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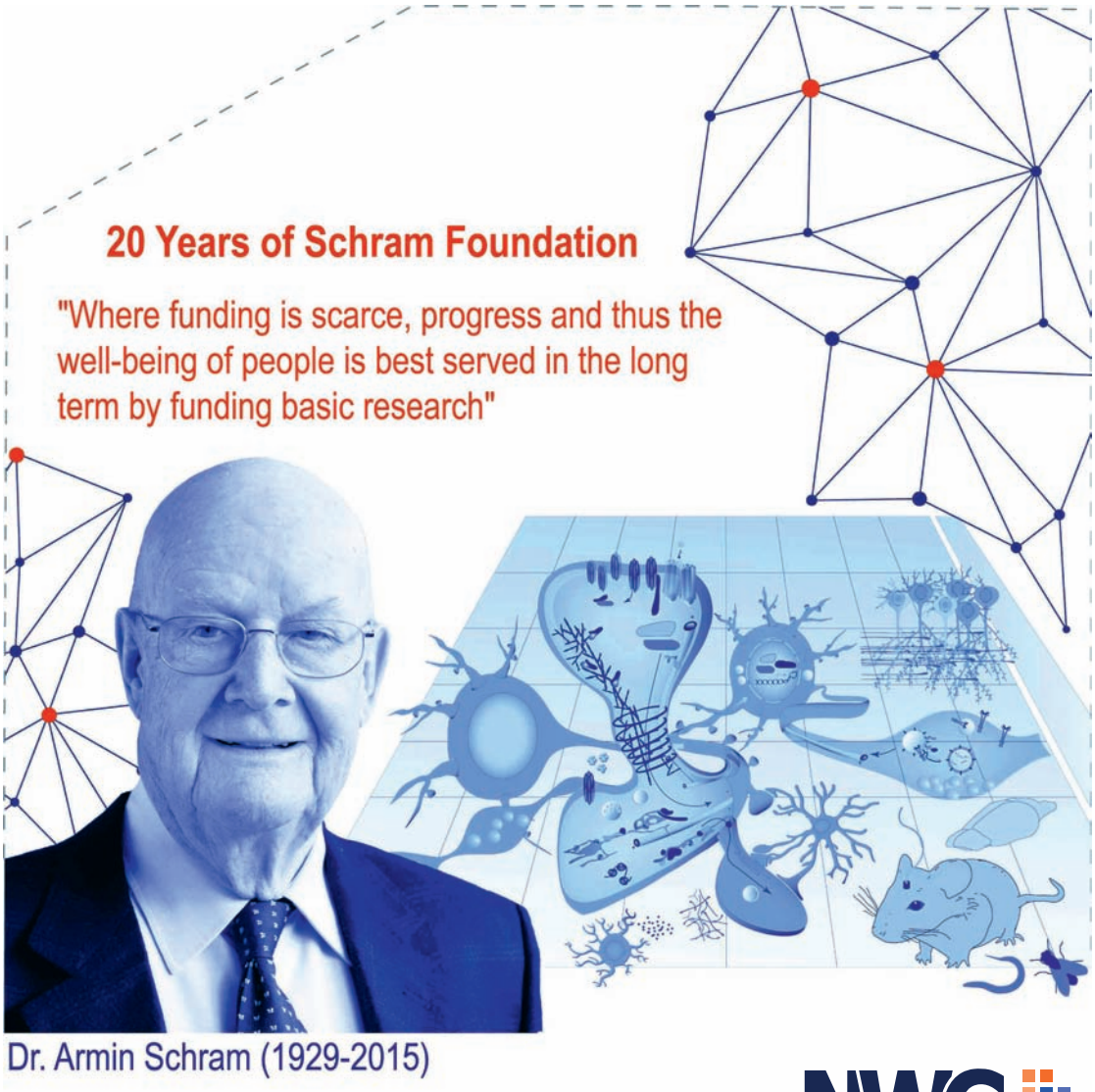

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Editorial

Eckart D. Gundelfinger*, Christian Rosenmund and Dorothea Schulte

Editorial

https://doi.org/10.1515/nf-2020-0026

Dear Reader,

Two decades ago, in 2000, Dr. Armin Schram (1929–2015), chemist by education and retired CEO of the RWE-DEA oil company, decided to establish a Foundation under his name, dedicated to neuroscience research under the roof of the German 'Stifterverband'. It was in the late summer of 2003 when the Foundation was given its current face. At that time, Armin Schram visited Heinrich Betz, then Director at the Max Planck Institute for Brain Research in Frankfurt, to invite him to become a scientific advisor of the Foundation and to discuss with him the funding modalities. "It was an unforgettable experience", Heinrich Betz remembers his first meeting with Armin Schram, "as a layman he had acquired enormous knowledge about modern brain research, and he was absolutely enthusiastic about the arsenal of methods and technologies available now to gain deeper insights into the molecular mechanisms underlying brain function." It was a wise decision on their part to set up a competitive program that supports pioneering DFG-style projects, primarily for applicants in the early stages of scientific independence. The projects should deal with curiosity-driven research in molecular and cellular neuroscience and could be unorthodox. Meanwhile 26 such projects have been or are currently being funded.

Armin Schram was always tightly affiliated with the German Neuroscience Society (NWG). Since 2009 the Schram Foundation Symposium is a steady satellite event of the biennial NWG Meetings. In 2011 Armin Schram became the first honorary member of the Society and in 2019 the NWG has included a 'Schram Lecture' delivered by a distinguished neuroscientist into its meeting program.

This special issue of NEUROFORUM is dedicated to the legacy of Armin Schram. An overview article by Schulte et al. sketches the outstanding scientific achievements of 20 years of research supported by the Schram Foundation with topics ranging from cell fate determination during brain development to synaptic function and plasticity, and the formation and function of neuronal networks. This is followed by a series of articles presenting the progress of various Schram-funded projects addressing questions like "How to keep synapses functional" (Natalia Kononenko & Volker Haucke) and "How decades of curiosity-driven research on synapses lead to an understanding of disease mechanisms" (Ira Milesovic), "How optogenetics can be used to study worm behavior at a cellular level *in vivo*" (Alexander Gottschalk), "How angiogenic factors can influence brain development" (Carmen Ruiz de Almodovar and lab members) and "How memory engrams emerge during learning and how they are influenced by interneurons" (Jonas Sauer & Marlene Bartos). This special volume is rounded off by contributions from Marilen Macher, member of the Board of Trustees of the Schram Foundation, which take a look at the founder, the development of the foundation and current funding opportunities for neuroscientists through foundations that are members of the German Stifterverband.

We hope that you will enjoy browsing through this issue, and that it will raise your interest in the Schram Foundation and the exciting science that originated from Armin Schram's enthusiasm for and commitment to our science. We would greatly appreciate to meet you during upcoming Schram Foundation events, such as the seventh Schram Foundation Symposium or the second Schram Lecture during the next biennial NWG Meeting in Göttingen. In particular, ambitious young scientists may feel stimulated to apply for a membership in the "Schram Family" by winning one of the future Schram Foundation Grants (presumably announced in 2022).

We thank Heinrich Betz and Marilen Macher for comments and for sharing memories from early times of the Schram Foundation. Finally, we want to thank the NWG and the Editorial Board of NEUROFORUM, in particular the Editor in chief, Petra Wahle, and the editorial officer, Susanne Hannig, for the opportunity to assemble this special issue and for their support.

Faithfully yours,
Eckart D. Gundelfinger, Magdeburg
Christian Rosenmund, Berlin
Dorothea Schulte, Frankfurt/M

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Review article

Dorothea Schulte*, Christian Rosenmund and Eckart D. Gundelfinger

Synapses, networks, brain development – funding basic neuroscience research in Germany by the Schram Foundation

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Abstract: Research driven solely by curiosity and the desire to understand fundamental principles of brain function. The freedom to address important questions with bold, sometimes risky experiments. A platform for open scientific exchange and discussions at highest academic level to provide new impulses to the field. And a growing number of scientists who share the passion for neuroscience and who join forces to tackle some of the big mysteries that surround the brain. These visions together with the deep conviction that basic research is the fundament needed for any progress in applied science motivated Dr. Armin Schram to create the foundation that carries his name. They are also the ideals that the foundation still pursues, and to date, 26 research proposals designed by individual researchers or small teams have been, or are, supported in this spirit. Here, we introduce the reader to the individual scientists who were awarded grants by the Schram Foundation over the years, highlight some of the many discoveries made in the course of their studies and list some of the key publications that arose from this work.

Keywords: basic neuroscience research; brain development; network; Schram Foundation; synapse.

Zusammenfassung: Forschungsförderung, die sich der neurobiologischen Grundgenforschung auf höchstem wissenschaftlichem Niveau verpflichtet sieht, sowie ein Forum, das offene wissenschaftliche Diskussionen fördert, Impulse setzt und die Forschungslandschaft in Deutschland nachhaltig stärkt – das waren die Visionen, die Dr. Armin Schram zur Gründung der nach ihm benannten Stiftung bewegten. In diesem Geiste wurden seither 26 Projekte gefördert, die sich aus den unterschiedlichsten Blickwinkeln der Erforschung von Entwicklung, Funktion, Homöostase und Altern des Gehirns widmen. Im Folgenden umreißen wir einige der wichtigsten Entdeckungen, die dank Förderung durch die Schram-Stiftung möglich wurden, und stellen die vielfältigen Förderaktivitäten der Stiftung kurz vor.

Schlüsselwörter: Neurowissenschaftliche Grundlagenforschung; Hirnentwicklung; Synapse; Netzwerk; Schram Stiftung.

Introduction

Working with animal models as diverse as mice, rats, chick, Mongolian gerbils, the fruit fly *D. melanogaster* or the nematode *C. elegans*, and drawing on a broad spectrum of techniques, projects supported by the Schram Foundation have tackled some of the central questions in molecular neuroscience: How is neuronal activity modulated at the level of individual synapses? How do neuronal networks form, become stabilized or adapt to ever-changing environmental conditions? How do genetic and epigenetic mechanisms influence nervous system development, homeostasis and aging? How do these building blocks cooperate to create what we call behavior? And finally, which techniques and methods are needed to accelerate neuroscientific research and how can they be developed?

Below, we have selected some of the most prominent discoveries, which were made with support of the Schram Foundation. This collection gives a good impression of the many activities of the foundation, yet it is far from complete. For a more comprehensive overview of the scientific output of research projects that had received support from

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the Schram Foundation, the reader is invited to visit the foundation's homepage at https://www.schram-stiftung.de/ (see Table 1).

The basic interface of neuronal communication: the synapse

The central units for information transmission and processing in the brain are the chemical synapses, the contacts between neurons, which allow regulated neurotransmitter release from the presynapse and detection at the postsynaptic site. Several of the projects that were supported by grants from the Schram Foundation addressed the question of how synapses operate and how their activity changes to allow for plasticity and ultimately learning and memory. Many excitatory transmitter release sites utilizing glutamate as neurotransmitter contact spines, small protrusions from neuronal dendrites. Focusing on the BAR (Bin/Amphiphysin/Rvs)-domain protein syndapin I, the project led by **Britta Qualmann** (Figure 1) took a cell biological approach and examined how membrane shaping at these spines can be mediated by cytoskeletal forces and membrane-associated proteins. Syndapins partially insert into one leaflet of the cell membrane and can remodel membranes by scaffolding. They thus combine cytoskeletal and membrane shaping mechanisms. Britta Qualmann and her coworkers identified syndapin I as a crucial postsynaptic coordinator in the formation of excitatory synapses. Syndapin I–enriched membrane nanodomains thereby serve as important organizing platforms, which shape dendritic membrane areas into synaptic subcompartments (Schwintzer et al., 2011; Schneider et al., 2014).

The project led by **Volker Hauke** dealt with the longstanding question of how synapses are kept up to speed (see also this issue). He and his coworkers focused on two complementary questions: First, how are key presynaptic components such as synaptic vesicles and active zone proteins formed, transported and assembled into nascent synapses? Second, how are synaptic vesicles regenerated after fusion? Among others, their work established that synaptic vesicles locally reform by adapter proteins that recognize specific components of the vesicle and sort them in a coordinated manner. Synapses thereby capitalize on clathrin-independent endocytosis and clathrin/AP-2–dependent reformation of synaptic vesicles from endosome-like vacuoles to maintain excitability (Kononenko et al., 2014).

The regulation of synaptic vesicle biogenesis and degradation is also addressed by the newly awarded grant to **Eugenio Fornasiero**. This project will develop new tools, based on protein stability measurements, imaging technologies and computational modeling, to decipher the precise molecular composition of synaptic vesicles and apply this knowledge to questions related to neuronal aging.

Membrane recycling mechanisms at the synapse were also at the center of the project headed by **Ira Milosevic** (see also this issue). Focusing on the key endocytic protein endophilin-A, she and her team described that, in addition to its essential role in endocytosis, endophilin-A has a role in the priming and fusion of secretory vesicles (Gowrisankaran et al., 2020). Endophilin-A deficiency causes dysregulation of autophagy and the ubiquitin-proteasome system (Murdoch et al., 2016). Synapses without endophilin-A accumulate clathrin-coated vesicles, an observation that led to the discovery that clathrin can control vesicle acidification by sterically blocking vacuolar ATPase activity (Farsi et al. 2018).

Besides membrane dynamics, the composition of the local extracellular matrix (ECM) at the synapse also profoundly influences synaptic function. **Renato Frischknecht** investigated the contribution of the perisynaptic ECM to network activity and memory formation. He and his colleagues observed that the perisynaptic ECM is modified during homeostatic plasticity and discovered activity-dependent mechanisms of ECM turnover. By training Mongolian gerbils in an auditory cortex–dependent discrimination and reversal learning task, they found that ECM removal promoted performance during reversal learning (Happel et al., 2014; Valenzuela et al., 2014). The local ECM at synapses thus contributes to neuronal network performance and memory consolidation.

Our ability to learn and memorize depends on internal brain states, such as attention and arousal, which are mediated by the action of neuromodulators. One such neuromodulator, noradrenaline, has long been known to facilitate NMDA (*N*-Methyl-D-Aspartat) receptor–dependent long-term synaptic potentiation (LTP), yet the precise mechanisms behind this effect have remained elusive. Supported by the Schram Foundation, **Oliver Schlüter** unraveled the identity of the potassium channel in the dendrite on which noradrenaline acts. Specifically, he discovered that the signaling scaffold protein SAP97 links the noradrenaline receptor beta2-adrenergic receptor to the inactivation of voltage-gated Kv1.1 potassium channels in the dendrite of hippocampal CA1 pyramidal neurons. This study provides a nice demonstration of how local changes in dendritic excitability can support the impact of NMDA-receptor activation during LTP (Liu et al., 2017).

Synapses do not work as isolated entities but must engage in continuous communication with the cell body and cell nucleus. Two of the first projects funded by the Schram Foundation addressed the mechanisms involved.

Table 1: Research projects funded by the Schram-Foundation.

2004

Sox9 vermittelte Genexpressionsänderungen als Ursache der Differenzierung neuraler Stammzellen zu zentralnervösen Gliazellen

Prof. Dr. Michael Wegner, Friedrich-Alexander-Universität Erlangen-Nürnberg, Emil-Fischer-Zentrum/Institut für Biochemie.

Caldendrin und Jacob – Eine Protein-Interaktion zur Kopplung synaptischer Ca^{2+}- Signale an die dendritische Morphogenese?

Prof. Dr. Michael R. Kreutz und Dr. Christina Spilker, Leibniz-Institut für Neurobiologie, Magdeburg, Projektgruppe Neuroplastizität; aktuell: Leibniz-Institut für Neurobiologie Magdeburg und Zentrum für Molekulare Neurobiologie Hamburg (ZMNH).

RNA-Transport in Dendriten

Prof. Dr. Michael Kiebler, Medizinische Universität Wien, Abteilung für neuronale Zellbiologie; aktuell Biomedizinisches Zentrum München, Ludwig-Maximilians-Universität München.

2006

Die Rolle von Genexpressionsprogrammen beim Aufbau neuronaler Verschaltungen

Prof. Dr. Bernd Knöll, Eberhard-Karls-Universität Tübingen, Interfakultäres Institut für Zellbiologie, Abteilung Molekularbiologie; aktuell: Universität Ulm, Institut für Physiologische Chemie.

Regulation der molekularen, strukturellen und physiologischen Differenzierung durch physiologische elektrische Aktivitätsmuster im neonatalen Säugercortex

Prof. Dr. Heiko J. Luhmann, Johannes-Gutenberg-Universität Mainz, Institut für Physiologie und Pathophysiologie.

Prof. Dr. Volkmar Leßmann, Otto-von- Guericke-Universität Magdeburg, Institut für Physiologie.

Prof. Dr. Petra Wahle und Dr. Silke Patz, Ruhr-Universität Bochum, Allgemeine Zoologie und Neurobiologie.

Untersuchungen zur strukturellen Plastizität von Nervenzellverbindungen als Basis für Lern- und Gedächtnisprozesse

Prof. Dr. Britta Qualmann; Friedrich-Schiller-Universität Jena, Institut für Biochemie I.

Transkriptionelle Kontrolle der Entwicklung sympatischer und parasympatische Nervenzellen

Prof. Dr. Hermann Rohrer, Max-Planck-Institut für Hirnforschung, Frankfurt am Main.

Table 1: (continued)

2009

Molecular mechanisms underlying region-specific microcircuit formation in the brain
Prof. Dr. Thomas Hummel, Westfälische Wilhelms-Universität Münster, Institute of Neuro- and Behavior Biology und Universität Wien, Abteilung für Neurowissenschaften und Entwicklungsbiologie.

Rolle endozytischer Adaptor- und akzessorischer Proteine bei der Sortierung und Rezyklierung synaptischer Vesikelproteine
Prof. Dr. Volker Haucke, Freie Universität Berlin, Institut für Chemie und Biochemie; aktuell: Leibniz Forschungsinstitut für molekulare Pharmakologie, Berlin.

Optogenetics-assisted analysis of small neuronal networks and identification of novel proteins affecting recycling of synaptic vesicles in Caenorhabditis elegans
Prof. Dr. Alexander Gottschalk, Goethe-Universität Frankfurt am Main, Institut für Biochemie, Molekulare Membranbiologie und Neurobiologie.

The cellular mechanisms by which chromatin plasticity affects neuronal gene-expression in the ageing brain
Prof. Dr. André Fischer, European Neuroscience Institute (ENI) Göttingen; aktuell: Universitätsmedizin Göttingen und Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE) Göttingen.

2011

Kerntranslokation als Mechanismus der neuronalen Differenzierung
Prof. Dr. Jens C. Schwamborn, Universitätsklinikum Münster, Institut für Zellbiologie (ZMBE); aktuell: Université du Luxembourg, LCSB, Department of Developmental and Cellular Biology, Luxemburg.

Poly ADP Ribosylierung as novel control mechanism in adult and embryonic neurogenesis
Prof. Dr. Dorothea Schulte, Klinikum der Goethe-Universität Frankfurt am Main, Neurologisches Institut (Edinger Institut).

Role of the Perisynaptic Extracellular Matrix in Synaptic Plasticity and Network Activity
Dr. Renato Frischknecht, Leibniz-Institut für Neurobiologie (IfN) Magdeburg; aktuell: Friedrich-Alexander-Universität Erlangen-Nürnberg, Abteilung für Tierphysiologie.

Dissecting the dentate gyrus circuitry: Influence of dendritic versus perisomatic inhibition on network oscillations
Prof. Dr. Marlene Bartos, Albert-Ludwigs-Universität Freiburg i. B., Physiologisches Institut, Lichtenberg-Professur.

2014

Angiopoietine und ihre Tie-Rezeptoren in der Entwicklung neuronaler Netzwerke im Hippocampus
Prof. Dr. Carmen Ruiz de Almodovar, Ruprecht-Karls-Universität Heidelberg, Biochemiezentrum; aktuell: Medizinische Fakultät Mannheim der Universität Heidelberg.

Dynamische Membranen der Synapse: die Rolle subkompartimentaler Endosome in gesunden und kranken Nervenzellen
Dr. Ira Milosevic, European Neuroscience Institute (ENI) Göttingen.

Mechanismen von dendritischer Kv1.1-Inaktivierung, um "spike-timing"-abhängige synaptische Potenzierung zu bahnen
Prof. Dr. Oliver Marcus Schlüter, European Neuroscience Institute (ENI) Göttingen; aktuell: Universitätsmedizin Göttingen, Abteilung für Psychiatrie und Psychotherapie und Department of Neuroscience, University of Pittsburgh, Pittsburgh, PA, USA.

Neuronale Schaltkreise für Erleichterungslernen bei Drosophila
Dr. Ayse Yarali, Leibniz-Institut für Neurobiologie (IfN) Magdeburg.

2017

Regulierung der Genexpression in humanen induzierten Neuronen durch Faktoren der Musterbildung
Prof. Dr. Marisa Karow, Ludwig-Maximilians-Universität München, Biomedizinisches Centrum (BMC), Physiologische Genomik; aktuell: Friedrich-Alexander-Universität Erlangen-Nürnberg, Institut für Biochemie.

Determining the function of local inhibitory circuits in the synaptic dynamics of hippocampal pyramidal neurons during learning and memory
Dr. Alessio Attardo, Max-Planck-Institut für Psychiatrie, Dept. Stress Neurobiologie und Neurogenetik, München.

Chromatin und epigenetische Regulation während der neuronalen Migration
Dr. Tran Tuoc, Universitätsmedizin Göttingen, Institut für Neuroanatomie, Göttingen; aktuell: Ruhr-Universität Bochum, Medizinische Fakultät, Abteilung für Humangenetik.

α2δ-Untereinheiten spannungsgesteuerter Kalziumkanäle bestimmen die erregende und hemmende Konnektivität in neuronalen Netzwerken
Prof. Dr. Martin Heine, Leibniz-Institut für Neurobiologie (IfN), Magdeburg; aktuell: Johannes-Gutenberg-Universität Mainz, Institut für Entwicklungsbiologie und Neurobiologie.

2020

Untersuchung der Genregulation durch Polycomb-Proteine in neuralen Vorläuferzellen während der Entwicklung des humanen Neocortex
Dr. Mareike Albert, CRTD / DFG – Forschungszentrum, für Regenerative Therapien Dresden.

Regulation of synaptic vesicle biogenesis and degradation in neuronal transport: novel tools for studying the vesicle life cycle
Dr. Eugenio F. Fornasiero, Universitätsmedizin Göttingen, Institut für Neuro- und Sinnesphysiologie.

Structural, Molecular, and Functional determinants of enteroendocrine cell mediated gut-to-brain signaling
Dr. Cordelia Imig und Dr. Benjamin H. Cooper, Max-Planck-Institut für Experimentelle Medizin / Molecular Neurobiology, Göttingen, und University of Copenhagen, Department of Neuroscience, Kopenhagen, Dänemark.

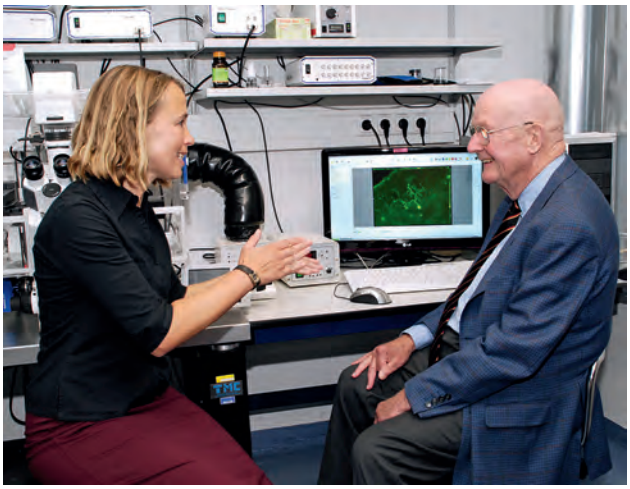

Figure 1: Prof. Britta Qualmann discussing her results with Dr. Armin Schram during his visit at University Hospital Jena in 2011. Picture courtesy of Britta Qualmann (Foto: Riese/UKJ).

Michael Kiebler discovered homologs of the invertebrate RNA-binding protein Staufen in mammals and made significant contributions to understanding their function at synapses. Supported by the Schram Foundation, he and his coworkers found that in rodent hippocampal neurons, Staufen 2 is critically involved in dendritic spine morphogenesis and contributes to memory formation and plasticity. Mechanistically, Staufen controls the transport and activity-dependent translation of mRNAs in distinct regions of the cell. Staufen proteins thereby facilitate locally restricted protein synthesis and consequently allow for spatially controlled adaptations within the cell (Fritzsche et al., 2013; Goetze et al., 2006; Heraud-Farlow et al., 2013).

