

Experiments from Doyle et al. as well as our own group show that B lymphocytes are not randomly spread in the ischemic hemisphere but rather form organized cell clusters reminiscent of B cell follicles in secondary lymphoid

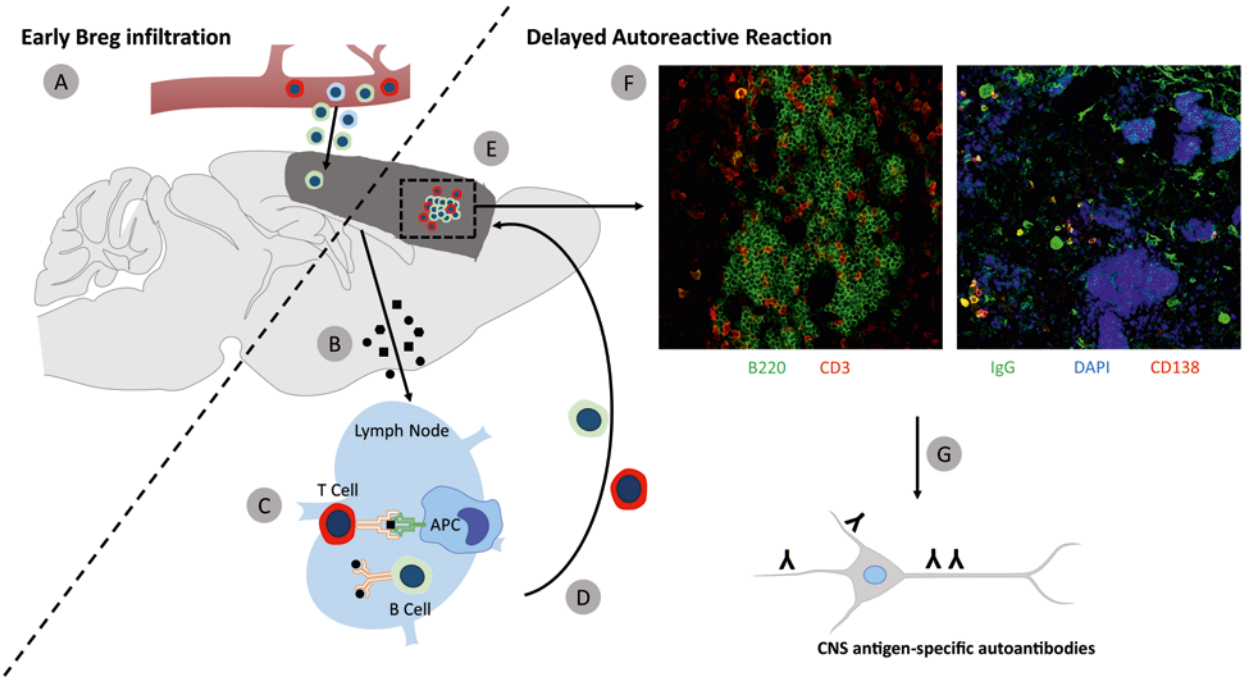

Figure 1: B Cells after stroke. A. Early after stroke, different immune cell subsets, such as T cells (red), B cells (green), and myeloid cells (blue) infiltrate into the ischemic brain region. Amongst them, IL-10 producing regulatory B cells exert a beneficial role by inhibiting the inflammatory process. B. Due to the tissue damage, brain-specific antigens are released and drained towards the cervical lymph nodes. C. In the lymph node, the antigens can be presented to T cells by APCs or directly recognized by autoreactive B cell clones. D/E. The activated autoreactive B and T cells then infiltrate in a delayed manner into the ischemic brain region and start forming ELS. F. Histological analysis of the mouse stroke brain 7 weeks after surgery. Left picture: Large B cell clusters (B220+, green) have formed in the infarct core and are surrounded by T cells (CD3+, red). Right picture: The B cells in the clusters express IgG (green) and surrounding the cluster IgG-producing plasma cells (CD138 and IgG double-positive, red/green respectively) can be observed. G. The antibodies produced in the local immune reaction in the ischemic brain can bind to neuronal antigens and cause delayed cognitive decline.

organs (Doyle et al., 2015) that were termed ectopic lymphoid structures (ELS). As described below, similar structures have been identified in MS, SCI and other models of autoimmunity and chronical inflammation (Aloisi and Pujol-Borrell, 2006; Corsiero et al., 2016; Pitzalis et al., 2014). Here, it has been demonstrated that ELS produce fully matured, CNS-specific B cells and antibody-secreting cells. The reactivity of these antibodies remains to be determined, but published data suggest that autoreactive antibodies recognizing CNS antigens are produced locally after stroke (Dambinova et al., 2003; Kalev-Zylinska et al., 2013; Ortega et al., 2015; Weissman et al., 2011). There is ample evidence that such antibodies might inflict dysfunction and even damage to the CNS. This can occur through binding cognate structures like receptors as well as by antibody-dependent cell-mediated cytotoxicity, complement-mediated mechanisms or direct induction of apoptosis. The latter is known to occur in MS or SCI as well.

On the other hand, antibodies against peripheral nerve antigens have been associated with beneficial effects in traumatic injury to peripheral nerves. Endogenous, self-reactive antibodies accumulate in injured nerve tissue and opsonize degenerating myelin debris. This opsonization facilitates phagocytosis of the myelin debris by macrophages and thus promotes nerve regeneration. The authors suggest that similar mechanisms might occur in the CNS (Vargas et al., 2010).

Research in a mouse model of SCI demonstrated that B cells proliferate and produce CNS-reactive antibodies in peripheral lymphoid organs. Intracerebral injections of these CNS-reactive antibodies caused robust glia activation and neuronal cell death, demonstrating their neurotoxic effect. Such autoreactive antibodies were also found to be increased in human SCI patients in the subacute phase (Arevalo-Martin et al., 2018). Moreover, they observed that B lymphocytes infiltrate in a rather delayed manner into the injured spinal cord and form ELS in this disease setting (Ankeny et al., 2006). Based on their previous findings, Popovich et al. went on to show that mice lacking B cells develop much smaller lesions after spinal cord contusion and recover considerably faster than their wild-type coun-

terparts. They attribute this phenomenon to direct neurotoxic effects of the antibodies as well as their ability to activate complement and innate immune cells (Ankeny et al., 2009). It is also possible that autoantibodies do not lead to cell death, but influence neuronal function through binding of surface receptors (Vincent et al., 2011). This is well described for the case of autoimmune encephalitis. Patients suffering from this disease show symptoms like psychosis, seizures, dyskinesia and autonomic dysfunction. In the case of autoimmune encephalitis, it has been shown that NMDA receptor autoantibodies are sufficient to elicit this pathology through binding and altering the function of the NMDA receptor (Kreye et al., 2016). Hence, it is likely that autoreactive antibodies produced after stroke have an impact on the long-term outcome, notably the occurrence of delayed cognitive deficits.

Lessons from multiple sclerosis and its experimental model

Since relatively little is known about the formation and function of ELS in stroke pathology parallels may be drawn from recent findings in the field of multiple sclerosis (MS) and its experimental autoimmune encephalomyelitis (EAE) model. MS is a chronic inflammatory disease of the CNS with various manifestation forms. Although major progress has been achieved in the past 2 decades, the underlying pathomechanisms are far from being fully understood. In the past, brain-infiltrating autoreactive myelin-specific CD4+ T-helper (Th) cells have been considered the main drivers of neuroinflammation, demyelination and neurodegeneration in MS. The abundance of CD4 T cells and their products in MS lesions (Wu and Alvarez, 2011), as well as large-scale genomic studies (Beecham et al., 2013) and the observation that MS-like symptoms can be induced in the EAE model by transferring CNS-autoreactive Th cells (McPherson et al., 2014) support this paradigm. However, as B cell-depleting approaches are currently among the most effective treatment options for MS (Gelfand et al., 2017; Hauser et al., 2008; Menge et al., 2016; Naismith et al., 2010), B lymphocytes and their involvement in MS pathomechanisms have gained increasing interest in recent years. They may contribute to neuroinflammation by producing autoreactive antibodies and proinflammatory cytokines or presenting auto-antigens. It has been known for a long time, that CNS-specific antibodies and activated B lymphocytes can be found in MS patients' CSF (Dobson et al., 2013; Kabat et al., 1948; Reiber et al., 1998). Their involvement in complement-dependent demyelination and loss of oligodendrocytes in MS lesions is a more recent discovery, however (Liu et al., 2017). Concordantly, antibodies found in MS patients were directed against myelin membrane lipids but also intracellular protein expressed ubiquitously (Brändle et al., 2016; Brennan et al., 2011). It has been shown, that removing antibodies from the peripheral blood by therapeutic plasma exchange is effective in some patients (Keegan et al., 2005). On the other hand, B cell depletion with anti-CD20 antibody effectively reduces relapses in MS without affecting intrathecal autoantibody titers (Piccio et al., 2010). Therefore, it is likely that B cells contribute to MS pathology beyond the production of autoantibodies. B cells are also known to secrete different cytokines, depending on their polarization state. Pro-inflammatory B cells have been described to induce interferon (IFN) γ, tumor necrosis factor α (TNFα), lymphotoxin α (LTα) and IL-6 production in active MS or EAE (Bar-Or et al., 2010; Barr et al., 2012), whereas anti-inflammatory cytokines such as IL-10 and transforming growth factor β (TGFβ) are expressed by regulatory B cells during early EAE induction(Matsushita et al., 2008).

Very recently it was demonstrated that IgA-producing plasma cells migrate from the gut to the CNS attenuating disease activity in the EAE model. Mice that were deficient of plasmablasts and/or plasma cells showed earlier and more severe EAE onset that could be rescued by adoptive transfer of plasma cells. This protective effect was attributed to IL-10 secretion by the plasma cells populating the CNS (Rojas et al., 2019). These new insights into the role of the gut-brain axis in regulating immune responses during neuroinflammation guide increasing interest towards the gut microbiome as a therapeutic target in stroke (Winek et al., 2016). Nevertheless, several studies indicate that pro-inflammatory B cells outweigh their regulatory counterpart in MS (Bar-Or et al., 2010; Barr et al., 2012; Ireland et al., 2012) promoting neuroinflammation. Interestingly, the imbalance between pro- and anti-inflammatory B cell cytokine expression was found to be highest in patients with more disabling MS subtypes (Piancone et al., 2016), indicating a direct link of cytokine expression levels to disease severity. B cells are also important antigen-presenting cells. In case of CNS-autoreactivity, B cells internalize and process myelin components such as MOG and MBP which then are presented on the cell surface, where antigen recognition and interaction with co-stimulatory proteins such as CD80 lead to activation of T cells. Increased CD80 expression in B cells has been observed in MS patients (Aung and Balashov, 2015) and could be reversed by IFN β therapy (Genç et al., 1997). Under healthy conditions, the blood brain barrier limits

lymphocyte entry into CNS tissue. Antigen recognition and presentation by lymphocytes takes place in perivascular compartments called Virchow-Robin space. Highly organized, B cell rich lymphoid structures were described in the Virchow-Robin space in MS patients already in 1979 (Prineas, 1979). Only recently, however, have these findings regained researchers' attention as several studies observed inflammatory B cell infiltrates in the meninges of some early- and late-stage MS patients (Lucchinetti et al., 2011; Serafini et al., 2004). These structures vary in complexity from simple B cell aggregates to highly organized lymphoid structures (Serafini et al., 2004). Recent studies also provide evidence for germinal center formation in ELS (Lehmann-Horn et al., 2016; Serafini et al., 2004). Germinal centers are the site in lymphoid tissue where B cells expand and immunoglobulin gene hypermutation and selection is induced (Jacob et al., 1991; MacLennan, 1994; MacLennan and Gray, 1986). In other words, B cells with low affinity to CNS-antigens proliferate and differentiate to highly specific memory B cells and antibody producing plasma cells. As a result, long-lived autoreactive B cells and plasma cells can maintain chronic inflammation in the brain for many years (Corsiero et al., 2016). Therefore, it seems that formation of meningeal ELS with germinal centers play a pivotal role in the development of MS. This assumption was reinforced by recent experiments that show a reduction in axonal damage by depleting CNS B cell aggregates with anti-CD52 antibody treatment in mice (Simon et al., 2018). In this context, it seems plausible, that B cells play a major role in MS pathology through various pathways.

Table 1: Immune responses in stroke compared to multiple sclerosis. Similarities and differences between the B cell immune response after stroke or during MS/EAE are listed in the following table.
♦ marks patient data, whereas • indicates results from experimental animal research.

Immune responses in stroke compared to multiple sclerosis		
	Stroke	**MS**
Regulatory B cells	• IL-10-producing B cells limit infarct volume, mortality and immune cell infiltration (Ren et al., 2011)	• B regs are protective in early phase of EAE-induction by suppressing IL-17 and IFNγ (Matsushita et al., 2008)
	• Enhance Treg response (Bodhankar et al., 2013)	
Effector B cells/ Plasma cells	♦/• Antibody production (Dambinova et al. 2003; Ortega et al., 2015)	♦/• Function: Secretion of IFNγ, TNFα, LTα, IL-6 Antibody production Antigen presentation (Staun-Ram & Miller, 2017)
	• Minor or no effects known in the acute phase (Schuhmann et al. 2017; Doyle et al., 2015)	• Promote T cell infiltration and expansion (Matsushita et al., 2008)
	• Delayed cognitive impairment (Doyle et al., 2015)	
CSF pleocytosis	♦ Lymphocytic pleocytosis in 18.1 % of patients (Prüss et al., 2012)	♦ Activated B lymphocytes in 59 % of patients (Reiber et al., 1998)
Oligoclonal bands in CSF	♦ Found in 24.8 % of stroke patients (Prüss et al., 2012)	♦ Found in 87.7 % of MS patients (Dobson et al., 2013)
Frequently found auto-antibodies	♦ In Serum: Myelin basic protein (22 %) Proteolipid protein (17 %) NMDA-receptor (44 %) (Dambinova et al., 2003; Becker et al. 2011)	♦ In CSF: Myelin membrane lipids Ubiquitous intracellular protein (Brändle et al., 2016 Brennan et al., 2011)
Antibody effects	♦ MBP antibodies in Serum associated with cognitive decline (Becker et al., 2011)	♦ Antibody-mediated demyelination (Lucchinetti et al., 2000)
Antigen-priming	• CNS antigen is presented in cervical lymph nodes after stroke (Planas et al., 2012; van Zwam et al., 2009)	♦ B cell maturation in cervical lymph nodes (Stern et al., 2014)
ELS localization	• Infarct lesion (Doyle et al., 2015)	♦ Leptomeningeal (Serafini et al., 2006)

Conclusion

As outlined above, B lymphocytes seem to play an increasingly appreciated role in the pathology of stroke. In the acute phase, regulatory B lymphocytes may be recruited to the injured CNS and dampen inflammatory processes. On the other hand, a delayed activation and infiltration of autoreactive B cells possibly has a detrimental effect on cognitive function through the production of CNS-specific autoantibodies. The production and role of CNS-autoantibodies have been well studied in the case of MS/EAE. Nevertheless, recent findings as well as old publications suggest that similar phenomena occur after ischemic stroke. Further studies are needed to shed light on the reactivity of locally produced antibodies and their mechanisms of action. Once better understood, B lymphocytes might become an attractive therapeutic target to treat post-stroke cognitive decline and increase the quality of life of stroke survivors.

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Glossary

APCs	Antigen presenting cells
CNS	Central nervous system
CSF	Cerebrospinal fluid
EAE	Experimental autoimmune encephalitis
ELS	Ectopic lymphoid structures
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
MBP	Myelin basic protein
MCAO	Middle cerebral artery occlusion
MOG	Myelin oligodendrocyte glycoprotein
MS	Multiple sclerosis
MZ	Marginal zone
NMDA	N-Methyl-D-Aspartate
PD-1	Programmed death-1
SAP	Stroke-associated pneumonia
SCI	Spinal cord injury
TGFβ	Transforming growth factor β
Th	T helper
TNFα	Tumor necrosis factor α
LTα	Lymphotoxin α

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Daniel Berchtold
Charite Universitaetsmedizin Berlin
Experimental Neurology
Chariteplatz 1
10117 Berlin
e-mail: daniel.berchtold@charite.de
ORCID ID: 0000-0002-7865-4157

Daniel Berchtold studied Biomedical Sciences at the University of Freiburg, Switzerland and Molecular Medicine at the Charité – University Medicine Berlin. He is currently doing his PhD thesis on the role of autoantibodies and delayed autoreactive B cell responses in post-stroke cognitive decline.

Luis Weitbrecht
Charite Universitaetsmedizin Berlin
Experimental Neurology
Charitéplatz 1
10117 Berlin
e-mail: luis.weitbrecht@charite.de

Luis Weitbrecht studies medicine at the Charité University Medicine Berlin. He is currently working on his MD/PhD thesis, investigating T lymphocytes and their role in autoreactive B cell responses after stroke.

Dr. Christian Meisel
Charité Universitaetsmedizin Berlin
Institute of Medical Immunology
Charitéplatz 1
10117 Berlin
e-mail: Christian.Meisel@laborberlin.com

Christian Meisel studied medicine at the Humboldt University Berlin and was Marie Curie fellow at the Wellcome Trust Centre for Human Genetics, Oxford, UK. Since his medical thesis on differential regulation of pro- and anti-inflammatory cytokines in immune cells his major research interests address the mechanisms, diagnostics and immunomodulatory therapeutic interventions in sepsis- and injury-induced immune dysfunction. He is group leader at the Institute for Medical immunology, Charité University Medicine Berlin, and Medical Director at the MVZ, Labor Berlin – Charité Vivantes GmbH.

Dr. Andreas Meisel
Charité Universitaetsmedizin Berlin
Department of Neurology
Center for Stroke Research
NeuroCure Clinical Research Center
Charitéplatz 1
10117 Berlin
e-mail: Andreas.Meisel@charite.de

Andreas Meisel studied medicine at the Humboldt University Berlin and was EMBO fellow at the Biocenter, University of Basel, Switzerland. After his medical thesis on molecular *decision making* of bacterial restriction-modification systems he turned his attention to experimental and clinical neurosciences focussed in immune responses of stroke and other neurological disorders. Currently he is Professor of Neurology at the NeuroCure Clinical Research Center (NCRC), Director of the Center for Stroke Research Berlin (CSB) and serves as a consultant at the Department of Neurology, Charité University Medicine Berlin.

Review Article

Thomas Wallach, Vitka Gres, Philipp Henneke, Seija Lehnardt*

Guardians of neuroimmunity – Toll-like receptors and their RNA ligands

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Abstract: RNA-sensing Toll-like receptors (TLRs) are mostly associated with the recognition of viruses. However, over the last years it has become clear that the function and relevance of these receptors are far more complex. They are essential for the recognition of bacteria, fungi and parasites, leading to transcriptional activation of central nervous system (CNS) resident and invading myeloid cells during infectious meningitis and encephalitis. Moreover, host-derived RNA species interact with TLRs. Injured CNS neurons release small RNAs, *e.g.* microRNAs, into the extracellular space. Neighboring neurons and microglia take up these RNA molecules via the endosomal route, which provides the opportunity for activation of endosomal TLRs. This process contributes to neuroinflammation and further neuronal injury, thus closing the vicious cycle of CNS damage, as it may occur in numerous CNS disorders including neurodegenerative diseases.

Keywords: Toll-like receptors, neuroinflammation, microRNA, endosomes, streptococci

***Corresponding author: Prof. Dr. Seija Lehnardt**, Institute of Cell Biology and Neurobiology and Department of Neurology, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany, e-mail: seija.lehnardt@charite.de
Dr. Thomas Wallach, Institute of Cell Biology and Neurobiology, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany,
e-mail: thomas.wallach@charite.de
Vitka Gres, Institute for Immunodeficiency, Center for Chronic Immunodeficiency (CCI), Medical Center and Faculty of Medicine, University of Freiburg, Freiburg, Germany; Faculty of Biology, University of Freiburg, Freiburg, Germany,
e-mail: vitka.gres@uniklinik-freiburg.de
Prof. Dr. Philipp Henneke, Institute for Immunodeficiency, Center for Chronic Immunodeficiency (CCI), Medical Center and Faculty of Medicine, University of Freiburg, Freiburg, Germany; Center for Pediatrics and Adolescent Medicine, Medical Center and Faculty of Medicine, University of Freiburg, Freiburg, Germany,
e-mail: philipp.henneke@uniklinik-freiburg.de

Zusammenfassung: RNA-erkennende Toll-like-Rezeptoren (TLRs) sind primär als Immunrezeptoren für Virusbestandteile bekannt. Während der letzten Jahren wurde jedoch deutlich, dass die funktionale Bedeutung dieser Rezeptoren weitaus komplexer ist. Sie sind für die Erkennung von Bakterien, Pilzen und Parasiten wichtig und führen während infektiöser Meningitiden und Enzephalitiden zur transkriptionellen Aktivierung von residenten und rekrutierten myeloischen Zellen im zentralen Nervensystem (ZNS). Darüber hinaus wurden endogene, d. h. vom Wirtsorganismus abstammende RNAs als Interaktionspartner von TLRs identifiziert. Im ZNS setzen geschädigte Neurone RNA Moleküle, z. B. microRNAs, in den extrazellulären Raum frei. Diese Oligonukleotide werden anschließend von benachbarten Neuronen und Mikroglia über Endosomen aufgenommen, wo sie endosomale TLRs aktivieren können. Dieser Prozess induziert neuroinflammatorische Prozesse, die weitere neuronale Schäden nach sich ziehen. Der resultierende Teufelskreis trägt vermutlich zur Entwicklung von ZNS-Erkrankungen einschließlich neurodegenerativer Prozesse bei.