Cellular events that lead to long-lasting memories require processes that occur in seconds but also on very long-time scales. That gene expression changes are involved has long been postulated. The project led by **Michael Kreutz** and **Christina Spilker** asked how synaptic events couple to transcriptional responses in the cell nucleus**.** They identified the neuronal Ca^{2+} sensor caldendrin, a postsynaptic density component, and Jacob, a caldendrin-binding partner, as key players in the communication from the dendrite to cell nucleus (Figure 2). Upon activation of NMDA-type glutamate receptors, Jacob is recruited to neuronal cell nuclei where it induces rapid transcriptional

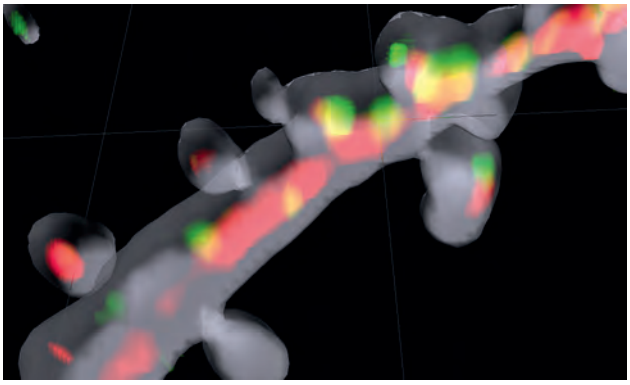

Figure 2: Communication between postsynapse and nucleus illuminated. 3D Imaris reconstruction of a dendritic segment filled with a volume marker (shown in gray) of a hippocampal pyramidal neuron. In red transport packages for importin-mediated long-distance transport are shown, and in green the synaptonuclear protein messenger Jacob can be seen on the way to the nucleus. Picture courtesy of Anna Karpova and Michael Kreutz, LIN, Magdeburg.

changes, which ultimately result in synaptic scaling and a drastically altered morphology of the dendritic tree. Caldendrin binds to Jacob's nuclear localization signal in a Ca^{2+}-dependent manner, thereby controlling Jacob's ability to enter the cell nucleus. In addition, Michael Kreutz and his colleagues established that Jacob is phosphorylated by synaptic, but not extrasynaptic, NMDA-receptor activation and that Jacob's differential phosphorylation determines whether NMDA-receptor activation promotes cell survival and enhances synaptic plasticity or induces cell death (Dieterich et al., 2008; Karpova et al., 2013).

While the work highlighted above deals with the events taking place at synapses in the central nervous system, the grant recently awarded to the research team of **Cordelia Imig** and **Benjamin H. Cooper** enters truly new territories by dissecting fundamental synaptic signaling mechanisms at the synapse formed between enteroendocrine cells and sensory neurons. Enteroendocrine cells sense nutrients and metabolites in the gut and produce a range of gut hormones. Information exchange along the gut–brain axis is receiving increasing attention recently as it is crucial not only for feeding-related physiological responses, like appetite and satiety, but has also been linked to more complex traits such as anxiety-like behaviors.

You never walk alone: neuronal networks

Although the events taking place at individual synapses are the basis of learning and memory, it is the orchestrated activity of many neurons and the computational capacity of the resulting neuronal network that drives information processing and higher cognitive functions. Formation and stabilization of neuronal networks in rodents was investigated by several projects and from very different angles.

The strength of a given synapse in its neuronal network is primarily shaped by two parameters: the release probability of individual synaptic vesicles and the number of release sites that exist within each active zone. In his currently ongoing project, **Martin Heine** investigates how the composition and biochemical properties of voltage-gated calcium channels affect these processes. Focusing on $Ca_V2.1$, one of the major voltage-gated calcium channels responsible for fast synaptic transmission in the mammalian nervous system, he reported on the physiological consequences of alternative splicing of $Ca_V2.1$ transcripts, leading to channel isoforms with different intracellular domains. Depending on the nature of their intracellular domain, these alternative $Ca_V2.1$ isoforms exhibit diverse mobilities and dynamic organization within the presynaptic membrane, which alters the release probability of synaptic vesicles from these sites. This in turn profoundly affects the strength of synaptic transmission and consequently short-term plasticity and network properties (Heck et al., 2019). This study demonstrated not only that calcium channels at the presynapse are mobile and undergo permanent movements within nanodomains of the presynaptic membrane but also that alternative splicing of a single exon can have far-reaching consequences for the performance of the neuronal network as a whole (Heck et al., 2019; Figure 3). Calcium channels are crucial for neurotransmission, but are they also utilized to tune how excitation and inhibition in networks interact? Martin Heine and his team found that in the developing network the balance of excitation and inhibition is indeed regulated through varying the specific content of voltage-gated calcium channels (Bikbaev et al., 2020).

GABAergic inhibitory interneurons play a key role in sculpting the representation of afferent information in principal cells. They are highly diverse and include the diversification in perisoma-inhibiting GABAergic interneurons, which control the timing and frequency of action potential generation in their target cells, and dendrite-targeting GABAergic cells whose functional characterization is lacking behind. Supported by the Schram Foundation, **Marlene Bartos** examined how dendrite-targeting GABAergic interneurons shape synaptic output properties in the dentate gyrus of mice (see also this issue). She and her coworkers discovered that one subtype of dendrite-targeting GABAergic interneurons, somatostatin-expressing

cells, fall into different classes with distinct functional and dynamic synaptic output properties, which relate to the nature of their target cells. They undergo synaptic plasticity at their glutamatergic inputs, and the long-lasting potentiation of their inputs plays a key role in cognitive functions, like the recognition of replaced objects in the environment (Booker et al., 2020; Elgueta and Bartos, 2019).

In keeping with the saying 'seeing is believing,' **Alessio Attardo** has developed deep-brain 2-photon microscopy as a tool to visualize the dynamics of neuronal connections in living mice over weeks to months (Ulivi et al., 2019). Supported by the Schram Foundation, he currently applies this technique to the CA1 region of the hippocampal formation and tracks how the connectivity of excitatory and inhibitory neurons changes when animals undergo hippocampus-dependent learning tasks. Including optogenetics and chemogenetics, he also probes the effect of activation or inactivation of different classes of genetically defined local inhibitory neurons on synaptic dynamics, learning and memory.

Network formation viewed from a very different perspective was the topic of the project headed by **Carmen Ruiz de Almodovar** (see also this issue). As has become increasingly clear during the past decades, classical molecules that regulate neurodevelopment also play an important role in regulating the development of the vascular system. Adopting a converse approach, Carmen Ruiz de Almodovar asked whether angiogenic factors may also impinge on the nervous system. Although vascular endothelial growth factor (VEGF) and its receptor *VEGFR2* were originally identified as angiogenesis-regulating receptor–ligand pair, *VEGFR2* exhibits surprisingly restricted and dynamic expression on neurons of the CA3 region of the developing mouse hippocampus. Stimulation of *VEGFR2*-expressing hippocampal neurons with VEGF or targeted deletion of *VEGFR2* in developing neurons both altered axonal branching and synapse formation. This finding established the prototypical angiogenic receptor VEGF as an important regulator of neuronal network formation (Luck et al., 2019).

A grant given to **Petra Wahle**, **Silke Patz**, **Heiko Luhmann** and **Volkmar Leßmann** dealt with the molecular, structural and physiological differentiation of the neonatal mammalian cortex. The Wahle and Patz groups identified which subunits of the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid)-type glutamate receptors promote dendritic growth of cortical pyramidal cells and interneurons

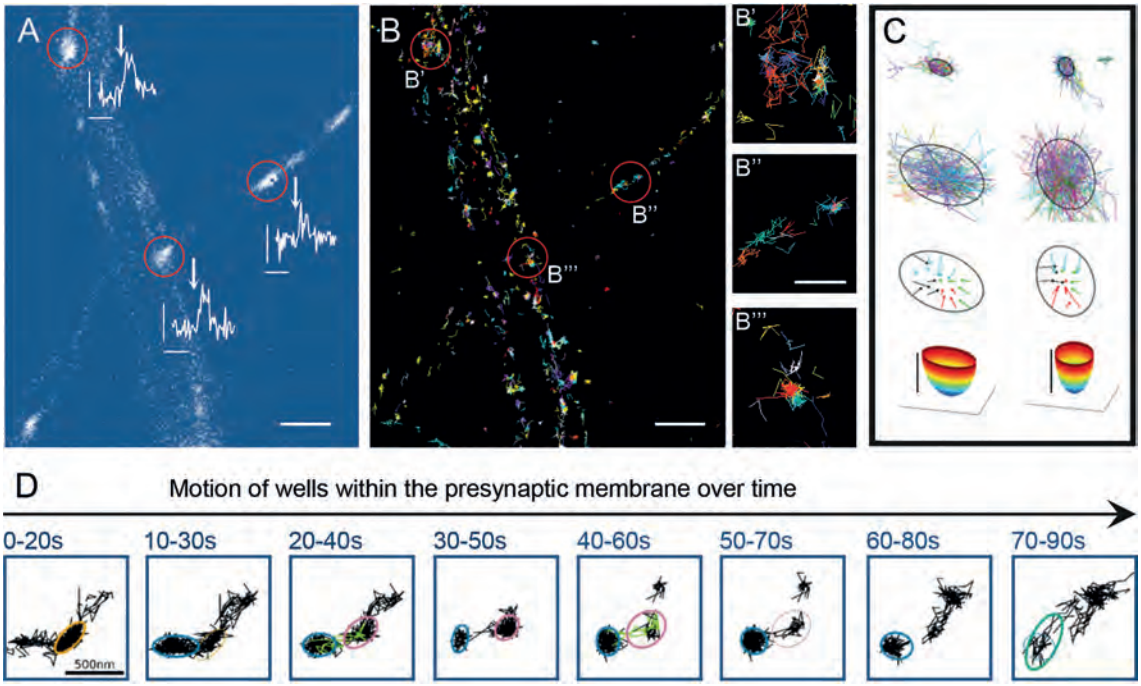

Figure 3: Local organization of voltage-gated calcium channels ($Ca_V2.1$) within the axonal membrane. (A) Axonal segments of rat hippocampal neurons expressing GCamp5::synaptophysin, the stimulation with four extracellular Action potential-like stimulations (scale bars 0.1 $F/\Delta F$, 400 ms). (B) Trajectories of $Ca_V2.1$ channels; indicated are the synaptic locations (scale bar 2 µm). (B′–B‴) higher magnifications of the synaptic regions marked in (B), demonstrating a mixed population of highly confined and mobile channels (scale bar 0.5 µm). (C) Examples of local confined $Ca_V2.1$ channels within energy wells keeping channels for a few 100 ms within the well. (D) In addition to motion inside the well, $Ca_V2.1$ channel wells move within the presynaptic membrane, disappearing and reappearing again. Picture courtesy of Martin Heine, Gutenberg University, Mainz.

(Hamad et al., 2011) and showed that elevated levels of the proinflammatory cytokine Leukemia inhibitory factor (LIF) during brain development cause malfunctions of GABAergic neurons (Engelhardt et al., 2018). The Lessmann and Luhmann laboratories focused on the interplay of programmed cell death and developmental survival of neurons. Work on organotypic cultures showed that the activation of ionotropic glutamate receptors, GABA-A receptors, voltage-controlled calcium channels and electrical synapses (gap junctions) promotes the survival of neonatal cortical neurons (Golbs et al., 2011). This survival is mainly mediated by the brain-derived neurotrophic factor (BDNF) (Kuczewski et al., 2008).

Owing to the relative simplicity and accessibility of their nervous systems, invertebrates are excellent models to study neuronal network formation. The fruit fly *D. melanogaster* and the nematode *C. elegans* have so far taken the center stage in research projects funded by the Schram Foundation. In sharp contrast to the complex nervous systems of vertebrates, the nervous system of the adult *C. elegans* hermaphrodite has been fully mapped and the complete wiring diagram is known. Supported by funds from the Schram Foundation, **Alexander Gottschalk** developed a multispectral optical illumination system that allows precise spatiotemporal control over the activation of optogenetic tools in freely behaving animals (Stirman et al., 2012) (see also this issue). The term optogenetics refers to the targeted expression of genetically encoded light-sensitive ion channels or proton pumps, with the aim to functionally characterize single neurons or neuronal networks. Applying these tools to a nociceptive treatment regime, Alexander Gottschalk and his team dissected a neural circuit surrounding the neuron termed PVD and identified the channels, which by acting on PVD regulate behavioral outputs (Husson et al., 2012).

Painful events not only are answered by avoidance reactions but also establish memories. A neutral stimulus given close to a noxious experience can be remembered in opposite ways: Cues that precede pain or overlap with it are remembered as predictors of punishment and are later avoided. Cues that follow pain are perceived as relief and are therefore recalled positively. The project headed by **Ayse Yarali** examined the minimal circuit that supports the formation, storage and retrieval of these opposite memories in the mushroom

bodies of *D. melanogaster*. These paired structures integrate multimodal inputs and fulfill important functions in learning and memory. Applying an optogenetic approach in fruit flies, Ayse Yarali's team identified two types of dopaminergic neurons, each comprising one paired cell per hemisphere, which upon photostimulation evoke a reaction resembling the punishment-versus-relief memories that are reinforced by real noxious events (König et al., 2018).

While the two projects above dealt with the plasticity of already established neuronal networks, the project led by **Thomas Hummel** investigated the first steps of neuronal circuit formation during development. Among the great wonders of embryogenesis are the apparently self-organizing processes through which structure, order and complexity emerge. Thomas Hummel and coworkers discovered a simple but ingenious principle that drives this process. Opting for the *D. melanogaster* visual system as model, they found that the afferents of photoreceptor cells sequentially segregate into distinct layers of their target region depending on the relative time when the cells had undergone their final division. They identified a transcription factor, Sequoia, whose absolute protein load in individual photoreceptor cells reflects their relative birth order and which organizes growth cones in a dosage-sensitive manner. Small differences in the amount of Sequoia protein between individual photoreceptors organize their growth cones within the same layer, whereas large differences segregate growth cones between layers. The birth order of photoreceptor neurons thus establishes a prepattern, which dictates the assembly of synaptic connections during visual map formation (Kulkarni et al., 2016).

Finding one's identity: cell fate specification

The performance of a neuronal network not only depends on the size, strength and kind of its synapses or the number and nature of its connections. Critically important for every network are the types of neurons it consists of and the glia that associate with them. Neuronal and glial cell types are highly diverse, differing in their size, morphology, and physiological and molecular properties. Understanding how individual cell types are produced at the right time and place and in the right relative proportions is therefore a key question in developmental neurobiology. Having been awarded one of the first Schram grants, **Michael Wegner** set out to decipher the transcription factors that control the generation of oligodendrocytes, the myelin-forming macroglia that facilitate the fast, saltatory nerve conduction characteristic of the vertebrate central nervous system. He uncovered a network of Sox-type transcription factors, centered around the Sox-family member SOX9, that allows for the timely progression of oligodendrocyte development in the spinal cord. He established that SOX9 is essential for gliogenesis and that it is required, jointly with SOX10, for survival and migration of oligodendroglial precursor cells (Finzsch et al., 2008). SOX9 and SOX10 regulate expression of the distantly related *Sox5* and *Sox6* genes, which in turn modulate the activity of *Sox9* and *Sox10* in a negative feedback loop and thereby determine the timing of oligodendroglial differentiation (Stolt et al., 2006). These studies shed light on the interdependent levels of transcriptional regulation that are needed to advance the production of a single cell type, myelinating oligodendrocytes.

A rather unexpected mechanism by which the development of myelinating oligodendrocytes is regulated in the corpus callosum of juvenile mice was revealed by the work of **Bernd Knöll**. He and his coworkers observed that targeted deletion of the transcription factor *SRF* in neurons interfered with oligodendrocyte development in a non–cell autonomous manner. Consistently, neuronal deletion of *SRF* resulted in myelination defects and axon degeneration, whereas forced activation of *SRF* in neurons affected the maturation of neighboring oligodendrocytes. Paracrine regulation of oligodendrogliogenesis by neuronal *SRF* involves two secreted molecules, connective tissue growth factor (CTGF), which is repressed by *SRF*, and insulin like growth factor 1 (IGF-1), which stimulates oligodendrocyte maturation but is antagonized by CTGF. This double-negative regulation places oligodendrocyte maturation under the control of nearby neurons (Stritt et al., 2009).

The network of transcription factors controlling autonomous nervous system development was investigated in the project led by **Hermann Rohrer**. The autonomous nervous system is derived from a transient cell population called neural crest. It regulates involuntary physiologic processes and contains three anatomical distinctions, the sympathetic, parasympathetic and enteric nervous system. Hermann Rohrer and his team demonstrated that the transcription factors AP-2α/AP-2β exert an early prespecifying function for sympathetic progenitor cells and a later survival function for sympathetic neurons (Schmidt et al., 2011). Likewise, transcription factors of the *HoxB* cluster exert an early influence on the prespecification of the sympathetic versus sensory neuron lineages of the neural crest and support and maintain the expression of sympathetic neuron genes (Huber et al., 2012).

Transcription factors bind enzymes, which chemically alter DNA or proteins, and recruit these enzymes to specific

sites in the genome. This process, known as epigenetic modification, introduces heritable but reversible changes in DNA or histones, the building blocks of nucleosomes. For transcription to occur, nucleosomes must be destabilized on DNA by the activity of nucleosome-remodeling ATPases. Nucleosome remodeling and histone modifying activities jointly reorganize the chromatin structure in a way that either facilitates or inhibits gene expression. The Schram Foundation supported several projects that examined the effect of these activities on development and aging of the nervous system. Studying the role of the BAF (BRG1- or BRM-associated factor) nucleosome-remodeling complex in the developing mouse neocortex, **Tran Tuoc** discovered that nucleosome remodeling is closely integrated with the activity of histone demethylases during corticogenesis. He found that BAF complexes can simultaneously silence the expression of genes required for the proliferation of cortical progenitor cells and stimulate the expression of genes associated with the differentiation and migration of young neurons. Mechanistically, this involved recruitment of histone-demethylating enzymes with opposing functions, KDM6A/B and KDM1A, respectively. By acting both as activators and repressors of gene expression, BAF complexes thus ensure the generation of the appropriate numbers of neurons as well as their proper migration during cortical histogenesis (Narayanan et al., 2018; Nguyen et al., 2018) (Figure 4).

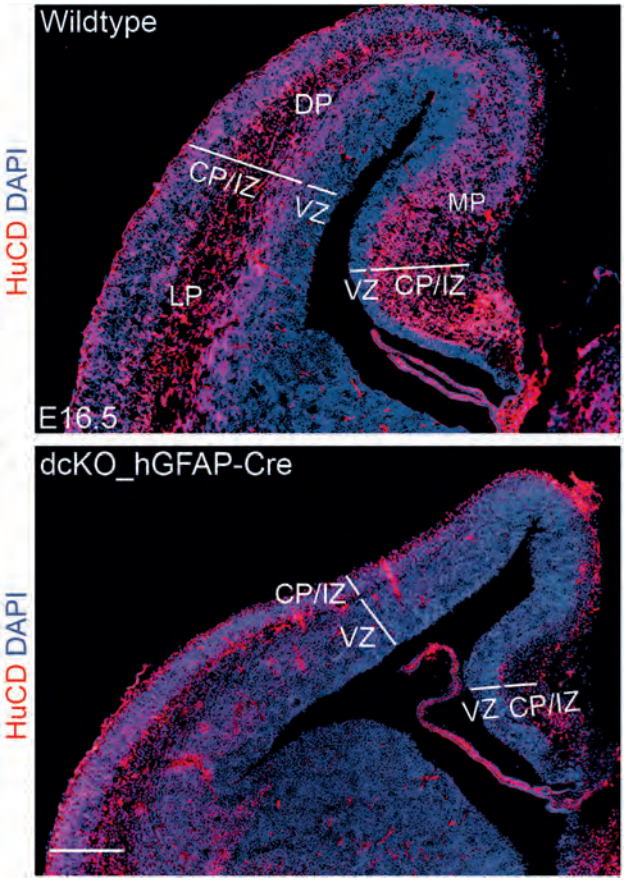

Figure 4: Loss of BAF155 and BAF170 in the early cortical anlage leads to a diminished thickness of the cortical plate at later embryonic stages. Immunofluorescence staining for the neuron-specific RNA-binding protein HuC/D (ELAV; red) in the cortices of E 16.5 wild-type mice and littermates double mutant for BAF155 and BAF170 under control of the human GFAP promoter. CP, cortical plate; DP, dorsal pallium; IZ, intermediate zone; LP, lateral pallium; MP, medial pallium; VZ, ventricular zone; Scale bar represents 100 mm. Picture courtesy of Tran Tuoc, Göttingen.

With the help of her recently awarded Schram grant, **Mareike Albert** will study the function(s) that Polycomb group (PcG) proteins, a family of histone-methylating enzymes and potent epigenetic repressors, have in the developing human cortex. In mice, PcG proteins contribute to all phases of cortical development. Yet, given the striking differences between the rodent and primate neocortex, lessons learned in murine models cannot be simply applied to humans. Mareike Albert will apply CRISPR/Cas9-based genome-editing tools to human brain organoids to functionally interrogate the role of histone methylation during human brain development.

The importance of epigenetic regulation for brain development cannot be discussed without acknowledging its role in aging. **André Fischer** established that age-associated memory impairment is tightly linked with altered epigenetic plasticity. In a study supported by the Schram Foundation, he discovered that aged mice display a specific deregulation of the epigenetic mark histone H4 lysine 12 (H4K12) acetylation and that this deregulation correlated with hippocampus-specific changes in gene expression programs associated with memory consolidation. Restoration of physiological H4K12 acetylation reinstated the expression of learning-induced genes and led to the recovery of cognitive abilities (Peleg et al., 2010). In another study, he and his team established that lysine acetyltransferase 2a (KAT2A), an enzyme that catalyzes the attachment of acetyl groups on histone and nonhistone proteins, regulates a highly interconnected gene expression network in the hippocampus and thereby impacts synaptic plasticity and long-term memory consolidation (Stilling et al., 2014).

Although most neurons in the central nervous system are generated during embryogenesis and in early postnatal life, a small but physiologically important number of neurons is continuously added during adulthood in a process known as adult neurogenesis. Production of neurons in the adult brain occurs in response to environmental stimuli and, hence, reflects the physiological state of the individuum. The molecular players that drive adult neurogenesis must therefore quickly and efficiently react to changing extrinsic cues.

Dorothea Schulte examined how transcriptionally silent genes become activated when adult neural stem cells exit dormancy and begin to differentiate toward neurons. She and her team discovered a molecular cascade, involving the transcription factors MEIS2 and PBX1 and the nuclear enzyme PARP1, which induces the decompaction of transcriptionally silent chromatin at the regulatory regions of neuron-specific genes, thereby facilitating the rapid execution of neuronal gene expression programs (Hau et al., 2017). These chromatin dynamics are set into motion by the translocation of MEIS2 into the cell nucleus, which is controlled by MEIS2′ post-translational modification downstream of signals from the stem cell niche (Kolb et al., 2018).

Temporal control over stem cell activation by nuclear translocation of a neurogenic cell fate determinant was also investigated in the project headed by **Jens Christian Schwamborn**. He demonstrated that the multifunctional protein TRIM32 undergoes differentiation-associated translocation into the nucleus when neural progenitors mature to olfactory bulb interneurons. TRIM32 participates in cytoplasmic and nuclear functions that are necessary for neuronal differentiation, consistent with the notion that its gradual nuclear accumulation reflects a gradual maturation of adult born neuroblasts (Hillje et al., 2013).

A fundamental question in cell biology is whether the acquisition of a given cell fate during embryonic development is fixed or reversible. Mounting evidence over the last years has shown that the forced expression of lineage-specific transcription factors in various differentiated cell types can promote the reversal of cellular fates, a process recognized as cellular reprogramming. In her ongoing project, **Marisa Karow** converted human pericytes, mural cells that wrap around blood vessels in the brain, into neurons by the overexpression of two neurogenic transcription factors, *Ascl1* and *Sox2*. Using single-cell RNA sequencing to dissect transcriptome changes and reconstruct lineage reprogramming trajectories, Marisa Karow and colleagues discovered that successful reprogramming involves the recapitulation of developmental programs via stem cell–like intermediates (Karow et al., 2018).

Closing remarks

Owing to space limitations, this collection of results and concepts is inevitably incomplete. Nevertheless, it gives a brief but comprehensive overview over the many fundamental discoveries that research grants awarded by the Schram Foundation have made possible over the years. Also worth of note is that grants are predominantly given to young researchers, many of them at the transition from postdoctoral fellow to independent group leader or on the brink of taking their first academic position. In fact, in several cases the Schram Foundation gave the very first research funds to these projects and thereby contributed in an essential way to the start of new, long-term areas of research. As one Schram fellow put it, "There are many challenges associated with starting an independent group and developing the own research profile. With the Schram Stiftung backing my work, some challenges simply turned into opportunities." Considering that most previous Schram fellows have taken permanent academic positions at domestic universities and institutions, the foundation's impact on neuroscience research in Germany goes well beyond the immediate duration of the funded projects. It is thus fair to say that the Schram Foundation, during the relatively short time of its existence, has made remarkable contributions to the neuroscience research landscape in Germany. Undoubtedly it will continue to do so in the future.

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Bionotes

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Review article

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Neuronal functions of clathrin-associated endocytic sorting adaptors – from molecules to disease

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Abstract: Communication in the central nervous system is based on the transmission of electrical signals at specialized junctions between nerve cells termed synapses. During chemical neurotransmission, tiny membrane spheres called synaptic vesicles that are packed with neurotransmitters elicit a postsynaptic response by fusing with the presynaptic membrane and releasing their content into the synaptic cleft. Synaptic vesicle fusion is followed by the reuptake of the membrane by endocytosis and the local reformation of functional synaptic vesicles within the presynaptic compartment to sustain further rounds of neurotransmitter release. Here, we provide an overview of the clathrin-associated endocytic adaptor proteins that help to sort and recycle synaptic vesicles during presynaptic activity. These adaptors also serve additional functions in the turnover of defective or aged synaptic components and in the retrograde axonal transport of important signaling molecules by regulating the formation or transport of autophagosomes. Endocytic adaptors thus play multiple roles in the maintenance of synaptic function. Defects in their expression or function can lead to neurodegenerative and neurological diseases.

Keywords: clathrin adaptors; endocytosis; neurological and neurodegenerative diseases; neurotransmission; synaptic vesicle.

Zusammenfassung: Kommunikation im Zentralnervensystem basiert auf der Umwandlung elektrischer in chemische Signale an spezialisierten Kontaktstellen zwischen Nervenzellen, die Synapsen heißen. Während der chemischen Erregungsübertragung fusionieren winzige Membransphären, synaptische Vesikel genannt, welche mit Neurotransmitter Molekülen beladen sind, mit der präsynaptischen Membran, um so ihren Inhalt in den synaptischen Spalt freizusetzen und eine postsynaptische Antwort auszulösen. Der Fusion synaptischer Vesikel folgt die Wiederaufnahme der Membran und die lokale Rückbildung funktioneller synaptischer Vesikel im präsynaptischen Kompartiment, um weitere Runden der Neurotransmitterfreisetzung aufrecht zu erhalten. Hier geben wir einen Überblick über die mit Clathrin assoziierten endozytotischen Adaptoren, welche die Komponenten synaptischer Vesikel sortieren und recyceln, um so die korrekte Wiederherstellung funktioneller synaptischer Vesikel sicherzustellen. Diese Adaptoren üben ferner zusätzliche Funktionen im regulierten Umsatz defekter oder alter Komponenten der Synapse und im retrograden Transport wichtiger Signalmoleküle aus, indem sie die Bildung oder den Transport von Autophagosomen regulieren. Endozytotische Sortieradaptoren spielen demzufolge multiple Rollen bei der Aufrechterhaltung der synaptischen Funktion. Defekte in ihrer Expression oder Funktion könnten zu neurodegenerativen und neurologischen Krankheiten führen.

Schlüsselwörter: Clathrin Adaptoren; Endozytose; neurologische und neurodegenerative Erkrankungen; Neurotransmission; Synaptische Vesikel.

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Introduction and objectives

The chemical way of neuronal communication involves the exchange of neurotransmitter messengers between neurons at specialized contact sites called synapses. Synapses are comprised of a presynaptic compartment

that contains small synaptic vesicles (SVs), filled with neurotransmitter molecules such as glutamate or γ-aminobutyric acid (GABA), and an opposing postsynaptic compartment that harbors neurotransmitter receptors that control the activity of the postsynaptic cell. Neurotransmitter release is triggered by an action potential, which upon its arrival at the presynapse activates voltage-gated calcium channels enriched at a specialized presynaptic part called active zones (AZs). Resulting calcium influx elicits the rapid (in less than a millisecond) fusion of SVs docked at the AZ membrane to release their neurotransmitter content into the synaptic cleft (Sudhof, 2013). The fusion of a single SV thus is a correlate of a neurotransmitter quantum, proposed by Bernhard Katz in the early 1950s to be the physical unit of neurotransmission (Fatt and Katz, 1952). To prevent the expansion of the presynaptic plasma membrane and to locally replenish the pool of SVs, exocytosis is coupled to compensatory internalization of SV membranes and SV reformation by endocytosis (Kononenko and Haucke, 2015; Rizzoli, 2014; Saheki and De Camilli, 2012). Over the lifetime of a nerve cell, SVs undergo hundreds of cycles of exocytosis, endocytosis and SV reformation (Truckenbrodt et al., 2018). Naturally, this has to occur with a high fidelity as the failure to sort and retrieve SV components results in functional impairments of neurotransmission and causes malfunction of neuronal networks.

The objective of this review is to provide an up-to-date overview of proteins, which function as endocytic sorting adaptors at the synapse, and highlight their roles in the maintenance of synaptic function in health and disease.

Endocytosis in neurons

The ability to internalize pieces of the plasma membrane is not a unique property of neurons. In fact, neurons express many of the same proteins known to carry out different forms of endocytosis in non-neuronal cells. Those include clathrin-mediated endocytosis (CME) described in more detail below, macropinocytosis and other forms of clathrin-independent membrane internalization and phagocytosis that is mainly used by immune cells to engulf and digest large particles such as bacteria. Apart from cycles of exocytosis/endocytosis of SVs, neurons employ endocytosis to regulate their cell surface content, e.g. ion channels or nutrient transporters, and to regulate cell polarity and cell signaling processes. For example, developmental neurotrophin signaling is regulated by endocytic proteins such as endophilin (Barker et al., 2002; Burk et al., 2017), while the clathrin adaptors assembly protein (AP) complex-2 (AP-2) (Fiuza et al., 2017; Kastning et al., 2007; Kittler et al., 2005), Huntingtin-interacting protein 1 (HIP1) (Metzler et al., 2003, 2007) and endophilin (Zhang et al., 2017) have been implicated in determining the surface levels of postsynaptic ionotropic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)- or N-Methyl-D-aspartate (NMDA)-type glutamate and $GABA_A$ neurotransmitter receptors.

Work by us and others has suggested that neurons ensure the maintenance of functional SVs of the correct size and composition by initially performing clathrin-independent retrieval of SV membranes (Soykan et al., 2016, 2017; Watanabe et al., 2014), followed by sorting and recycling of SV components by CME (Heerssen et al., 2008; Kononenko et al., 2014). Clathrin-independent endocytosis of SV membranes requires linear F-actin filament polymerization by actin-nucleating formins as well as the activity of Bin/amphiphysin/Rvs (BAR) domain proteins such as endophilin, an upstream regulator of the lipid phosphatase synaptojanin, and the oligomeric GTPase dynamin (Ferguson et al., 2007; Soykan et al., 2017; Watanabe and Boucrot, 2017; Watanabe et al., 2018; Wu et al., 2016).

CME and endocytic protein sorting

Vesicle formation by CME allows selective internalization of plasma membrane proteins, followed by their sorting and incorporation into a newly generated vesicle, for example, SV in the case of neurons (Kononenko and Haucke, 2015; Rizzoli, 2014; Saheki and De Camilli, 2012) (Figure 1). It involves the formation of a characteristic lattice-like coat that is mainly comprised of the name-giving protein clathrin, a three-legged scaffold protein highly enriched in nerve terminals. In fact, clathrin was first purified from the brain by Barbara Pearse, more than 10 years after Keith Porter had described clathrin-coated vesicles and pits in electron micrographs from cells and tissues (Roth and Porter, 1964). Subsequent work over decades has unraveled the complex machinery underlying the formation of clathrin-coated vesicles during endocytosis in various cells and tissues.

Mechanistically, CME is initiated by the recruitment of early-acting endocytic proteins, termed adapters, such as Fer/Cip4 homology domain-only (FCHO) proteins, EPS15/ EPS15R, stonins and AP-2, a heterotetramer of two large (α, β2) and two small subunits (μ2, σ2), as well as curvature-inducing proteins (e.g. epsins and AP180 or clathrin assembly lymphoid myeloid [CALM] protein) to

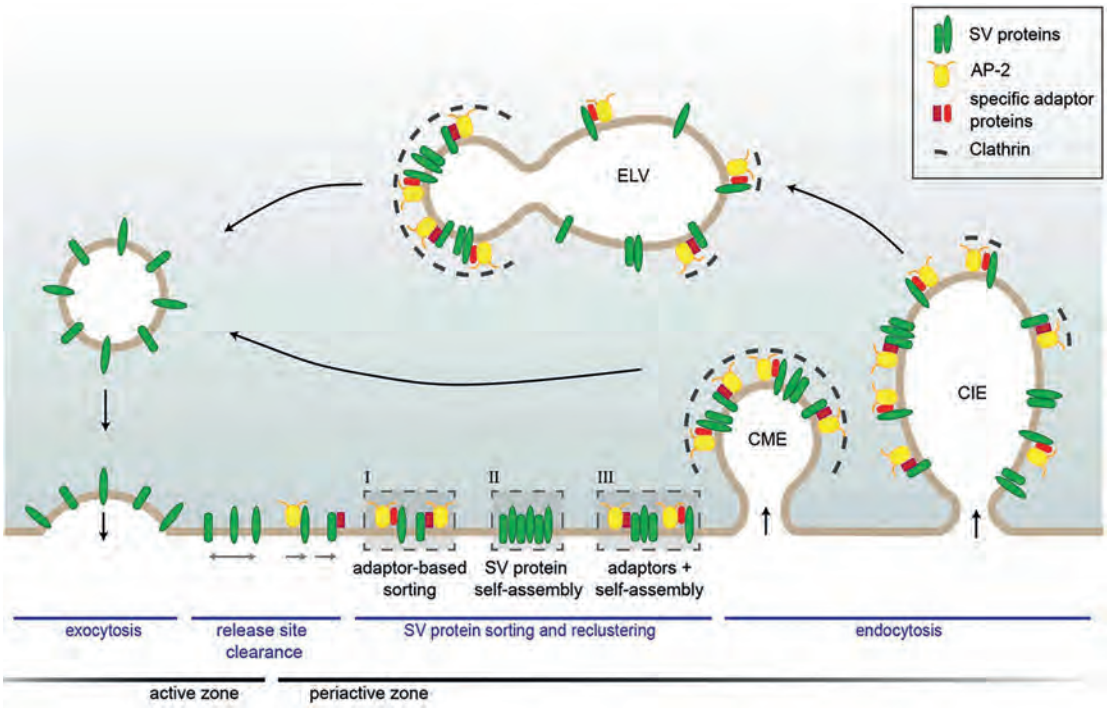

Figure 1: Model of SV protein sorting by endocytic adapters at the presynapse. After full collapse fusion, freely diffusing SV proteins are confined and recaptured by endocytic sorting adapters at the periactive zone. At the plasma membrane, SV proteins might either be clustered by AP-2 and additional cargo-specific adapter proteins (I); they might interact with each other and thereby self-assemble into clusters (II) or form mixed clusters of self-assembled SV proteins together with sorting adapters (III). These clusters can directly be endocytosed from the plasma membrane by CME to reform SVs. However, cargo-specific sorting proteins together with AP-2 and clathrin can also operate on endosome-like vacuoles (ELVs) after clathrin-independent endocytosis (CIE) to recycle SVs with correct protein composition. Reproduced from the study by Kaempf and Maritzen (2017). SV, synaptic vesicle; AP-2, assembly protein complex-2; CME, clathrin-mediated endocytosis.

the plasma membrane. These adapters link clathrin to the underlying membrane via their association with charged plasma membrane lipids and couple the assembly of the clathrin coat with the selection of transmembrane cargo proteins, i.e. receptors and their ligands. The assembled endocytic clathrin coat progressively bends inward, into the direction of the cytoplasm, until eventually, the so-called endocytic pit is connected to the membrane only via a narrow stalk that is severed by mechanochemical forces executed by the protein dynamin, likely aided by other membrane-bending proteins such as endophilin, and the actin cytoskeleton (McMahon and Boucrot, 2011).

Endocytic adaptors are crucial in this process since they conduct the selection of membrane proteins destined for endocytosis. For example, specific adaptors enable liver cells to internalize the low-density lipoprotein receptors (i.e. these are then the “cargo” of the forming endocytic vesicle) to clear cholesterol from the circulation and loss of these adaptors causes hypercholesterolemia and atherosclerosis in humans (Mishra et al., 2002). A similar cargo-selective function is carried out by endocytic sorting adaptors in neurons, in particular during the exocytic/endocytic cycling of SVs that we will focus on now.

Neurons capitalize on endocytic sorting adaptors to reform functional SVs

The number of SVs available for fusion defines the efficacy of neurotransmitter release and fine-tunes neuronal function. A single SV is a complex organelle that contains several dozens of SV membrane proteins, many of which are present in just a few copies. These SV proteins are crucial for calcium-sensing, docking and fusion, endocytosis and other forms of membrane traffic at the presynapse (Takamori et al., 2006). Following calcium-triggered SV exocytosis, SV proteins are integrated into the presynaptic plasma membrane, from where they need to be removed by endocytosis. Although this compensatory endocytosis of collapsed SV membranes may not require clathrin coats *per se*, SV proteins must be sorted into the forming endocytic

structure from which new SVs are eventually regenerated in a clathrin-mediated budding process that bears similarity to clathrin-coated vesicle formation in non-neuronal cells as first shown by Heuser and Reese (1973). A partially unsolved riddle is how synapses are capable of maintaining the composition of their SVs over multiple rapid rounds of exocytosis and endocytosis. Work in recent years has established a crucial role of endocytic adaptors in maintaining the protein composition of SVs by sorting their transmembrane proteins during the exo-endocytic cycle.

Multiple SV proteins have been shown to be recognized by specific dedicated endocytic sorting adaptors. The first specific adaptor for SV protein sorting was revealed by genetic screens in *Drosophila* (Grigliatti et al., 1973), where some mutant flies were reported to become paralyzed at elevated temperature as if they were "stoned". The term "stoned" was used to describe the mutant locus, which subsequently was found to code, among others, for a protein called stoned B, a binding partner of SV calcium sensor protein synaptotagmin 1 (Maritzen et al., 2010; Phillips et al., 2010). Work by us and others identified stonin 2 as the mammalian paralog of *Drosophila* stoned B, which acts as a selective adapter for the retrieval of synaptotagmin 1 from the presynaptic plasma membrane (Diril et al., 2006; Jung et al., 2007; Kononenko et al., 2013). Interestingly, loss of stonin 2 does not affect the process of SV endocytosis *per se* but results in the selective accumulation of synaptotagmin 1 molecules at or near the presynaptic AZ. This function of stoned B/stonin 2 is conserved throughout evolution from worms and flies to humans (Jung et al., 2007; Maritzen et al., 2010; Mullen et al., 2012; Phillips et al., 2010). Recent work suggests that a related adaptor protein SGIP1 may serve a partially overlapping function in synaptotagmin 1 sorting at the synapse (Lee et al., 2019). In a similar way, it has been found that the SV soluble *N*–ethylmaleimide sensitive factor attachment protein receptor (SNARE) protein synaptobrevin/vesicle-associated membrane protein 2 (VAMP2) is recognized and sorted by a neuron-specific endocytic adaptor AP180 and its ubiquitous paralog CALM protein (Koo et al., 2015; Maritzen et al., 2012), while the vesicular glutamate transporter 1 required for the refilling of SVs with glutamate contains motifs recognized by endocytic proteins AP-2 and endophilin (Voglmaier et al., 2006).

How sorting of other SV proteins such as the vacuolar ATPase or SV2A/SV2B is accomplished remains unknown. For some SV proteins, piggy-back riding mechanisms involving the association with other SV proteins have been proposed. For example, sorting of synaptotagmin is chaperoned by its association with SV2A, which itself can bind to AP-2 (Kaempf et al., 2015), and synaptobrevin/VAMP2 is sorted in a complex with synaptophysin (Gordon and Cousin, 2016). An equally important but unresolved question is how the sorting by this diverse set of endocytic adaptors is coordinated to maintain the SV protein stoichiometry during multiple rounds of exo-endocytosis. Future studies will need to address this.

Noncanonical functions of endocytic adaptors in autophagy at the presynapse

Neurons like most other cells employ several strategies for removing damaged or misfolded proteins that include the ubiquitin-proteasome system, and the autophagy-lysosomal pathway. In autophagy, a double membrane organelle referred to as the autophagosome is formed from other membranes. The autophagosome delivers its engulfed cytoplasmic material to the lysosome for degradation (Ariosa and Klionsky, 2016). This function of autophagy is especially crucial in neurons, and defects of neuronal autophagy are associated with neurodegeneration and aging-associated memory decline (Gupta et al., 2013; Menzies et al., 2017). Recent work has suggested that synaptic autophagosome formation is crucially regulated by endocytic proteins (Murdoch et al., 2016, Soukup and Verstreken, 2017). For instance, endophilin, a possible sorting adaptor for the vesicular glutamate transporter 1, has been shown to be required for the stimulation-induced formation of autophagosomes. The switch of endophilin between a function in SV endocytosis and the formation of autophagosomes is regulated by its phosphorylation by the kinase LRRK2, a protein genetically linked to Parkinson's disease (PD) (Arranz et al., 2015; Matta et al., 2012; Soukup and Verstreken, 2017). A similar switch between a role in SV endocytosis and autophagy has also been postulated for the endocytic adaptor AP-2, which has been shown to promote the retrograde axonal transport of autophagosomes that carry neurotrophin signals to the neuronal soma (Kononenko et al., 2017) (Figure 2). In summary, the study of SV endocytosis and the role of endocytic adaptors has revealed exciting and unexpected connections of endocytic adaptors for SV recycling to the autophagy.

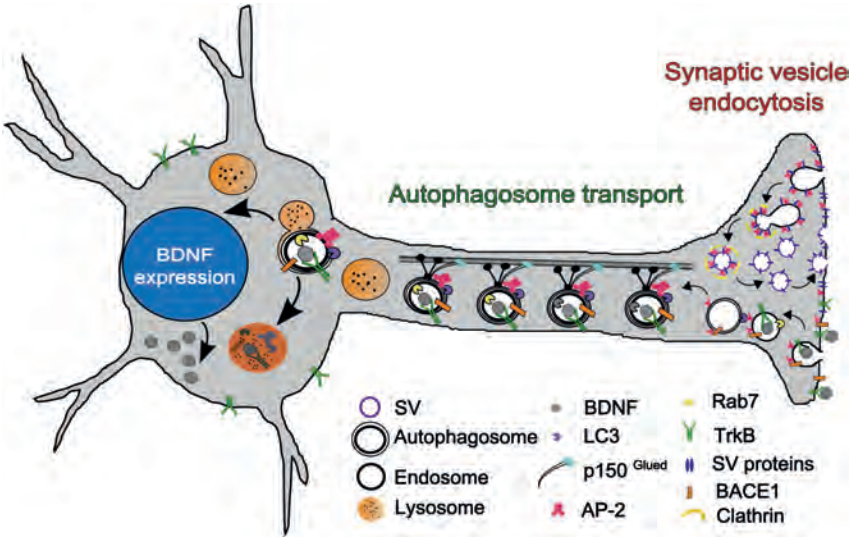

Figure 2: Hypothetical model explaining the dual role of AP-2 in SV recycling and autophagosome transport. At the presynapse, AP-2 is required to regenerate SVs from plasma membrane-derived endosomal vacuoles formed by clathrin-independent endocytosis of SV membranes postfusion. AP-2 may also aid sorting of select SV proteins at the plasma membrane. In addition, AP-2 serves a nonendocytic function in axonal trafficking of TrkB-containing signaling autophagosomes and in turnover of BACE1 via autophagy. SV, synaptic vesicle; AP-2, assembly protein complex-2.

Endocytic sorting adaptors in neurological disorders – where we go from here

Alterations in the function of endocytic sorting adaptors have been implicated in various forms of neurological disorders. How these relate to their functions in SV endocytosis or autophagy in most cases is unclear. For example, several endocytic adaptors, such as stonin 2, AP180 and CALM, has been linked to autism spectrum disorders (ASDs) and schizophrenia in humans (Ben-David and Shifman, 2012; Breedveld et al., 2010; Luan et al., 2011), although no mechanistic studies have been conducted to support this. SGIP1, a stonin 2-related adaptor for synaptotagmin 1 has been implicated in alcohol use disorder and as a factor affecting the human electroencephalogram, suggesting a role in the regulation of brain activity (Hodgkinson et al., 2010). Conversely, α-synuclein, a protein genetically linked to PD in humans has been found to play a role in SV recycling and the regulation of SV pool sizes (Vargas et al., 2014, 2017). Endocytic adaptors may also function at the presynapse to prevent the epilepsy. For example, loss of the endocytic proteins amphiphysin (Di Paolo et al., 2002), synaptojanin (Hardies et al., 2016), syndapin 1 (Koch et al., 2011) or the synaptobrevin/VAMP2 adaptor AP180 (Koo et al., 2015) in mice results in excitatory/inhibitory imbalance and seizures. Mutations in synaptojanin 1 have also been associated with early-onset Parkinsonism and generalized seizures in humans (Krebs et al., 2013). Moreover, recent work from us has uncovered that a missense mutation in one of the subunits of the AP-2 causes developmental and epileptic encephalopathy in children (Helbig et al., 2019). How exactly epilepsy arises in this case is unknown but may conceivably involve the missorting of SV proteins such as the vesicular GABA transporter, a known AP-2 cargo at inhibitory synapses.

The VAMP/synaptobrevin adaptors AP180 and CALM (PICALM) have also been genetically associated with Alzheimer's disease (AD) (Gusareva et al., 2014). CALM expression is inversely correlated with levels of phospho-tau and the autophagosomal marker LC3 in the AD brain (Ando et al., 2016), where it may function to prevent the amyloid β generation by promoting the trafficking and autophagic degradation of crucial components of amyloidogenic pathway (Kanatsu et al., 2014; Tian et al., 2013; Zhao et al., 2015) and/or by regulating the sorting of endolysosomal VAMP/synaptobrevin required for functional autophagy (Moreau et al., 2014). Autophagosomes, in addition to their canonical role in degradation in all cells, may also promote survival by carrying neurotrophin signals to the neuronal soma (Deinhardt et al., 2006). We have shown that in neurons, the endocytic adaptor AP-2 serves an additional nonendocytic function in retrograde transport of neurotrophin-containing autophagosomes that depends on its ability to associate with autophagosome proteins and dynein motor proteins. Neuron-specific AP-2 knockout mice suffer from severe neurodegeneration and reduced neuronal complexity (Kononenko et al., 2017). AP-2 also may play a role in AD by promoting the degradation of BACE1, a protease known to function in amyloidogenic pathway to generate the toxic amyloid β isoforms (Bera et al., 2020) (see Figure 2). Finally, sorting nexins such as SNX27, an endosomal BAR domain protein associated with the retromer complex that acts downstream of endocytic clathrin adaptors, counteract neurodegeneration in PD by facilitating the recycling of receptors such as AMPA- and NMDA-type glutamate and serotonin receptors (Gallon et al., 2014;

McMillan et al., 2016; Patel et al., 2018). Thus, endocytic sorting adaptors may counteract neurodegeneration by additionally promoting protein turnover via autophagy, while endosomal sorting adaptors such as SNX27 maintain synaptic function via recycling membrane cargo. We predict that future studies will uncover further novel associations between SV sorting adaptors and neurological disorders ranging from epilepsy and autism to neurodegeneration.

Glossary

AP-2	Adaptor protein complex-2
AZ	Presynaptic active zone
Cargo	Proteins and lipids taken up from the plasma membrane and trafficked within the call
CME	Clathrin-mediated endocytosis, a pathway canonically used by cells for uptake of nutrients
GABA	γ-Aminobutyric acid
LC3	Microtubule-associated protein 1 light chain 3
Membrane retrieval	Endocytosis of plasma membrane
SV	Synaptic vesicle

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Review article

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Mechanisms of synaptic vesicle recycling provide a platform to explore mechanisms of neurodegeneration

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Abstract: The synaptic vesicle (SV) cycle, a trafficking pathway by which SV fuses with the plasma membrane to release neurotransmitters at the neuronal synapse, resides at the heart of neurotransmission. SV fusion consumes vesicle membrane and proteins, whose availability is limited, and these components must be recycled quickly to prevent synaptic fatigue. Biochemical, genetic and physiological approaches over the past five decades have led to a discovery of a large directory of proteins and lipids central to the SV cycle and several models on how these constituents account for the synapse function. The complexity of the SV cycle is starting to be comprehended, which opens new perspectives for our understanding of neuronal physiology and provides mechanistic explanations for several neurological and neurodegenerative diseases. Here, selected classic and recent insights into the mechanisms of two key SV trafficking steps (exocytosis and endocytosis) are reviewed, as well as their links to selected brain pathologies.

Keywords: drug targets; endocytosis; exocytosis; lipids; neurodegeneration; neurological diseases; PI(4,5)P2; synaptic vesicle cycle; vesicle acidification.

Zusammenfassung: Der synaptische Vesikelzyklus ist ein Transportweg, in dessen Verlauf die synaptischen Vesikel (SV) mit der Plasmamembran verschmelzen und Neurotransmitter an der neuronalen Synapse freisetzen. Er steht im Zentrum der Neurotransmission. Die SV-Fusion verbraucht die Vesikelmembran und Proteine, deren Verfügbarkeit begrenzt ist, so dass diese Komponenten schnell recycelt werden müssen, um eine synaptische Ermüdung/Erschöpfung zu verhindern. Biochemische, genetische und physiologische Ansätze der Grundlagenforschung haben in den letzten fünf Jahrzehnten die zentrale Bedeutung des SV-Zyklus für die Synapsenfunktion dokumentiert und zu einer Reihe von Modellen wie am Zyklus beteiligte Proteine dazu beitragen geführt. Die Komplexität des Zyklus wird allmählich verstanden, was neue Perspektiven für unser Verständnis der neuronalen Physiologie eröffnet und mechanistische Erklärungen für verschiedene neurologische und neurodegenerative Krankheiten liefert. Hier werden klassische und neuere Erkenntnisse über die Mechanismen von zwei wichtigen Phasen des SV-Zyklus, Exozytose und Endozytose, sowie ihre Verbindungen zu ausgewählten Hirnpathologien untersucht.