Schlüsselwörter: Toll-like Rezeptor, Neuroinflammation, microRNA, Endosomen, Streptokokken

Background

Activation of microglia, the major innate immune cell in the brain, and the subsequent recruitment of immune cells occur in essentially all diseases of the central nervous system (CNS). Although inflammation occurs nearly universally in CNS injury, it is unclear how this response is evoked after brain damage. Immune receptors and their associated signaling pathways are important for crosstalk between immune cells and non-immune CNS cells (Heneka et al., 2014). In particular, specific pattern recognition receptors, such as the Toll-like receptors (TLRs), may play a major role in CNS disorders (Kielian, 2009). In principle, these receptors are assumed to have evolved in order to protect the host from invading pathogens including bacteria and viruses. Thus, they provide a first line of

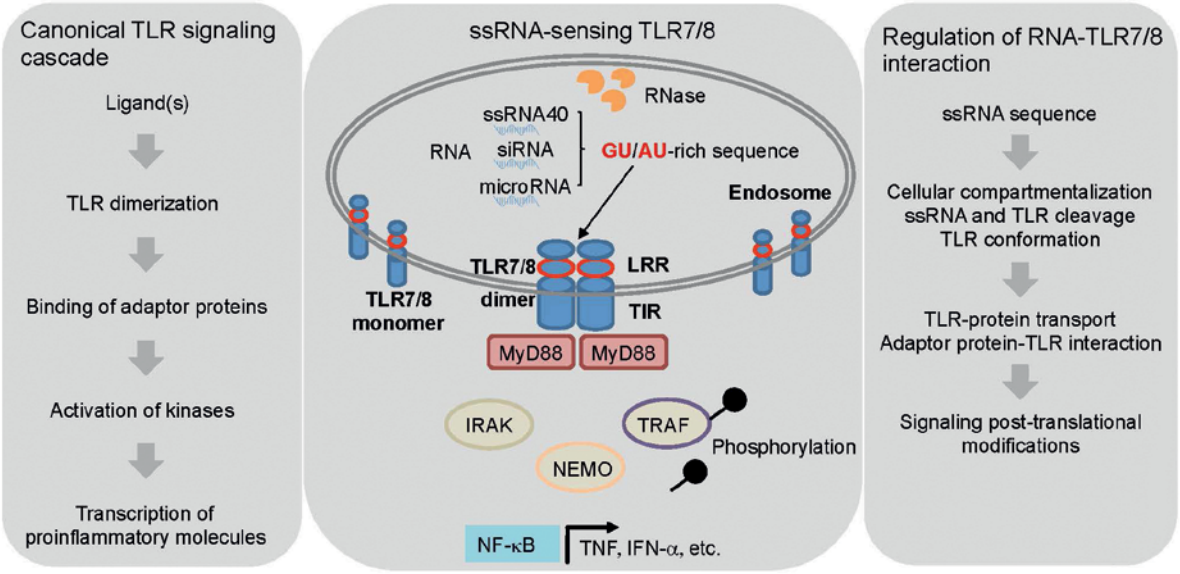

Figure 1: Signaling and regulatory pathway of ssRNA-sensing TLR7 and TLR8. LRR, leucine-rich repeats; TIR, Toll/IL-1 receptor domain; ssRNA, single-stranded RNA; siRNA, small interfering RNA; miRNA, microRNA.

innate immune defense in the brain (Heneka et al., 2014). Over the past 20 years, a plethora of cell types, not only immune cells, have been found to express TLRs under both physiological and pathological conditions. Their activation not only results in an inflammatory response, but also impacts on cell survival, migration, plasticity, cell communication, etc. To date, 10 TLRs have been identified in human and 13 in mouse, of which TLR1 to TLR9 share a high degree of genetic and structural conservation. While the mouse genome encodes TLR1 to TLR13, among them TLR10 being considered non-functional, the human genome lacks functional TLR11, TLR12, and TLR13 (Hidmark et al., 2012; Kawai and Akira, 2010; Roach et al., 2005). A subgroup of TLRs, including TLR1, TLR2, TLR4, TLR5 and TLR6, is primarily expressed at the cell surface and recognizes lipidated and proteinaceous bacterial components. The nucleic acid-sensing receptors TLR3, TLR7, TLR8, TLR9, and TLR13 are located in endosomes (Hidmark et al., 2012; Kawai and Akira, 2010). In the CNS, TLRs are broadly expressed. While microglia, astrocytes and neurons express TLR1 to TLR9, oligodendrocytes were shown only to express TLR2 and TLR3 (Rietdijk et al., 2016; Hanisch et al., 2008; Lehmann et al., 2012; Xu et al., 2015).

In general, TLRs comprise monomeric proteins harboring an ectodomain composed of leucine-rich repeats (LRRs), which mediates ligand specificity, a transmembrane domain, and a cytoplasmic Toll/IL-1 receptor (TIR) domain that interacts with adaptor proteins within the cytosol. The canonical TLR signaling pathway consists of ligand binding, TLR dimerization or multimerization, docking of adaptor proteins such as myeloid differentiation primary response 88 (MyD88) to the TIR domain, followed by activation of downstream protein kinases, e. g. IRAK, NEMO and TRAF. This signaling cascade results in activation of transcription factors such as NF-κB and, consequently, in the production and release of chemokines and cytokines including tumor necrosis factor (TNF) as well as interferons (INF) (Kawai and Akira, 2010) (**Figure 1**).

TLR7 and TLR8 recognize ssRNA

In 2004, Heil and colleagues found that mouse TLR7 and human TLR8 in macrophages and dendritic cells (DCs) were activated by a single-stranded RNA (ssRNA) known as ssRNA40, derived from the human immunodeficiency virus-1 (HIV-1). Furthermore, they identified a specific RNA-activating consensus sequence composed of GUUGUGU repeats (G, guanine; U, uridine), which could be directly linked to the degree of TLR activation (Heil et al., 2004). Several studies linked GU-rich RNA motifs to species-specific TLR7/TLR8 recognition (Diebold et al., 2004; Heil et al., 2003; Heil et al., 2004; Jurk et al., 2002; Lund et al., 2004). Interestingly, not only viral, but also small interfering RNAs (siRNAs) containing such consensus variants can activate TLR7/TLR8 (Sioud, 2005, 2007; Sioud et al., 2007). Moreover, Forsbach et al. systemati-

cally evaluated nucleotide trimers and tetramers, finding those that were GU-rich or AU-rich to preferentially activate human TLR7 and TLR8, respectively. Indeed, diverse motifs were shown to have specific receptor preferences, resulting in the release of inflammatory factors including TNF and/or IFNs, indicating a specific RNA sequence-dependent mechanism triggering inflammation (Forsbach et al., 2008). Subsequently, Mancuso et al. found TLR7 to be important for the induction of type I INFs in bone marrow-derived DCs by streptococci (Mancuso et al., 2009). Thus, endosomal TLR sensing was suggested to be important for myeloid cell-mediated immunity against bacteria.

Human TLR8 was found to be a promiscuous receptor for uridine-rich 23S ribosomal (r) RNA from *S. aureus*, *E.coli*, *S. pyogenes*, and *S. agalactiae*, resulting in the induction of cytokines by mononuclear phagocytes (Ehrnstrom et al., 2017; Eigenbrod et al., 2015; Kruger et al., 2015). Furthermore, TLR8-mediated sensing of *S. aureus* RNA results in the formation of IFN-β and IL-12, which could be antagonized by concomitant TLR2 signaling (Bergstrom et al., 2015). Moreover, a TLR8 polymorphism is associated with immunity induced by a bacillus Calmette-Guérin (BCG) vaccine, by whole bacteria and by bacterial RNA, activating human and porcine antigen-presenting cells to propagate T follicular helper (TFH) cell differentiation (Ugolini et al., 2018). Finally, allergy-protective properties of the probiotic bacterium, *Lactococcus lactis*, have been linked to its RNA recognition by DC via TLR8 (humans) and TLR13 (mice).

TLR13 recognition of bacterial RNA

Whereas TLR7 was found to recognize bacterial RNA in mouse DCs, it appeared to be redundant in the activation of mouse macrophages (Mancuso et al., 2009). Still, the endoplasmic reticulum protein, UNC-93B, a signaling intermediate downstream of endosomal TLRs, contributed to the response by macrophages toward ssRNA from a broad range of bacteria, although all of the previously described nucleic acid-sensing TLRs (TLR3, 7, 8 and 9) were dispensable in this context (Deshmukh et al., 2011). The puzzle was solved with the identification in macrophages and DCs of TLR13 as an endosomal receptor for staphylococcal 23*S* ribosomal RNA (rRNA) (Hidmark et al., 2012; Oldenburg et al., 2012). TLR13 was found to be essential for the recognition of streptococci by phagocytes including microglia and intestinal macrophages. By contrast, TLR13 was largely dispensable for the induction of cytokines by streptococci in inflammatory monocytes. This indicates that the engagement of endosomal TLRs by streptococci and other bacteria depends on the macrophage differentiation status rather than on origin and tissue-specific cues (Kolter et al., 2016). Notably, the specific TLR13-binding sequence in bacterial rRNA overlaps with the binding site for macrolides, lincosamides and streptogramines (MLS antibiotics). Since 23S rRNA modifications resulted in circumvention of bacterial recognition by TLR13, antibiotic resistance could be linked to a lack of TLR13 signaling (Oldenburg et al., 2012). At a structural level, ssRNA induces TLR13 dimerization in a stem-loop-like structure, distinct from that in the bacterial ribosome. RNA-specific interactions with the concave surface of TLR13 enable the discrimination from DNA (Song et al., 2015). Whereas the principle role of TLR13 in the recognition of bacteria, in particular staphylococci and streptococci, seems now beyond doubt, its contribution to host defense and immunopathology *in vivo* has not been fully resolved (Hafner et al., 2019; Kolter et al., 2016). Moreover, although TLR13 expression was detected in several cell types of the brain, including neurons and immune cells, it remains enigmatic at this stage how endosomal TLRs contribute to bacterial meningitis and meningoencephalitis (Mishra et al., 2008).

Activation of TLRs by endogenous ligands in the CNS

TLRs play an essential role not only in regulating innate immunity against pathogens but also in cellular responses to endogenous stimuli. Widely accepted is their role in the inflammatory response as a consequence of tissue damage, e. g. in autoimmune and tumor diseases. Endogenous ligands for TLRs expressed in immune cells, both inside and outside the CNS include components of the extracellular matrix, such as hyaluronan and versican (activators of TLR4 and TLR2 signaling, respectively), heat shock proteins (HSP) HSP60 and HSP70 (activators of TLR2 and TLR4 signaling), and RNA molecules including mRNA and microRNA (miRNA) that are recognized by TLR3 and TLR7, respectively (Beg, 2002; Kariko et al., 2004; Lehnardt et al., 2008; Lehmann et al., 2012; Hu et al., 2015). In previous work we demonstrated that activation of microglial TLR4 and MyD88 by HSP60 released from injured neurons leads to further CNS injury (Lehnardt et al., 2008). Likewise, neuronal miRNAs such as *let-7* are capable of inducing neurodegeneration through TLR7 expressed in both microglia and neurons (Lehmann et al., 2012). Thus, in the CNS, recognition of endogenous molecules by TLRs and their

subsequent signaling cascades may contribute to chronic inflammatory diseases and further pathological processes in the context of brain disorders (Lehnardt, 2010; Lehnardt et al., 2008; Lehmann et al., 2012). Indeed, there is growing evidence that TLR signaling not only contributes to CNS infection, but also to brain diseases in which no obvious pathogen-derived molecules are detected. For example, the possibility that different TLRs contribute to neurodegenerative diseases are being intensively studied in mouse models of Alzheimer's disease (AD), Parkinson's disease, and amyotrophic lateral sclerosis (Heneka et al., 2014). It is well established that activation of microglia and chronic neuroinflammation occur at primary stages of these diseases. However, the molecular and cellular mechanisms of TLR activation by host-derived ligands, which ultimately lead to specific tissue injury in the respective brain disorder, remain unclear. It is conceivable that TLRs play different roles in the CNS under different pathological conditions. Endogenous TLR activators may activate multiple signaling pathways and induce transcription of distinct gene classes, as observed for pathogen-derived ligands. Furthermore, endogenous ligands, which are potentially released from injured CNS cells, may trigger further neuronal inflammation and damage, irrespective of the origin of the respective brain dysfunction. Thus, identification of TLRs and their endogenous ligands in the context of CNS injury is essential, both for understanding CNS disease mechanisms and development of therapeutic options.

miRNAs are new endogenous TLR ligands promoting CNS injury

MiRNAs are small noncoding RNAs of 19–24 nucleotides that typically bind to 3' untranslated regions (UTR) of their target RNA. Thus, they are post-transcriptional regulators that in most cases inhibit translation. In humans, about 5000 sequences have so far been discovered that fulfill the classification of a miRNA – and the number is still growing. Moreover, the level of gene regulation by miRNAs is immensely complex since, based on their sequence, a single miRNA can bind many RNAs. In addition, one 3' UTR can be occupied by several miRNAs. Notably, it was recently shown that miRNAs within exosomes and vesicles, as well as being part of protein complexes, can be secreted by cells into the extracellular space (Gaudet et al., 2018) (**Figure 2**). Due to as yet unidentified features, miRNAs are seemingly stable in body fluids including blood and cerebrospinal fluid (CSF). This enables them to mediate communication between cells and different tissues, and to serve as valid biomarkers for diverse diseases including inflammatory and neurodegenerative disorders (Bekris et al., 2013; Cogswell et al., 2008). So far, it remains speculative whether structural and/or sequence-based features of miRNAs, or their presence, e. g. in protein complexes, contribute to their firm stabilization in the extracellular space (Gaudet et al., 2018).

The human brain expresses several hundred miRNAs that are specific to a given lineage or cell type (Lagos-Quintana et al., 2002). They exert key roles in CNS development and physiological function, as well as in various CNS disorders including traumatic brain injury, stroke, brain tumors, and neurodegenerative diseases such as AD (Junn and Mouradian, 2012). Given that miRNAs are present extracellularly, and that neurodegenerative processes are associated both with TLR-mediated neuroinflammation and altered miRNA profiles in brain cells, we have raised the question whether miRNAs can also serve as signaling molecules for TLRs. Since the miRNA let-7b, which was first discovered in *C. elegans* and is also highly conserved in human tissues (Reinhart et al., 2000), is (i) highly abundant in the brain and (ii) contains the TLR recognition motif GUUGUGU (Lehmann et al., 2012), we hypothesized that in the brain this miRNA can function as an endogenous ligand for ssRNA-sensing TLRs. Indeed, both microglia and peripheral macrophages respond to extracellularly delivered let-7b by releasing TNF in a time- and dose-dependent fashion, and this response strictly requires TLR7. Furthermore, CNS injury is accompanied by neuronal release of let-7b, which can in turn induce further neuronal apoptosis, dependent on TLR7 expression in these cells (Lehmann et al 2012). The pathophysiological relevance of these findings was confirmed *in vivo,* when intrathecal injection of let-7b into mice resulted in loss of neurons and an increase in caspase-3-positive cells in the cerebral cortex. Importantly, the number of let-7b copies was elevated in CSF from AD patients compared to healthy individuals, implying an effect of this miRNA in a neurodegenerative disease context (Lehmann et al., 2012). In line with these findings, the high degree of sequence homology among the nine different let-7 miRNA family members (all of them contain 3' GU-rich sequences), together with increased copy numbers of both let-7b and let-7e from extracellular vesicles, were detected specifically in the CSF of AD patients (Derkow et al., 2018). Recent studies in our lab revealed that injured and dying neurons release several miRNAs, not only let-7 miRNAs, which can serve as signaling molecules for TLR7 and/or TLR8. These miRNAs can then enter neighboring neurons and microglia that both express the receptors described above. Following TLR7/8

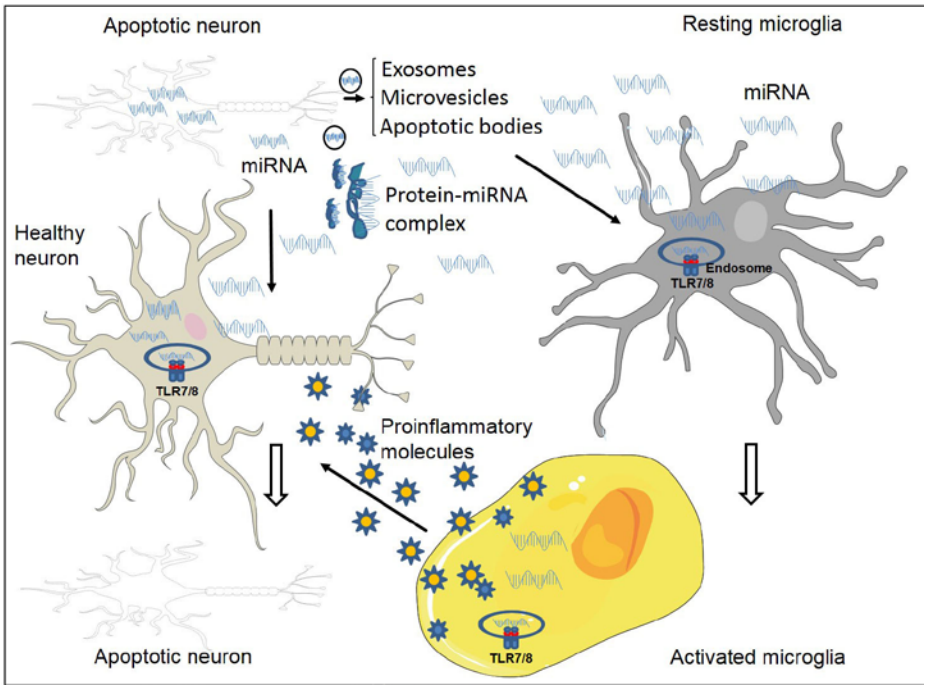

Fig. 2: Role of miRNAs as signaling molecules for TLRs in CNS inflammation. Neurons suffer an initial insult and undergo cell death, thereby releasing ssRNAs, such as miRNAs, into the extracellular space. These host-derived molecules bind to TLR7/8 expressed in (i) microglia that in turn release numerous proinflammatory mediators (e. g. TNF, yellow stars; IL-6, blue stars), or (ii) in neighboring neurons. In the case of microglial activation, this neuroinflammatory response causes injury of neighboring neurons leading to the release of endogenous TLR ligands. In the case of TLR7/8 stimulation in neighboring neurons, apoptosis and cell-autonomous neurodegeneration are induced, also leading to the release of endogenous TLR ligands and thereby closing the vicious cycle of neuronal injury.

activation in the CNS, neurons undergo both cell-autonomous apoptosis and cell death mediated by pro-inflammatory and neurotoxic inflammatory molecules released from activated microglia (Lehmann et al., 2012; Lehnardt, unpublished data) (**Figure 2**). Principles governing the biological mechanism by which miRNAs serve as TLR signaling molecules were further characterized by the work of Fabbri and colleagues. These authors demonstrated that miRNA-21 and miRNA-29a are secreted by lung tumor cells via exosomes, followed by uptake into macrophages, which in turn leads to murine TLR7 and human TLR8 activation and subsequent secretion of TNF and IL-6 (Fabbri et al., 2012). In this lung tumor model, the miRNA-TLR7/8 interaction contributes to disease progression, as well as metastasis formation. Since then, numerous studies have identified several extracellular miRNAs, including miRNA-34a, miRNA-29b, and let-7c, as endogenous ligands for TLR7 expressed in e. g. cardiomyocytes, spleen cells, and neurons, which lead to chemokine and cytokine release after receptor activation (Feng et al., 2017; Salama et al., 2014; Yelamanchili et al., 2015). In addition, Salvi and colleagues showed that miRNAs, such as miR574, are present in exosomes and initiate INF-α release from human plasmacytoid dendritic cells (pDCs) through TLR7 signaling (Salvi et al., 2018). Interestingly, the role of miRNA-TLR signaling in neuroinflammatory processes was further characterized in the brain of rhesus macaques. Here, miRNA-21 copies were found to be increased in extracellular vesicles derived from simian immunodeficiency virus (SIV)-infected neurons, resulting in neurotoxicity through the TLR7 signaling pathway. In this context, IL-6 and TNF were released from microglia and macrophages upon miRNA-21 treatment (Yelamanchili et al., 2015). Furthermore, Liu and colleagues showed that murine neuronal TLR7 detects let-7c and miRNA-21, leading to changes in dendritic morphology (Liu et al., 2015). Taken together, extracellular miRNAs were recently uncovered as novel endogenous ligands for TLRs, indicating a role for these small RNA molecules in disease initiation and progress, as well as potential therapeutic targets (Gaudet et al., 2018; Iranifar et al., 2019).

Structural aspects of ssRNA-TLR interaction and immune function

Over the last years, several groups have succeeded in crystallizing nucleic acid-sensing TLR fragments from different species (Shimizu, 2017). With respect to ssRNA-sensing TLRs, crystallographic studies on monkey TLR7 and human TLR8 revealed structures at atomic resolution. Studies by Tanji et al. and Zhang et al. showed that two ligand binding sites in these receptors co-exist, which can synergistically lead to receptor activation. In monkey TLR7, one site can be bound by a free guanosine, and this interaction alone can lead to receptor homodimerization. The other binding site preferentially binds uridine-containing ssRNA and enhances the binding of guanosine to the first site. Therefore, TLR7 is a dual sensor for guanosine and uridine-harboring ssRNAs. Within human TLR8, the first binding site at the dimerization interface prefers uridine to guanosine, while the second site binds ssRNA fragments and leads to receptor activation (Tanji et al., 2015; Zhang et al., 2016). In contrast, the synthetic guanosine analogs loxoribine and resiquimode are able to activate TLR7 signaling without additional ssRNA interactions in the second binding site (Majer et al., 2017). With respect to the binding of miRNAs to TLR7 and TLR8, it is of interest that these small oligoribonucleotides hold different sequence features that can potentially bind to both sites within the respective receptor, resulting in an immune response (Lehnardt, unpublished observation). Thus, it is tempting to hypothesize that miRNAs can potentially function as activating chimeras by presenting uridine- and guanosine-containing sequences to the respective ssRNA-sensing TLRs. So far, it is not clear how a single ssRNA or miRNA molecule is connected to the binding sites, and how this structure may be composed. In crystallization experiments, only short uridine- and guanosine-containing RNA fragments were co-crystallized with TLRs, leading to the assumption that RNA could be fragmented by regulatory RNases in endosomes to be presented to the TLRs (Tanji et al., 2015) (see **Figure 1**). It is also likely that a combinatorial effect of sequences from different miRNAs could trigger the TLR response. Therefore, analyzing the TLR-ssRNA structure at atomic resolution may be an essential step in the design and identification of potentially therapeutic substances that might reduce collateral inflammation damage in a neuroinflammatory context.

Future perspectives

It is now well established that the interaction of exogenous and endogenous RNA species with endosomal TLRs contributes to early recognition of pathogens, immune regulation, and end organ damage. Over the last five years, miRNAs have been identified as new endogenous TLR ligands, thereby unraveling a new mechanism for regulating inflammation not only in the brain, but also in several other organs in the context of human disease. GU-/AU-rich sequences are needed for the activation of distinct binding sites within TLR7/8. However, it is not exactly clear how and where different ssRNAs including miRNAs are presented to such interfaces within and/or outside of the CNS. Moreover, it is not yet fully understood what miRNA structural features are essential determinants of TLR activation (and the subsequent peripheral and neuroimmune responses that follow), nor whether a given TLR activation requires the concerted binding of different miRNAs. Thus, further investigation of the RNA-TLR interaction is required to uncover potential structural features of RNA species that govern binding to TLRs and the conformational changes that result therein. For example, it is possible that particular secondary structure motifs stabilize a respective miRNA, especially since miRNAs seem to have a role in both cell-to-cell and long-distance communication within the organism. Further, for a better understanding of miRNAs' role as TLR ligands, their binding kinetics should be characterized using biophysical and imaging approaches. Although some miRNAs have been shown to be secreted within exosomes and/or microvesicles, the temporal and spatial kinetics of the release, uptake into cells and transport to endosomes are unknown. Finally, the complex regulation of an immune response within and outside of the brain is most certainly not mediated only by a single ligand, such as a specific ssRNA molecule, or a single immune receptor class. Analysis of the combinatorial release of different ssRNAs/miRNAs, with e. g. different sequential features, and investigation of parallel immune receptor interaction/activation may be the key to understanding pathological processes and to defining novel biomarkers for numerous human diseases including CNS disorders.

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Glossary

CNS	central nervous system
TLR	toll-like receptor
LRR	leucine-rich repeats
TIR	toll/IL-1 receptor
Myd88	myeloid differentiation primary response 88
TNF	tumor necrosis factor
INF	interferon
DC	dendritic cells
ssRNA	single-stranded RNA
HIV-1	human immunodeficiency virus-1
siRNAs	small interfering RNAs
BCG	bacillus Calmette-Guérin
TFH	T follicular helper
rRNA	ribosomal RNA
HSP	heat shock proteins
miRNA	microRNA
AD	Alzheimer's disease
UTR	untranslated region
CSF	cerebrospinal fluid
pDC	plasmacytoid dendritic cell
SIV	simian immunodeficiency virus

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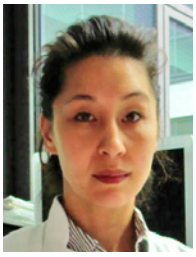
Bionotes



Prof. Dr. Seija Lehnardt
Institute of Cell Biology and Neurobiology and Department of Neurology, Charité-Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin
e-mail: seija.lehnardt@charite.de

Prof. Dr. med Seija Lehnardt is a neurologist with a strong interest in neuroimmunology and neurodegeneration. She has studied Medicine at the Charité-Universitätsmedizin Berlin, obtained her doctoral degree (2002) and habilitation (2008) at the same institution and was a postdoctoral fellow with Timothy Vartanian at the Harvard Medical School, Boston. She continued to work on neuroimmunological aspects in CNS diseases as a resident and consultant in the Department of Neuropediatrics and Department of Neurology at the Charité. Since 2009 she holds a professorship for neurodegeneration at the Charité-Universitätsmedizin Berlin. Her research team focuses on immune cell-mediated and cell-autonomous CNS injury in the context of neurodegenerative diseases.

Dr. Thomas Wallach
Institute of Cell Biology and Neurobiology, Charité – Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany
e-mail: thomas.wallach@charite.de

Dr. Thomas Wallach, Ph.D. – studied Biology at the Free University Berlin, Germany and The Rockefeller University, New York, NY, U.S.A. (2002–2007). He obtained his Ph.D. in Biology at the Humboldt University of Berlin for his work on molecular mechanisms of circadian clocks at the Institute for Medical Immunology Charité – Universitätsmedizin Berlin (2007–2012). He continued to work as a postdoc in the field of molecular chronobiology focusing on the circadian oscillator using systems biology approaches. In 2018 he moved to the Institute of Cell and Neurobiology within the Charité where he is now working on neuroinflammatory and neurodegenerative diseases. His focus lies on the identification and characterization of microRNAs as signaling molecules and their disease association.

Vitka Gres
Institute for Immunodeficiency, Center for Chronic Immunodeficiency (CCI), Medical Center and Faculty of Medicine, University of Freiburg, Freiburg, Germany, Breisacher Str. 115, 79106 Freiburg, Germany
e-mail: vitka.gres@uniklinik-freiburg.de

Vitka Gres, Dipl.ing. – studied Molecular Biotechnology at the University of Zagreb, Croatia (2009–2016), after which she worked as a research assistant at Instituto Gulbenkian de Ciencia in Lisbon, Portugal working on neuroimmune mechanisms underlying obesity (2017–2018). In 2018 she started her Ph.D. in Biology at Albert-Ludwigs-University of Freiburg working on CNS macrophages and microglia response to Group B Streptococcus infection.

Prof. Dr. Philipp Henneke
Professor, Head of Pediatric Infectious Diseases and Rheumatology, Institute for Immunodeficiency, Center for Chronic Immunodeficiency & Center for Pediatrics, University Medical Center Freiburg, Breisacher Straße 115 · 79106 Freiburg, Germany
e-mail: philipp.henneke@uniklinik-freiburg.de

Philipp Henneke received his medical degree from the FU Berlin (Germany). He pursued training in pediatrics and pediatric infectious diseases at the University Children's Hospitals in Berlin and Freiburg, Germany. From 1999 onwards, he was postdoctoral fellow at Boston University. In 2002, he was appointed Assistant Professor at the University of Massachusetts Medical School, and Lecturer at Harvard Medical School. Since 2003 he has been Assistant Professor, and since 2012 Full Professor at the University of Freiburg (Germany), where he heads the Division of Pediatric Infectious Diseases and Rheumatology. His research focus lies on the innate immune response to streptococci, staphylococci and mycobacteria and on the development of mononuclear phagocytes. His research group receives major grants e. g. from the German Research Council and the German Ministry of Research. He has received several research awards, such as the ESPID Young Investigator Award and the Research Award of the German Society of Pediatric Infectious Diseases. Philipp Henneke is Associate Chief Editor of the Pediatric Infectious Disease Journal and Associate Editor of the Journal of Immunology.

Review Article

Chotima Böttcher, Roman Sankowski, Josef Priller, Marco Prinz*

CNS myeloid cell heterogeneity at the single-cell level

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Abstract: The cellular composition of the central nervous system (CNS) is highly complex and dynamic. Regulation of this complexity is increasingly recognized to be spatially and temporally dependent during development, homeostasis and disease. Context-dependent cellular heterogeneity was shown for neuroectodermal cells as well as the myeloid compartment of the CNS. The brain myeloid compartment comprises microglia and other CNS-associated macrophages. These are brain-resident cells with critical roles in brain development, maintenance, and immune responses during states of disease. Profiling of CNS myeloid cell heterogeneity has been greatly facilitated in the past years by development of high-throughput technologies for single-cell analysis. This review summarizes current insights into heterogeneity of the CNS myeloid cell population determined by single-cell RNA sequencing and mass cytometry. The results offer invaluable insights into CNS biology and will facilitate the development of therapies for neurodegenerative and neuroinflammatory pathologies.

Keywords: Single-cell sequencing, mass cytometry, brain, immunity, neurodegeneration, glioma.

***Corresponding author: Prof. Dr. med. Marco Prinz**, Institute of Neuropathology, Faculty of Medicine, University of Freiburg, Breisacher Str. 64, D-79106 Freiburg, Germany; Signalling Research Centres BIOSS and CIBSS, University of Freiburg, Freiburg, Germany; Center for Basics in NeuroModulation (NeuroModulBasics), Faculty of Medicine, University of Freiburg, Freiburg, Germany, e-mail: marco.prinz@uniklinik-freiburg.de
Dr. Chotima Böttcher, Department of Neuropsychiatry and Laboratory of Molecular Psychiatry, Charité – Universitätsmedizin Berlin, Berlin, Germany, e-mail: chotima.boettcher@charite.de
Dr. Roman Sankowski, Institute of Neuropathology, Faculty of Medicine, University of Freiburg, Freiburg, Germany; Berta-Ottenstein-Programme for Clinician Scientists, Faculty of Medicine, University of Freiburg, Freiburg, Germany, e-mail: roman.sankowski@uniklinik-freiburg.de
Prof. Dr. med. Josef Priller, Department of Neuropsychiatry and Laboratory of Molecular Psychiatry, Charité – Universitätsmedizin Berlin, Berlin, Germany; DZNE and BIH, Berlin, Germany; University of Edinburgh and UK DRI, Edinburgh, UK, e-mail: Josef.Priller@charite.de

Zusammenfassung: Die zelluläre Zusammensetzung des zentralen Nervensystems (ZNS) ist hochkomplex und dynamisch. Sie unterliegt einer räumlichen und zeitlichen Regulation im Rahmen der Hirnentwicklung, Homöostase und bei Erkrankungen. Kontext-abhängige zelluläre Heterogenität wurde sowohl für neuroektodermale, als auch für myeloische ZNS-Zellen gezeigt. Das myeloische ZNS-Kompartment besteht aus Mikroglia und anderen ZNS-Makrophagen. Dabei handelt es sich um ortsständige Zellen, die wichtige Aufgaben während der Hirnentwicklung und -instandhaltung sowie bei Immunantworten im Rahmen von Hirnerkrankungen übernehmen. Die Analyse der Heterogenität myeloischer ZNS-Zellen wurde in den vergangenen Jahren von der Entwicklung neuartiger Hochdurchsatz-Methoden begünstigt. Diese Übersichtsarbeit beschreibt neueste Erkenntnisse über die Heterogenität myeloischer Zellen im ZNS, die mittels Einzelzell-RNA-Sequenzierung und Massenzytometrie gewonnen wurden. Diese Erkenntnisse vertiefen nicht nur den Kenntnisstand über die Biologie des ZNS, sondern werden auch zur Therapieentwicklung im Bereich der Neurodegeneration und Neuroinflammation beitragen.

Schlüsselwörter: Einzelzell-Zelle-Sequenzierung, Massenzytometrie, Gehirn, Immunität, neurodegeneration, glioma

1 Introduction

The myeloid compartment of the central nervous system (CNS) is increasingly recognized as a key player in CNS homeostasis and pathology. In the brain, this compartment comprises a diverse population of tissue-resident macrophages including parenchymal microglia and non-parenchymal macrophages that reside in perivascular (Virchow-Robin) spaces, choroid plexus, and meningeal compartments (Prinz and Priller, 2014; Prinz et al., 2017). Parenchymal microglia and most of the CNS-associated macrophages (CAMs, these are meningeal, perivascular and choroid plexus macrophages) originate from prenatal yolk sac- or fetal liver-derived myeloid precursors

that enter the CNS during early embryonic development (Ginhoux et al., 2010; Goldmann et al., 2016; Kierdorf et al., 2013; Mizutani et al., 2012; Schulz et al., 2012). Development of both parenchymal microglia and CNS macrophages relies largely on similar transcription factors such as PU.1 (Goldmann et al., 2016). CNS myeloid cells differentially regulate immune responses at the boundaries and in the parenchyma of both healthy and diseased brain, and are involving in the maintenance of CNS integrity and function (Goldmann et al., 2016; Parkhurst et al., 2013; Sierra et al., 2010). They sense danger signals at brain interfaces such as the blood-brain barrier (BBB; perivascular macrophages) or the blood-cerebrospinal fluid barrier (choroid plexus macrophages). Furthermore, spatial heterogeneity and functional differences of the resident brain macrophages, especially microglia, were detected on the basis of transcriptomic bulk system analysis in the murine brain (Grabert et al., 2016).

During CNS pathology, cells from the periphery can enter the CNS, differentiate into brain parenchymal and/or non-parenchymal macrophages, and integrate into the existing CNS myeloid compartment (Böttcher et al., 2013; Mildner et al., 2007; Priller et al., 2001). These hematogenous brain macrophages share some phenotypic signatures with resident CNS myeloid cells. In the past, it was technically challenging to distinguish these infiltrating cells from their CNS counterparts and models such as bone marrow chimerism were used (Mildner et al., 2007; Priller et al., 2001). Nowadays, comprehensive approaches for cell profiling at the single-cell level have been developed to unravel the transcriptomic, phenotypic and functional complexity of the CNS myeloid compartment. Recently, single-cell RNA sequencing (scRNA-Seq) and Cytometry by Time-of-Flight (CyTOF) or mass cytometry have been used by us and other groups to profile the heterogeneity of the murine and human CNS myeloid compartments in health and disease. scRNA-Seq examines single-cell transcriptomes by sequencing the mRNA of thousands of genes, while CyTOF measures dozens of proteins in individual cells (**Figure 1**). As both methods offer complementary information, side-by-side application of both offers the prospect of identifying disease-associated myeloid populations that can potentially be therapeutically targeted.

In this review, we report an overview of CNS myeloid heterogeneity determined by single-cell RNA sequencing, as well as single-cell multiplexed mass cytometry.

2 Single-cell RNA-sequencing

Recent years have witnessed pioneering studies mapping the murine and human brain tissues with single-cell RNA-sequencing (scRNA-Seq). This technique offers an efficient way to distinguish cell types based on their transcriptomes. Thus, it serves a long-unmet need to examine heterogeneity of cell populations, i. e. differential gene expression due to functional specialization or pathological perturbations. Facilitated by declining sequencing costs, ambitious consortium approaches are currently performed using scRNA-Seq to construct a single-cell atlas of the human body (Regev et al., 2017). The goal is to eventually understand healthy and diseased human tissues at an unprecedented level. Notably, atlases of a whole mouse and other model organisms have already been achieved (Han et al., 2018; The Tabula Muris Consortium et al., 2018). Early scRNA-Seq studies of the brain have applied exploratory approaches by analyzing all cell types of a given brain region, e. g. somatosensory cortex and hippocampus (Zeisel et al., 2015). Initially, the cells analysed numbered in the hundreds. Within a few years, technological advances increased the scale up to over 500,000 cells from all nervous tissues of the mouse (Zeisel et al., 2018). The resulting high-dimensional datasets offered even more information than was sought by a given study. Due to this richness of information, it has become common for studies with scRNA-Seq data to release online data viewers to facilitate independent exploration of data (Han et al., 2018; The Tabula Muris Consortium et al., 2018; Zeisel et al., 2015) (Table 1).

The brain myeloid field has benefitted from the availability of scRNA-Seq data from mouse and human brains and the concomitant sharing of data. A number of exploratory studies included data on microglia and other CNS-associated macrophages (CAMs) (Campbell et al., 2017; Darmanis et al., 2017, 2015; Gokce et al., 2016; Hochgerner et al., 2018; La Manno et al., 2016; Macosko et al., 2015; Moffitt et al., 2018; Tepe et al., 2018; Tirosh et al., 2016; Vanlandewijck et al., 2018; Venteicher et al., 2017; Zeisel et al., 2018, 2015; Zhong et al., 2018; Zywitza et al., 2018; Campbell et al., 2017; Darmanis et al., 2017, 2015; Gokce et al., 2016; Hochgerner et al., 2018; La Manno et al., 2016; Macosko et al., 2015; Moffitt et al., 2018; Tepe et al., 2018; Tirosh et al., 2016; Vanlandewijck et al., 2018; Venteicher et al., 2017; Zeisel et al., 2018, 2015; Zhong et al., 2018; Zywitza et al., 2018; Jordão et al., 2019; Masuda et al., 2019). While the aforementioned studies conducted scRNA-Seq on fresh tissues, RNA sequencing of nuclei extracted from frozen tissues has enabled the study of immune and non-immune cells of archived cryopreserved tissues (Habib et al., 2017;

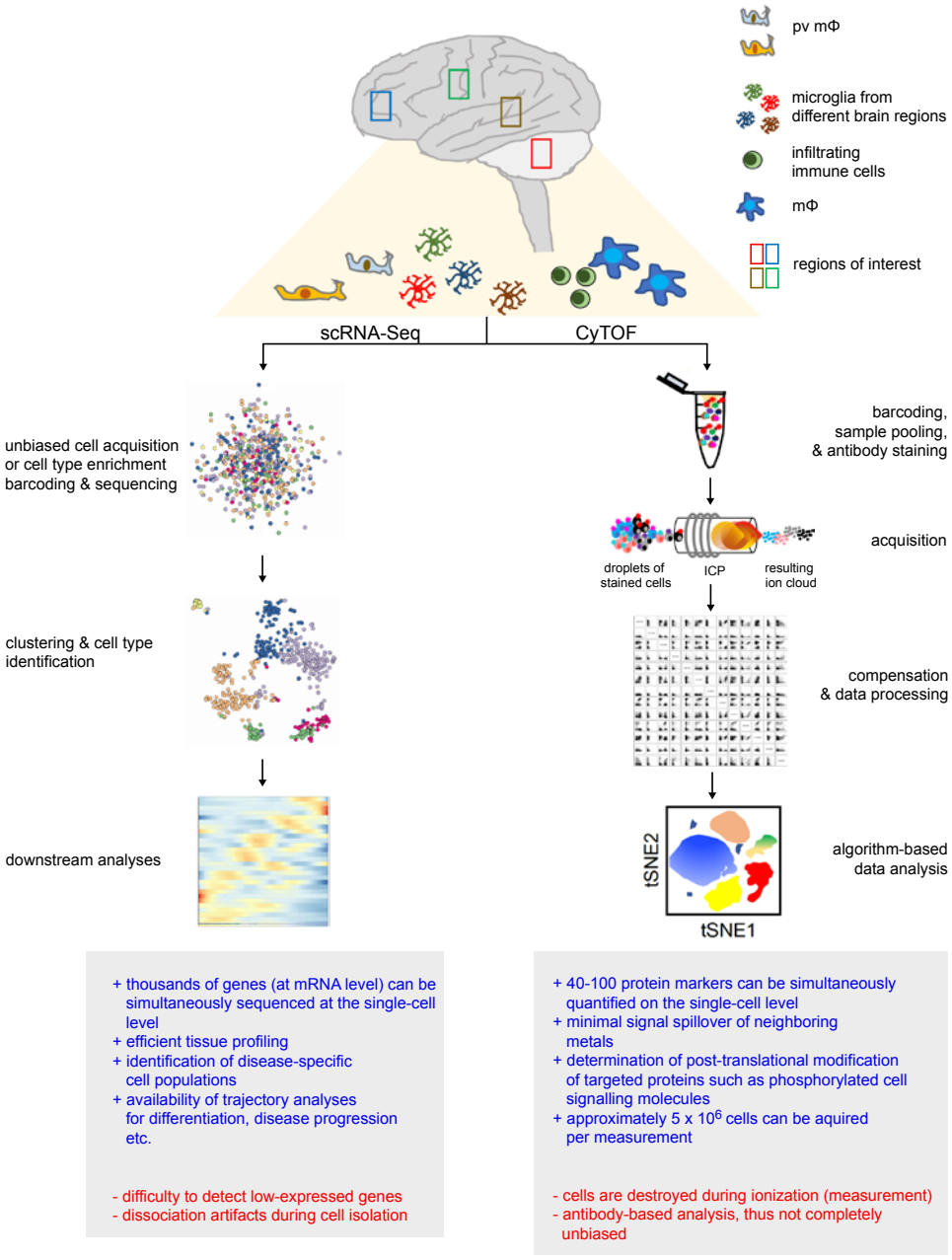

Fig. 1: Comparative schematic representation between single-cell RNA sequencing and CyTOF analysis. Isolated single cells including perivascular macrophages (pv mΦ), microglia from different brain regions (coded accordingly by colour; differently colour-coded rectangles represent different regions of interest), infiltrating immune cells and macrophages (mΦ) can be analyzed in parallel by scRNA-Seq and mass cytometry (CyTOF). General workflows of scRNA-Seq and CyTOF are shown on the left and right sides, respectively. scRNA-Seq consists of either unbiased acquisition of all cells or cell type enrichment using FACS (different cell types are represented by coloured dots). Cell types are differentiated *in silico* based on transcriptomic similarities and visualized using dimension reduction techniques, such as t-Distributed Stochastic Neighbor Embedding (t-SNE) that facilitate human-readable visualization of complex data in two dimensions (t-SNE1 and t-SNE2). Note that, as t-SNE places similar cells in close proximity to each other, dots of the same color now form distinct clouds representing cell populations. Finally, a number of downstream analyses enables the researcher to conduct fine-grained examinations beyond cell type distinction, such as the evaluation of gradual transcriptional changes across 'pseudo-time' that represent differentiation trajectories or different stages of disease etc. (here, with a heatmap of gene clusters across pseudo-time). For CyTOF analysis, samples are generally barcoded, pooled and stained with panels of selected antibodies. Stained cells are then acquired into CyTOF via a nebulizer. A fine spray of droplets are then completely atomized and ionized in an inductively coupled plasma (ICP). The resulting ion cloud is then measured in a Time-of-flight (TOF) chamber. The data are then compensated. The panel of two-dimension plots are shown as a symbol representing how compensation matrix was calculated, thereby signal intensity of each metal-channel is plotted against the other metals. Compensated data are then processed (debarcoding and data pooling) prior to algorithm-based data analysis. Advantages (text in blue) and disadvantages (text in red) of both techniques are summarized.