Schlüsselwörter: Endozytose; Exozytose; Identifizierung potentieller Wirkstoffe; Lipide; Neurodegeneration; neurologische Erkrankungen; PI(4,5)P2; synaptischer Vesikelzyklus; Vesikel Azidifizierung.

Introduction and objectives

As a result of membrane-centred organization of life, cells have acquired routes by which biomolecules are shuffled between compartments or released to the extracellular space by exocytosis. For neuronal cells, which externalize signalling molecules (neurotransmitters and peptides) quickly and on demand, this process is typically initiated by an elevation in the intracellular calcium concentrations. Neurotransmission capitalizes on the existence of synaptic vesicles (SVs) that store neurotransmitters and define the properties of neuronal synapses, like quantal release, signal directionality and modulation. Since small presynaptic boutons can store limited amounts of SVs, proteins and membranes used during exocytosis must be swiftly recycled: accumulated SVs would be quickly consumed without the existence of robust mechanisms for the local (re)formation of new vesicles (Milosevic, 2018; Saheki and De Camilli 2012). For continuous synaptic function, it is critical that the activity-enforced demands are matched precisely by the local membrane recycling mechanisms.

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It is now recognized that the SV cycle is much more complex than previously thought (Chanaday et al., 2019). However, basic curiosity-driven research over the past 50 years has led to immense breakthroughs towards understanding synaptic physiology and functions of lipids and proteins central to the SV cycle. The body of knowledge that even subtle imbalances in neurotransmitter release or alterations in SV recycling result in disorders is increasing (e.g. Hussain et al., 2019; Lauwers et al., 2016; Li and Kavalali, 2017; Soukup et al., 2018; Verhage and Sørensen, 2020). With this, the opportunities to build on the basic mechanisms of synapse function to tackle complex brain diseases in novel ways are also rising and suggest a dawn of a new era. Dr Armin Schram once stated 'Where funding is scarce, progress and thus the well-being of people is best served in the long term by funding basic research'. This visionary perspective is likely true for many fields of research, but it resonates particularly well with the present stage of synaptic transmission research where we start capitalizing on basic knowledge to bring major benefits to medicine.

The objective of this review is to summarize the selected features of presynaptic release and SV recycling components, both lipidaceous and proteinaceous, and provide examples where the mechanistic insights into this field may be of use to tackle pathologies of selected neurological and neurodegenerative diseases.

Exocytosis at the presynaptic bouton – a protein view

At the synapse, exocytosis comprises directed translocation of SVs to the active zone of the presynaptic bouton, the contact with the plasma membrane (termed 'docking'), the preparation of SVs for the fusion (termed 'priming') and the calcium-triggered fusion of membranes, resulting in the release of neurotransmitters (Sudhof, 2013). This fast and coordinated process builds on dozens of proteins and lipids, yet the main role is played by the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins, a minimal machinery for the fusion of lipid bilayers (Milosevic and Sørensen, 2015; Sudhof, 2013). Synchronized by the calcium influx through voltage-gated channels, SNARE complexes made between vesicular Vesicle Associated Membrane Protein 2 (VAMP2)/synaptobrevin-2 and the plasma membrane–resident synaptosomal-associated protein 25 (SNAP-25) and syntaxin-1 pull the respective membranes into a close contact and lead to 'zippering' of their SNARE domains (Sørensen et al., 2006). Due to the need for high spatial and temporal coordination of synaptic activity, SNARE proteins are controlled at several steps of their generation and function (Milosevic and Sørensen, 2015). This includes the formation of the SNARE complex by activating syntaxin-1 bound to Sec1/Munc18 (SM)-like proteins (de Wit et al., 2009; Lai et al., 2017; Rizo and Sudhof, 2012; Shen et al., 2007). Next, the Munc13 proteins mediate the opening of syntaxin-1 and sequential binding of synaptobrevin-2/VAMP2 and SNAP-25 (Lai et al., 2017). A half-zippered (intermediate) SNARE complex is a characteristic of the primed SV (Sørensen et al., 2006), ready to be released when the final stage of the SNARE assembly is initiated by a calcium sensor from the synaptotagmin family, following the influx of calcium and promoting fusion of the SV with the plasma membrane (Sudhof, 2012, 2013).

SNARE-regulating proteins are vital for the exquisite regulation of exocytosis at the synapse. Hence, it is not surprising that the pathogenic mutations in the SNARE regulators have been described (Verhage and Sørensen, 2020). Notably, some of these proteins function exclusively as inhibitors of exocytosis, e.g. tomosyns/STXBP5 and amisyn/STXBP6. Tomosyn-1, a WD40-repeat protein, is thought to suppress synaptic transmission by inhibiting SV docking and priming (Ben-Simon et al., 2015; Fujita et al., 1998; Gracheva et al., 2006; Yizhar et al., 2004). Amisyn interferes with the priming of secretory vesicles, likely as a vertebrate-specific competitor of synaptobrevin-2/VAMP-2 (Constable et al., 2005; Kondratiuk et al., 2020). A recent study has shown that amisyn contains an N-terminal pleckstrin-homology domain that mediates its transient association with the plasma membrane by binding to lipid phosphatidylinositol-4,5-bisphosphate ($PI(4,5)P_2$; Kondratiuk et al., 2020). The importance of the negative regulation of exocytosis exerted by both amisyn and tomosyn-1 is underscored by their association to neurodevelopment and neurological diseases, including autism-spectrum disorder (ASD; Castermans et al., 2008; Cukier et al., 2014; Davis et al., 2009). Understanding the mechanisms by which such negative regulators interfere with neuronal exocytosis will advance our comprehension of synaptic physiology during different stages of brain development as well as pathological processes and may open new avenues for diagnostics and treatments of disorders such as ASD.

Exocytosis at the presynaptic boutons – a lipid view

In parallel with the discovery of the proteinaceous machinery, the lipid requirements for the SNARE-mediated

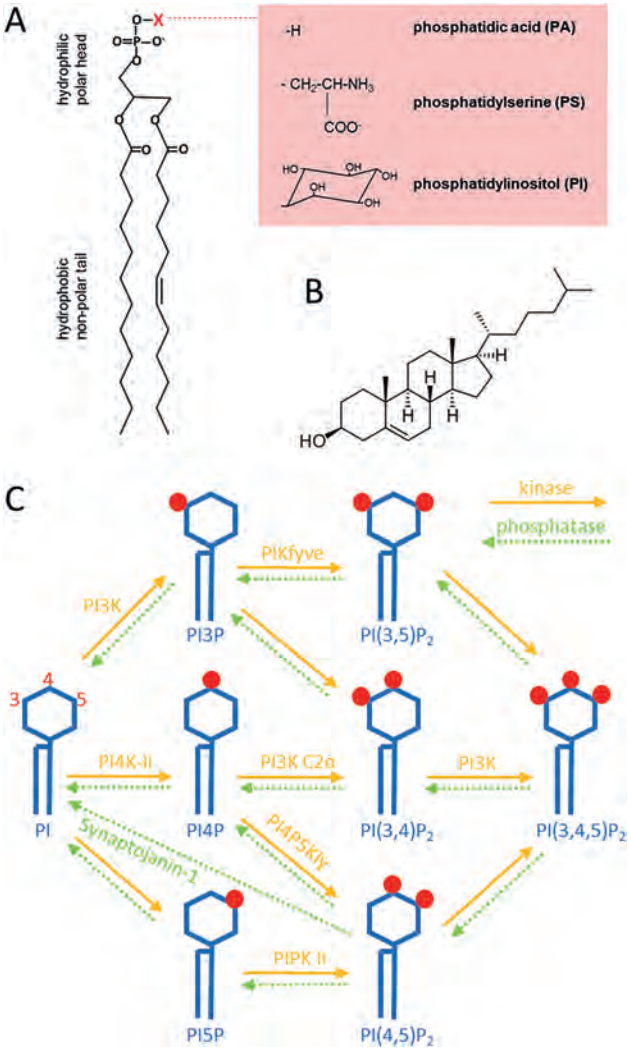

Figure 1: Structure and metabolism of key lipids involved in synaptic vesicle recycling. (A and B) Structure of lipids detailed in this study, i.e. glycerophospholipids (A) and cholesterol (B). (C) The phosphatidylinositide cycle, depicting the biosynthesis of phosphatidylinositides: phosphatidylinositol (PI) is the precursor of all phosphatidylinositides, the head groups of which have a different number of phosphate groups. The individual phosphatidylinositides are maintained at steady-state levels by continuous phosphorylation and dephosphorylation reactions performed by kinases and phosphatases.

exocytosis were emerging. Lipids are the core components of the fusing membranes; thus, changes in their composition, abundance or localization promptly modify the intrinsic fusogenic properties of membranes (Figure 1A and B). Lipids also activate and recruit proteins to the local environments where exocytosis happens (Chasserot-Golaz et al., 2010).

The discovery of Hokin and Hokin (1953) that the secretory cell stimulation leads to an increased production of phosphatidylinositides (PIs) has triggered the characterization of PI roles in exocytosis. The research is still ongoing, in part due to the PI versatility and rapid turnover (Milosevic and Sørensen, 2015). $PI(4,5)P_2$ is the main PI with a role in regulated exocytosis (Figure 1C) (Aikawa and Martin, 2003; Milosevic et al., 2005). Several-decade-long work on this topic resulted in a model in which PIs are delivered to the vesicle membrane via phosphatidylinositol transfer proteins (PITPs), phosphorylated to PI(4)P by vesicular protein phosphatidylinositol-4-kinase (PI4K-II), and then converted to $PI(4,5)P_2$ by phosphatidylinositol-4-phosphate-5-kinase Iγ (PI4P5KIγ) recruited from the cytoplasm (Figure 1C) (Hay et al., 1993; Martin, 2012; Milosevic and Sørensen, 2015). The production of $PI(4,5)P_2$ by PI4P5KIγ is tightly regulated by calcium, phosphorylation, a small GTPase Arf6 and phosphatidic acid (PA; Figure 1A), a product of phospholipase D activity (Aikawa and Martin, 2003; Jang et al., 2012; Martin, 2012). Further, an increase in the plasmalemmal $PI(4,5)P_2$ level led to a larger pool of primed vesicles and potentiated exocytosis, whereas reduction in $PI(4,5)P_2$ reduced exocytosis, demonstrating that the balance between the plasmalemmal $PI(4,5)P_2$ generation and degradation rates regulates vesicle priming (Milosevic et al., 2005). The mechanisms of how $PI(4,5)P_2$ drives the recruitment of secretory vesicles have been reported (Honigmann et al., 2013). Also, many exocytic proteins are known to interact with this phospholipid: $PI(4,5)P_2$ was also associated with vesicle docking and fusion based on these interactions. Namely, $PI(4,5)P_2$ binds to calcium-dependent activator protein for secretion (CAPS) and synaptotagmin-1 (Bai et al., 2004; Loyet et al., 1998), as well as Rab3 effector rabphilin 3 (Chung et al., 1998) and Mints (Okamoto and Sudhof, 1997). This dynamic interplay between $PI(4,5)P_2$ and exocytic proteins creates opportunities for therapeutic interventions to tackle exocytic defects.

Besides $PI(4,5)P_2$, its hydrolysis products and other PIs have a role in exocytosis and may function as diagnostic markers for selective brain disorders. Phospholipase C–driven hydrolysis of $PI(4,5)P_2$ results in diacylglycerol production needed for secretory vesicle priming through Munc13's activation and an opening of syntaxin-1 (Bauer et al., 2007). A FYVE finger-containing phosphoinositide kinase PIKfyve generates $PI(3,5)P_2$ from PI(3)P to inhibit exocytosis (Figure 1C) (Osborne et al., 2008), while PI(3)P itself seems to promote exocytosis (Meunier et al., 2005). Lastly, $PI(3,4,5)P_3$ also influences syntaxin-1 clustering and neurotransmission at the synapse (Khuong et al., 2013).

Cholesterol and phosphatidylserine (PS) are considered to play a role in spatial organization of exocytic sites

(Figure 1A and B) (Ammar et al., 2013). PS, mainly present in the inner plasma membrane leaflet, is needed for binding of synpatotagmin-1 and hence for triggering fusion (Ory et al., 2013). Depletion of cholesterol inhibits exocytosis and alters SNARE protein clusters (Lang et al., 2001). In addition to the aforementioned PI4P5KI*γ* regulation, PA produced by phospholipase D1 under the secretory vesicle recruits syntaxin-1 and contributes to fusion (Jang et al., 2012; Zeniou-Meyer et al., 2007). Finally, fatty acids, like omega-3, omega-6 and arachidonic acid, seem to stimulate the SNARE complex formation (Darios et al., 2007), while arachidonic acid also supports docking (Garcia-Martinez et al., 2013).

Due to fast turnover, various lipid species have a potential to rapidly adapt synaptic functions. As the knowledge on the roles of lipids in brain pathologies is emerging (e.g. Hussain et al., 2019; Lauwers et al., 2016), the interplay between exocytosis and lipids will likely attract more attention in the future, in the context of neurological and neurodegenerative diseases. This is another example of how the mechanisms unveiled by 'basic science' are starting to be employed to explain, diagnose or treat pathologies.

Endocytosis at the presynaptic bouton – a protein view

Almost five decades after a suggestion that the SVs are formed and recycled locally at the synapse (Heuser and Reese, 1973), the mechanisms of endocytosis are still debated (Watanabe and Boucrot 2017). Several mechanisms for SV endocytosis are likely at play: clathrin-mediated endocytosis, ultrafast endocytosis, bulk endocytosis in the case of high synaptic activity and, possibly, a brief and transient SV contact the plasma membrane through a fusion pore (called 'kiss-and-run') (Chanaday et al., 2019; Watanabe and Boucrot 2017). Even in the case of ultrafast and bulk endocytic forms, the subsequent clathrin-mediated endocytosis is considered to be key for the formation of homogeneously sized SVs with defined protein and lipid compositions (Watanabe and Boucrot 2017). The machinery involved in clathrin-mediated endocytosis is complex but well studied (Milosevic, 2018), often with a focus on clathrin and adaptor proteins (e.g. AP-2, AP180), as reviewed by Kononenko and Haucke in this issue, or dynamin protein family needed for vesicle scission (Ferguson and De Camilli, 2012). The endocytic process also depends on the action of the Bin/Amphiphysin/RVS (BAR) superfamily members involved in lipid bilayer deformation and reshaping (Milosevic, 2018). These proteins induce membrane curvature, stabilize curvature generated by other forces and/or recruit cytoplasmic proteins to membranes of a particular shape. Hallmarks of the BAR superfamily are endophilins-A, a family of evolutionarily conserved proteins for sensing and generating membrane curvature (Saheki and De Camilli, 2012). Endophilins-A recruit the phospholipid phosphatase synaptojanin-1 to the bud necks prior to fission by the GTPase dynamin (which also interacts with endophilin-A, but can act independently of it; Milosevic et al., 2011). Actions of endophilin and synaptojanin-1 are needed to promote vesicle uncoating by recruiting DnaJ protein auxilin, as detailed below (Milosevic et al., 2011).

Despite the fact that the coupling between endocytosis and exocytosis has been evolutionary perfected (Gundelfinger et al., 2003), two processes are studied mainly independently. However, it is essential that newly added SV proteins and lipids are rapidly removed from the fusion sites so that the new SV can dock and fuse there (Hosoi et al., 2009). Hence, exocytosis and endocytosis should be studied concomitantly whenever feasible.

Endocytosis at the presynaptic bouton – a lipid view

Similar to their important role in exocytosis, PIs have an essential role in endocytosis. Many endocytic proteins have domains that recognize particular PIs, and the changes in PI levels profoundly affect many cellular activities, including endocytosis (Falkenburger et al., 2010). For example, adaptor proteins (e.g. epsin, AP-2, AP180, Hip1) are recruited in part by $PI(4,5)P_2$. Besides being needed for exocytosis, $PI(4,5)P_2$ also has a central role in endocytosis (Posor et al., 2015). Specifically, $PI(4,5)P_2$ participates at the several steps of the clathrin-mediated endocytosis: cargo sorting and coat recruitment (through adaptor proteins), endocytic pit fission (through dynamins) and vesicle uncoating (through endophilins and synaptojanin-1) (Cremona and De Camilli, 2001; Posor et al., 2015; Saheki and De Camilli, 2012). This dual role of $PI(4,5)P_2$ in exocytosis and endocytosis has been a basis for a model in which a PI cycle underlies the SV cycle (Figure 2; Cremona and De Camilli, 2001).

Another PI besides $PI(4,5)P_2$, phosphatidylinositol-3,4-bisphosphate [$PI(3,4)P_2$], formed by phosphatidylinositol-3-kinase C2*α*, is employed in clathrin-mediated endocytosis (Figure 1C) (Posor et al., 2013). Timed generation of $PI(3,4)P_2$ is a base for an enrichment of sorting

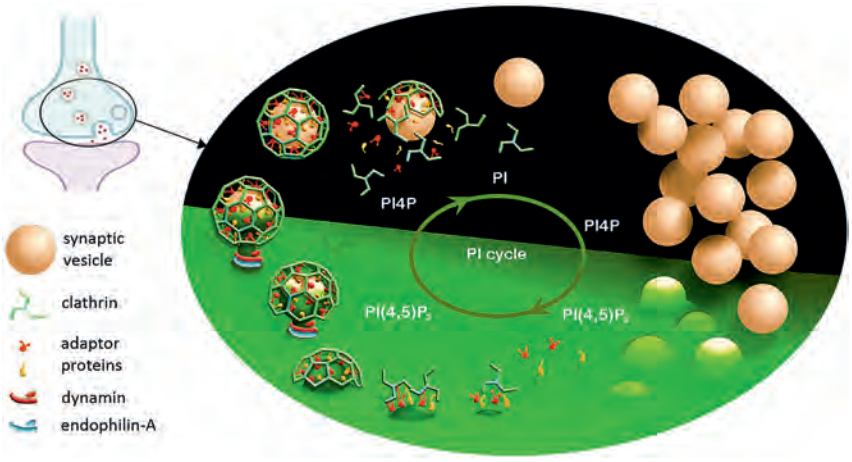

Figure 2: The model of a phosphoinositide cycle nested within the synaptic vesicle cycle. Links between membrane traffic at the synapse and PI(4,5)P_2 synthesis and dephosphorylation.

nexin 9 (SNX9), a BAR protein, and clathrin-coated pit progression during endocytosis (Posor et al., 2013, 2015). Lately, the interplay between endocytosis and lipids is raising attention in the context of neurological and neurodegenerative diseases (Hussain et al., 2019).

Synaptic vesicle recycling and the aetiology of Parkinson's disease

For decades, defects in synaptic transmission have been implicated in neurodegeneration. Neurodegenerative diseases impose a significant burden on families and societies and will become of greater concern as life expectancy increases and populations around the world continue to age. Notably, ageing is the primary risk factor for most neurodegenerative diseases, including Parkinson's disease (PD) used here as an example. PD is a debilitating, progressive, age-related disorder affecting ~2% of the population over 65 years (this number is expected to double by 2030; Pilsl and Winklhofer, 2012). The pathology of PD is characterized by degeneration and death of dopaminergic neurons in the relevant brain region, i.e. the substantia nigra, and by formation of α-synuclein and ubiquitin-positive Lewy body aggregates, causing neurological impairments (Poewe et al., 2017). Exactly what causes the degeneration and loss of these neurons is unknown. Emerging consensus points that at least some forms of PD are mediated by ubiquitin-proteasome pathway disruption and mitochondrial dysfunction (Youle and van der Bliek, 2012), yet these have not lent themselves to straightforward characterization of specific mechanisms thus far.

Curiously, several reports link endocytosis to PD and neurodegeneration (e.g. Cao et al., 2017; Edvardson et al., 2012; Krebs et al., 2013; Matta et al., 2012; Milosevic et al., 2011; Murdoch et al., 2016; Shi et al., 2009; Trempe et al., 2009). Several case studies described mutations in two clathrin uncoating factors, synaptojanin-1 and auxilin, as a cause of early-onset PD (Edvardson et al., 2012; Krebs et al., 2013). Auxilin is recruited to the clathrin-coated vesicles directly after action of synaptojanin-1, a phospholipid phosphatase with an identified PD-causing mutation (Figure 1C), which is found to impair clathrin uncoating and trigger dystrophic changes in dopaminergic axons (Cao et al., 2017). Synaptojanin-1 is brought to the neck of clathrin-coated pits by endophilin-A, an endocytic adaptor with membrane curvature–sensing and curvature-generating properties (Milosevic et al., 2011). Interestingly, endophilin-A itself is linked to PD and neurodegeneration: its levels are altered in the cortex of PD patients and associated with PD progression (Shi et al., 2009), and it directly interacts with two hallmark PD proteins, the E3 ubiquitin ligase Parkin (Trempe et al., 2009) and the leucine-rich repeat kinase LRRK2 (Matta et al., 2012), the most commonly disrupted gene in familial PD. A partial loss of endophilin in mice results in progressive neurodegeneration, ataxia and premature death (Milosevic et al., 2011). It also alters neurotransmission, SV recycling and SV acidification; elevates Parkin levels; induces the FoxO3a-Fbxo32 network in the brain and causes dysregulation of autophagy and the ubiquitin-proteasome system (Cao et al., 2014; Milosevic et al., 2011; Murdoch et al., 2016). Altogether, it is striking that several genes and proteins directly or indirectly related to endocytosis provide a mechanistic clarification to the pathology underlying PD that is expected to be further explored in the coming years.

One of the greatest challenges at present is to identify markers for neurodegenerative disease stages, which would allow PD and other neurodegenerative

disease–modifying therapies to be started earlier (Lauwers et al., 2016; Poewe et al., 2017). The presynaptic lipidaceous and proteinaceous components are appealing putative diagnostic and/or therapeutic targets since they allow for dynamic adjustments of neurotransmission, either by potentiation or inhibition. The goal in the latter case is not to completely inhibit but rather introduce subtle modifications of the system that counter disease mechanisms without interfering with the postsynaptic receptor signalling. Like this, the synaptic transmission accuracy could still be maintained at the postsynaptic side. SV cycle components may also present targets for drug screens and/or designs in a way that synaptic transmission is selectively altered in subtle ways that alleviate disorder symptoms but have limited side effects. Such approaches will likely intensify in the near future, building on the extensive knowledge already available.

In conclusion, our advanced understanding of SV cycle, synaptic transmission mechanisms and their emerging links to pathogenesis of several neurodegenerative and neurological disorders made it timely to build on the 'basic science' findings to explore new diagnostic markers and treatments. Reciprocally, studies on how pathological mutations modify presynaptic functions will also advance molecular characterizations of the SV cycle. Dr Schram's view that the support of 'basic science' will lead to the greater good is starting to pay off.

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Bionote

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Ira Milosevic obtained her Diploma in Molecular Biology from the University of Zagreb, Croatia, in 2001. She completed her MSc and PhD studies on the exocytic mechanisms within the International Max Planck Research School (IMPRS) at the Georg August University Göttingen, Germany, in 2003 and 2006, respectively. After that, she studied endocytosis at the neuronal synapse with Pietro De Camilli at the Yale University School of Medicine in New Haven, CT, USA. Supported by an Emmy Noether Young Investigator Award, Ira started her independent research at the European Neuroscience Institute (ENI) in Göttingen in 2013. Her present interests include the mechanism of synaptic transmission and pathological aspects of synaptic vesicle recycling that lead to neurodegeneration.

Review article

Alexander Gottschalk*

Optogenetic analyses of neuronal networks that generate behavior in *Caenorhabditis elegans*

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Abstract: In compact brains, circuits consisting of few neurons fulfill functions of entire brain systems in mammals. Thus, studying these small circuits can provide insights and guidelines also for the study of the human brain. We developed methods and approaches to use optogenetics in the nervous and neuromuscular system of the nematode *Caenorhabditis elegans*. These include single-cell expression and/or photoactivation of optogenetic tools, to control the function of individual neurons, and behavioral, electrophysiological or electron microscopic analyses of circuit function and synaptic transmission. We studied a number of circuits involved in locomotion, navigation and food searching; we addressed new genes in synaptic vesicle recycling, and we identified a novel pathway of neuromodulatory presynaptic plasticity. In our laboratory, support by the Schram foundation allowed me to explore new avenues of research especially during the early years of my career.

Keywords: behavior; connectome; electron microscopy; electrophysiology; optogenetics; synaptic transmission.

Zusammenfassung: In kompakten Nervensystemen übernehmen Schaltkreise aus einigen wenigen Neuronen die Funktionen ganzer Hirnsysteme in Säugetieren. Daher kann die Untersuchung solcher kompakter Gehirne Leitlinien auch für die Untersuchung des menschlichen Gehirns liefern. Wir haben optogenetische Methoden für die Untersuchung des neuromuskulären Systems des Nematoden *Caenorhabditis elegans* entwickelt, u.A. für die Expression und Aktivierung von optogenetischen Werkzeugen in einzelnen Nervenzellen, sowie für Verhaltens-, elektrophysiologische und elektronenmikroskopische Analysen. Mit diesen Methoden untersuchten wir eine Reihe von Schaltkreisen für die Bewegung, Navigation und Futtersuche. Wir analysierten außerdem verschiedene Gene mit Funktionen im Recycling von synaptischen Vesikeln, und identifizierten einen neuen Mechanismus der neuromodulatorischen Plastizität. Die Schram Stiftung half bei der Implementierung dieser Projekte und der Entwicklung neuer Forschungsgebiete in meinem Labor.