Tab. 1: Overview of open-access single-cell sequencing data viewers.

Resource URL	Cell types	Method
http://brainrnaseq.org/	Mouse and human microglia	Bulk RNA-Seq and Smart-Seq2
http://research-pub.gene.com/BrainMyeloidLandscape/	Mouse brain $CD45^+$ sorted cells	Bulk RNA-Seq
http://celltypes.brain-map.org/rnaseq	Different datasets	Single-cell and single-nucleus RNA-Seq
https://portals.broadinstitute.org/single_cell	Different datasets	Single-cell and single-nucleus RNA-Seq
http://betsholtzlab.org/VascularSingleCells/database.html	Mouse brain and lung cells	Smart-Seq2
http://www.gbmseq.org/	Human brain tumors	Smart-Seq2
http://linnarssonlab.org/data/	Different datasets	Fluidigm C1 & 10x Genomics
http://www.microgliasinglecell.com/	Mouse microglia	10x Genomics
https://tabula-muris.ds.czbiohub.org/	Different mouse tissues	Smart-Seq2 & 10x Genomics
http://bis.zju.edu.cn/MCA	Different mouse tissues	Microwell-Seq

Jäkel et al., 2019; Renthal et al., 2018). The main shortcoming of exploratory studies is their limited ability to resolve heterogeneity of CNS myeloid cells due to the low numbers of these cells. This shortcoming was addressed through scRNA-Seq of FACS-sorted microglia and CAMs under homeostatic and pathological conditions in developing and adult humans and mice. Microglia and CAMs were shown to be derived from the yolk sac or fetal liver during embryonic development and to show lasting transcriptional changes in the adult animals that were influenced by the microbiome and maternal infections (Goldmann et al., 2016; Li et al., 2019; Matcovitch-Natan et al., 2016; Thion et al., 2018; Erny et al., 2015). In adult mice, microglia and CAMs show pronounced heterogeneity in healthy animals and drastically change during demyelination, characterized by increasing expression levels of *Apoe, Axl, Igf1, Lyz2, Itgax, Gpnmb and Apoc1* and strongly down-regulated microglial markers such as *TMEM119* and *P2RY12* (Hammond et al., 2019; Jordão et al., 2019; Masuda et al., 2019; Li et al., 2019). Increased transcription of *Apoe* and reduced expression of microglial genes was confirmed in brain tissue from patients with multiple sclerosis (Masuda et al., 2019). Neurodegeneration-associated microglia were found to display a largely consistent proinflammatory phenotype that is distinct from demyelination-associated microglia (Keren-Shaul et al., 2017; Masuda et al., 2019; Mathys et al., 2017; Tay et al., 2018).

Major insights into the astonishing heterogeneity of CNS myeloid cells during development, as well as in healthy and disease states were recently gleaned using scRNA-Seq. These findings paved the way for a deeper understanding of the human brain immune system with a potential for future diagnostic use.

3 Revealing myeloid heterogeneity by mass cytometry

Unlike scRNA-Seq, elucidating total proteomics in a single cell has remained challenging. A high-dimensional single-cell phenotypic analysis, which is widely used for cellular profiling in neurosciences, is CyTOF. CyTOF combines metal isotope-labelling technology, flow cytometric analysis with time-of-flight mass spectrometry to identify and quantify cells. Using CyTOF technology, cellular targets are labelled with metal-conjugated antibodies and detected and quantified by time-of-flight mass spectrometry. Taking advantage of the low signal overlap between metal isotopes, CyTOF allows simultaneous cell identi-

fication and quantification on the basis of more than 45 marker targets on a single cell. Data obtained from CyTOF measurements are processed and analysed in an unsupervised manner using algorithm-based data analysis. The combination of a comprehensive array of protein markers and unsupervised data analysis provides a powerful strategy for cell identification and quantification in a complex system like the CNS.

Utilizing this technique, key transcriptomic signatures of mouse (Galatro et al., 2017) and human microglia (Gosselin et al., 2017) were confirmed at the protein level in individual cells. We found the microglial phenotypic signature to be distinct from peripheral myeloid cells isolated from the peripheral blood or cerebrospinal fluid (Böttcher et al., 2019). Compared to peripheral cells, microglia expressed higher levels of $P2Y_{12}$, TMEM119, CD64, TGF-β1, CCR5, CD32, CD172a, CD91, HLA-DR, CD11c, CX3CR1, CD115 and TREM2, whereas expression of CD44, CCR2, CD45, CD206, CD163, CD274, CD14 and CD16 was lower in the microglial population (Table 2). These results agree with previous microglial profiling studies using multi-parameter mass cytometry (more than 35 markers). The resulting high-dimensional data demonstrated that murine microglia are heterogenous and show distinct phenotypes from circulating monocytes and other tissue-resident macrophages (Becher et al., 2014; Korin et al., 2017; Ajami et al., 2018). On the basis of multi-parameter characterization and computational unbiased data analysis, we could clearly distinguish parenchymal microglia from perivascular macrophages, which highly expressed CD45, CD206 and CD163. Interestingly, we detected the expression of EMR1 (F4/80) on microglia. In humans, this protein was demonstrated to be highly specific to eosinophils and was suggested to be absent from monocytes, macrophages and dendritic cells (Hamann et al., 2007). Similar to the previous results obtained from transcriptomic profiling of the mouse brain (Grabert et al., 2016), massive single-cell immune profiling by CyTOF revealed regional heterogeneity of human microglial phenotypes between the subventricular zone, thalamus, cerebellum, as well as temporal and frontal lobes of the human brain. We detected higher expression of markers involved in microglial activation (these were CD68, CD86, CD45, CX3CR1, CD11c, CD64, EMR1 and HLA-DR) in microglia from the subventricular zone and thalamus, compared with that from cerebellum, temporal and frontal lobes (Table 2). The human microglial subset, which was predominantly found in the frontal and temporal cortex, expressed the mannose receptor, CD206, a marker of M2-polarized macrophages, whereas microglia found in other regions were negative for this marker. Whether this microglial heterogeneity implies a region-specific function and/or a region-dependent vulnerability of microglia in different neurodegenerative disorders remains to be investigated.

Tab. 2: Overview of CNS myeloid cell phenotypes.

CNS cell types	High expressed markers	Low/negative expressed markers
Microglia (MG)	$P2Y_{12}$, TMEM119, CD64, TGF-β1, CCR5, CD32, CD172a, CD91, HLA-DR, CD11c, CX3CR1, CD115, EMR1, TREM2	CD44, CCR2, CD45, CD206, CD163, CD274, CD14, CD11b, CD16, CD33
SVZ- and THA-MG	CD68, CD86, CD45, CX3CR1, CD11c, CD64, EMR1,HLA-DR	
FL- and TL-MG	CD206	
perivascular macrophage	CD45, CD206, CD163, CD14, HLA-DR, CD68, CD33, EMR1, CD64, CD11c, CD11b, IRF8, TGF-β1, CD115	$P2Y_{12}$, TMEM119, CX3CR1, CD16
infiltrating myeloid cells	CD44, CCR2, CD45, CD206, CD163, CD274, CD14, CD16, CD32, CD172a, CD18	$P2Y_{12}$, TMEM119, TREM2, EMR1

SVZ = Subventricular zone; THA = Thalamus; FL = Frontal lobe; TL = Temporal lobe
Summarized from Böttcher et al., 2019.

4 Conclusion

Using unsupervised high-dimensional scRNA-seq and CyTOF analyses, CNS myeloid cell heterogeneity is currently being unravelled at the single-cell level in both mouse and human brains. However, open questions including the spatial characteristics of and the dynamic interactions between different myeloid cell subsets and the surrounding neuronal and non-neuronal cells remain to be answered. Imaging mass cytometry (IMC) is a technology that couples mass cytometry to immunohistochemical methods with high-resolution laser ablation (Keren et al., 2018). This technology enables high-dimensional cell profiling of a wide spectrum of cell types on tissue sections using the same principle as mass cytometry. Likewise, spatial transcriptomics approaches investigate cellular transcriptomics *in situ* (Lein et al., 2017). To this end, a recent study utilized tissue clearing to achieve three-dimensional single-cell spatial transcriptomics (Wang et al., 2018). As both IMC and spatial transcriptomics can be applied to fresh as well as archived tissues, they hold great promise to uncover spatial phenotypic and functional heterogeneity of the CNS myeloid compartment in health and disease.

Since scRNA-seq and CyTOF record complementary information, the combination of both methods appears promising. This has already shown promise for dendritic cells (See et al., 2017), and also for healthy and glioma-associated microglia (own unpublished data).

The findings on CNS myeloid heterogeneity may imply microglial region-dependent vulnerability and regionally differential involvement in neurological and psychiatric diseases. Better understanding of heterogeneity-associated functional differences of CNS myeloid cells, as well as the mechanisms involved in heterogeneity may have clinical implications for diagnosis and treatment of CNS disorders.

List of abbreviations used in this text

CNS	central nervous system
CAMs	CNS-associated macrophages
BBB	blood-brain barrier
scRNA-Seq	single-cell RNA sequencing
CyTOF	cytometry by time-of-flight
IMC	imaging mass cytometry

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Bionotes

Dr. Chotima Böttcher
Department of Neuropsychiatry and Laboratory of Molecular Psychiatry, Charité – Universitätsmedizin Berlin, Berlin, Germany
e-mail: chotima.boettcher@charite.de

Chotima Böttcher is senior scientist of Laboratory of Molecular Psychiatry at the Charité – Universitätsmedizin Berlin, Germany. Dr. Böttcher obtained her PhD at Institute of Pharmacy, Faculty of Natural Sciences at Martin-Luther-University Halle-Wittenberg, Halle/Saale, Germany in 2005. In the first year of her postdocteral fellow, she continued her study on biosynthesis of mammalian morphine at Martin-Luther-University Halle-Wittenberg and later at Donald Danforth Plant Science Center, MO, USA. In 2006, she moved to the group of Dr. Priller (Laboratory of Molecular Psychiatry) at the Charité – Universitätsmedizin Berlin and has started her new research field –systems immunology in neuroscience, with particular emphasis on myeloid cells including monocytes and brain microglia/macrophages.
Currently, her research aims to identify cellular complexity and heterogeneity of the myeloid compartment of the human central nervous system (CNS) and to further investigate how these signatures alter during neurodegeneration/neuroinflammation. In the Laboratory of Molecular Psychiatry, high-dimensional single-cell immune profiling technique such as mass cytometry (cytometry by time-of-flight, CyTOF) has been established for an investigation of phenotypic profiles of circulating myeloid cells in the peripheral blood and the cerebrospinal fluid, as well as for immune phenotyping of tissue-resident macrophages such as the CNS microglia and macrophages.

Dr. Roman Sankowski
Institute of Neuropathology, Faculty of Medicine, University of Freiburg, Freiburg, Germany
e-mail: roman.sankowski@uniklinik-freiburg.de

Roman Sankowski studied Medicine at Philpps-University of Marburg. During his PhD at the Feinstein Institute for Medical Research he studied the effect of neuroinflammation on spatial learning. Since 2016, he is Neuropathology resident at Freiburg University Medical Center. His research focuses are brain myeloid cells in health and disease.

Prof. Dr. med. Josef Priller
Department of Neuropsychiatry and Laboratory of Molecular Psychiatry, Charité – Universitätsmedizin Berlin, Berlin, Germany
e-mail: Josef.Priller@charite.de

Prof. Dr. med. Marco Prinz
Institute of Neuropathology
University of Freiburg
Breisacher Str. 64,
D-79106 Freiburg, Germany
e-mail: marco.prinz@uniklinik-freiburg.de

Marco Prinz is Professor of Neuropathology and Chair of the Institute of Neuropathology at the University of Freiburg, Germany. Dr. Prinz obtained his MD at the Charitè, Humboldt-University Berlin in 1997. He performed his residency in Neuropathology at the University Hospital Zurich, Switzerland and studied there the role of the peripheral and CNS-restricted immune system for the pathogenesis of neurodegenerative diseases such as prion diseases. He was recruited to the University of Freiburg, Germany, in 2008 and was promoted to the rank of Full Professor and Chair of the Institute of Neuropathology.
Dr. Prinz laboratory studies the mechanisms that regulate the development and function of the mononuclear phagocyte lineage in the central nervous system including microglia, perivascular and meningeal macrophages. His laboratory has made seminal discoveries in CNS macrophage biology revealing their embryonic origin and their local maintenance in situ.
Currently, his research group aims to understand myeloid cell biology in the CNS during health and disease and studies the impact of the immune system on the pathogenesis of neurological disorders such as neurodegenerative diseases, ultimately aimed at recognizing novel therapeutic strategies and targets to treat these central nervous system diseases.

Review Article

Charlotte Mezö, Omar Mossad, Daniel Erny*, Thomas Blank*

The gut-brain axis: microglia in the spotlight

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Summary: Microbiome research has grown significantly in the last decade, highlighting manifold implications of the microbiota to the host's health. The gut microbiota is connected to the brain through several avenues that allow their interaction. Thus, recent studies have attemtpted to characterize these connections and enhance our understanding of the so called 'gut-brain-axis'. Microglia, the central nervous system resident macrophages, are crucial for the proper development and maintenance of brain functions. As immune cells, they are in the spotlight for relaying signals between the microbiota and cells of the brain. In this review, we contemplate on interactions between the gut microbiota and microglia, and their influence on brain functions in health and disease.

Keywords: central nervous system, germ-free, gut microbiota, neuroimmune, short-chain fatty acids

Zusammenfassung: Die Erforschung des Mikrobioms hat in den letzten Jahrzenten deutlich zugenommen, was die vielfältigen Auswirkungen der mikrobiellen Flora auf die Gesundheit hervorhebt. Die Darmflora kann über mehrere Wege die Funktion des Gehirns beeinflussen. Jüngste Studien versuchten diese Verbindungen zu charakterisieren, um somit das Verständnis über die sogenannte Darm-Hirn-Achse zu verbessern. Mikroglia, die Makrophagen im Parenchym des zentralen Nervensystems, spielen eine zentrale Rolle bei der Entwicklung und Aufrechterhaltung der Hirnfunktionen. Als Immunzellen stehen sie im Fokus für die Signalweiterleitung zwischen der mikrobiellen Flora und den Zellen des Gehirns. Im vorliegenden Übersichtsartikel betrachten wir die Interaktionen zwischen der Darmflora und Mikroglia und dessen Einfluss auf die Hirnfunktionen sowohl im gesunden als auch im erkrankten Gehirn.

Schlüsselworte: zentralen Nervensystems, Keimfrei, Darmflora, Neuroimmune, kurzkettige Fettsäuren

***Corresponding author: Dr. med. Daniel Erny**, Institute of Neuropathology, University Hospital of Freiburg, Breisacher Str. 64, 79106 Freiburg, Germany, e-mail: daniel.erny@uniklinik-freiburg.de
Dr. rer. nat. Thomas Blank, Institute of Neuropathology, University Hospital of Freiburg, Breisacher Str. 64, 79106 Freiburg, Germany, e-mail: thomas.blank@uniklinik-freiburg.de
M.Sc. Charlotte Mezö, Institute of Neuropathology, University Hospital of Freiburg, Breisacher Str. 64, 79106 Freiburg, Germany, e-mail: charlotte.mezoe@uniklinik-freiburg.de
M.Sc. Omar Mossad, Institute of Neuropathology, University Hospital of Freiburg, Breisacher Str. 64, 79106 Freiburg, Germany, e-mail: omar.mossad@uniklinik-freiburg.de

Introduction and objectives

Invisible communities of microbes are by far the most dominant lifeform on Earth. Microbes are deeply integrated into all types of living niches. Millions of years of co-evolutionary symbiosis have shaped the human body, enabling it to harbor one of the most intricate, yet underexplored ecosystems. We are constantly discovering and characterizing distinct microbial populations living in different orifices of our bodies. The largest population resides in the nearly anoxic gastrointestinal tract, particularly in the large intestine. More than 100 trillion microbes are estimated to live in the human gut alone. This is about the same number of all human cells in the body (Turnbaugh et al., 2007).

It is no wonder that the microbiota supplies the human body with many benefits, making it a crucial element for maintenance of the host's health. For example, the microbiota is important for maintenance of intestinal barrier integrity, inhibition of pathogen adhesion to intestinal surfaces, vitamins B & K synthesis, as well as the metabolism of bile acids, sterols and xenobiotics (Cummings and Macfarlane, 1997). Disturbances and alterations of the gut microbiota (a state called dysbiosis) have been related to a wide variety of diseases such as asthma (Stokholm et al., 2018), cardiovascular disease (Howitt and Garrett, 2012), skin immunity and tissue repair (Linehan et al., 2018), as well as type 1 and 2 diabetes (Murri et al., 2013; Qin et al., 2012). Microbes salvage energy from unabsorbed food providing the body with short-chain fatty acids (SCFAs) and control the maturation and functional regulation of the peripheral immune system (Bäckhed et al., 2005). This latter interaction is especially critical because the microbiota may also drive immune development in the brain. The neuroimmune system, primarily comprised of glial cells, is

distinct from the peripheral immune system in part due to anatomical barriers and developmental sequence. Microglia are the only parenchymal macrophages of the central nervous system (CNS); they constitute 5–10 % of total brain cells (Prinz et al., 2017) and arise from the yolk sac erythromyeloid precursors (Ginhoux et al., 2010; Gomez Perdiguero et al., 2015; Kierdorf et al., 2013; Prinz et al., 2017). They conduct the CNS orchestra by phagocytosing dead cells, by secreting trophic factors like brain-derived neurotrophic factor to support developing cells and by moderating angiogenesis (Kierdorf and Prinz, 2017). Contrary to long-standing belief, the brain is not immune privileged in a classical sense and shows constant communication with the periphery. Microglia are a good indicator of this interaction, where excess dietary lipids in a high-fat diet mouse model resulted in an activated state (Baufeld et al., 2016).

In this review, we shed light on recent findings entailing the impact of gut microbiota on the host CNS in health and disease, with specific focus on CNS tissue resident macrophages, the microglia.

Gut-brain axis

In the last decade, accumulating evidence has highlighted the interaction between gut microbiota and the host's CNS via the so-called *gut-brain axis*. The microbiota is crucial for maintaining CNS functions including neuronal signaling, brain circuitries and behavior, all of which are mediated through neuronal, endocrine, metabolic and immune pathways (Collins et al., 2012; Cryan and O'Mahony, 2011; Sarkar et al., 2016). Afferent fibers from the vagus nerve (VN) transmit information from the gut to the CNS. Hypothalamic neurons in the brain controlling food intake can sense nutrients within seconds after entering the duodenum (Beutler et al., 2017; Su et al., 2017). More surprisingly, a new study by Kaelberer and colleagues showed that signals from a small enteroendocrine cell population that they termed 'Neuropod cells' can reach the brainstem in a matter of milliseconds (Reinshagen, 2019). It was reported that germ-free (GF) mice showed increased permeability of blood brain barrier (Braniste et al., 2014) and increased myelination of axons in the prefrontal cortex (Hoban et al., 2016). Here, the authors speculate that increased neuronal signalling in the prefrontal cortex (PFC) of GF mice might underlie the observed increase in myelination. Dysbiosis of the gut microbiota can influence social interactions (Desbonnet et al., 2014), stress responsiveness (Desbonnet et al., 2010) and is linked to several CNS disorders (Erny et al., 2017). Bacterial fermentation products include active metabolic mediators like SCFAs that are known to modify several cellular processes including gene expression, chemotaxis, differentiation, proliferation and apoptosis (Corrêa-Oliveira et al., 2016). SCFAs are involved in activation of G-protein coupled receptors (Vinolo et al., 2012), inhibition of histone deacetylases (Donohoe et al., 2012) and stabilization of hypoxia-inducible factor (Kelly et al., 2015). Another signal possibly affecting the CNS, both directly and indirectly, is the release of microbial-associated molecular patterns (MAMPs) in the gut. MAMPs include bacterial lipoproteins, double stranded RNA and lipopolysaccharides (LPS). These molecules can be detected by immune cells through toll-like receptors (TLRs), mediating myeloid differentiation primary response 88 (MyD88)-dependent nuclear factor 'kappa-light-chain-enhancer' of activated B-cells (NF-κB) activation, which is involved in host defense responses.

Microbiota modulates microglial maturation and function under healthy conditions

Findings by Thion et al. suggest that signals from the microbiota can shape prenatal differentiation of microglia in the CNS (Thion et al., 2018). In this study, they dissected the microglia differentiation phases by assessing their transcriptional profiles and chromatin accessibility landscapes. At E14.5, embryonic microglia from offspring of germ-free (GF) dams showed only minor differences in gene expression (19 differentially expressed genes) compared to the specific pathogen free (SPF) condition, which encounter signals from the maternal complex microbiome. However, at a later prenatal time point (E18.5), a downregulation of genes (e. g. *Aoah*, *Ly86*, and *Cd28)* and transcription factors (e. g. *Irf8*, *Stat1, Klf2/4/6* and *Jun/Fos)* that modulate microglial differentiation, or that are involved in inflammatory responses became apparent. Notably, the impact of microbiota on microglial features has shown sex-specific differences. While alterations in morphology and the transcriptomic profile of microglia during the embryonic phase (E18.5) were more pronounced in male offspring of GF dams, female offspring showed alterations in microglial profile at a far later time point (Thion et al., 2018). Erny et al. described the impact of host microbiota on microglia in adult mice (Erny et al., 2015). They showed that surface proteins, like CSF1-R, F4/80 and CD31, which are downregulated in adult compared to embryonic microglia (Kierdorf et al., 2013), are up-regulated in microglia

from adult GF or antibiotics-treated SPF mice. In accord with the up-regulation of genes promoting cell survival and proliferation, like *Csf1r*, *Ddit4* and *TGF-ß1*, microglia from GF mice showed higher cell density in several brain regions. Microglia from adult GF mice failed to elicit an appropriate activation response to immunostimulants such as LPS and lymphocytic choriomeningitis virus. Interestingly, oral supplementation of the GF mice with SCFAs largely reversed these immature features. Yet, mice lacking the SCFAs receptor Free fatty acid receptor 2 (FFAR2), which is not expressed in the adult brain, also showed immature microglial phenotype similar to GF mice, suggesting a relay of the signal through peripheral cells or organs (Erny et al., 2015). To further investigate the extent by which microbiota complexity mediates the maturation of microglia, Erny et al. examined altered Schaedler flora (ASF) mice (Stecher et al., 2010) that have a strongly diminished and narrowly-defined microbiota harboring a subset of bacterial species. Microglia from ASF mice displayed morphological and gene expression features similar to those from GF mice. Recolonization with a diverse microbiota from SPF mice was able to fully restore the mature microglia phenotype. These findings suggest that microglia require constant input from a complex microbiota to maintain their homoeostatic phenotype and function in adulthood (**Figure 1**).

Gut microbiota – microglia interaction in neurodegenerative diseases

Several studies demonstrated that alterations in the gut microbiota are associated with neurodegenerative diseases, including Alzheimer's disease (AD) (Minter et al., 2016; Vogt et al., 2017) and Parkinson's disease (PD) (Keshavarzian et al., 2015; Scheperjans et al., 2015) and other neurological diseases, such as multiple sclerosis (Berer et al., 2011) and autism spectrum disorder (Tabouy et al., 2018). A recent study showed that metabolites of dietary tryptophan, produced by the gut microbiota, controlled microglial activation through the aryl hydrocarbon receptor, modulating the release of TGF-α and VEGF-ß. These two factors modulated astrocytic activation and exacerbated CNS inflammation in a mouse model of multiple sclerosis (**Figure 1**) (Rothhammer et al., 2018). We will now have a closer look at the impact of gut microbiota on the two most common neurodegenerative diseases, AD and PD.

Alzheimer´s disease

AD is the most common type of dementia and is characterized by a progressive decline in cognitive function. Pathological hallmarks of AD are extracellular plaques composed of amyloid beta (Aβ) peptides, derived by enzymatic cleavage of the amyloid precursor protein and intracellular neurofibrillary tangles formed by hyperphosphorylated tau proteins (Selkoe and Hardy, 2016). Aβ depositions trigger an immune activation, which leads to synapse loss and neuronal death, and consequently results in impaired cognitive function (Selkoe and Hardy, 2016). Besides genetic risk factors, there is some evidence that the host's gut microbiota might play a critical role in AD pathogenesis as well (Fåk et al., 2017; Minter et al., 2016). Recent studies in AD patients highlighted the relation between brain amyloidosis and pro-inflammatory gut bacteria in cognitively impaired patients (Cattaneo et al., 2017). Analysis of fecal microbiota from AD patients compared to healthy control patients revealed reduced microbial diversity (Vogt et al., 2017). Fecal microbiota composition of transgenic mouse models of AD suggested less diversity when compared to non-transgenic littermates with potential impact on Aβ depositions (Fåk et al., 2017; Minter et al., 2016). Transgenic AD mice raised under GF conditions had less Aβ depositions than SPF AD mice. In AD, microglia can bind soluble Aβ oligomers via cell-surface receptors, such as TLRs, which results in inflammatory immune response and enhanced Aβ clearance by phagocytosis and degradation to restore tissue homeostasis. Sustained microglia activation and exposure to pro-inflammatory cytokines and chemokines in turn induces neurotoxicity, leading to neurodegeneration and consequently contribute to disease progression (Seeher and Brodaty, 2017). These results suggest that gut microbiota shapes host innate immunity, which in turn influences AD pathology. But how microglia functions are affected by gut microbiota during AD is not yet resolved.

Parkinson's disease

Parkinson's disease (PD) is a progressive movement disorder, which is associated with the accumulation of α-synuclein (α-syn) aggregates. As a result, intracellular deposits (Lewy bodies) are formed, leading to degeneration of dopaminergic neurons in the substantia nigra of the midbrain (Healy and Schapira, 2012).

In almost 80 % of PD patients, gastrointestinal abnormalities, including impaired gastric motility, small intesti-

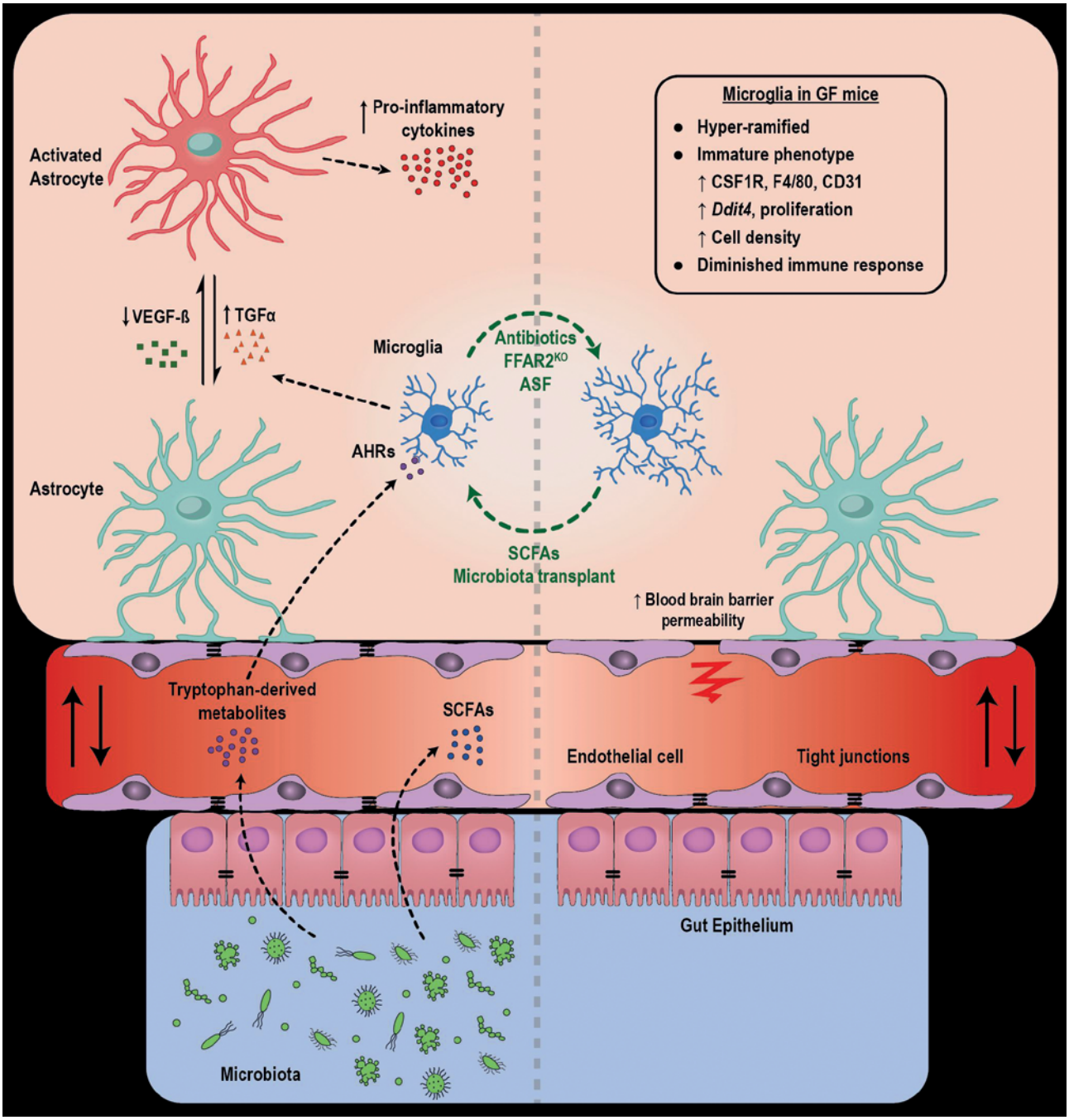

Fig. 1: Microbiota-derived signals regulate microglial maturation and function. Microglia require constant input from a complex microbiota to maintain their homeostatic phenotype. Microglia from germ-free (GF) mice show a rather immature phenotype, with increased cell density and hyper-ramification; they upregulate several immaturation markers and elicit a dampened response to immune stimulation. Treating those mice with short chain fatty acids (SCFAs) or recolonizing the gut with a complex gut microbiota transplant can restore them to a mature phenotype. Interestingly, free fatty acid receptor 2 knockout (FFAR2^{ko}) mice, antibiotics-treated mice or mice harboring microbiota with reduced complexity (altered Schaedler flora, ASF) showed, at least partially, a GF-like immature microglia phenotype. In addition, microbiota secretes tryptophan-derived metabolites, which in turn act on aryl-hydrocarbon receptors (AHRs) on the microglia to modulate astrocytic activation in the context of neuroinflammation.

nal bacterial overgrowth and *Heliobacter pylori* infections are present (Pfeiffer, 2012; Scheperjans et al., 2015). Braaks and colleagues proposed that α-syn aggregates reach the brain by propagating from the enteric system via the vagus nerve (VN), thereby spreading in a prion-like manner in cases of sporadic PD (Braak et al., 2004). This hypothesis is supported by presence of α-syn aggregates in the gut and enteric neurons, before they are detectable in the CNS (Bencsik et al., 2014; Braak et al., 2004). In PD patients, abnormal intestinal permeability was observed, which may promote the propagation of α-syn to the CNS (Forsythe et al., 2014). Furthermore, full truncal vagotomy, a surgical procedure that functionally denervates and disconnects multiple organs, including the stomach, liver, gallbladder, pancreas, small intestine, and proximal colon, resulted in decreased risk of PD progression, suggesting the involvement of the VN as a conduit for the spreading of α-syn from the ENS to CNS (Svensson et al., 2015).

In terms of microbial composition, analysis of fecal samples from PD patients showed altered gut microbial composition, related to the clinical phenotype of the disease (Keshavarzian et al., 2015; Scheperjans et al., 2015). A subset of patients showed elevated abundance of *Enterobacteriaceae*, which was associated with severity of motor symptoms. Whereas SPF-raised PD mice showed high level of α-syn aggregation in the brain, together with motor dysfunctions, GF PD mice clearly exhibited less aggregations and motor deficits. By applying SCFA, those effects were restored (Sampson et al., 2016). In this study, mice which had received fecal microbes from PD patients, displayed an altered SCFA profile, with a lower concentration of acetate and higher relative abundances of propionate and butyrate. However, in PD patients, Unger and colleagues observed a general decrease in fecal SCFA concentrations, suggesting that all SCFAs play a role in health maintenance (Unger et al., 2016). The aggregation of α-syn in PD is responsible for microglia activation (Kim et al., 2013; Sanchez-Guajardo et al., 2013), as indicated by elevated expression of pro-inflammatory cytokines *Tnfα* and *Il6* in microglia of mice kept under SPF conditions. Microglial activation in PD mice was clearly attenuated in GF animals, which underscores previous findings that GF microglia have diminished immune response in absence of gut microbiota (Erny et al., 2015). It is presently reasonable to assume that gut microbiota promotes α-syn-dependent immune activation of microglia, but in how far the gut microbiota elicits or even drive PD pathology is yet to be determined.

Conclusion

Although the field of gut-brain research is still in its early phase, the microbiota has rapidly been emphasized as a key contributor to CNS homeostasis. Signals from the gut take part in fine tuning microglia during development and in adulthood. Dysbiosis is associated with several CNS disorders, where microglia display impaired function. Yet further studies are still needed to understand the exact mechanisms by which gut microbiota and microbial metabolites, such as SCFAs, contribute to microglia maturation and function. This would pave the way for developing new non-invasive therapeutic approaches that can potentially modulate the illness by promoting microglial phenotype in CNS diseases.

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Glossary

Aβ:	Beta amyloid;
AD:	Alzheimer's disease;
AHR:	Aryl-hydrocarbon receptor;
α-syn:	α-synuclein;
ASF:	Altered Schaedler flora;
CNS:	Central nervous system;
ENS:	Enteric nervous system;
FFAR2:	Free fatty acid receptor 2;
GF:	Germ-free;
Il6:	Interleukin 6;
Irf8:	IFN regulatory factor 8;
LPS:	Lipopolysaccharides;
MAMPS:	Microbial-associated molecular patterns;
PD:	Parkinson's disease;
SCFA:	Short-chain-fatty acids;
TNFα:	Tumor necrosis factor α;
VN:	Vagus nerve

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Bionotes

M.Sc. Charlotte Mezö
Institute of Neuropathology, University Hospital of Freiburg, Breisacher Str. 64, 79106 Freiburg, Germany
e-mail: charlotte.mezoe@uniklinik-freiburg.de

Charlotte Mezö studied biology at the Eberhard-Karls University of Tübingen, Germany. After she received her master's degree with a specialization in neurobiology in 2017, she moved to Freiburg, Germany and started her Ph.D. at the Faculty of Biology at the Albert-Ludwigs University. Since then she works as a Ph.D. student at the Institute of Neuropathology in the lab of Marco Prinz. Her work focuses on microbiota-microglia interactions in disease.
The funding is provided by CRC/TRR 167 (https://www.sfb-trr167.uni-freiburg.de/projects).

M.Sc. Omar Mossad
Institute of Neuropathology, University Hospital of Freiburg, Breisacher Str. 64, 79106 Freiburg, Germany
e-mail: omar.mossad@uniklinik-freiburg.de

Omar Mossad was born and raised in Egypt, where he attained a bachelor's degree in Pharmacy and Biotechnology. He then moved to Bonn, Germany to pursue his Masters in Neurosciences. There, he developed a deep interest for microglia, which led him to Freiburg for graduate studies. He started his Ph.D. in 2016 in the Faculty of Biology at the Albert-Ludwigs University of Freiburg, Germany. He works in the Blank lab at the Institute of Neuropathology, where he focuses on microbiota-microglia interactions in homeostasis.
His project is funded by CRC/TRR 167 (https://www.sfb-trr167.uni-freiburg.de/projects).

Dr. med. Daniel Erny
Institute of Neuropathology, University Hospital of Freiburg, Breisacher Str. 64, 79106 Freiburg, Germany
e-mail: daniel.erny@uniklinik-freiburg.de

Daniel Erny studied medicine at the Albert-Ludwig University of Freiburg, Germany where he received his M.D. in 2013 at the Institute of Neuropathology in the lab of Marco Prinz. Since 2013 he works as a resident at the Institute of Neuropathology. In 2016 he became a member of the local Clinician Scientist Program (Berta-Ottenstein-Programme), and since 2017 he works as a principle investigator and is funded by the DFG in a joint project with Dr. Thomas Blank in the CRC/TRR 167 (https://www.sfb-trr167.uni-freiburg.de/projects). His research focuses on how the gut microbiota controls microglia maturation and function in the CNS.

Dr. rer. nat. Thomas Blank
Institute of Neuropathology, University Hospital of Freiburg, Breisacher Str. 64, 79106 Freiburg, Germany
e-mail: thomas.blank@uniklinik-freiburg.de

Thomas Blank studied biology at the University of Osnabrück, Germany (1987–1992), and received his Ph.D. in 1997 from the MPI of Experimental Medicine, Göttingen, Germany, with a specialization in cellular and molecular neurosciences. He studied the neurophysiology of drug addiction at the Scripps Research Institute, San Diego, California, as a post-doctoral researcher. He then investigated the impact of stress on learning and memory as a group leader back at the MPI of Experimental Medicine, Göttingen, Germany and as an Associate Research Professor at the University of Hawaii Medical School, Honolulu, Hawaii. In 2009 he joined the Institute of Neuropathology at the University Hospital in Freiburg, Germany, where he studies as a group leader focusing on the interaction between the immune system and CNS function.

Review Article

Hermann Wagner*

Personal View: The barn owl — a specialist for studying sensory systems

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Abstract: In this personal view article, the impact of an auditory specialist, the barn owl, to our understanding of sensory processing, especially auditory processing, is discussed from the perspective of a long-lasting career.

In times when research on model systems such as the mouse or the fruit fly, both generalists for most of the behaviors examined, celebrates big successes, one may ask what the work on animals occupying specialized niches, "specialists", can contribute to advance our knowledge about sensory systems. A specialist in this context is an animal that occupies a certain ecological niche and shows corresponding adaptations in anatomy and physiology. This article presents a personal view on the impact of the work on such a specialist. In my article I shall focus on audition in the barn owl, a specialist for hunting by listening.

I started my scientific career in 1979, working with houseflies, and have worked with barn owls since my time as a postdoc at the California Institute of Technology ("Caltech", Pasadena, CA, USA) in 1985. My interest in specialists derived from my work as an ornithologist when I realized that adaptations like the long and curved bill of the curlew help animals to occupy certain ecological niches. I wanted to understand in a formal sense, and in comparison to engineering, how evolution shapes such specializations.

Keywords: Sound localization, barn owl, interaural time difference

***Corresponding author: Prof. Dr. Hermann Wagner**, RWTH Aachen University – Zoology, Worringerweg 3, Aachen 52056, Germany, e-mail: wagner@bio2.rwth-aachen.de

General thoughts about sensory adaptations

Sensory function includes the conversion of an external stimulus to a perception and eventually an action. The process typically includes a series of computational steps – often in parallel pathways and with feedback loops. In my view, the study of sensory function requires an integration of the fields of physiology, ethology, neurobiology, computational neuroscience (in 1979 still called biological cybernetics), and bionics.