Schlüsselwörter: Elektronenmikroskopie; Elektrophysiologie; Konnektom; Optogenetik; synaptische transmission; Verhalten.

Introduction

The nematode *Caenorhabditis elegans* has a compact nervous system of 302 neurons, whose connectivity has been determined by serial electron microscopy (EM) in the 70s and 80s of the twentieth century, and the data were revisited last year (Cook et al., 2019; White et al., 1986). Also, new connectomes, acquired with modern EM techniques, across development of the animal, have been determined, providing comprehensive 'wiring diagrams' of the *C. elegans* brain (Witvliet et al., 2020). This information forms a basis to the understanding of the function of neuronal circuits in the generation of behavior of 'the worm.' Due to the compactness of the *C. elegans* nervous system, single neurons often need to fulfill the function of entire circuits in higher animals, though at a lower level of complexity. Until recently, the function of individual *C. elegans* neurons was inferred from animals in which these neurons were eliminated by laser ablation and by observing the altered behaviors that resulted from the loss of the neuron. More recent methods to address the loss of (single, or entire classes of) neurons involve expression of caspases (Chelur and Chalfie, 2007) or other proteins that affect cell viability or neuronal function, like constitutively active mutated potassium channels (Kunkel et al., 2000) or photosensitizers, that generate toxic reactive oxygen species upon illumination (Qi et al., 2012). Also, optogenetic methods allow the interference with neuronal function, for example, *via* light-activated anion channels or

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ion pumps, that cause hyperpolarization during illumination (Bergs et al., 2018; Govorunova et al., 2015; Zhang et al., 2007), or inactivators of the synaptic machinery for transmitter release, like photoactivated botulinum neurotoxin (PA-BoNT) (Liu et al., 2019). Of course, also light-triggered activators like channelrhodopsin (ChR2) are used to probe neuronal function in *C. elegans* (Fang-Yen et al., 2015; Nagel et al., 2005).

Supported in part by the Schram foundation, my lab developed integrated approaches of using optogenetics, electrophysiology, imaging and behavioral analysis, as well as electron microscopy, to analyze functional neuronal networks that generate behavior, the molecules they use and also, how synaptic transmission is achieved at the physiological, ultrastructural and molecular level (Figure 1). Paradigmatically, the potential of the *C. elegans* system will be discussed in this overview article.

Single-neuron photoactivation in free-moving animals

Distinct neuronal connections that are visible in the wiring diagram can be explored by the methods described above, provided one can express or activate the tools in a cell-specific manner. Optogenetics enables this to some extent, e.g. by a special microscope and tracking system that can illuminate specific regions of the *C. elegans* body, thus restricting illumination in time and space, but also in different light colors, to cells of interest (Stirman et al., 2011) (Figure 2).

Nociceptive neurons and molecules acting within them

This methodology was used to probe a network of neurons downstream of, and the function of ion channels acting within, a nociceptor neuron termed PVD (Husson et al., 2012) (Figure 3). PVD is a harsh-touch sensor that evokes escape behavior, allowing the animal to avoid harmful stimuli. For the size of *C. elegans*, PVD is a huge neuron, covering almost the entire body with a complex, branched dendritic arbor. *C. elegans* was until recently believed to transmit electrical signals passively (Bargmann and Kaplan, 1998; Liu et al., 2018). Yet, since a nociceptor neuron must act rapidly, the question arose whether PVD has specific mechanisms to ensure fast

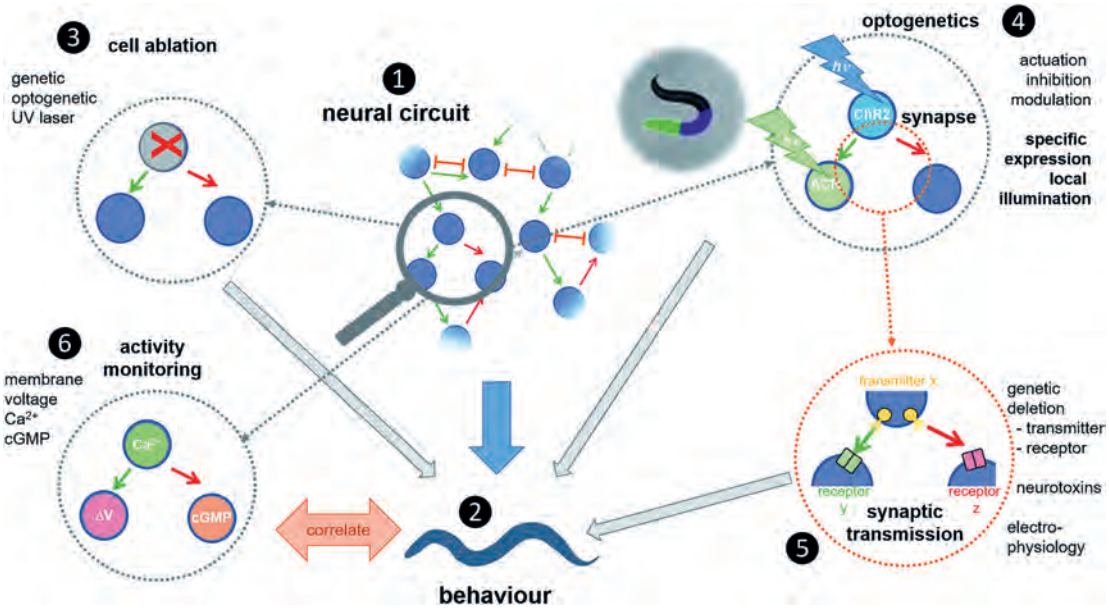

Figure 1: Functional connectomics approaches to study neural circuits and their role in generating behavior in the nematode *Caenorhabditis elegans*.
Circuits (1) drive behavior (2), and to understand the function of individual neurons in this, several approaches need to be combined: Cell ablation (3), to eliminate the neuron of interest; optogenetics (4), to stimulate, inhibit or modulate the neuron acutely (local illumination, inset from Stirman et al., 2012), and to understand its synaptic connections to its partners; synaptic details (5, e.g. transmitter and receptors used) need to be determined, by blocking transmission or by electrophysiology. Experiments to monitor evoked or intrinsic activity, correlated with behavior, involve imaging of second messengers and/or membrane voltage (6). Manipulations in (3–5) affect behavior, and information gathered in all approaches can be combined in models to enable a comprehensive understanding of the circuit.

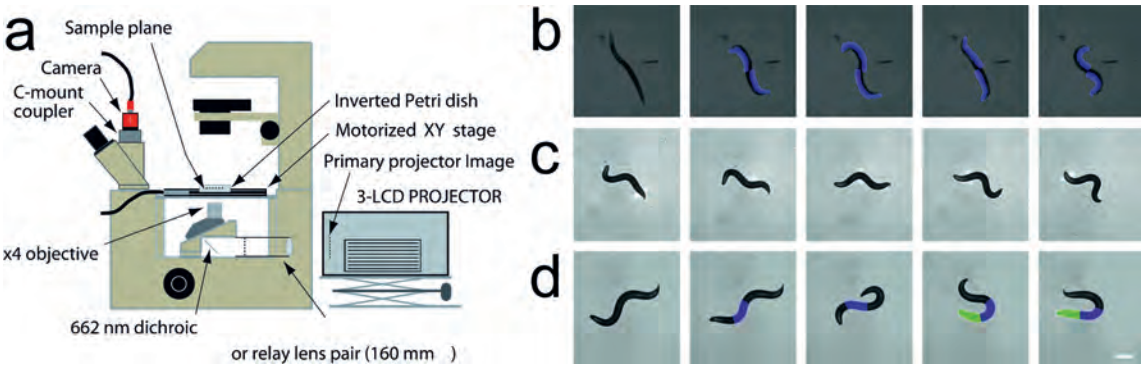

Figure 2: A tracking and illumination system for specific optogenetic manipulation of neurons in freely moving *C. elegans*. (a) Microscope setup, including a projector used to shine light patterns into the microscope, based on the body posture of the animal, obtained from a live video, while the animal is automatically tracked. (b–d) Example geometries, light colors and stimulus sequences to free-moving animals. In (b), this animal, expressing Channelrhodopsin (ChR2) in its body wall muscles, has been paralyzed by addition of ivermectin, which hyperpolarizes motor neurons. The animal can be forced to move by alternating illumination of anterior and dorsal halves, on opposing sides of the body, thus photodepolarizing muscle cells in a localized fashion. In (c), a bar of light is moved from tail to head, which hits ChR2 expressed in mechanosensory neurons, evoking forward locomotion. Once it reaches the anterior half, reverse locomotion results. In (d), the animal expresses ChR2 for photodepolarization in the mechanosensory neurons, and the green light activated hyperpolarizing proton pump Mac in interneurons that normally mediate reverse locomotion. First, blue light evokes a reversal, which is inhibited by concomitant green illumination of the head, in this optogenetic 'circuit-breaker' experiment. From Stirman et al. (2011, 2012).

electrical transmission from the most distal dendritic branches to the axon and synaptic terminals. Optogenetics and RNAi were used to identify ion channels expressed in PVD that may act in the propagation of electrical signals. This led to the identification of two channels, which either amplify signals within PVD (i.e., GTL-1, a TRPM channel) or enhance its output at the synaptic terminal (i.e., ASIC-1, an acid-sensing ion channel) (Figure 3a). Do such channels contribute to mechanisms of habituation in sensory systems? This may be assessed by voltage imaging or electrophysiology. The PVD neuron can be repeatedly photostimulated and always generates a strong behavioral response, as expected for a nociceptor. Similar experiments using gentle-touch neurons demonstrated a profound habituation, as these (repeated) signals represent irrelevant stimuli (Figure 3b and c). Interestingly, PVD optogenetic stimulation would evoke forward escape behavior, while harsh mechanical stimuli mostly evoked reversal escape. The wiring diagram shows synapses from PVD to both forward and reverse pre-motor interneurons, PVC and AVA, respectively (Figure 3d). By ablation of PVC, the response to optogenetic activation of PVD was switched from forward to reverse escape behavior, thus showing that the PVD-PVC synapses are functional (Figure 3e). Thus, during mechanical stimulation, PVD signals and signals from other touch sensors are integrated in the interneuron circuit to determine whether forward or reverse escape behavior is most appropriate.

Single-cell expression of optogenetic tools

Often, neurons in *C. elegans* are located close to each other in the dense nerve ring or ventral nerve cord, thus precluding achieving single-neuron activation or inhibition by spatiotemporally restricting light and optogenetic tools. We thus implemented conditional expression of optogenetic tools (Schmitt et al., 2012), based on methods for cell-specific, conditional expression from two promoters, overlapping in the cell of interest (Figure 4), as established by several labs (Davis et al., 2008; Macosko et al., 2009).

Analysis of neuronal circuits in the generation of behavior

Neuronal circuits as defined by the anatomical connections of chemical and electrical synapses appear hard-wired and thus may be very limited in their activities and potential computations. However, even the genetically determined *C. elegans* circuits are plastic and are not restricted to a single, hard-wired connectivity. Instead, an additional 'wireless' network of neuromodulators is in effect that can alter the function of neurons or even individual synapses such that several functional networks can be superimposed on a single anatomical network (Bargmann and Marder, 2013).

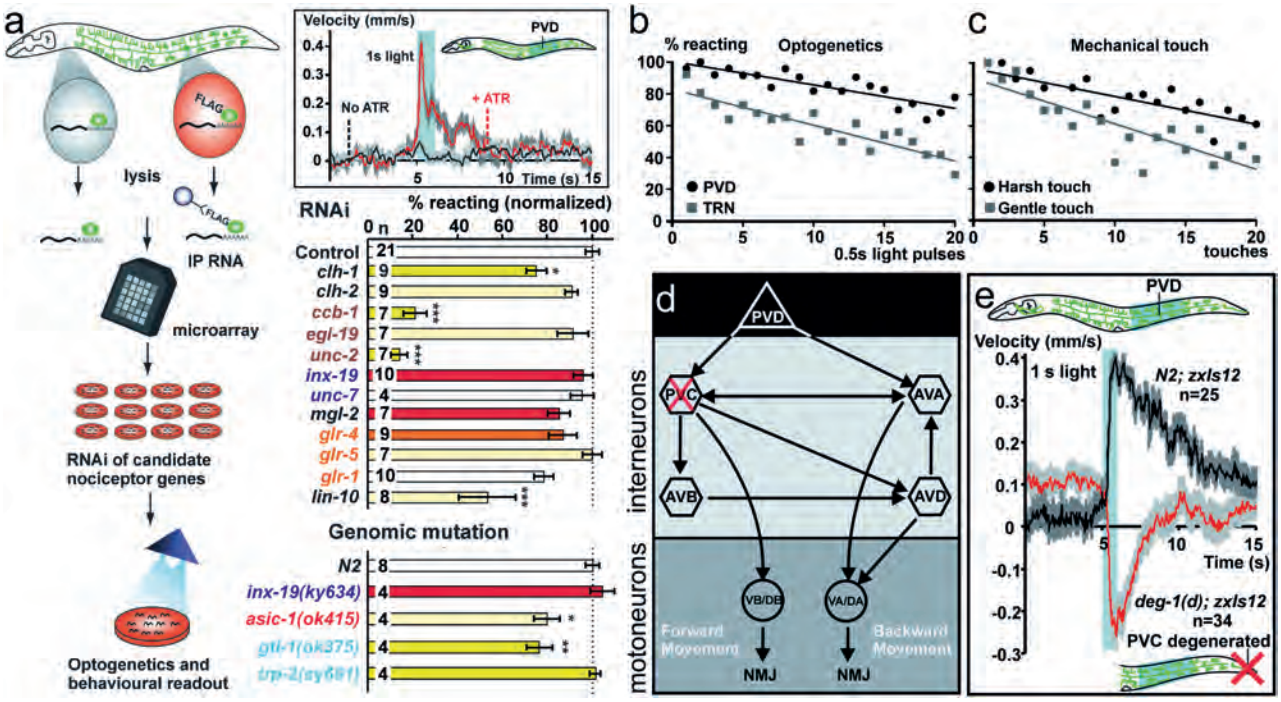

Figure 3: A reverse genetic screen for modifiers of nociceptive signaling. (a) *C. elegans* possesses nociceptive neurons, normally responding to harsh touch, termed PVD. To identify ion channels acting in these neurons, downstream of the nociceptive mechanoreceptors, mRNAs were isolated from PVD by our collaborator, David Miller (Vanderbilt University, Nashville, USA), and identified by microarray analysis. We used double-stranded RNA-mediated interference (RNAi) to knock down candidate genes and analyzed the optogenetically evoked activation of PVD and subsequent escape behavior in these animals (inset, showing increased velocity when PVD is photostimulated via Channelrhodopsin [ChR2; red graph]; no response occurs when all-trans retinal [ATR] is absent, which renders ChR2 non-functional). This highlighted several ion channels (and other genes) required for PVD function. A transient receptor potential (TRP) channel, GTL-1, and an acid-sensing ion channel (ASIC-1) amplified signals or mediated their transmission following PVD photodepolarization. (b, c) Habituation of repeated, optogenetically (b) or mechanically (c) evoked escape behavior following PVD (harsh-touch) or gentle touch receptor neuron (TRN) activation. While gentle touch responses habituate, harsh touch responses (as threat signals) do not. (d) Network downstream of PVD neurons. PVD innervates both forward (PVC) and reverse (AVA) pre-motor interneurons, causing escape behavior. Interestingly, under normal conditions, the photostimulated PVD-evoked behavior is forward escape (as quantified in e). To show whether synapses to AVA are also functional, we genetically ablated PVC (red cross in d). These animals now showed backward escape responses (red graph). From Husson et al. (2012).

An antagonistic network of peptidergic neurons in the regulation of food-motivated behavior

One such network regulates *C. elegans* locomotion as well as navigation behavior, for example, when the animal encounters or searches food (Oranth et al., 2018) (Figure 5). Two peptidergic neurons, called 'AVK' and 'DVA', which have opposing influence on the motor nervous system, either enhance or reduce the extent of body bending and the rate of directional changes. This way, the animal can stay more local (i.e. when food is present), or can initiate long-range search behavior. Further, these two interneurons integrate information from sensory neurons, which detect the presence of food, and signal via dopamine and two opposing dopamine receptors in AVK and DVA, respectively. The neuropeptides released by these two neurons are either excitatory (DVA) or inhibitory (AVK). AVK function gets inhibited in the presence of food (or by optogenetic inhibition using halorhodopsin – NpHR), and the resulting disinhibition of motor neurons leads to increased body bending and dwelling behavior. Similar neuropeptides and dopamine regulate motivated behavior in mammals.

A locomotion stop neuron

Another circuit we studied involves the peptidergic neuron RIS (Steuer Costa et al., 2019) (Figure 6a). The RIS neuron was previously implicated in sleep regulation. Upon photoinhibition of RIS, the animals stopped all muscular behaviors, including locomotion (Figure 6b). This was a rapid and brief response, only as long as the photostimulus,

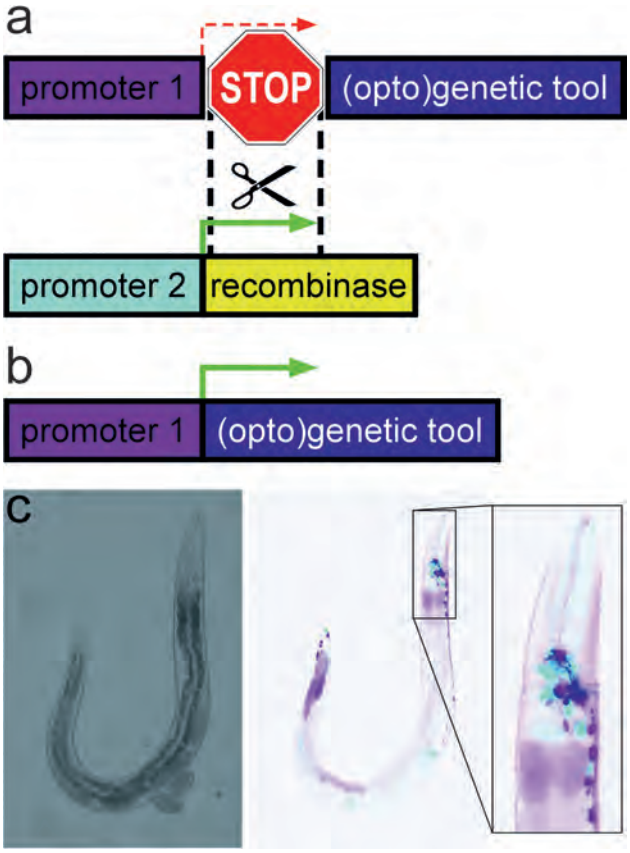

Figure 4: Conditional approach to achieve cell-specific expression of (opto)genetic transgenes. (a) Two promoters with overlapping expression in the cell of interest are used to express (promoter 1) the cDNA for an optogenetic tool, behind a stop cassette, flanked by recombination sites, or (promoter 2) a recombinase like FLP or *Cre*. (b) The stop cassette prevents expression unless it is removed by the recombinase. (c) Example expression patterns of two promoters (pink and cyan), overlapping in the cells of interest (SMB neurons, blue), in the animal's head. Differential interference contrast (DIC) micrograph, (false-colored) overlaid fluorescence micrograph, as well as enlarged section showing the head. Modified from the studies by Oranth et al. (2018) and Schmitt et al. (2012).

unlike a typical sleep episode. We thus wondered if the RIS neuron, which is involved in sleep mainly during development (Turek et al., 2016), functions also in the fine-control of locomotion in adult animals. Animals frequently change their direction, and this involves slowing and stopping, before a reverse locomotion can be initiated, and finally forward locomotion resumes in a different direction. This directional change of locomotion is orchestrated by a motor program (Donnelly et al., 2013; Roberts et al., 2016), and we speculated that a brief episode of RIS function may induce this locomotion stop during the reversal behavior. RIS activity in free-moving animals (Figure 6d), i.e. the onset of the rise of cytosolic Ca^{2+}, preceded the slowing event; thus RIS plays an active role in this behavior or is part of a sequence of neuronal events involving additional cells. The connectome of RIS (Figure 6c) indicates which neurons these could be. How does RIS actually affect locomotion stop? We found that activity of cholinergic motor neurons became desynchronized during RIS activation, which releases neuropeptides and gamma-amino butyric acid (GABA). This appeared to stop locomotion without losing the muscular tone (unlike in sleep), such that movement can quickly resume. RIS shows locally different Ca^{2+} signals in the axon and a branch of the axon, depending on whether just slowing or slowing and reversal occur (Figure 6e). Since the branch receives innervation by three neurons (Figure 6c), we speculate that upstream, as well as RIS-intrinsic events, are integrated at branch and axon, to determine different output of the neuron.

The missing link: AS motor neurons in coordination of locomotor circuits

The locomotion circuits contain a class of cholinergic motor neurons, termed AS (Figure 7a). These had not been analyzed previously, due to the lack of promoters specifically expressed in these neurons. Using a combination of promoters as well as transcriptional activators and repressors, optogenetic tools could be expressed in a 'subtractive' way, specifically only in the AS motor neurons (Tolstenkov et al., 2018; Wei et al., 2012). We characterized locomotion behavior in animals in which AS neurons were ablated, acutely photoinhibited, or photostimulated, and monitored AS neuron activity in moving animals. Inhibition of AS neurons blocked locomotion. They activate the musculature asymmetrically, i.e. only the dorsal muscles, and they trigger GABAergic neurons that concomitantly release GABA on the ventral side. This way, AS neurons may regulate navigation and bending, as a means of fine-tuning the general motor program (Figure 7a and b). The AS neurons are connected to premotor interneurons for reverse (AVA) and forward (AVB) locomotion, by chemical and electrical synapses (Figure 7b).

Analyses of chemical synaptic transmission at the neuromuscular junction

The second interest of my lab is the analysis of synaptic transmission at the neuromuscular junction (NMJ). The

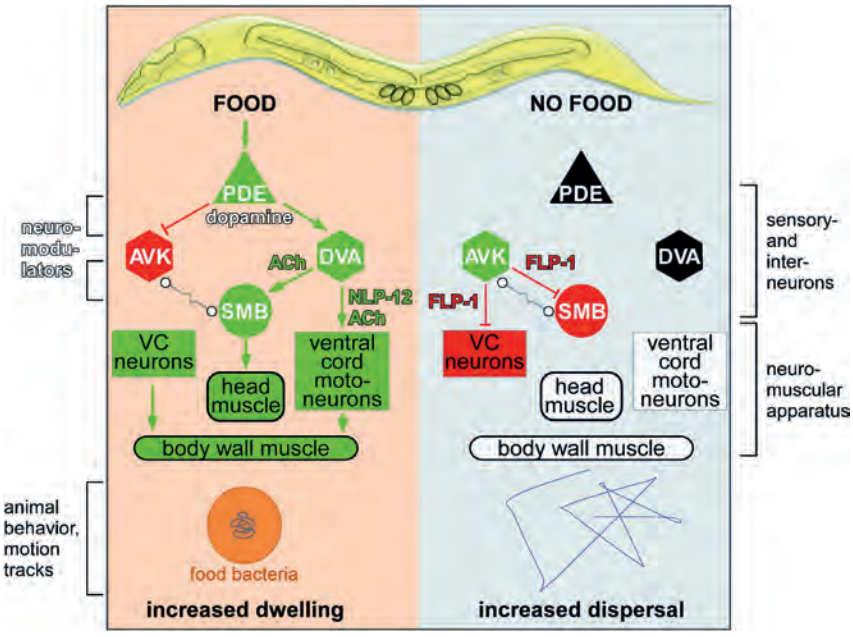

Figure 5: A neuronal circuit for navigation during food search behavior.
C. elegans navigates its environment to find, or remain on, a food source (bacterial lawn). It can also initiate search behavior, once it lost a food source. As we could show, this behavior is in part orchestrated by neuropeptidergic interneurons (AVK and DVA, symbolized by hexagons, releasing FLP-1 or NLP-12 (neuropeptide-like protein) neuropeptides, respectively) that antagonize each other in their effects on the motor system (circles, boxes), thus promoting dwelling (in the presence of food, left) or long-range search behavior (without food, right). Food is sensed by dopaminergic, sensory PDE neurons (triangles). Signals are inhibitory (red) or excitatory (green), sometimes depending on the postsynaptic receptors used. Activity states of the neurons in one or the other condition are also indicated by color. Food inhibits AVK, which stops releasing the inhibitory FLP-1 neuropeptide, thus disinhibiting motor neurons. Modified from Oranth et al. (2018).