Different senses are useful in different ecological niches. Vision is good for diurnal, fast moving animals such as flies. Audition is good where vision fails, like in darkness. The barn owl (henceforth the "owl") is a predator that is specialized for hunting in the dark by listening. Therefore, one would expect its auditory system to be well developed in terms of anatomy, physiology, and computational efficiency. At the same time, owls should show the general structure of the avian auditory system. Both are indeed the case. While the owl's auditory pathway resembles those of other birds, many nuclei of the auditory pathway are enlarged compared to generalists such as the chicken. The nuclei also show a clearer geometrical structure that often is reminiscent of the ice-cube model developed for the organization of the visual cortex. This holds for the Nucleus laminaris, in which the first binaural interaction in the processing of temporal information takes place, as well as the main auditory midbrain nucleus, the Inferior colliculus (IC). Such clear and well-developed anatomical structures help in physiological recordings (see below). A further advantage is that in the owl the tuning in cells of the IC is so stable that one of my colleagues said while watching my experiment 'oh, this looks just like in the publications, which is not so in other animals'. Likewise, in another situation, when a neuron did not respond as I expected, I claimed that the person who had programmed the stimulus would have made a mistake – and this was true. Last but not least, behavioral experiments can be easily performed with the owl. All these advantages make the owl an excellent model system for auditory research.

When I started to work on owls, auditory physiology was driven by an engineering approach, trying to understand the computational processes underlying audition in a bottom-up way. Neuro-ethologists, on the other hand, were using a top-down approach. They claimed that what needs to be explained in terms of behavior influences the design of sensory systems. The advisor of my doctoral thesis at the Max-Planck Institute for Biological Cybernetics in Tübingen, Werner Reichardt, had taught me the engineering approach. For me as a biologist, it seemed a logical step for the postdoctoral phase to join a lab where I could combine the engineering and neuro-ethological approaches. The perfect place for this was the laboratory of Mark Konishi at Caltech. Indeed, at Caltech I found the necessary scientific breadth as represented by Mark Konishi, David van Essen, Carver Mead, Dick Lyon and later Christof Koch, whom I had already known from my time in Tübingen. Together with my fellow postdocs Catherine Carr, Terry Takahashi, Susan Volman, Ichiro Fujita and Alison Doupe, and doctoral students Rich Mooney, Ralph Adolphs and Jamie Mazer, we were a group of young researchers who were eager to understand more of how behavior shapes nervous systems. Also, while I had done purely behavioral work for my doctoral thesis, I wanted to learn new methods, especially neurophysiology. The standard approach for examining neural function at that time was extracellular single-cell recording. Optical-recording techniques were just being developed as was intracellular recording from freely moving and behaving animals. Research in vertebrates was dominated by rats, cats and monkeys, with major contributions also from work in frogs, pigeons and chickens. Mice were only used by a few researchers. In mainstream auditory research, the two main model systems were the cat and the owl. These two systems represented also the two different approaches as outlined above. The research on cats was mainly based on the engineering approach, while the research on owls was driven by the neuro-ethological approach.

My first steps to study sound localization

Sound-localization research tries to understand how auditory space is represented in the brain and how the brain generates behavior for stimulus localization. Auditory space needs to be derived from the signals reaching the ears. The auditory signal may be characterized by the variation of amplitude with time. Time (T) may also be expressed by phase (ϕ) and frequency (F), simplified: $T=\phi/F$. Each ear receives a signal and processes it in terms of amplitude, frequency and phase. Since we listen with two ears, the comparison of the signals reaching the two eardrums also carries important spatial information. For example, cues like the interaural time difference (ITD) and the interaural level difference play major roles in coding for sound azimuth in humans. Theoreticians had suggested that in neural processing the external ITD should be compensated for by an internal delay so that the signals from both sides reaches the detector neuron coincidently and that the binaural interaction at this neuron is non-linear, reflecting a multiplication. The result of the binaural interaction may be quantified experimentally by a so-called ITD vs response curve, in brief ITD-curve. While recording at one location and varying the stimulus ITD, the time averaged response recorded during stimulation is plotted as a function of the ITD in the ITD curve (Figure 1A, D). Some colleagues claim that the frequency-specific equivalent of the ITD, the interaural PHASE difference (IPD), and not the interaural TIME difference, is important in sound localization. I always remained skeptical about the importance of IPD, because in my view sound localization is a broadband phenomenon, and phase does not work well over a large frequency range. This holds specifically for the barn owl, because narrowband stimuli lead to the appearance of auditory illusions, so-called phantom sources, and the owls are indeed attracted not only to the real but also to phantom sources, which in real life may reduce success in prey capture. In other words, IPD would not be an optimal solution from an evolutionary point of view. Nevertheless, in the owl, like in other animals, virtually every nucleus up to and including the initial stations of the IC is characterized by neurons narrowly tuned to frequency. The part of the IC that is characterized by narrow frequency tuning was called the frequency-specific region (Figure 1C). The ITD curves recorded from neurons in this region are characterized by periodic responses with several equivalent response peaks (Figure 1A). The frequency-specific region is separated from another part of the IC, the space-specific region in the external nucleus of the IC (ICx). ICx neurons are called space-specific neurons, because they are tuned to one location in space. Many of these neurons are arranged in a map of auditory space (Figure 1E). The map mainly represents contralateral space. The neurons in the space map are broadly tuned to frequency, and their ITD curves exhibit one main response peak and several smaller side peaks (Figure 1D).

What interested me when I started my postdoc was how the transition from the frequency-specific region to the space-specific region takes place in terms of computation and anatomy. To answer this question, Terry Taka-

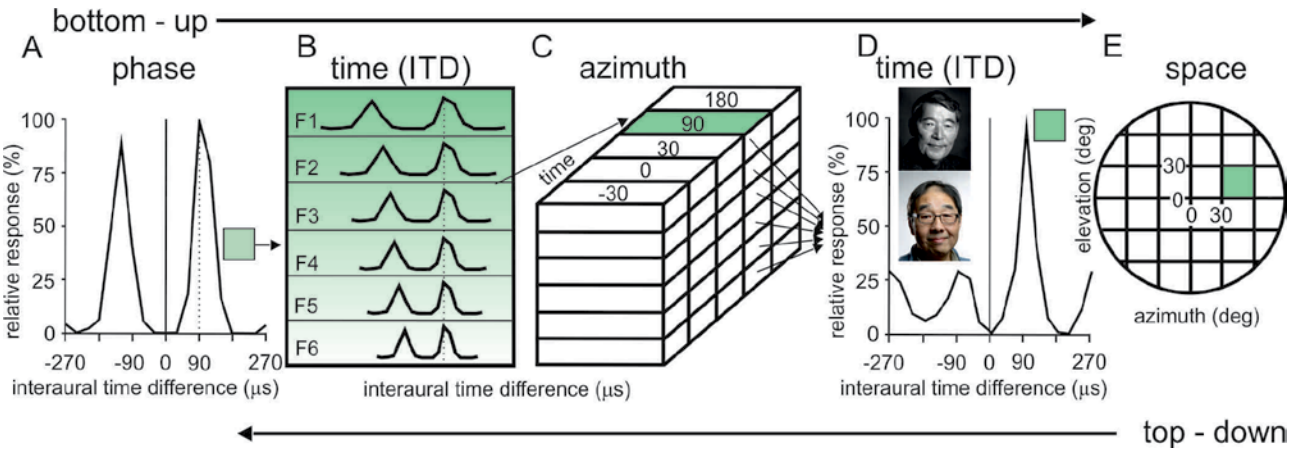

Figure 1: Processing of interaural time difference (ITD) in the inferior colliculus (IC). A) ITD curve of a neuron narrowly tuned to frequency in the central nucleus of the IC (ICc). B) Functional column in ICc. While frequency changes in dorsoventral direction, one of the response peaks of the ITD curve is shared by all neurons as indicated by the dotted line. C) Ice-cube model of ICc (for explanation see text). D) ITD curve of a neuron broadly tuned to frequency in the external nucleus of the IC (ICx). E) Space map in ICx. Photos: Mark Konishi (top), Terry Takahashi (bottom).

hashi and I developed a testable theory, across-frequency integration in a delay-specific way. The results of this work led to my first paper in auditory physiology (Wagner et al., J Neurosci 7: 3105, 1987). We showed that many neurons of the frequency-specific region, precisely the lateral shell of the central nucleus of the IC, are arranged in a functional column (Figure 1B). Each of these neurons is narrowly tuned to a given frequency F_x, and each exhibits periodic ITD tuning with several equivalent response peaks. One of the peaks of the ITD curve is shared by all neurons in a column. This is the functionally relevant peak and represents the ITD that is represented by the column (Figure 1B). The nucleus contains many such columns, each of which represents a specific ITD (Figure 1C). The neurons belonging to one column project to one neuron in ICx (Figure 1C, D). This projection makes the ICx neuron broadly tuned to frequency, and it allows the appearance of the main peak in the ITD curve (Figure 1D). Terry Takahashi and I continued the work on the functional organization of the central nucleus of the IC. In a second paper, we demonstrated a contralateral projection within this nucleus that is organized to conserve spatial information in terms of time and frequency (Takahashi et al., J Comp Neurol 281: 545, 1989).

Both papers demonstrated the advantages of working with the owl as outlined above. The apparent crystalline-like functional organization prompted me to develop an ice-cube-like scheme with the intention to capture this high ordering (Figure 1C). In this scheme, each small rectangle represents a neuron. The neurons in each row are tuned to the same frequency, whereas the neurons in each column represent one ITD. I doubt that we would have succeeded in detecting the functional columns without this clear geometrical structure in the nucleus.

Continuing with sound-localization research in my own lab

After two and a half years at Caltech, I was prepared to move on. Kuno Kirschfeld at the MPI for Biological Cybernetics (Tübingen) gave me the chance to develop my own research project. With this step, I depended fully on my own ideas, and I had to set up a new lab. Indeed, I saw many fellow postdocs failing at this step. Setting up a physiology lab from scratch is especially tedious. So the cleverest people started to work in parallel on "easier" fields, like anatomy, while the physiology lab was not yet running, and bridged the time of low production in this way.

My first project back in Germany tackled the question of whether the map of auditory space was behaviorally relevant. The ICx is part of the tectofugal auditory pathway, and not of the forebrain pathway which is considered the main auditory pathway in mammals. I did experiments to test whether lesions in the space map would cause behavioral deficits. The idea was that a restricted lesion in the ICx would only destroy a restricted representation of auditory space (Figure 2A). I reasoned that a stimulus coming from a position that is represented in the lesioned region of the map would still be heard by the owl. However, the bird would no longer know where the stimulus came from,

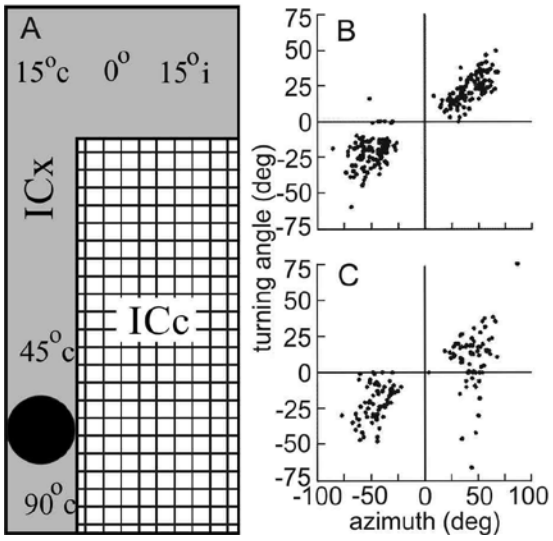

Figure 2: Design and result of lesion experiment. A) Scheme of the design. View of a horizontal section of the inferior colliculus (IC). The region marked by the crossing lines is the central nucleus of the IC (ICc), the grey region the external nucleus of the IC (ICx). The map of space is indicated by the numbers that reflect degrees in a spherical coordinate system with "i" corresponding to ipsilateral space and "c" corresponding to contralateral space. The lesion (dark spot) erased a small region of the map. B) The behavior of the owl before the lesion. The angle of the azimuthal stimulus is plotted on the x axis and the direction of the head after the turn, the turning angle, is plotted on the y axis. C) The behavior of the owl after a lesion in the map that represented 60 degrees in right auditory space. Note the behavioral deficit that is restricted to a small region around 60 degrees in azimuth.

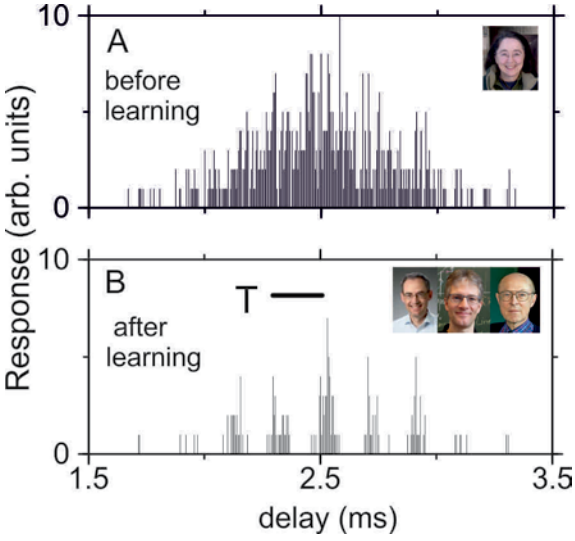

Figure 3: Temporal precision and temporal sharpening as a result of Hebbian learning. A) A sketch of the initial temporal precision in Nucleus laminaris as inspired by data of Catherine Carr. B) After Hebbian learning, the axons from the input neurons that carry the "wrong" temporal information were eliminated. The detector neuron reaches the necessary temporal precision by responding only in restricted phases of the stimulus. The horizontal line corresponds to one period of the stimulus, which was supposed to be a tone of 5 kHz in this case. Photos: Catherine Carr (in A), in B) from left to right Wulfram Gerstner, Richard Kempter, Leo van Hemmen.

and, thus, the bird might either not react at all or turn in a random way. While the owl turned towards sounds from different directions before the lesion (Figure 2B), after the lesion random turning occurred for stimuli emanating from those regions of space that were represented by the lesioned region (Figure 2C). For such stimuli, the owl also reacted with a longer response delay. While the random turning disappeared after a short time, the deficit in response delay remained. This left open the possibility that the disappearance of the deficit was due to a reorganization of the auditory pathway. After the lesion, the forebrain pathway might have overtaken the computation of turning amplitudes, and the longer pathway to the forebrain might have been the reason why the response latency remained increased. I always wanted to test this reorganization hypothesis experimentally, but never managed to do so. One reason was that when we started to investigate the computations in the forebrain, we got distracted by the computational properties of these neurons.

My interest in understanding the computations underlying the representation of ITD was not restricted to the computations in the IC, but extended from the step of detection to behavior. My approach was driven by the conviction that in the end an animal must be able to find a location in space, independent of what cues it uses. This insight was based on theoretical studies that I conducted with David Fleet (then Kingston, now Toronto) on a similar problem in stereo vision. We found that in hierarchical processing, the first interaction is not really crucial, but may be adjusted in later steps by integration (Fleet et al., Vision Res 36: 1839, 1996). Thus, it is consistent with theory that in the owl, the first interaction in the computation of a correlate for azimuth is based on time delays, while in mammals it may also be based on phase delays. The first to demonstrate the time-based computation in the owl was Catherine Carr. Wulf Gerstner, Richard Kempter, Leo van Hemmen and I used some of her data to develop a model that showed how the necessary temporal precision may be reached with Hebbian learning (Figure 3). This happened during my time at the TU Munich, where Geoff Manley had given me the opportunity to continue my research during my Heisenberg fellowship, after my position in Tübingen

had ended. I was very happy when Catherine Carr invited me to her laboratory to experimentally tackle the question of how the auditory system in the barn owl detects ITD. This research, also including Richard Kempter and Christine Köppl and many graduate students, started in Munich, but continued after my move to Aachen in 1995, where I took the Chair of Zoology and Animal Physiology at RWTH Aachen University. The collaborations were and are very fruitful. They generated many publications (MEDLINE search "Wagner H, Kempter R" finds most of them) that led to a deeper understanding of the processes not only involved in the computation of ITD, but also more general issues like the properties of field potentials.

Up to now I have focused on the stationary cues that are important in sound localization. Yet, the world is not static, it is dynamic. How does the auditory system represent motion? Coming from vision and cybernetics, I speculated that the acoustic motion detector might follow rules similar to those proposed for visual motion detection. Motion in the sense used here is a vector where a stimulus changes position and should not be confused with flicker that is characterized by changes in illumination at a given position. To extract the direction of motion, a signal has to be detected at two different positions, the information from these two positions has to be processed asymmetrically and combined in a non-linear way. The detectors work such that responses in the preferred direction are favored, while responses in the opposite direction, the null direction, are absent or suppressed. My thesis advisor, Werner Reichardt, had proposed one such scheme in which the non-linear interaction was multiplicative, boosting the response in the preferred direction, while Horace Barlow and William Levick had proposed another scheme which included inhibition in the null direction. Terry Takahashi and I found cells in the IC that changed their response when motion direction changed. The computation in these cells was very similar to the Barlow-Levick type of motion detector in the visual system. We tried out several approaches to substantiate our findings on the behavioral level, but were not really successful. It was at this time (1993) when Baruch Minke entered my office and told me that I should collaborate with a young Israeli researcher, Eli Nelken, who was about to start an acoustic laboratory at Hebrew University, Jerusalem. I was excited about this possibility. We received funding from the German-Israeli Foundation. All this happened while I was at a decisive point of my career and was moving a lot. Baruch Minke set the impulse while I was still in Tübingen, we submitted the proposal after I had moved to Munich in 1994, and we received the money after I had settled in Aachen a year later. In Aachen, I also got help from my colleague Michael Vorländer who had just taken the chair of the Institute of Technical Acoustics. Michael and his people constructed interfaces for (virtual) stimulus presentation for us that we used for most of the remaining time in Aachen. With the new opportunities, we tried many things to unravel acoustic-motion behavior, but none of them worked convincingly. So in the end, we gave up on this direction. I should mention that it then took more than 10 more years until Georg Klump's group in Oldenburg was successful to demonstrate acoustic-motion direction in the barn owl behaviorally. The collaboration with Eli Nelken triggered a continuing connection and friendship with Israeli researchers that last to this day.

Putting my research into a broader perspective

So far, I mentioned a few corner stones that characterized mainly my early career. There would be many more things to say, but space does not allow. Thus, I only mention in passing further efforts in studying audition. Together with Eli Nelken I examined correlates of binaural (un) masking in the barn owl. We compared the computations in the auditory pathway with those in the visual pathway and processing in the forebrain and tectofugal pathways. We studied the influence of adaptation and attention on sound localization, and also how the owl processes ambiguous stimuli. Last but not least, Onur Güntürkün helped us in understanding the anatomy. These studies were flanked by studies of the visual system of the barn owl extending from properties of the eyes via stereo vision to cognitive function. I was also interested in the silent flight of barn owls and collaborated with engineers in this attempt. Lately we examined the high flexibility of the neck and the evolution of ear asymmetry. It may become clear from this list that in the end, I was really interested in how the owl was adapted to the ecological niche it occupies in a broad sense.

I already stressed that in my view, understanding a problem in a broad sense requires the combination of behavior, electrophysiology, theory and application. While I felt competent for the first two goals, I was looking for help with the latter two. Luckily I found collaborators who shared my interest. I was able to work with theoreticians such as Sonja Grün, Ad Aertsen, David Fleet, Eli Nelken, Leo van Hemmen, Wulf Gerstner, Richard Kempter, Christian Leibold, Ehud Rivlin, Ohad Ben-Shahar, Alon Wolf and Hartmut Führ, with engineers such as Wolfgang Schröder, and with Gerhard Lakemeyer, a computer sci-

entist working on autonomic agents. Many of my publications are joint publications with such colleagues. I profited a lot from these collaborations. What was important in all these collaborations was that the people coming from the different fields first had to get a common ground for discussion. This meant that everyone had to read both experimental and theoretical literature and that terms had to be clarified. For example, when I first talked to David Fleet, we had to sort out what we mean by the ITD curve. While for me as experimentalist it was clear that I recorded my data at one position in the brain and varied the stimulus, David's first comment was that a theoretician would never do it this way. A theoretician would consider a stimulus at a given position and would look at the response at different locations in the space map. Importantly for our work, after we sorted out these problems, the collaborations were always very fulfilling.

With the advent of the new media, the way in which information is collected has changed, but what did not change is that scientists need to get a deep understanding of the available literature, not only in their often very narrow own field, but also beyond. This is what we tried to offer our students in Aachen, although it was clear that students of computer science might find it hard to understand neuroscience talks and neuroscience students might struggle to follow a talk on computational principles.

Possible future directions

While the field of sound localization has been fulfilling for me so far, I also see a bright future. For example, one may go beyond the two approaches mentioned above by adding aspects of information in a formal way. In other words, it would be helpful to formally derive what information is available to an animal and study how the animal uses this information. In other matters, Matthias Dietz (Oldenburg) has started an effort to integrate the different theoretical and experimental approaches available in the field into a joint platform which would allow a comparison of the power of the different approaches as is already done, for example, in artificial speech-recognition systems. These efforts have already started to boost the field. I hope to be able to contribute to these new developments, although we cannot continue with owl research in Aachen, because our owl colony no longer exists.

When I started my career, hypothesis driven research was the gold standard, and non-hypothesis driven approaches or big data approaches like "omics" approaches were rare or impossible, due to insufficient computational power. Optogenetic tools and the possibility of manipulating genomes and generating mutants in which behavior can be influenced in a controlled way were not yet available. These new tools, together with the realization that modern biology needs more mathematical support as is provided by computational neuroscience, have allowed clarification of many processes that could not be well formulated earlier. With these new tools, many new doors have opened to dissect and understand the processes that underlie sensation and behavior in general. Where will the field move in the future? Coming from the specialist side and seeing the advantage of working with a specialist animal, my hope is that with genetic tools becoming cheap and available for all, work on specialized animals will again thrive. This would allow us to examine well working solutions that often test out the theoretically possible and could offer better insight in the optimal design of systems than generalist models do. By analogy, if an engineer wants to understand the limits of a car engine, she/he should study a Formula 1 racing car and not a passenger car that you can buy from a dealer. Work on champions can help to improve our understanding not only from a basic science point of view, but also from a view of application as in the rising field of bionics. However, it was not always easy to convince granting agencies and colleagues of this view. So it may also happen that research on specialists will disappear entirely. I was lucky during my career and hope my successors will be so too. Essential for this will be that basic research will continue to be funded in Germany. My hope would also be that more researchers will take a broad view and not resort to more and more narrow questions according to the German saying that "someone knows everything of nothing".

I was always driven to understand how evolution shapes survival in a comprehensive way. I chose the barn owl as study object, because I find its appearance, its ecological niche, its sensory capabilities, and its aptitude as a role model for biomimetic applications fascinating. While I have only addressed acoustics in this article, and especially sound localization, I also studied vision, the silent flight and questions of natural protection. The latter interest brings me back to the beginning, when I went out to the field as a young ornithologist to observe curlews and lapwings. I hope that I can continue to do this for many years to come.

List of word abbreviations

T	time
F	frequency
ϕ	phase
IC	inferior colliculus
ITD	interaural time difference
IPD	interaural phase difference
ICc	central nucleus of the IC
ICx	external nucleus of the IC

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Bionotes

Prof. Dr. Hermann Wagner
RWTH Aachen University – Zoology,
Worringerweg 3, Aachen 52056, Germany
e-mail: wagner@bio2.rwth-aachen.de

Prof. Dr. Hermann Wagner studied biology in Ulm, Erlangen and Tübingen, Germany. After his doctoral thesis at the Max-Planck-Institute of Biological Cybernetics, he went to the California Institute of Technology for a postdoc. He returned to the Max-Planck Institute as research fellow ("Assistant"), obtained his Habilitation, worked at the TU Munich as Heisenberg fellow, and took the chair of Zoology/Animal Physiology at the RWTH Aachen. He officially retired at the end of August 2018. He is member of the Leopoldina and the Academy of Science and Literature in Mainz.

presentation of scientific institutions

Sabine Dannenberg and Denise Manahan-Vaughan*

NEURONEXXT – Network for Women in Neuroscience

Career platform for international visibility and networking

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The platform "NEURONE^{XX}T – Network for Women in Neuroscience" was recently established for female neuroscientists at all levels of academia from the postdoctoral through senior professorship career stages. The primary aim is to significantly raise the international visibility of female neuroscientists, thereby facilitating the identification of candidates for career appointments, as well as the identification of future collaboration partners, conference speakers, or mentors. The platform was created by Prof. Dr Denise Manahan-Vaughan and Dr Sabine Dannenberg as an initiative of the Collaborative Research Centre 874 "Integration and Representation of Sensory Processes" and is funded by the Ruhr University Bochum.

The motivation in creating NEURONE^{XX}T was derived from the fact that despite more than a decade of academia striving to improve equality measures, very little has changed in the upper ranks of academia: the percentage of women in leading and senior positions is still strikingly low and stands in stark contrast to the relatively high numbers of female neuroscientists at the doctoral and postdoctoral career levels. There is a similar problem evident in research consortia. One causative reason for this is the low visibility of a great majority of female neuroscientists. This confounds expectations that the most excellent researchers climb the career ladder – irrespective of gender.

It is therefore the goal of NEURONE^{XX}T to provide women from all neuroscientific disciplines, employed in academic and non-academic fields worldwide, with a platform that draws attention to their work and shares their research findings. NEURONE^{XX}T aims to help female neuroscientists to create new networking possibilities, thus supporting their career development and fostering new opportunities for collaborations with other researchers. NEURONE^{XX}T is aimed at women at all stages of their postdoctoral careers, right through to senior professorship and leadership levels: researchers can showcase their qualifications, competencies and research interests by creating a profile in the platform.

NEURONE^{XX}T is aimed at two target groups: Female neuroscientists who work in a neuroscientific field can create a profile in order to share their contact details and their research focus. Furthermore, any individual that is interested in identifying female neuroscientists in a specific research discipline, or for specific appointments, can register with NEURONE^{XX}T in order to search the database.

NEURONE^{XX}T will thus serve as a platform for active recruitment politics for professorships and leading positions in science and research, and for the identification of highly qualified scientists and experts for committees, advisory boards, commissions and grant reviews, as well as for the selection of guest speakers within the scope of symposia and conferences. Furthermore, the platform will enable the creation of academic and research networks with other female neuroscientists. The platform is open for registrations at: www.nexxt.rub.de

***Corresponding author: Prof. Dr. Denise Manahan-Vaughan**, Department of Neurophysiology, Medical Faculty, Ruhr-Universität Bochum, Universitätsstr. 150, 44780 Bochum, Germany, e-mail: denise.manahan-vaughan@rub.de
Dr. Sabine Dannenberg, Research Department of Neuroscience, Ruhr-Universität Bochum, Universitätsstr. 150, 44780 Bochum, Germany, e-mail: sabine.dannenberg@rub.de

Bionotes

Prof. Dr. Denise Manahan-Vaughan
Department of Neurophysiology, Medical Faculty, Ruhr-Universität Bochum, Universitätsstr. 150, 44780 Bochum, Germany
e-mail: denise.manahan-vaughan@rub.de

Denise Manahan-Vaughan is a Neurophysiologist, Neuroscientist and Head of the Department of Neurophysiology within the Medical Faculty of the Ruhr-Universität Bochum (www.rub.de/neurophys). She is also director and Dean of Studies of the International Graduate School of Neuroscience (www.rub.de/igsn), speaker (primary coordinator) of the DFG-funded Collaborative Research Centre on Integration and Representation of Sensory Information Processes (www.rub.de/sfb874) and speaker of the Research Department of Neuroscience (www.rd.rub.de/neuro/) at the same university. After completion of her primary degree in Natural Sciences (majoring in Physiology) at Trinity College Dublin (TCD), Ireland, she obtained a PhD in Neuropharmacology at TCD and then moved to Germany to the Leibniz Institute for Neurobiology in Magdeburg. Having obtained an Habilitation degree in Physiology from the Otto von Guericke University in Magdeburg, she moved to the Johannes Müller Institute for Physiology at the Charité, Berlin, before moving to Bochum in 2003. Her research focusses on understanding the relationship between hippocampal and cortical synaptic plasticity and long-term associative and spatial memory.

Dr. Sabine Dannenberg
Research Department of Neuroscience, Ruhr-Universität Bochum, Universitätsstr. 150, 44780 Bochum, Germany
e-mail: sabine.dannenberg@rub.de

Sabine Dannenberg studied Biology at the Ruhr-Universität Bochum. Her doctoral thesis at the Department of Zoology and Neurobiology focused on electrophysiological studies of the superior colliculus in primates. She was subsequently a research scientist in the Department of General Zoology and Neurobiology at the Ruhr-Universität Bochum led by Professor Klaus-Peter Hoffmann, before becoming Science Manager of the Research Department of Neuroscience in 2009 (www.rd.rub.de/neuro/). Since 2010 she is also the coordinator of the SFB 874 at the Ruhr-Universität Bochum (www.rub.de/sfb874).

Nachrichten

Die jNWG

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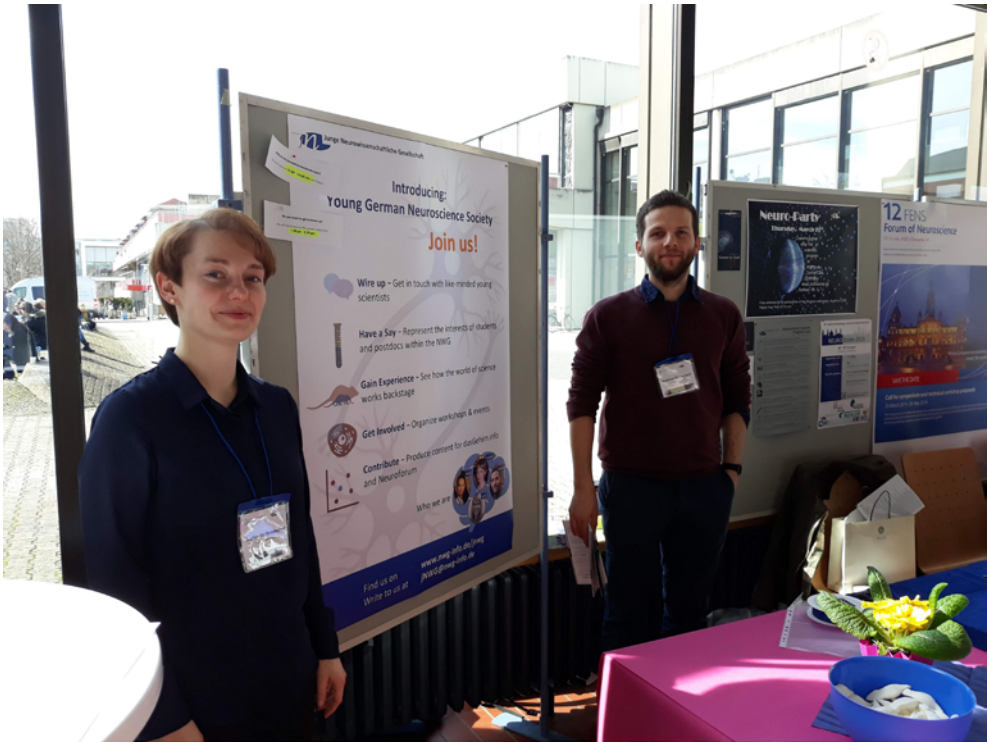
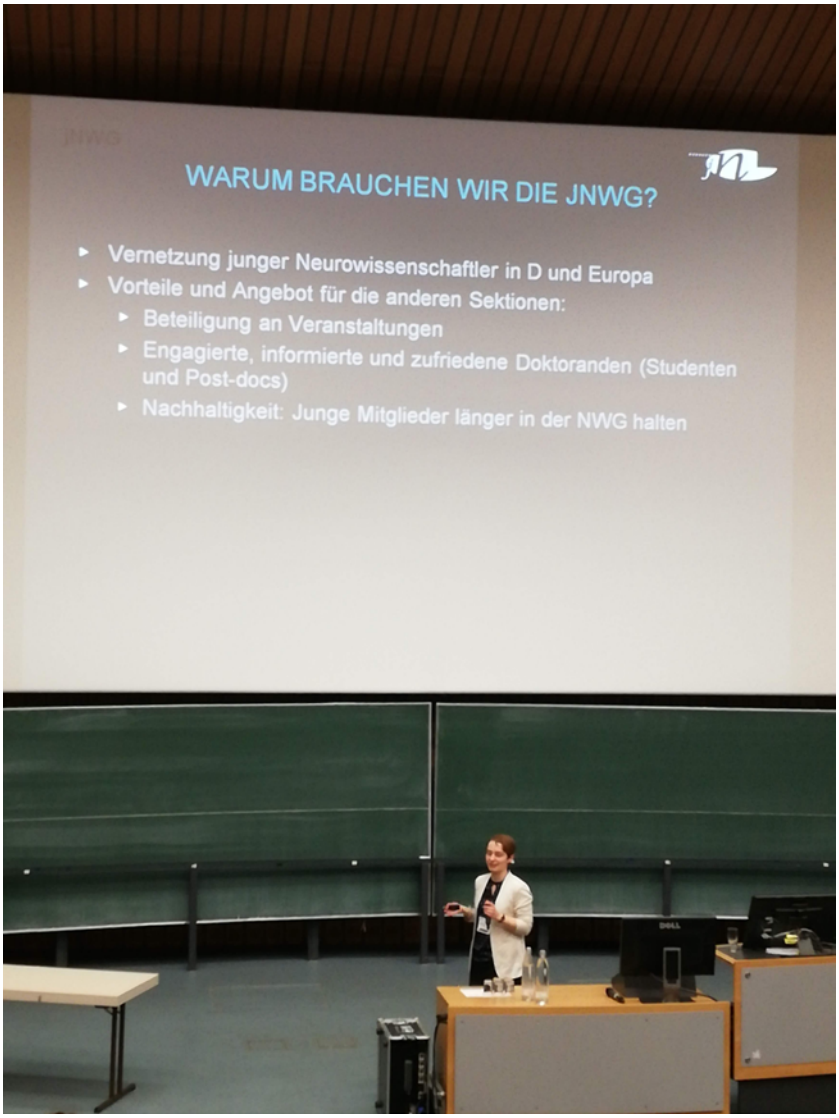
Junge Forscherinnen und Forscher langfristig für die Neurowissenschaften zu gewinnen ist die wichtigste Grundlage für innovative und zukunftsorientierte Forschung und erklärtes Ziel der NWG (§ 2 der Satzung). Auf der diesjährigen Mitgliederversammlung in Göttingen wurde deshalb die Gründung einer eigenen Sektion für junge NWG-Mitglieder beschlossen, die sich genau dies zum Ziel gesetzt hat. Die neue Sektion trägt den Namen „junge Neurowissenschaftliche Gesellschaft (jNWG)“ und bietet als nunmehr zehnte Sektion eine Plattform zur Kommunikation, Vernetzung sowie Karriereentwicklung aller jungen WissenschaftlerInnen in den Neurowissenschaften in Deutschland.

Bislang fehlte eine eigene Vertretung junger WissenschaftlerInnen in der NWG, die sich explizit ihrer Förderung und der gezielten Weiterbildung und -entwicklung widmet. Wir als jNWG werden diese Lücke füllen und wollen eine Repräsentation des neurowissenschaftlichen Nachwuchses verschiedenster thematischer Facetten innerhalb der NWG und darüber hinaus darstellen.

Im Rahmen der Göttinger Tagung 2019 haben wir uns mit jungen interessierten WissenschaftlerInnen getroffen um über unsere eigenen Ideen hinaus Wünsche und Bedürfnisse der Nachwuchs-Generation zu diskutieren. Dabei wurden unter anderem Wünsche nach einer Kommunikationsplattform zum wissenschaftlichen und persönlichen Austausch und fachlichen und soft-skill Workshops geäußert. Wir möchten als Stimme der jungen NeurowissenschaftlerInnen verstanden werden und haben die Erkenntnisse aus diesen Gesprächen in unser Programm aufgenommen.

Seit der Göttinger Tagung im März 2019 freuen wir uns als jNWG über sehr viel ermunternden Zuspruch sowie junge WissenschaftlerInnen, die die jNWG nicht nur unterstützen, sondern auch aktiv mitgestalten möchten. Daher möchten wir mit einem *kick-off meeting* im September 2019 (https://nwg-info.de/de/jnwg/veranstaltungen) die zukünftigen jNWG-Aktivitäten organisieren und die bisherigen Ideen weiterentwickeln. In Kleingruppen werden wir uns thematisch vertiefen und eine Agenda bis zur kommenden Göttinger Tagung erarbeiten. Dabei werden wir unter anderem Themen wie die jNWG-Medienpräsenz,





die Kommunikationsplattform für den forschenden Nachwuchs und die Vernetzung mit anderen Nachwuchsorganisationen angehen. Außerdem möchten wir die Organisation der ersten eigenen wissenschaftlichen jNWG-Tagung starten, die im kommenden Jahr stattfinden und die bisher nur für Doktoranden angebotene NeuroDoWo ablösen wird. Unsere ausführliche Zielsetzung finden Sie auch unter https://nwg-info.de/jnwg/aktivitaeten. Unterstützt werden wir beim kick-off außerdem von renommierten Gästen, die mit uns – ganz im Sinne der Nachwuchsförderung – ihre persönlichen Karriereerfahrungen und -tipps teilen werden.

Interessiert? Dann melden Sie sich für das *kick-off meeting* bis zum 30.07.2019 unter https://nwg-info.de/de/jnwg/veranstaltungen an! Weitere Informationen zur Jungen NWG finden Sie außerdem im jNWG-Bereich der NWG-Website.

P.S.: We do not want to be limited to German speaking neuroscientists. Therefore, we also encourage every young scientist working in, or feeling connected to, neuroscience in Germany to participate! Please find further information, in particular regarding our kick-off meeting on our website https://nwg-info.de/jnwg

Mit den besten Wünschen, Ihre jNWG.

Nachrichten

https://doi.org/10.1515/nf-2019-0017

Call for Symposia: 14. Jahrestagung der NWG in Göttingen, 24.–27. März 2021

Die nächste Göttinger Tagung der Neurowissenschaftlichen Gesellschaft wird vom 24.–27. März 2021 stattfinden. Vorschläge für Symposien können bis 17. Februar 2020 eingereicht werden.

Die Göttinger Tagung der Neurowissenschaftlichen Gesellschaft findet alle zwei Jahre in der Tradition der Göttinger Neurobiologentagung alternierend zum FENS Forum statt. Die Mitglieder der NWG und alle interessierten Neurowissenschaftler sind eingeladen, Vorschläge für Symposien aus allen Bereichen der Neurowissenschaften einzureichen.

Folgende Kriterien sind dabei zu beachten:

- die Organisatoren der Symposien in 2021 dürfen kein Symposium bei der Göttinger Tagung 2019 organisiert haben
- die eingeladenen Redner für die Symposien 2021 dürfen nicht schon in 2019 einen Vortrag gehalten haben (siehe Proceedings 2019 auf der Website der Göttinger Tagung 2019: nwg-goettingen.de/2019)
- der Titel sollte griffig sein und 100 Zeichen inkl. Leerzeichen nicht überschreiten
- eine kurze Beschreibung (max. 5.000 Zeichen inkl. Leerzeichen) des Symposiums in Englisch wird gefordert
- Mitglieder des Programmkomitees können zwar eingeladene Redner in einem Symposium sein, dürfen aber nicht als Haupt- bzw. alleinige Organisatoren auftreten.
- die Dauer eines Symposiums beträgt 2 Stunden
- die maximale Rednerzahl liegt bei 4 Rednern
- zudem sind zwei Kurzvorträge von Studenten (d. h. junge Wissenschaftler ohne abgeschlossene Promotion) einzuplanen. Diese sind bei der Einreichung noch nicht zu benennen, vielmehr bewerben sich die Studenten im Herbst 2020 bei der Abstract-Einreichung darum und werden dann vom Organisator ausgewählt.

Die NWG strebt eine Verbesserung des Anteils von Frauen als Organisatorinnen und Sprecherinnen an. Eine ausgewogene Beteiligung aller Geschlechter ist ein Kriterium bei der Begutachtung der Symposiumsvorschläge.

Die NWG kann keine finanziellen Mittel für die Organisation der Symposien garantieren. Sie wird zwar wie in der Vergangenheit einen Antrag auf Unterstützung der Jahrestagung an die DFG stellen, aber auch im Falle einer positiven Begutachtung durch die DFG werden die beantragten Mittel **nicht** für eine Kostendeckung ausreichen, da diese Mittel **nur** für ausländische Redner und nach den Vorgaben der DFG verwendet werden können.

Wir bitten daher dringend zu berücksichtigen, dass die Organisatoren – gegebenenfalls zu großen Teilen – für die Finanzierung ihres Symposiums selbst Sorge tragen müssen (Differenz aus Reisekosten, die nicht erstattbar waren; Registrierungsgebühren; nationale Redner).

Für die Einreichung von Symposien steht auf der Website der NWG ab Mitte Oktober ein Formular zur Verfügung.

Bei Fragen wenden Sie sich bitte an die Geschäftsstelle (Stefanie Korthals, korthals@mdc-berlin.de).

Protokoll der Mitgliederversammlung Donnerstag, 22. März 2019

Hörsaal 11
auf der 13. Göttinger Jahrestagung der Neurowissenschaftlichen Gesellschaft e.V.

Versammlungsleiter ist der Präsident der Neurowissenschaftlichen Gesellschaft, Prof. Dr. Eckhard Friauf

Protokollführer ist der Generalsekretär der Neurowissenschaftlichen Gesellschaft, Prof. Dr. Christian Steinhäuser.

Die Zahl der erschienenen Mitglieder beträgt 63.