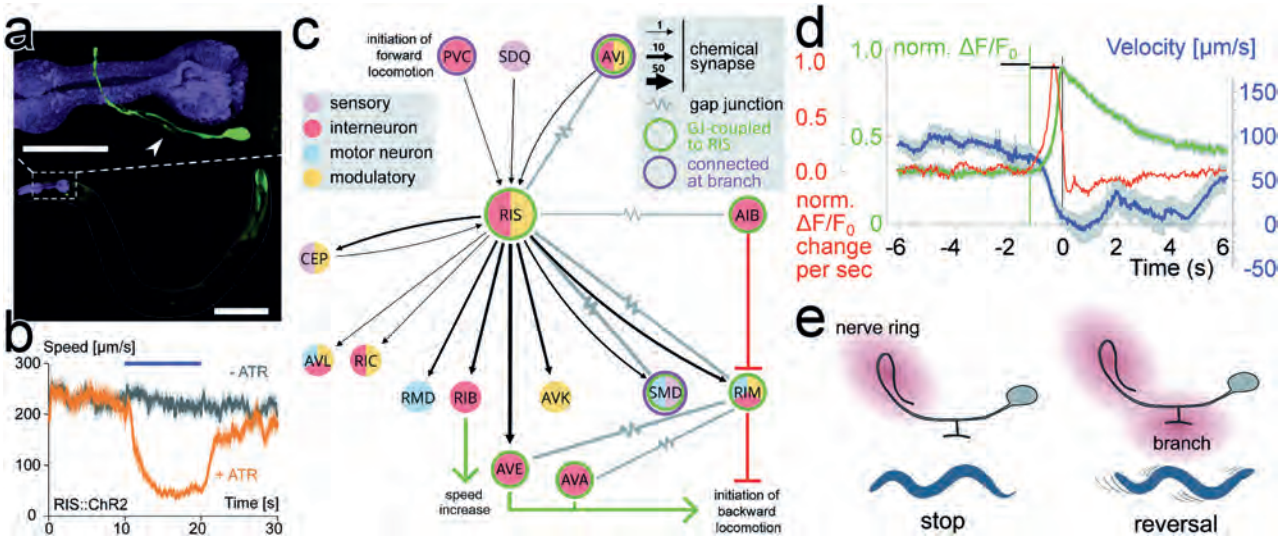

Figure 6: A single neuron, RIS, transiently inhibits the motor circuit for initiation of slowing or reversal behavior. (a) RIS (green) is a single neuron located in the animals' head, with a branched axon (arrowhead). (b) Locomotion speed is reduced when RIS is photoactivated via ChR2. (c) RIS connectome, showing input from (upper half) and output to (lower half) all its partners, with cell types and nature and the number of synaptic connections indicated. (d) Ca^{2+} activity in the axon of the RIS neuron (green) was measured in free-moving animals, and peak Ca^{2+} events were used to align these events and the concomitant locomotion velocity (and direction) of the animals (blue curve; negative indicates reverse locomotion). The Ca^{2+} events and the first derivative (rise rate of the Ca^{2+} signal, red curve) precede the slowing and onset of the slowing event, sometimes leading to a reversal (on average, a transient stop results). (e) RIS has an axon extending to the nerve ring, and a branch that dips into the ventral nerve cord. Ca^{2+} signals in the nerve ring always occurred when the animals slowed down but were accompanied by additional Ca^{2+} activity in the branch upon a reversal. Modified from the study by Steuer Costa et al. (2019).

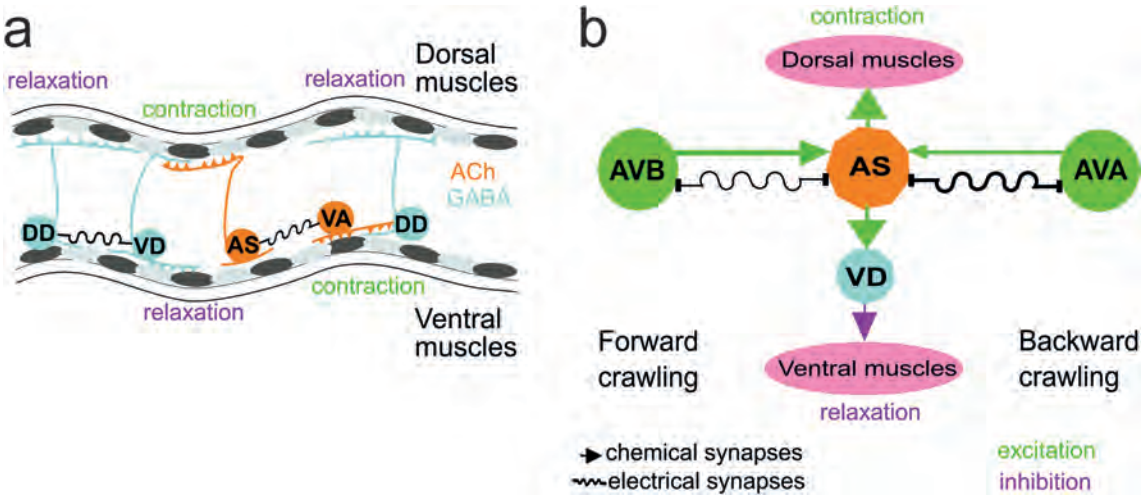

Figure 7: The previously unstudied motor neuron class AS is important for the coordination of the locomotion motor program and possibly for navigation.
(a) Integration of AS motor neurons in the motor nervous system of *C. elegans*, found in sub-circuits, repeated 6–11 times in a segmented fashion along the body. Note that only some neuron classes are shown. Anterior is to the left. (b) AS neurons are connected to premotor interneurons for reverse (AVA) and forward (AVB) locomotion via gap junctions. They are asymmetrically innervating only dorsal muscle, as well as ventral inhibitory GABAergic neurons (VD class); thus, AS neuron activation causes as strong bias to dorsal bending. From the study by Tolstenkov et al. (2018).

C. elegans NMJ is a well-accessible synapse, enabling pharmacological assays which report on postsynaptic and presynaptic functionality. Also, the NMJ arrangement of motor neurons and muscles is easily addressed by fluorescence microscopy in the live animal. The *C. elegans* NMJ is special in that the muscle cells, which extend dendritic spine-like protrusions, directly receive cholinergic and GABAergic input; thus, they integrate excitatory and inhibitory motor activity (Figure 8a). This is opposed to the mammalian system, where activity of excitatory and inhibitory interneurons is integrated by (excitatory) motor neurons. However, for *C. elegans* this means that stimulation of cholinergic neurons will also trigger GABA neurons (on the contralateral side), as this reciprocal innervation is required for coordination of body undulations. The different classes of cholinergic and GABAergic neurons and their ventral or dorsal innervation patterns have been characterized, and to some extent, specific genetic markers exist (see above). Electrophysiology of the NMJ is demanding, due to the dissection of the small animal (Figure 8b).

We developed additional – optical – assays to analyze NMJ activity (Figure 8a, c and d). To this end, we expressed (red) fluorescent sensors of cytosolic Ca^{2+} (e.g. RCaMP; Akerboom et al., 2013) or of membrane potential (rhodopsin-based voltage indicators like Arch(D95N), QuasAr or electrochromic FRET sensors; Gong et al., 2014; Azimi Hashemi et al., 2019; Kralj et al., 2012), in muscle cells (or neurons), and used optogenetic actuators like ChR2 in the motor neurons, to trigger acetylcholine (ACh) or GABA release. The resulting effects can be imaged in the postsynaptic muscle. This is not as acute as electrophysiological recordings. Yet, particularly voltage imaging enables an important alternative to electrophysiology since it can be performed in the intact animal, thus not requiring use of artificial solutions that may not reflect the endogenous ionic compositions and could thus falsify results. The all-optical electrophysiology at the NMJ also enables a more accurate analysis of the integration of excitatory and inhibitory signals in muscle cells, as no dorsoventral motor neuron commissures are severed, like in the 'filet' preparation for electrophysiology. These methods allow a straightforward comparison of wild type and mutants in presynaptic or postsynaptic proteins required for synaptic transmission, neurotransmitter detection or synaptic vesicle recycling (Kittelmann et al., 2013; Liewald et al., 2008; Wabnig et al., 2015).

Analysis of synaptic transmission by combined optophysiology and electron microscopy

These methods allowed analyzing proteins required for synaptic vesicle (SV) endocytosis and recycling. Particularly under conditions of prolonged stimulation, the synapse needs to efficiently recycle SV membrane and

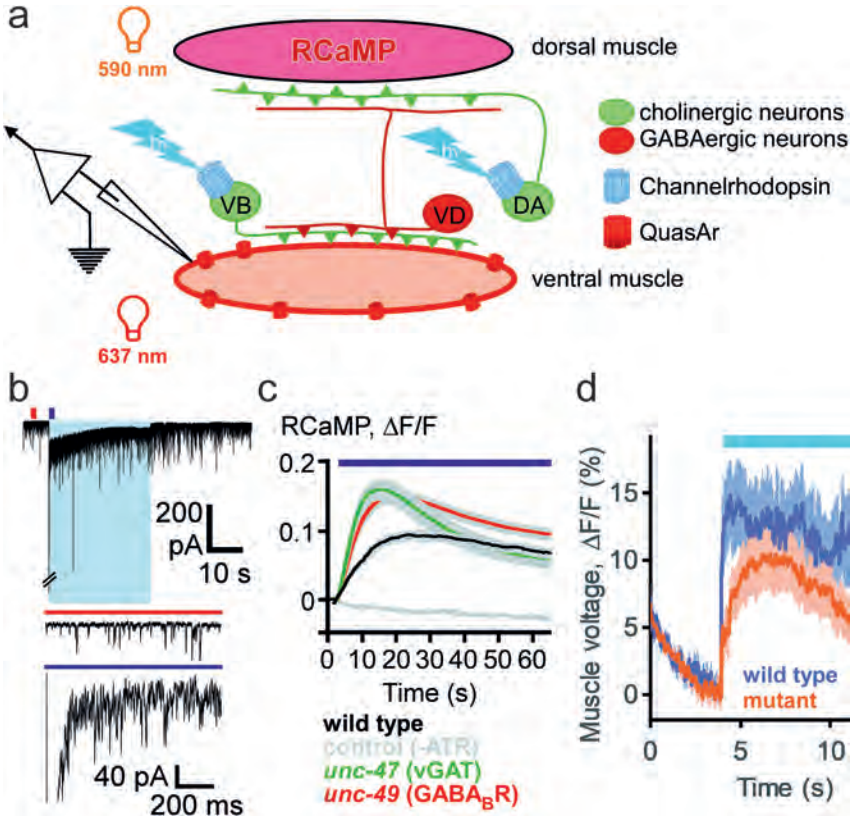

Figure 8: Optophysiology methods to study synaptic transmission at the NMJ. (a) NMJ organization of *C. elegans* (simplified). Cholinergic neurons innervate muscle, as well as GABAergic neurons that innervate the opposite side. Optogenetic tools for actuation (ChR2) expressed and activated in cholinergic neurons cause release of acetylcholine but also trigger GABAergic neurons. As an alternative to patch recordings, postsynaptic RCaMP (a red-fluorescent Ca^{2+} indicator) or QuasAr (a rhodopsin-based fluorescent voltage indicator) can be combined with presynaptic ChR2 photostimulation to monitor NMJ transmission. (b) Example electrophysiology (evoked post-synaptic currents) of light-evoked transmission (blue shaded region). Red and blue bars indicate region enlarged in the lower panels. (c) Mean ± SEM RCaMP fluorescence ($\triangle F/F$) in muscle, before and during a photostimulus to cholinergic motor neurons (blue bar). Compared are wild type and two mutants lacking GABA transmission, resulting in higher signals in muscle. (d) Experiment similar to (c), but using QuasAr voltage sensor and comparing wild type and a mutant with a presynaptic defect. Modified from the study by Kittelmann et al. (2013), Wabnig et al. (2015), and Bergs and Gottschalk, unpublished (d).

proteins, in order to keep up with ongoing release of neurotransmitter. To study these processes in more detail, optical stimulation of neurons is combined with electron microscopy of rapidly, high-pressure frozen, freeze-substituted and metal-stained animals, during or following light stimulation of the motor neurons (Kittelmann et al., 2013; Yu et al., 2018) (Figure 9a).

This methodology was also developed by other labs for use in mammalian neurons and termed 'flash-n-freeze' electron microscopy (Watanabe et al., 2013a, b). In my lab, we further analyzed the effects of cyclic adenosine monophosphate (cAMP) signaling in cholinergic motor neurons, following optogenetic stimulation of cAMP generation by photoactivated adenylyl cyclase (bPAC) (Steuer Costa et al., 2014). We found that cAMP has several effects (Figure 9b). It mobilized synaptic vesicles from the reserve pool, and it causes increased fusion due to the higher amount of fusion-competent vesicles. Furthermore, we found that cAMP causes the fusion of neuropeptides from dense core vesicles, which could not be evoked by mere depolarization using ChR2. The neuropeptides had an autocrine effect in motor neurons, which caused the filling of existing SVs with additional neurotransmitter (ACh), thereby increasing quantal size (Steuer Costa et al., 2017) (Figure 9c and d). This was due to increased activity of the vesicular ACh transporter (vAChT) and markedly enlarged the SVs. This novel neuromodulatory presynaptic plasticity may enable the motor system to integrate signals as determined by different internal states, or signals in response to external stimuli, which cause cAMP

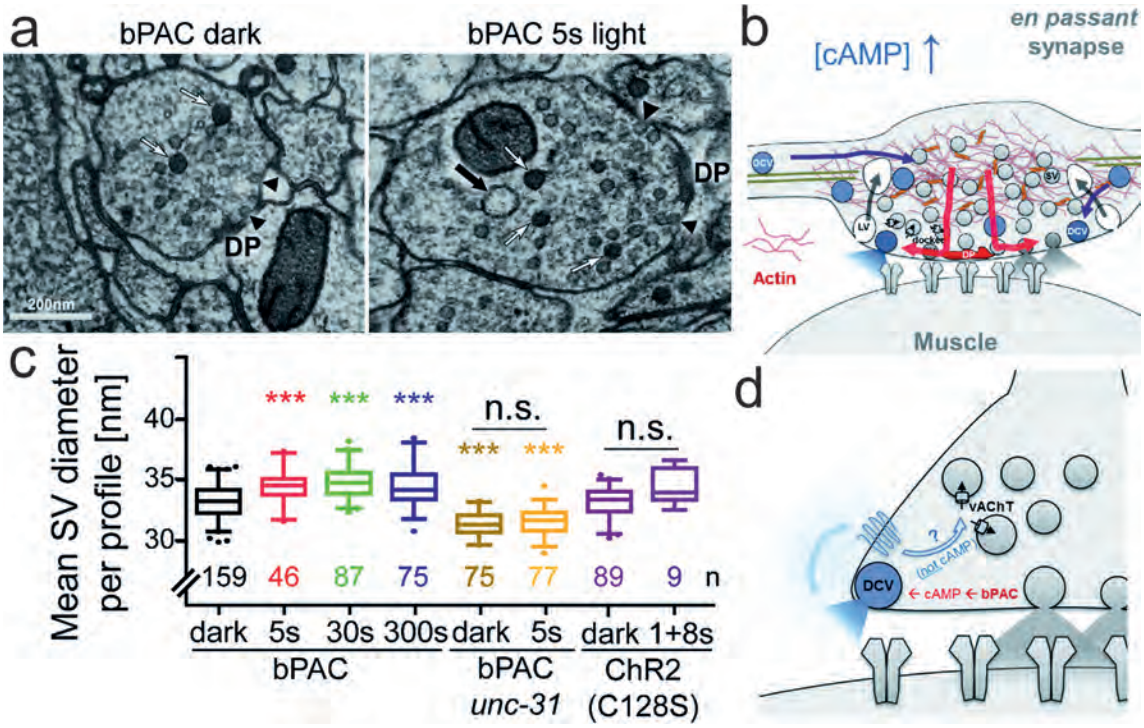

Figure 9: Cyclic adenosine monophosphate (cAMP) signaling enhances synaptic transmission at the *C. elegans* NMJ via neuropeptidergic signals and increased synaptic vesicle (SV) filling. (a) Transmission electron micrographs (40-nm sections) from cholinergic synapses expressing the blue light–activated adenylyl cyclase bPAC, without and with 5-s photostimulation. Arrowheads: Docked SVs; white arrows: dense core vesicles (DCVs), containing neuropeptides; DP: dense projection, protein machinery at the center of the active zone; black arrow: endosome, evoked by SV fusion and compensatory endocytosis. (b) Summary of cAMP evoked effects in the synapse: SVs are mobilized from the actin cytoskeleton and more readily released upon neuronal depolarization; increased DCV fusion causes neuropeptide release, and enhanced endocytosis causes formation of endosomes. (c) Diameter of SVs, averaged per profile, shows that photoevoked cAMP signaling causes a significant increase in SV diameter. This is not found in response to ChR2 photostimulation and is absent in *unc-31* mutants lacking Calcium-dependent activator protein for secretion (CAPS), a protein required for DCV fusion. Moreover, CAPS mutants have significantly smaller SVs, indicating that neuropeptide signaling may generally regulate SV size (filling state). (d) cAMP causes release of neuropeptides, which are sensed by autoreceptors and trigger a second, non-cAMP signaling pathway affecting SV filling via the vesicular acetylcholine transporter (vAChT). Modified from the study by Steuer Costa et al. (2017).

increase in the motor neurons, thus enabling a more potent output of the motor system.

Conclusion

C. elegans is an elegant system to develop integrated approaches for studies of neuronal networks and synaptic transmission by optogenetics, which allows us to make important contributions to the field in clarifying the role of some circuits and analyzing novel aspects of transmission between its neurons. We could provide methods and reagents for other labs, to address additional parts of the *C. elegans* nervous system. The Schram foundation helped us to bring forward or sparked ideas for a number of projects, and this funding was particularly important during the early days of my lab.

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Bionote

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Alexander Gottschalk studied chemistry in Frankfurt and Marburg and initially specialized in biochemistry of spliceosomes, before he turned to the neurobiology of *Caenorhabditis elegans*. After a postdoc at the University of California, San Diego, where he studied nicotinic acetylcholine receptors, he returned to Goethe University as a junior professor. In Frankfurt he learned about a novel protein, which proved to be a light-activated ion channel – channelrhodopsin-2 (ChR2). With Georg Nagel and Ernst Bamberg, he could demonstrate that ChR2 can mediate rapid depolarization of muscles and neurons in response to light, in live animals, and that it can trigger coordinated behavior. This sparked a new direction for his lab, and he began to develop and apply numerous light-sensitive proteins as optogenetic tools in *C. elegans* and to use them for analyses of neuronal network function, as well as to study mechanisms of synaptic transmission. In 2009, he became a Heisenberg Professor, and since 2016, he is a full professor at Goethe University. Furthermore, he is the speaker of the DFG priority program SPP1926 – Next Generation Optogenetics – tools and application.

Review article

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Direct contribution of angiogenic factors to neurodevelopment: a focus on angiopoietins

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Abstract: Over the last two decades, it has become clear that classical molecules that regulate neurodevelopment also play an important role in directly regulating the development of the vascular system and *vice versa*. The prototypical angiogenic ligand vascular endothelial growth factor (VEGF) is by now also regarded as a molecular regulator of different neurodevelopmental processes, such as neuronal progenitor proliferation, migration and differentiation, dendritic and axonal branching and synaptogenesis. The direct effect of other classical angiogenic factors, such as angiopoietins and its receptor Tie2, on neurodevelopmental processes remains less defined. Recent work from our group indicates that the angiopoietin-Tie2 pathway does not only regulate blood vessel formation and stabilization but also simultaneously affect neuronal dendritogenesis in a cell-autonomous manner. In this mini-review, we will integrate our findings within the current understanding of the neurovascular link and within the previous knowledge of the potential effects of angiopoietins in the neuronal context.

Keywords: angiopoietins; dendritic branching; neurons; neurovascular; Tie2.

Zusammenfassung: Während der letzten beiden Jahrzehnte hat sich gezeigt, dass Moleküle die bekanntermaßen die neuronale Entwicklung regulieren, auch eine wichtige Rolle bei der Entwicklung des vaskulären Systems spielen. Der prototypische angiogene Wachstumsfaktor *Vascular Endothelial Growth Factor (VEGF)* wird heute auch als molekularer Regulator verschiedener neuronaler Entwicklungsprozesse angesehen, welche von der Proliferation, Migration und Differenzierung neuronaler Vorläuferzellen bis hin zur Verzweigung von Dendriten und Axonen sowie der Bildung von Synapsen reichen. Der direkte Effekt von anderen klassischen, angiogenen Faktoren, wie beispielsweise Angiopoietine und deren Rezeptor Tie2, auf die neuronale Entwicklung ist sehr viel weniger untersucht. In einer aktuellen Studie unserer Gruppe konnten wir zeigen, dass der Angiopoietin-Tie2 Signalweg nicht nur das Blutgefäßwachstum sondern simultan auch die neuronale Dendritogenese in einer zell-autonomem Weise reguliert. In diesem Mini-Review wollen wir unsere Ergebnisse in die heutigen Erkenntnisse über die Neurovaskuläre-Wechselwirkung sowie die bekannten Effekte von Angiopoietinen im neuronalen Kontext einbinden.

Schlüsselwörter: Angiopoietine; dendritische Verzweigung; Neurone; Neurovaskuläre-Wechselwirkung; Tie2.

The concept of the neurovascular link

Research of the past decades highlighted the term neurovascular link, as a research concept trying to understand the similarities and parallelisms between the vascular and the nervous system. Powered by advancing technologies and increasing experimental sensitivity, a number of research studies have demonstrated that both the neuronal and the vascular systems have many more similarities than previously anticipated. Signaling pathways that were classically discovered in either the vascular or the neuronal context are today known to be cross-expressed and functionally affect the respective other one (Paredes et al., 2018; Walchli et al., 2015). In particular, from the vascular perspective, the best-studied molecule is the prototypic angiogenic factor vascular endothelial growth factor (VEGF). Extensive research at the interface of neuro and vascular biology has shown that VEGF is able to directly act on neural cells and participate in the regulation of different neural-related processes (Carmeliet and Ruiz de Almodovar, 2013). Work from us and other research groups

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contributed to the characterization of VEGF as a factor regulating neurogenesis, axon guidance, neuronal migration, motoneuron vascularization and hippocampal dendritic and axon branching (Erskine et al., 2011; Harde et al., 2019; Himmels et al., 2017; Luck et al., 2019; Mackenzie and Ruhrberg, 2012; Ruiz de Almodovar et al., 2010, 2011; Schwarz et al., 2004). Particularly for the latter, with the support from the Schram Foundation, we were able to characterize that the VEGF receptor VEGFR2 is expressed in CA3 hippocampal neurons and that a direct VEGF/VEGFR2 signaling is required for proper CA3 axon branching during development (Luck et al., 2019). While the function of VEGF in the nervous system is starting to be well understood, little is known about whether other essential angiogenic pathways, such as the angiopoietin-Tie pathway, can act as potential factors signaling directly on neural cells, or as neurovascular communication signals.

The angiopoietin-Tie pathway

The Tie (Tyrosine kinase receptors with Immunoglobulin-like and EGF-like domain) receptors, Tie1 and Tie2, were first described in the 1990s to be highly expressed in the vascular system (Dumont et al., 1992; Partanen et al., 1992; Tanaka et al., 1993). Their amino-terminal ectodomain is composed of two Ig-like domains, followed by three epidermal growth factor (EGF)-like repeats, one Ig-like domain and three fibronectin-type III domains (Figure 1A) (Barton et al., 2006; Fiedler et al., 2003; Macdonald et al., 2006). Shortly after the discovery of the receptors, researchers identified specific ligands for Tie2 named angiopoietins, with the main two being Ang1 and Ang2 (Davis et al., 1996; Maisonpierre et al., 1997; Reiss et al., 2007). Angiopoietins are glycosylated, secreted proteins composed of a N-terminal superclustering domain followed by the coiled-coil oligomerization domain and the C-terminal fibrinogen-like domain (Figure 1C) (Davis et al., 2003; Kim et al., 2005). Even though Tie1 and Tie2 share a high sequence homology, it is only Tie2 that can bind directly and signal via angiopoietins (Barton et al., 2006). Tie1 on the other hand is important for modulating the signaling properties of Tie2 by forming a heterodimeric complex (Figure 1B) (Saharinen et al., 2005; Seegar et al., 2010). Tie1 additionally controls Tie2 surface presentation (Savant et al., 2015), and it further modulates Tie2 signaling via regulated cleavage of its extracellular domain (Kim et al., 2016; Korhonen et al., 2016) Until recently, Tie1 was considered an orphan receptor that acts as a coreceptor for Tie2. However, leukocyte cell-derived chemotaxin 2 (LECT2) was recently identified as a functional ligand of Tie1 (Xu et al., 2019). The crucial role of Tie receptors in the vascular system is highlighted in studies performed with knockout mice. The complete knockout of Tie2 leads to severe vascular and venous malformations, and mutant embryos die early (E10.5) due to defects in cardiac development (Chu et al., 2016; Dumont et al., 1994; Katoh et al., 1995). Similarly, in the absence of Tie1 mice have an increased and leaky vascular network and die latest at birth due to respiratory failure (Katoh et al., 1995; Puri et al., 1995; Yuan et al., 2007). In the adult, vascular loss of Tie receptors does not cause lethality but leads to reduced angiogenic sprouting and vascular density in case of Tie1 and to reduced arterial angiogenesis and increased venous sprouting in case of Tie2 (Chu et al., 2016).

Angiopoietins in the vascular system

Here we just give a brief overview of the main and classical roles of Ang1 and Ang2 signaling via Tie2 in the vascular system. However, it is important to mention that in the vascular system, angiopoietins can also signal in a Tie2-independent manner via integrins (Bae et al., 2020) For further details, we refer the reader to excellent reviews in the topic (Eklund and Saharinen, 2013; Koh, 2013; Saharinen et al., 2005, 2017). In the angiogenic context, the spatiotemporal expression and role of angiopoietins has been extensively characterized. Vascular endothelial cells comprise the predominant source of Ang2 (Fiedler et al., 2006); however, recent studies have shown that other cell types also express and signal via Ang2, including adipocytes (Bae et al., 2020) and tumor cells (Abdul Pari et al., 2020). On the other hand, Ang1 expression and secretion can be detected from various cells in the proximity of blood vessels, including pericytes and astrocytes (Acker et al., 2001; Davis et al., 1996; Kim et al., 2000). Angiopoietin ligands affect the vascular system in an opposing fashion, with Ang1 and Ang2 showing agonistic and antagonistic functions, respectively (Figure 1D). These opposing roles have been unraveled in studies performed both *in vivo* and *in vitro*. More specifically, Ang1 stimulation of endothelial cells *in vitro* has a proangiogenic effect, leading to a time- and dose-dependent phosphorylation of Tie2 receptors (Bogdanovic et al., 2006). Thus, Tie2 activation through Ang1 reduces vascular leakage and induces endothelial quiescence (Oh et al., 2015). In this line, Ang1-knockout mice show severe vascular malformations and die embryonically, resembling the phenotype observed upon Tie2 loss of function (Suri et al., 1996). Ang2, however, seems to act as a partial agonist. Ang2-knockout animals are

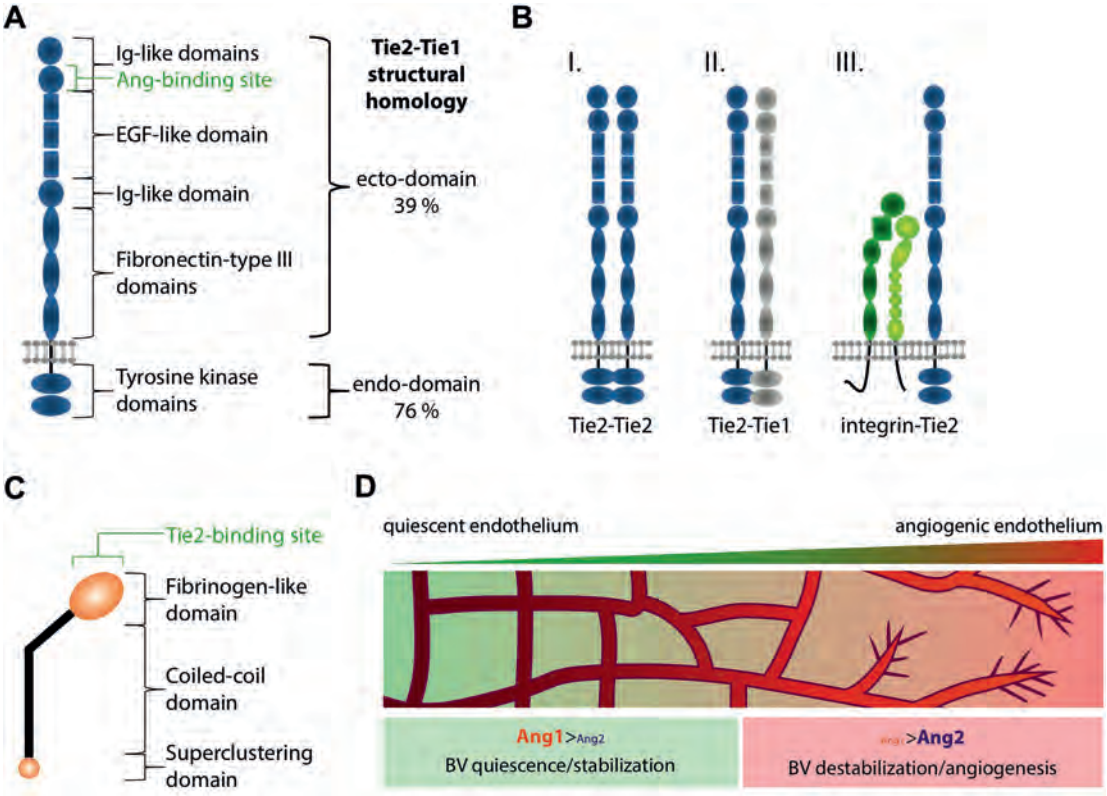

Figure 1: The angiopoietin-Tie2 signaling pathway. (A) The molecular structure of Tie2 (Tyrosine kinase receptors with Immunoglobulin-like and EGF-like domain) is composed of two N-terminal Ig-like domains, followed by three EGF-like repeats, one Ig-like domain and three fibronectin-type III domains. The C-terminal endo-domain shows kinase activity. (B) The Tie2 receptor can form homodimers (I.) or heterodimers with Tie1 (II.) as well as with integrins (III.), what changes its affinity to bind angiopoietin ligands and the downstream signaling. (C) Angiopoietins are glycosylated, secreted proteins containing three main domains, the N-terminal superclustering domain followed by the coiled-coil oligomerization domain and the C-terminal fibrinogen-like domain. (D) The balance of Ang1/Ang2 is indicative for the angiogenic potential in the vascular system. Ang1 induces vascular quiescence, whereas Ang2 promotes angiogenesis through endothelial destabilization. BV: blood vessels.

generally viable and are characterized by a reduced inflammatory response (Benest et al., 2013). The excess of Ang2 activates endothelial cells and induces angiogenesis, whereby it can counteract the effect of Ang1 (Maisonpierre et al., 1997; Witzenbichler et al., 1998). Therefore, the overexpression of Ang2 causes a phenotype similar to the loss of function of Ang1 and Tie2 (Maisonpierre et al., 1997).

Angiopoietins in the nervous system

Ang/Tie2 in neurogenesis and neuronal survival

In vitro studies show that Ang1 stimulation leads to proliferation and differentiation in neural progenitor cells (NPCs) and neuronal survival in sensory and cortical neurons (Bai et al., 2009; Lim et al., 2015; Rosa et al., 2010; Valable et al., 2003). In adulthood, NPCs of the subventricular zone (SVZ) neurogenic niche express Ang1, which was shown to act as a proneurogenic factor and regulate stem cell dynamics by promoting stem cell proliferation and differentiation in a Tie2-dependent manner (Rosa et al., 2010). Ang2 is expressed in the developing cortex, and specific knockdown of Ang2 in the cortex during embryonic development leads to impaired radial migration of cortical neurons (Marteau et al., 2011). In a pathological setting, Ang2 becomes more highly expressed in endothelial cells (Beck et al., 2000) and in NPCs after stroke and leads to endothelial cell proliferation (Beck et al., 2000) and neuronal differentiation in a Tie2--dependent manner (Androutsellis-Theotokis et al., 2009; Liu et al., 2009).

In summary, there is accumulating evidence suggesting that the angiopoietin-Tie2 pathway contributes to physiological neurogenesis as well as regulates disease progression in the adult central nervous system (CNS)

(Figure 2). Future experiments will be needed to better understand its function and its effect in pathology.

Ang/Tie2 in neuronal maturation

The contribution of Ang–Tie2 signaling to later neurodevelopmental processes, such as dendritogenesis and synaptogenesis, has so far received little attention. In this line, it was shown that Ang1 stimulation promoted neurite outgrowth of dorsal root ganglia *in vitro* in a Tie2-dependent (Kosacka et al., 2005) or Tie2-independent manner (Chen et al., 2009). Consistently, Ang1 overexpression in forebrain neurons during development resulted in increased dendritic length as well as qualitative changes in dendritic morphology, suggesting a possible regulatory function of angiopoietins during later stages of neuronal maturation (Ward et al., 2005). However, whether physiological levels of Ang1 would also regulate dendritogenesis and whether a direct signaling via Tie2 in neurons might be responsible for these observations are still open questions (Figure 2).

With the support of the Schram Foundation, our work contributed to answer these open questions as we identified a role for angiopoietin-Tie2 signaling in dendritogenesis of hippocampal neurons and Purkinje cells (PCs) during development. During development, astroglia cells in the hippocampus and cerebellum express Ang1, whereas Tie2 expression is found in CA1 pyramidal neurons and in PCs (Luck et al., unpublished). In the hippocampus, neural cell-specific deletion of Ang1 resulted in aberrant dendritic development with reduced branch complexity of CA1 neurons, which is in line with the abovementioned studies where Ang1 was administrated or overexpressed (Kosacka et al., 2005; Ward et al., 2005). Interestingly, the role of Ang1 during dendritogenesis does not seem to be restricted to hippocampal neurons as dendritic branching in PCs of the cerebellum was also affected in neural-specific Ang1-knockout mice. PC dendrites are unique, as they show a particular high degree of arborization that is characterized by a strong planar orientation and dendritic self-avoidance. Neural loss of Ang1 caused a reduced dendritic complexity of PCs with aberrant dendritic planarity and dendritic self-avoidance, suggesting a more general role of Ang1 on dendritic development (Figure 2). Consistently, specific deletion of Tie2 in PCs *in vivo* caused a reduced PC-dendritic development. Simultaneously, Ang2 is expressed in endothelial cells and Ang2-knockout mice present a similar PC branching phenotype (Luck et al., unpublished). This study showed that angiopoietins do not just regulate early angiogenesis and progenitor development but also contribute to later processes of differentiation and cellular maintenance.

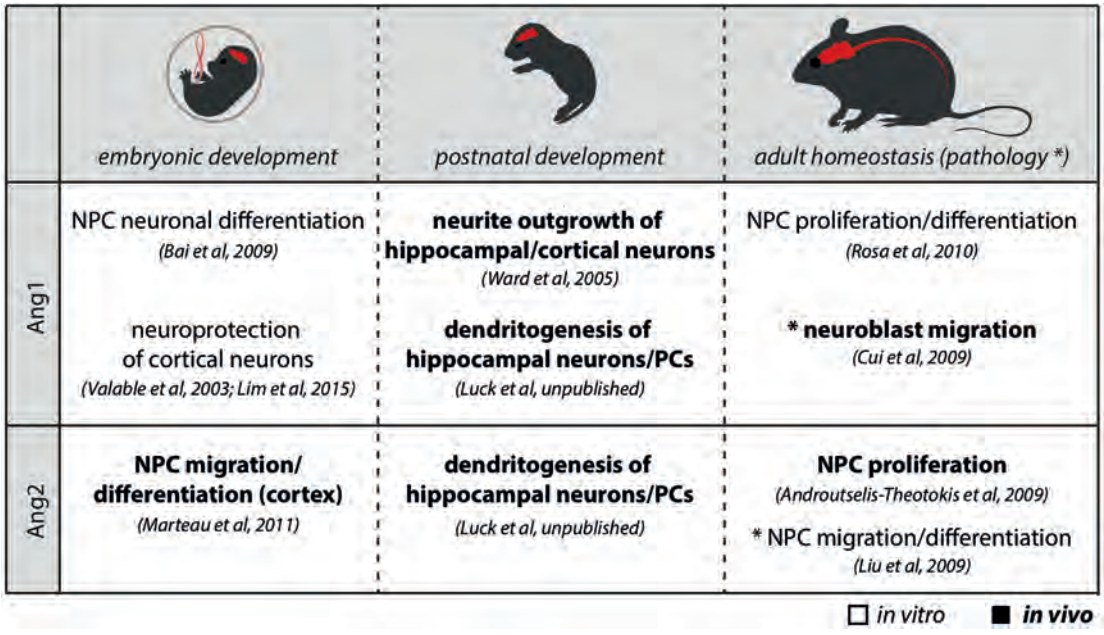

Figure 2: The role of angiopoietins in the CNS. The table summarizes the effect of Ang1 and Ang2 on neural cells of the CNS during development and adulthood. CNS is shown in red. NPCs: neural progenitor cells; PCs: Purkinje cells; CNS: central nervous system.

Concluding remarks

Research of the last decade has highlighted that to understand CNS development and functionality, one should study not only neuronal function but also other cellular components of the CNS, such as glia cells and blood vessels. Indeed, there are many examples where neurovascular communication is essential for proper CNS formation and function. Thus, understanding the intercellular communication and the molecular pathways of such interactions, among them VEGF and angiopoietins signaling pathways in neural cells, will bring further insights into the complex regulation of CNS formation and function.

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Review article

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The role of the dentate gyrus in mnemonic functions

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Abstract: The hippocampus is decisive for the storage of conscious memories. Current theories suggest that experience-dependent modifications in excitation–inhibition balance enable a select group of neurons to form a new cell association during learning which represents the new memory trace. It was further proposed that particularly GABAergic-inhibitory interneurons have a large impact on population activity in neuronal networks by means of their inhibitory output synapses. They synchronize active principal cells at high frequencies, thereby supporting their binding to cell assemblies to jointly encode information. However, how cell associations emerge in space and time and how interneurons may contribute to this process is still largely unknown. We started to address this fundamental question in the dentate gyrus (DG) as the input gate of the hippocampus, which has an indispensable role in conscious memory formation. We used a combination of *in vivo* chronic two-photon imaging of population activity in the DG and the hippocampal areas CA1–3 of mice exposed to a virtual reality, in which they perform a goal-oriented spatial memory tasks, with high-density *in vivo* recordings and multiple whole-cell recordings in acute slice preparations, to determine how memory engrams emerge during learning. We further examine how GABAergic interneurons may contribute to this process. We believe that these lines of research will add to a better understanding on the mechanisms of memory formation in cortical networks.

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Keywords: dentate gyrus; engram; gamma oscillations; interneuron; memory.

Zusammenfassung: Der Hippocampus ist für das Abspeichern bewusster Gedächtnisspuren entscheidend. Aktuelle Theorien besagen, dass erfahrungsabhängige Änderungen in dem Verhältnis von Exzitation zu Hemmung es auserwählten Neuronen ermöglichen neue Zellassoziationen während des Lernvorgans zu bilden, die die neue Gedächtnisspur repräsentieren. Es wird weiterhin angenommen, dass GABAerge hemmende Interneurone mit Hilfe ihrer inhibitorischen Ausgangsynapsen erheblichen Einfluss auf die Populationsaktivität neuronaler Netzwerke nehmen. Sie synchronisieren aktive Prinzipalzellen mit hohen Frequenzen und ermöglichen damit die funktionelle Kopplung aktiver Neurone zu Zellassoziationen, die gemeinsam Information kodieren. Wie sich diese Zellassoziationen zeitlich und räumlich ausbilden und welchen Beitrag hemmende Interneurone in diesem Prozess einnehmen ist allerdings weitgehend unklar. Wir begannen diese zentrale Frage im Gyrus Dentatus (DG) als der Eingangsregion des Hippocampus, der eine unersetzliche Rolle in der bewussten Gedächtnisbildung einnimmt, zu untersuchen. Wir setzen die zwei-Photonen angeregte bildliche Darstellung von Kalziumsignalen ein, um die Populationsaktivität von Prinzipalzellen im Hippocampus kopffixierter Mäuse, die zielorientierte räumliche Gedächtnisaufgaben in einer virtuellen Umgebung durchführen, zu messen und kombinieren die erfassten Daten mit elektrophysiologischen *in vivo* Ableitungen und simultanen Mehrfachableitungen von Neuronen in akuten Schnittpräparaten, um zu bestimmen, wie Gedächtnisspuren sich während des Lernprozesses ausbilden und welche Rolle Interneurone in diesem Prozess einnehmen. Wir sind davon überzeugt, dass diese multidisziplinäre Forschungsrichtung zu einem verbesserten Verständnis der Gedächtnisbildung in kortikalen Netzwerken beitragen wird.

Schlüsselwörter: Gyrus Dentatus; Engramm; Gammaoszillationen; Interneuron; Gedächtnis.

Introduction and objectives

Our daily life depends on the processing and storing of a continuous stream of information, which enables us to rapidly adapt our behaviour to changes in our environment. Current theories of memory formation suggest that experience-dependent modifications in the balance between excitation and inhibition enable a selected group of neurons to form a new cell association during the learning process, which represents the newly formed memory trace, the engram (Buzsáki & Draguhn, 2004; Eichenbaum, 1993; Leutgeb et al., 2005; Neunuebel & Knierim, 2014). The functional changes at the level of synapses, single cells and cell populations that are associated with the learning process are, however, largely unknown. This is a crucial issue because impaired memory is a global problem implicated in various diseases including post-traumatic stress disorder, anxiety and depression and can have various causes such as stress, sleep deficit or medication (Kheirbek et al., 2012). In order to be able to effectively treat memory disorders, we first need to understand how new memories are formed in the central nervous system. We aim to address this question in the rodent dentate gyrus (DG) as the main input region of the hippocampus, functionally vital for the acquisition of new memories. Functional and lesion studies in animals proposed several memory functions for the DG, including spatial pattern separation (Gilbert et al., 2001; Neunuebel & Knierim, 2014; Treves & Rolls, 1994), pattern completion (Nakashiba et al., 2012), novelty detection (Hunsaker et al., 2008) and binding of sensory information or objects to a spatial context (Lee and Jung, 2017). However, only recent technical advances in neuroscience, particularly in two-photon calcium imaging of population activity with single-cell resolution, allowed us to study the activity of identified DG neuron types in behaving animals during learning on subsequent days (Danielson et al., 2016; Diamantaki et al., 2016; Hainmueller & Bartos, 2018, 2020). These data together with state-of-the-art *in vitro* recordings of interconnected cells (Bartos et al., 2002, 2007; Elgueta & Bartos, 2019; Savanthrapadian et al., 2014; Strüber et al., 2015, 2017), and *in vivo* high-density single-unit and local field potential recordings provided information on the synaptic, cellular and network mechanisms, which may underlie the emergence of DG-dependent memory traces. Thereby, this work massively propelled our understanding on the mnemonic functions of the DG. Here, we will focus on our recent published investigations on two main objectives:

(1) Identification of the temporal and spatial emergence of learning-related cell associations representing new memories in the DG, and
(2) Examination on the potential contribution of GABAergic inhibitory cells in the formation of memory engrams by synchronizing cell assemblies at high gamma (30–150 Hz) frequencies.

We will show that chronic two-photon calcium imaging in head-fixed mice enables us to perform a multiple-day spatial memory task in a virtual environment and to record neuronal activity from the same set of neurons in all major hippocampal subfields. We provide evidence that pyramidal cells in the hippocampal areas CA1–3 show precise and highly environment-specific but continuously changing representations of the learned spatial sceneries. In contrast, granule cells (GCs), the glutamatergic principal cells of the DG, have a spatial code that is stable over many days with low place or context specificity. Moreover, we show that fast-spiking parvalbumin-expressing interneurons (PVIs) in the DG contribute to the synchronization of cell assemblies and thereby may add to the encoding of contextual information.

Structure and function of the DG

Memories about our interactions with the environment are fundamental for our daily behavior. Conscious or 'declarative' memories can be divided into semantic memories including factual knowledge (e.g., Berlin is the capital of Germany), whereas episodic memories represent unique experiences (e.g., my first train ride to Berlin). Episodic memories associate individual events with the spatial and temporal context in which they were experienced. Memories must be first encoded as a permanent 'engram,' maintained and 'consolidated' over time (Tonegawa et al., 2018) for its subsequent recall and their usage in daily cognitive processes. The hippocampus, located in the temporal lobes of the brain (Figure 1), is crucial for declarative memories. Hippocampal principal cells encode with their activity spatially defined places, distinct elements of the environment or the association of elements with a context (e.g., blackboard in a lecture hall; O'Keefe & Dostrovsky, 1971). The hippocampus is divided into three areas, the CA1, the CA3 and the DG, which are interconnected by excitatory synapses, thereby forming the canonical trisynaptic path (Figure 1). The DG is situated between the entorhinal cortex and the CA3 area, forming the first stage of the trisynaptic circuit. Current theories

propose that the DG receives a rich multimodal input from the entorhinal cortex which carries information on various modalities of the environment and its objects and translates the rich input stream into sparse and segregated ('orthogonalized') representations, a process called pattern separation (Leutgeb et al., 2007; Marr, 1971; Santoro, 2013; Treves & Rolls, 1994). By decorrelating the rich input stream into nonoverlapping sparse memories, the DG is proposed to allow a high resolution of information (Marr, 1971). Consistent with the sparse coding theory, GCs in the DG, discharge at low mean frequency (~0.5 Hz; Pernía-Andrade & Jonas, 2014) and labeling studies of immediate early genes indicate that small differences in spatial environments are represented by nonoverlapping GC ensembles (Ramirez et al., 2013).

The activity of GCs stays under tight inhibitory control of GABAergic interneurons. Among the various types of GABAergic cells in the DG (Hosp et al., 2014), particularly PVIs attracted highest attention owing to the strong perisomatic inhibition they provide onto GCs (Bartos et al., 2002, 2007; Vida et al., 2006). Enhanced activity of the entorhinal cortex onto GCs can induce long-lasting potentiation leading to input strengthening (Schmidt-Hieber et al., 2004) and enhanced activation of a selected group of GCs. They contact PVIs, which in turn provide feedback inhibition to the DG circuitry. Thus, once a selected group of GCs is recruited, this group will provide inhibition to the DG network and silence less-excited GCs. This mechanism may increase the signal-to-noise ratio in the network, ensure sparse coding and enhance the storage capacity of the DG. Thus, changes in excitation and inhibition on the level of single cells during learning may define who is the member of the cell assembly and who falls out.

Emergence of memory engrams in the DG

To study the emergence and dynamics of hippocampal memory engrams during the course of long-term learning, we established a *virtual environment-based, goal-oriented learning task for head-fixed mice* (Figure 2). By running on a spherical treadmill, mice move along a 4-m-long virtual linear track with four soymilk reward locations, displayed on monitors covering the mouse's visual field. After >10 days of familiarization to this track ('familiar' context), imaging sessions started in which mice run alternatingly in this familiar context and additionally on a 'novel' linear track with only two reward sites and different visual cues. Thus, mice have to discriminate between the two novel and familiar contexts and to identify the new rewarded sites on the novel track. We showed that mice recognize the reward site by consistently licking more often inside *versus* outside the reward zone. Initially, the reward-related licking is lower in the novel than in the familiar context. These differences vanish with learning over the next subsequent ~3 days, indicating that mice remember the new rewarded locations; they do learn (Hainmueller & Bartos, 2018). To measure the activity of hippocampal neurons, we inject unilaterally the recombinant adeno-associated virus (rAAV) encoding the fluorescent calcium indicator GCaMP6f in the hippocampus and panneuronally labelled cells of the DG, CA1 and CA3 of transgenic mice expressing the red fluorophore tdTomato (tdT) in PVIs. This allowed us to visually differentiate principal cells from PVIs. We chronically implanted an imaging window (Figure 2A and C) allowing us to image the same set of cells on subsequent days during learning (Dombeck et al., 2010).

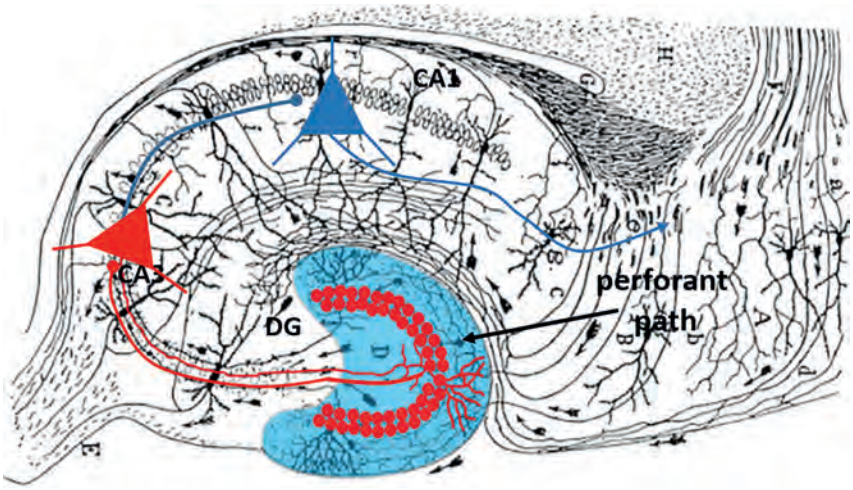

Figure 1: Hippocampal trisynaptic pathway. *Blue*, dentate gyrus (DG); *red* circles, granule cell (GC) somata forming mossy fiber axons projecting to CA3. Pyramidal cells in CA3 (*red triangles*) project to CA1 (*blue triangles*) with axons forming the Schaffer collateral pathway.