Die Versammlung wurde satzungsgemäß einberufen, die Tagesordnung war den Mitgliedern bei der Einberufung mitgeteilt worden.

Beginn: 13:30 Uhr
Ende: 14:30 Uhr

Tagesordnung:

1. Begrüßung durch den Präsidenten
2. Bestätigung des Protokolls der letzten Mitgliederversammlung
3. Bericht des Schatzmeisters/Bericht der Kassenprüfer
4. Mitteilungen
5. Bericht zur Göttinger Jahrestagung
6. Wahl des neuen Vorstandes
7. Aktivitäten der Gesellschaft
8. Verschiedenes

1. Begrüßung durch den Präsidenten

E. Friauf begrüßt die Anwesenden und eröffnet die Sitzung.

2. Bestätigung des Protokolls der letzten Mitgliederversammlung

Das Protokoll der letzten Mitgliederversammlung vom 8. Juli 2018 ist in der Ausgabe 3/2018 von Neuroforum erschienen. Es wird mit 63 Ja-Stimmen, 0 Enthaltung und 0 Nein-Stimmen angenommen.

3. Bericht des Schatzmeisters / Bericht der Kassenprüfer

A. Büschges erläutert die Einnahmen und Ausgaben der NWG bis zum Jahr 2019 und kommentiert einige Posten. Wie immer zeigt die Jahresabrechnung der NWG die zweijährigen, durch die Göttinger Tagung bedingten Schwankungen, d. h. in den geraden Jahren fließen die Teilnehmergebühren und andere Einnahmen auf das NWG-Konto, und in den ungeraden Jahren wird das Geld für die Göttinger Tagung wieder ausgegeben. Eine nicht wiederkehrende Einnahme ist im Jahr 2018 die Zuwendung von FENS für das FENS Forum 2018 in Höhe von 56.000 Euro.

Die Einnahmen und Ausgaben der NWG im Jahr 2018 wurden am 12. Februar 2019 von den Kassenprüfern Jens Dreier und Constance Scharff geprüft. Die Kassenprüfer bestätigen eine korrekte Kontenführung und empfehlen der Mitgliederversammlung, den Schatzmeister zu entlasten.

Die Mitgliederversammlung entlastet den Schatzmeister auf der Grundlage des Berichts der Kassenprüfer mit 62 Ja-Stimmen, 1 Enthaltung und 0 Nein-Stimmen.

Ein Mitglied dankt dem Vorstand für seine Arbeit und stellt den Antrag, dass die Mitgliederversammlung den Vorstand entlastet. Die Mitgliederversammlung entlastet den Vorstand mit 63 Ja-Stimmen, 0 Enthaltung und 0 Nein-Stimmen.

E. Friauf schlägt der Mitgliederversammlung als Kassenprüfer für die Prüfung der Jahresabrechnung 2019 Jens Dreier und Helmut Kettenmann, beide Berlin, vor. Beide sind bereit, die Kassenprüfung 2019 zu übernehmen. Die Mitgliederversammlung stimmt dem Vorschlag mit 63 Ja-Stimmen, 0 Enthaltung und 0 Nein-Stimmen zu.

A. Büschges stellt die Finanzprognose für die kommenden Jahre vor. Im Moment stagniert die Rücklagenbildung der Gesellschaft, mittelfristig wird das Vermögen gegen Null gehen und wird spätestens 2022 aufgebraucht sein. Gründe dafür sind u. a. die Stagnation der Mitgliederzahlen und der Rückgang der Teilnehmer bei der Göttinger Tagung. Deshalb schlägt A.Büschges (a) eine Erhöhung der Mitgliedsbeiträge (die letzte Erhöhung war 2012) und (b) eine Erhöhung der Tagungsgebühren für die Göttinger Tagung vor. Es wird diskutiert, ob die Gebühren nur für die Seniors oder für alle angehoben werden sollen. Für die Mitgliedsbeiträge soll eine neue Kategorie „Postdocs“ eingeführt werden.

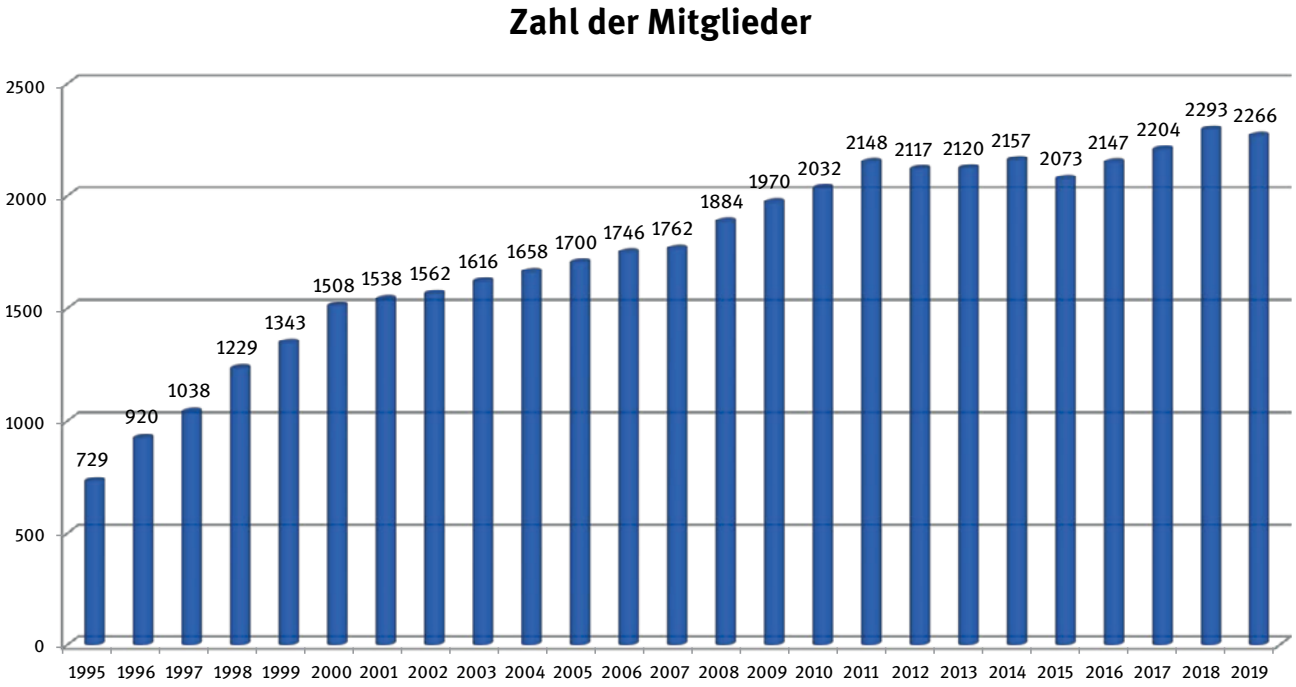


Abb. 1: Entwicklung der Mitgliederzahlen 1995 – 2019

Für die Mitgliedsbeiträge werden folgende Modelle zur Wahl gestellt:
Modell I: Studierende keine Erhöhung; Postdocs 80 €; Seniors Erhöhung um 20 € auf 90 € (16 Ja-Stimmen)
Modell II: Studierende Erhöhung um 10 € auf 40 €; Postdocs 80 €; Seniors Erhöhung um 30 € auf 100 € (36 Stimmen)
Keines der Modelle (7 Stimmen)
Enthaltungen (4 Stimmen)

Damit wird Modell II angenommen und die Beiträge ab 2020 sind wie folgt:
Jahresbeitrag Studenten: 40 €
Jahresbeitrag Postdocs: 80 €
Jahresbeitrag Seniors: 100 €

Für die Tagungsgebühren wird folgender Vorschlag gemacht:
Studierende Erhöhung um 10 € auf 85 €; Seniors Erhöhung um 30 € auf 140 €.
Dieser Vorschlag wird mit 57 Ja-Stimmen, 3 Gegenstimmen und 3 Enthaltungen angenommen.

4. Mitteilungen

Mitgliederzahlen

Die Mitgliederzahlen stagnieren, liegen aber seit 2010 bei über 2.000. Damit ist die NWG eine der größten Neurogesellschaften in Europa. Durch das FENS Forum war in 2018 ein mäßiger Anstieg zu verzeichnen, der sich inzwischen aber wieder abgebaut hat. Auch die Verteilung auf die Sektionen ist fast unverändert.

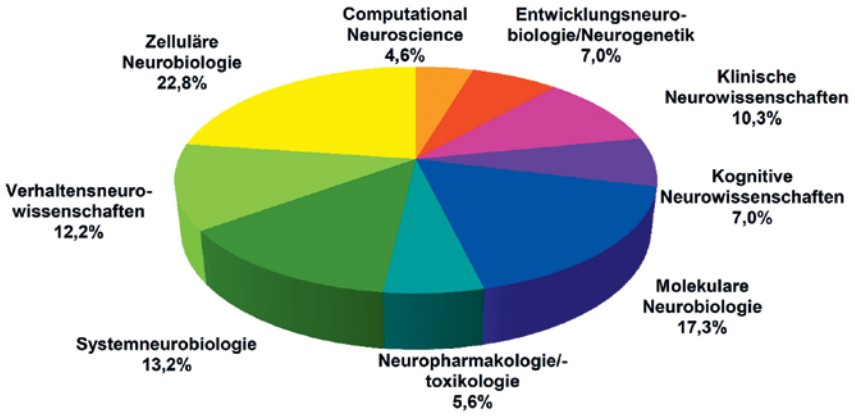
Bericht Partnerorganisationen / GBC

Der German Brain Council wurde neu in Deutschland gegründet und sieht seine Aufgabe darin, Lobbyarbeit in Brüssel zu leisten. Die NWG ist Gründungsmitglied.

5. Bericht zur Göttinger Tagung

Generell ist ein Trend hin zu kleineren Fachtagungen und weg von den großen, fachübergreifenden Tagungen zu verzeichnen. So ist auch die Teilnehmerzahl in Göttingen rückläufig (2019 ca. 19 % weniger als 2017). 20 % der Teilnehmer kommen nicht aus Deutschland.

Erstmals wurde für die Göttinger Tagung eine App angeboten. Deren Programmierung war sehr aufwendig. Die



	2018	2017	2016	2015	2014
Computational Neuroscience:	4,6%	4,5%	4,9%	5,1%	4,3%
Entwicklungsneurobiologie/Neurogenetik:	7,0%	8,9%	6,9%	6,7%	6,7%
Klinische Neurowissenschaften:	10,3%	10,3%	11,0%	11,3%	11,8%
Kognitive Neurowissenschaften:	7,0%	7,1%	6,9%	7,3%	5,9%
Molekulare Neurobiologie:	17,3%	16,8%	16,8%	16,6%	17,1%
Neuropharmakologie/-toxikologie:	5.6%	5,7%	6,2%	6,7%	7,1%
Systemneurobiologie:	13,2%	12,7%	13,0%	12,7%	13,2%
Verhaltensneurowissenschaften:	12,2%	11,9%	12,3%	11,8%	11,3%
Zelluläre Neurobiologie:	22,8%	22,0%	22,1%	21,8%	22,1%

Grün = Steigerung im Vergleich zum Vorjahr

Rot = Rückgang im Vergleich zum Vorjahr

Schwarz = keine Veränderung

Abb. 2: Sektionzugehörigkeit der Mitglieder

App muss an einigen Stellen noch verbessert werden, z. B. in puncto Lesbarkeit, Suchfunktionen, Datumsangabe bei Postern, Personensuche oder Speichern der Auswahl. R. Polder regt an, die Aussteller mit aufzunehmen.

6. Wahl des neuen Vorstandes

Der alte NWG-Vorstand wird bis zum Ende der Tagung am Samstag im Amt bleiben, dann übernimmt der neue. Zum Teil gehen Sektionssprecher in die zweite Runde. E. Friauf dankt dem Wahlkomitee für die Erstellung der Wahlliste. Die Ergebnisse der Wahl waren in Neuroforum 2/2019 zu finden.

7. Aktivitäten der Gesellschaft

Neuroforum

Seit Juli 2018 lenkt P. Wahle die Geschicke der Zeitschrift, die seit der ersten Ausgabe 2019 nur noch in englischer Sprache erscheint. Lediglich die Nachrichten aus der Gesellschaft erscheinen noch auf Deutsch.

Webportal „dasGehirn.info“

Das Internetportal und der Förderverein, der die Finanzierung des Portals zum Teil absichern soll und dessen einzige Aufgabe es ist, Spenden zu empfangen, wird von S. Blanke betreut. Die Förderung durch die Gemeinnützige Hertie-Stiftung endet im September 2019. Als neuer Hauptunterstützer hat die Klaus Tschira Stiftung eine Finanzierungg in Höhe von 450.000 € für drei Jahre zugesagt. E. Friauf dankt M. Madeja für sein jahrelanges Engagement für das Portal.

FENS Forum 2018

Das FENS Forum 2018 in Berlin unter Mitwirkung der NWG war der größte je veranstaltete FENS Kongress mit 7.300 Teilnehmern. E. Friauf dankt Helmut Kettenmann für sein Engagement bei der Organisation dieses FENS Forums.

Junge NWG

S. Seidenbecher stellt die Idee einer neuen Sektion „Junge NWG“ (jNWG) vor. Diese soll eine Interessensvertretung für junge Wissenschaftler innerhalb der NWG werden

mit dem Ziel, Netzwerke zu bilden, sich zu verlinken, Unterstützung bei der Karriereentwicklung zu geben, oder eigene Workshops, auch über die deutschen Grenzen hinaus, zu organisieren. Als „jung“ werden dabei Studenten, Doktoranden und Postdocs bis zu fünf Jahre nach der Promotion angesehen. Mit bereits vorhandenen „jungen“ Strukturen wie dem Neurowissenschaftlichen Doktoranden-Workshop (DoWo) oder der European Neuroscience Conference by Doctoral Students (ENCODS) sowie von der DFG geförderten Netzwerken soll Kontakt aufgenommen werden.

Die Mitgliederversammlung stimmt über den Vorschlag, die jNWG als neue, zehnte Sektion in die NWG aufzunehmen, ab. Der Vorschlag wird einstimmig mit 63 Ja-Stimmen, 0 Gegenstimmen und 0 Enthaltungen angenommen.

Breaking News Preise

Erstmalig in diesem Jahr sind drei Breaking News Preise (500 €, 300 € und 200 €) für die besten Kurzvorträge in der Breaking News Sessions vergeben worden. Mit in der Jury saßen zwei Mitglieder der jNWG. Der Preis wurde von einem NWG-Mitglied gestiftet und reicht mit 10.000 Euro für noch weitere 9 Tagungen.

DFG-Fachkollegienwahl

Eine Zusammenstellung der für die DFG-Fachkollegienwahl von der NWG in Abstimmung mit anderen Fachgesellschaften vorgeschlagenen Kandidaten macht deutlich, dass die NWG im klinischen Bereich kaum Kandidaten hat. A. Ludolph wird daher Kontakt mit anderen, klinisch orientierten Fachgesellschaften wie der Gesellschaft für Neuropathologie, der DGN oder der DGPPN aufnehmen, um hier klinisch orientierte Personengruppen anzusprechen. Erste Schritte in diese Richtung wurden bereits unternommen.

8. Verschiedenes

Nationale Forschungsdaten Infrastruktur (NFDI)

S. Grün stellt die Digitalisierungsinitiative des BMBF vor. Deren Ziel ist es, Daten zugänglicher zu machen und effizienter nach zu nutzen (**F**indable; **A**ccessible; **I**nteroperable; **R**eusable = FAIR). Es wird dafür drei Ausschreibungsrunden über 10 Jahre mit einem Finanzvolumen von 90 Mio geben. Mit diesen Mitteln sollen große Konsortien gebildet werden, in denen bestehende Säulen in der Datensicherung vernetzt werden. Ein solches Konsortium wird für die Neurowissenschaften zuständig sein, und die NWG ist zur Mitwirkung aufgefordert. Es sollen alle Arten von Daten gespeichert werden – Rohdaten und aufbereitete Daten. Ein Problem dabei sind Patientendaten, die eigentlich der Schweigepflicht unterliegen.

Neues NWG-Logo

Das seit Gründung der NWG gültige Logo wird durch ein neues, zeitgemäßes ersetzt. Letzte Detailabsprachen mit der Graphikerin müssen noch vorgenommen werden.

Ende der Sitzung: 14:30 Uhr

Prof. Dr. Eckhard Friauf
(Präsident)

Protokollführer
Prof. Dr. Christian Steinhäuser
(Generalsekretär)

Neueintritte

Folgende Kolleginnen und Kollegen dürfen wir als Mitglieder der Neurowissenschaftlichen Gesellschaft begrüßen:

Abdel-Hafiz, Dr. rer nat Laila (Düsseldorf)
Ba, Mame Aida (Heidelberg)
Braun, Lukas (Berlin)
Cassau, Sina (Halle (Saale))
Everlien, Isabelle (Heidelberg)
Franzke, Myriam (Würzburg)
Hafner, Dr. Anne-Sophie (Frankfurt / Main)
Held, Martina (Würzburg)
Hensel, Dr. rer. nat. Niko (Hannover)
Ibrahim, Hussam (Düsseldorf)
Ikefuama, Ebenezer Chinenye (Ulm)
Janssen, Jan Maximilian (Mannheim)
Jörk, Dr. Alexander (Jena)
Kunz, Dr. Lukas (Freiburg)
Lauterbach, Prof. Dr. Marcel (Homburg)
Maxeiner, Dr. Stephan (Homburg)
Mercan, Delek (Bonn)
Miljanovic, Nina (München)
Owald, David (Berlin)
Rautu, Iona-Sabina (Regensburg)
Rehman, Rida (Ulm)
Remmes, Dr. Jasmin (Münster)
Rieke, Johannes (Berlin)
Roesler, Mona (Hamburg)
Schlecht, Peter (Berlin)
Verma, Ph.D. Dilip (Münster)
Zunke, Dr. rer. nat. Friederike (Kiel)
Zhou, Fangmin (Hamburg)

Der Mitgliedsstand zum 21. Juni 2019 beträgt 2.266 Mitglieder.

Ausblick

Andreas Fink
The neuroscience of creativity

Ildiko Rita Dunay
The neurotrophic parasite Toxoplasma gondii is fine tuning our synapses

Tittgemeyer Thanarajah
Eating behavior/eating disorders and its neurobiological mechanisms

Simone Mayer
Single-cell transcriptomics in neuroscience - benefits and challenges

Michael Scheman
A little brain in the gut

Ferdinand Hucho
Personal View: The Evolution of Neurochemistry: Two questions - one answer

Neurowissenschaftliche Gesellschaft

Neurowissenschaftliche Gesellschaft e.V.

Beitrittserklärung
Hiermit erkläre ich meinen Beitritt zur Neurowissenschaftlichen Gesellschaft e.V.

Eintrag in das Mitgliederverzeichnis

Name

Vorname

Titel

Dienstadresse

Universität/Institut/Firma

Straße

PLZ, Ort

Tel./E-Mail

Privatadresse

Straße

PLZ, Ort

Tel.

Datum/Unterschrift des neuen Mitglieds

Ich unterstütze den Antrag auf Beitritt zur Neurowissenschaftlichen Gesellschaft e.V.:

Datum/Unterschrift

Ich unterstütze den Antrag auf Beitritt zur Neurowissenschaftlichen Gesellschaft e.V.:

Datum/Unterschrift

Neurowissenschaftliche Gesellschaft e.V.
Stefanie Korthals
Max-Delbrück-Centrum für Molekulare Medizin
Zelluläre Neurowissenschaften
Robert-Rössle-Straße 10

13092 Berlin

Ich optiere für folgende 2 Sektionen:
(bitte ankreuzen)

- ☐ Verhaltensneurowissenschaften
- ☐ Zelluläre Neurobiologie
- ☐ Entwicklungsneurobiologie und Neurogenetik
- ☐ Neuropharmakologie und -toxikologie
- ☐ Systemneurobiologie
- ☐ Molekulare Neurobiologie
- ☐ Klinische Neurowisschenschaften
- ☐ Computational Neuroscience
- ☐ Kognitive Neurowissenschaften

Ich bin Student ☐ ja ☐ nein
(Bescheinigung anbei)

Ich bin ☐ weiblich ☐ männlich

Jahresbeitrag:
(bitte ankreuzen)

- ☐ 70,– €/Jahr ordentliches Mitglied
- ☐ 30,– €/Jahr Studenten, Mitglieder im Ruhestand, Arbeitslose

Überweisung:
Bankverbindung: Berliner Bank AG,
IBAN: DE39 1007 0848 0463 8664 05
BIC: DEUTDEBD110

Einzug über VISA-Kreditkarte:
Einzug über EUROcard:

Kartennummer

Exp. Date

Betrag

Name

Unterschrift

Bankeinzugsermächtigung:
Ich ermächtige die Neurowissenschaftliche Gesellschaft e.V. von meinem Konto

bei der Bank

IBAN

BIC

einmal jährlich den Mitgliedsbeitrag in Höhe von € _____ einzuziehen

Ort, Datum

Unterschrift

Kontoinhaber

Anschrift