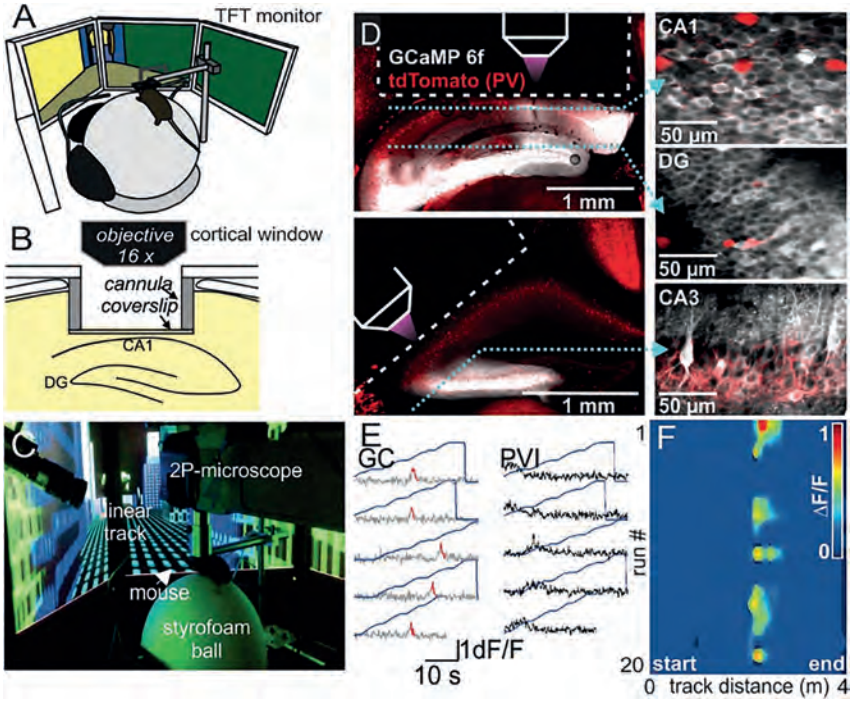

Figure 2: 2P-imaging of DG population activity in a virtual reality.
A–C Experimental design. Mice are head-fixed and run on a Styrofoam ball in a virtual reality presented with four monitors in front of the animal. The movement of the ball is detected and transferred in a movement of the virtual reality in real time. **B** Cranial window for DG imaging. **C** Linear track in the virtual reality. The ground and walls of the linear track are decorated with symbols, which visually allow the mouse to identify the virtual reality. The mouse is trained to obtain a reward at visually identifiable locations on the linear track. Lick frequency, running speed and pupil size are detected throughout the behavior. **D** rAAV-GCAMP6f expression in DG/CA1 of PV-tdT mice. *White*, GCAMP6f-expressing cells; *red*, PVIs. For DG and CA1 imaging, a cranial window was implanted above the hippocampus. For CA3 imaging, a cranial window was implanted above CA3 and the objective was tilted by 20°. **E** Ca^{2+} signals of a representative GC and a PVI; *blue line*, linear track. *Red*, Ca^{2+} transient in the GC. *Note* Ca^{2+} transients in PVIs have a slower time course than in GCs. **F** Activity in warm colors of a GC place cell recorded on 20 subsequent runs on the linear track.

Our data showed that consistent with previous findings, activity in the DG was sparse, markedly lower than the one in CA1 and CA3. Moreover, approximately 35% of these active neurons had place cell characteristics either in the familiar, novel or in both contexts (Hainmueller & Bartos, 2018). Place cells are active in a certain field of the world (O'Keefe & Burgess, 1996) and thereby encode with their activity a certain area of the linear track. Several of these place cells jointly formed a map of the virtual world. Pyramidal neurons in CA1–CA3 show precise and highly environment-specific but continuously changing representations of the learned spatial sceneries, whereas GCs of the DG had a spatial code that is stable over several days, with low place or environment specificity (Haimueller & Bartos, 2020). Finally, activity of DG GCs markedly declined on the first day of novelty exposure and then increased on the subsequent days, indicating that the memory engram emerged during the learning process. This decline in activity was not caused by PVIs (Hainmueller & Bartos, 2018) but more likely by reduced activity of the entorhinal cortex (Qin et al., 2018). Thus, our data showed that in contrast to previous views, spatial context representation is stable over time in the DG and the ability to discriminate between different contexts is low. In contrast, representation of spatial information is dynamic over time in the hippocampal CA1–CA3 and the ability to discriminate between spatial contexts is high. We therefore hypothesize that the DG provides a stable reference map of the global environment (e.g., lecture hall) to the hippocampus, in which it is combined with the temporally varying detailed contents (e.g., different students) constituting an experience.

GABAergic inhibition contributes to synchronization of cell assemblies

Elucidating the mechanisms underlying the formation of cell assemblies is crucial to understand the function of

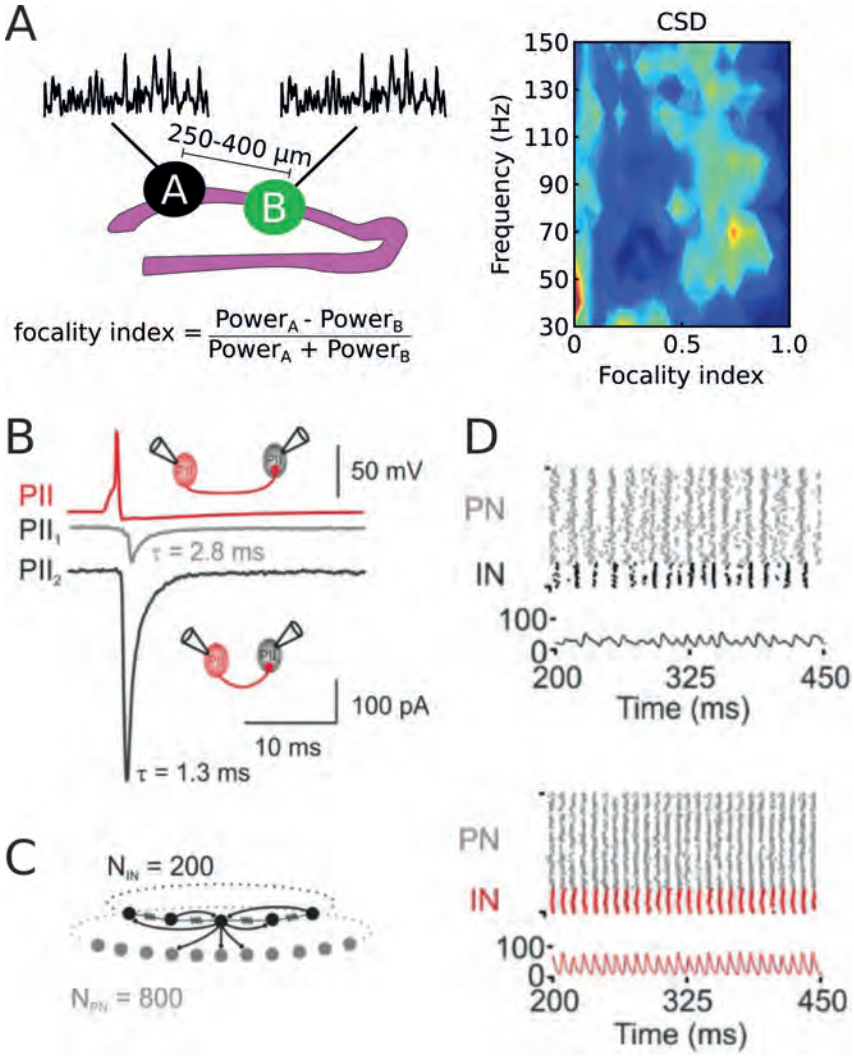

Figure 3: Measuring local gamma oscillations in the DG.
A Multisite local field potential (LFP) recording in the DG *in vivo*. Two LFP recording locations (A and B) are shown. *Right*, local high-gamma oscillations. Red colors show locally emerging oscillations as quantified by a focality index, which expresses how much the observed oscillation is restricted to a single electrode of the recording array. CSD: current source density. **B** *In vitro* paired whole-cell recordings between synaptically connected parvalbumin-positive interneurons (PIIs) reveal distance-dependent properties of amplitude and kinetics of inhibitory postsynaptic currents (IPSCs). The graph shows average IPSCs elicited by presynaptic action potentials (*red*) in a synaptically connected PII_2 at a short intersomatic distance (<100 µm; black trace). After the recording was obtained, the recording pipette was removed and a second postsynaptic PII_1 was recorded at a larger intersomatic distance (>100 µm; gray trace). Note: With increasing distance between presynaptic and postsynaptic partners, the amplitude and time course of IPSCs declines. **C** Structure of a network model composed by glutamatergic principal neurons (PNs, gray circles) and GABAergic inhibitory neurons (INs, black circles) with intersomatic distance of 50 µm between neighboring cells connected by synapses (lines with dots). **D** Network simulations with distance-dependent inhibition (*bottom*, red) create focal gamma oscillations upon excitatory stimulation with higher synchrony than in networks without distance-dependent inhibition (*top*, *black*). Individual dots represent individual action potentials generated by PNs (*gray*) or INs (black, *red*). *Bottom* trace, LFP trace. *Note*, network with distance-dependent inhibition shows highly synchronous LFP trace (bottom, *red*). Figure adapted from Strüber et al. (2017).

memory processes. In a behavioral task, in which rats have to repeatedly learn new spatial reward locations, local CA1 interneurons become associated with the emerging cell assembly, presumably by synaptic plasticity mechanisms that selectively strengthen excitatory connections among principal cells that encode the path to the reward locations (Dupret et al., 2013). In contrast, synaptic inputs onto interneurons from assemblies representing old target locations are weakened (Dupret et al., 2013). Thus, local GABAergic interneurons are considered to play an important role in the emergence of memory engrams during learning.

How do interneurons contribute to the organization of cell assembly firing? Oscillations of the local field potential are thought to provide temporal reference signals for assembly activity (Buzsáki & Draguhn, 2004). Gamma frequency oscillations (30–150 Hz) are a particularly interesting candidate in this regard. First, during exploratory behavior, gamma oscillations appear in the hippocampal formation typically nested in slower theta (6–12 Hz) oscillations. Second, gamma oscillations are prominently visible during cognitive efforts like the execution of spatial working memory (Yamamoto et al., 2014). Finally, the emergence of gamma activity depends on synaptic inhibition from and among GABAergic cells, particularly of PVIs (Sohal et al., 2009). Gamma activities can be further divided in two distinct functional components: low-gamma oscillations (30–75 Hz), which are thought to be generated in principle cell–interneurons synaptic loops, and high-gamma oscillations (75–150 Hz), which originate mostly from synaptic connections among interneurons (Bartos et al., 2002, 2007; Bieri et al., 2014; Colgin et al., 2009; Lasztóczi et al., 2016). Using current source density analysis applied to freely moving mice, we demonstrated that high-gamma oscillations are focal network phenomena, essentially occurring independently at several places within the DG, while low-gamma activities appear to be more distributed global network events (Figure 3; Strüber et al., 2017). These data suggest that local high-gamma activities might contribute to the segregation of active cell assemblies. Interestingly, using a combined *in vitro* whole-cell patch-clamp and computational approach, we could further show that the necessary prerequisites for the emergence of focal high-gamma activities might be embedded in the very fabric of the DG circuitry itself: GABAergic connections between PVIs show distinct distance-dependent changes in their basic properties, such as connection strength and decay kinetics, which support the occurrence of localized high-gamma activities in neuronal network models (Figure 3; Strüber et al., 2017). This functional configuration of the network might thus permit the parallel processing of spatially confined inputs by distinct gamma-modulated cell assemblies.

Conclusion and outlook

Our data propose that the DG provides a stable reference map of the global environment to the hippocampus, in which it can be flexibly associated with the temporally varying detailed contents such as objects or events that constitute a given experience in an environment (e.g., graduation ceremony on the Schlossberg in Freiburg). Activation of the map (e.g., by visiting the Schlossberg) may allow the reinstatement of that memory. DGs' sparse activity supports the representation of a multitude of reference maps and associated experiences. We propose that among several mechanisms, distance-dependent inhibition in the DG supports the formation of multiple noninterfering reference maps.

Although *in vivo* Ca^{2+} imaging and electrophysiology propelled our understanding on how hippocampal circuits contribute to memory processes, it will be important to determine the dynamics of memory-bearing engrams over time. Particularly, what synaptic, cellular and network processes underlie the formation of memory engrams and their recall? Which roles do specialized classes of neurons such as the various GABAergic inhibitory neuron types play in shaping, maintaining and dissolving engrams? The increasing availability of advanced molecular, imaging and electrophysiological methods will put the field in the position to improve our understanding of encoding and recall of memories and thereby may open new avenues of study and eventually therapies for hippocampal dysfunction.

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Bionotes

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Prof. Marlene Bartos studied Biology at the Technical University of Braunschweig and received her Ph.D. at the Technical University Munich 1994. With a DFG fellowship, she moved as postdoctoral fellow to the University of Pennsylvania, Philadelphia (USA). She was an assistant professor (C1) at the Albert-Ludwigs University in Freiburg where she received the Habilitation and *Venia Legendi* in Physiology.

In 2007, she was appointed as a full professor at the University of Aberdeen (UK) and received a Lichtenberg Professorship (W3) of the VW Foundation at Freiburg University in 2010. She is since 2018 the Director of the Institute for Physiology I at Freiburg University. Since 2014, she is a speaker of the Research Unit FOR2143 'Interneuron Plasticity,' and she received the ERC Advanced Grant in 2018. Dr. Bartos laboratory examines the synaptic, cellular and network mechanisms, which underlie the formation of memory traces in the hippocampus. Her laboratory made seminal discoveries on the emergence, stability and reliability of memory during learning in the hippocampus and the mechanisms, which may underlie cognitive disorders in genetic models of schizophrenia and depression. Currently, she examines the role of inhibitory cells and interneuron plasticity in the establishment of memory engrams.

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Dr. Jonas-Frederic Sauer studied Molecular Medicine at the University of Freiburg, conducted his Ph.D. research at the University of Aberdeen (UK) and University of Freiburg (Ph.D. 2012) and investigated cellular and network defects in psychiatric disorders as a postdoctoral fellow in the laboratory of Prof. Bartos. With funding from the DFG (2018), EUCOR-the European Campus (2018) and the Else Kröner-Fresenius Stiftung (2020), he examines how neuronal oscillations organize network activity in the healthy and diseased brain.

Presentation of scientific institutions

Marilen Macher*

Armin Schram: a sponsor of curiosity-driven brain research

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At the age of 71, Armin Schram realized his life's dream: In 2000, he established the Schram-Stiftung to promote science and research in the neurosciences.

Since his childhood and youth spent in St. Gilgen, Vienna, and Prague, Armin Schram was interested in the enormous performance power of the brain. Already as a child, he was fascinated by the waggle dance of the honeybees; as a teenager, he kept track of the research of the later Nobel Laureate Karl von Frisch. If the pin-size brain of the bees was capable of such great things, how much more must the human brain be able to achieve! "I am fascinated by brain research. The human brain is a miracle of nature", he said at the inauguration of the foundation.

Armin Schram was convinced that without knowledge of the molecular processes in the brain, we will not be able to gain a full understanding of its complexity and its specific performance. As a result, he focused on basic research, leaving clinical research to other sponsors. "Research that aims for quick-to-achieve, specific goals will find funding more easily, no matter whether the goal is humanitarian, cultural, or medical. Where funding is scarce, progress and thus the well-being of people is best served in the long term by funding basic research."

Armin Schram was also passionately interested in how scientific insights come about, frequently in ways that may not always look straight forward. He was very critical about the fact that our current science funding systems do not accept failures and dead ends, rather they primarily support research that promises short-term pay-off at manageable risks. He admired the tenacity of somebody like Ramón y Cajal, the founder of modern neuroscience. Cajal's assumption that nerve cells are independent entities, for which he received the Nobel Prize in 1906, was corroborated only half a century later through electron microscopy.

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Through his foundation, Armin Schram wanted to support researchers who are willing to break new ground and leave the "low-risk culture" behind. With its "Cellular Neurobiology" funding program, launched in 2004, the foundation addresses "Cajal's modern heirs, pioneers who appreciate lateral thinking" and are not afraid of risk, i.e. independent young scientists such as heads of junior research groups, junior professors or young university teachers. To date, the foundation has issued seven calls and supported a total of 26 projects. The donor greatly enjoyed watching the growth of his "Schram family" and meeting the grant recipients at the "Schram Symposia" that took place since 2009 as satellite events of the Biennial Meetings of the German Neuroscience Society (Neurowissenschaftliche Gesellschaft – NWG). At these symposia Schram-funded project leaders present their latest research results. The donor's "closing remarks" always addressed and encouraged the participants at a very personal level. However, not only the participation in the biennial symposia, was an important occasion to interact with his scientific family. Armin Schram was deeply and personally committed to advising and supporting all project leaders of the foundation. He appreciated the direct exchange with the funded scientists, visited the researchers in their labs in Frankfurt, Vienna or Jena to look over their shoulders, and was highly interested in the advanced techniques that they applied. And he was delighted when the NWG in 2011 made him an honorary member for his long-term and outstanding commitment to fundamental research in neuroscience.

Armin Schram was always aware that a foundation needs to rely on expert advice. As a result, he appointed two renowned neuroscientists, Heinrich Betz and Eckart Gundelfinger, to the foundation's advisory board. Together, they set the course for the foundation's program. Schram participated in the annual board meetings, demonstrating great expertise and spreading contagious enthusiasm. Well-prepared with a list of his favorites in the pocket, he only revealed his choice once the selection process of winning grants had been completed. Quite often, his "passionate layman's" assessment was in complete accord with the ranking made by the advisory board based on external reviews.

Since Heinrich Betz' retirement from the board, Christian Rosenmund and Dorothea Schulte have joined Eckart Gundelfinger in guiding the course of the foundation. In 2019, the board, together with the NWG, launched the "Armin Schram Lecture" to honor the donor and his accomplishments posthumously. The first lecture of this type was given by Volker Haucke from the Leibniz Research Institute for Molecular Pharmacology in Berlin-Buch, who was a grant recipient in 2009.

Armin Schram was confident that his foundation would not run out of research questions in the near future; like Cajal, he was convinced that the grey substance was so complicated "that it defies the tenacious curiosity of researchers — and will continue to do so for many centuries to come."

Dr. Armin Schram: from the energy industry to neuroscience

(*January 31, 1929, Prague — †January 15, 2015, St. Gilgen)

After completing his studies of chemistry at the Vienna University of Technology (today: TU Wien), Armin Schram in 1953 joined the Deutsche Erdöl Aktiengesellschaft. In 1968 he became a member of the managing board of the company, which since 1970 has operated under the name of Deutsche Texaco AG. In 1979, he was appointed CEO of Deutsche Texaco AG.

Armin Schram ended his active career in 1993 as chair of the board of RWE-DEA and as a member of the board of RWE AG. Armin Schram had close ties to the Stifterverband für die Deutsche Wissenschaft: As board member of RWE AG, he was a member of the Hamburg/Schleswig-Holstein state board of trustees; in addition, he was a personal member since 1981. In 2000, he established the Schram-Stiftung under the umbrella of the Stifterverband to promote science and research in medicine and neuroscience, in particular in the field of brain research.

The Deutsches Stiftungszentrum (DSZ) of the Stifterverband für die Deutsche Wissenschaft e.V.

The Schram-Stiftung is managed by the Deutsches Stiftungszentrum (DSZ). The DSZ is the service center of the Stifterverband for donors and foundations. For more than 60 years, the DSZ has supported and advised donors on issues related to establishing a foundation and nonprofit and charitable foundations on realizing and achieving the goals set down in their charters. Currently, the DSZ supports 680 civil-law and trust foundations having a total endowment of more than EUR 3.1 billion. The nonprofit foundations have available ca. EUR 155 million for their foundation purposes per year. In the life sciences, several foundations focus their activities on neurology and neuroscience:

Hermann und Lilly Schilling-Stiftung für medizinische Forschung

The foundation was set up in 1970 by Aloysia Schilling in memory of her husband Hermann Schilling (d. 1961), former State Finance Councillor of the Preußische Staatsbank and CEO of the Vereinigte Elektrizitäts- und Bergwerks-Gesellschaft (VEBA).
Endowment capital: EUR 25 million

Funding programs

Schilling professorship and research group
The foundation supports excellent, innovative, and structurally pioneering research projects at the intersection of pre-clinical and clinical research in the neurosciences. The grants provide basic researchers in medicine and science with the opportunity to set up their own research groups and realize forward-looking, innovative projects in translational, neuroscientific research over an eight-year period.

Schilling research prize
The 20,000-Euro research prize is awarded every two years by the NWG for outstanding achievements in the field of brain research. The prize is given to young scientists no older than 35.

Stiftung zur Förderung junger Neurowissenschaftler

The civil-law foundation was established in 1999 by Professor Dr. med. Klaus Felgenhauer (1933—2002), former director of the Neurology Clinic at the University of Göttingen.
Endowment capital: EUR 1.35 million

Funding programs

Felgenhauer award
The 10,000-euro prize, which is offered by the foundation in cooperation with the Deutsche Gesellschaft für Neurologie (DGN), is awarded for outstanding accomplishments in

Figure 1: Side conversation during the second Schram Symposium 2011 at the Max Planck Institute for Experimental Medicine in Göttingen. From left to right: Heinrich Betz (Board member), Marilen Macher (Board member, Stifterverband), Armin Schram (the Founder) and Klaus-Armin Nave (Director at the host institution).
Figure 1: blende-elf, Wolfgang Rink

neurobiologically oriented clinical research. Prize recipients are young scientists who should not be older than 30 years.

Felgenhauer symposium

The foundation selects a program proposal that has been accepted by the DGN for its Annual Meeting's "Open Stage: Neuroscience" format, designates it as "Felgenhauer Symposium," and awards it with a prize money of EUR 3,000.

Schram-Stiftung

The foundation was established in 2000 by Armin Schram.
Endowment capital: EUR 8 million

Funding programs

Schram grants in basic neuroscience

Every three years, the foundation offers its program "Brain Research – Research Grants in Basic Neuroscience" for independent young scientists who want to tackle and develop new research themes. Priority is given to innovative, partly high-risk projects using novel methodological approaches.

Schram symposium

At this satellite symposium of the NWG Annual Meeting in Göttingen, Schram grantees present their research results.

Armin Schram lecture

Keynote lecture as part of the NWG Annual Meeting in Göttingen.

Figure 2: Armin Schram in the audience of the third Schram Symposium 2013 in Göttingen.
Foto: Peter Himsel

Figure 3: Lecture during the fifth Schram Symposium 2017 in Göttingen. Foto: David Ausserhofer

Stiftung Deutsche Gesellschaft für Neurologie

The foundation was established in 2017 by the Deutsche Gesellschaft für Neurologie e.V. (DGN).
Endowment capital: EUR 1.07 million

Funding programs

Through its Stiftung Deutsche Gesellschaft für Neurologie, the DGN pursues a clear vision: neurology's independence from third parties as well as the long-term, sustainable promotion of the aims of the DGN.

The foundation supports measures that are conducive to diagnostic and therapeutic progress in the whole field of neurology. It promotes talented young neurologists, conference and workshop participation, and is able to fund science prizes or research projects that do not find the necessary financial support elsewhere.

Deutsche Demenzhilfe — DZNE-Stiftung für Forschung und innovation

The foundation was set up in 2018 by Dr. h. c. Erwin V. Conradi in memory of his wife Karin Christiane Conradi.

Endowment capital: EUR 1 million

Funding programs

The foundation aims to promote science and research, in particular in the field of neurodegenerative diseases; a main concern is funding the activities of the Deutsches Zentrum für Neurodegenerative Erkrankungen e.V. (DZNE) in Bonn. Here, a particular focus is on work that aims to translate the results from basic research into practice and the "Querdenker-Programm" (unorthodox thinker program) open to young researchers with innovative ideas outside the scientific mainstream.

Bionote

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Marilen Macher heads the Department Science and Environment at the Deutsches Stiftungszentrum (DSZ) in the Stifterverband. She studied biology in Cologne and Gießen and performed doctoral research at the Institute for Plant Ecology in Gießen. Since 1990 she has been working at the DSZ. She joined the Board of the Schram Foundation in 2000.

Nachrichten aus der Gesellschaft

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Göttinger Tagung der Neurowissenschaftlichen Gesellschaft 2021

Liebe NWG-Mitglieder,

Göttingen Meeting 2021 goes virtual!
Die NWG gehört glücklicherweise zu jenen Fachgesellschaften, deren nächste Tagung erst wieder für 2021 geplant war. So muss nicht wie beim FENS Forum 2020 das physische Meeting kurzfristig in ein virtuelles umgewandelt werden. Vielmehr kann sorgfältig geplant werden – "Glück im Unglück", trotz COVID-19, könnte man sagen. Denn allen Hoffnungen zum Trotz zeichnete sich seit dem Spätsommer ab, dass es auch im Frühjahr 2021 leider noch keine Tagungen wie in der Vergangenheit geben wird. So blieb dem NWG-Vorstand Zeit, sich reiflich Gedanken zur nächsten Göttinger Tagung zu machen, von anderen virtuellen Konferenzen zu lernen und Meinungen einzuholen. Es war keine Option die Tagung ausfallen zu lassen. Fast 50 Jahre Tradition seit der ersten Neurobiologentagung im Jahr 1973 wirft man nicht so einfach über Bord. Und eine Verschiebung ins Jahr 2022 hätte zu Konflikten mit anderen großen und kleinen Tagungen geführt.

Die NWG möchte ihren Mitgliedern und der Neuroscience Community im In- und Ausland auch 2021 eine Tagung anbieten, die wie gewohnt den weit gefächerten neurowissenschaftlichen Austausch ermöglicht. Vor dem Hintergrund virtueller Tagungen in den letzten Monaten und deren Vor- und Nachteilen wurde nun besonderes Augenmerk auf die Frage gerichtet, mit welchen Maßnahmen die Göttinger Tagung am besten die Bedürfnisse ihrer Teilnehmer berücksichtigen kann.

Göttingen 2021 wird nicht versuchen, ein analoges Meeting zum ursprünglich geplanten Zeitpunkt mit einem virtuellen Treffen zu spiegeln. Niemand möchte wie auf einer realen Konferenz 12 Stunden am Tag bei einem virtuellen Meeting anwesend sein, sprich in diesem Fall vor dem Rechner sitzen. Und, so bedauerlich das auch ist, die so wichtigen sozialen Aspekte einer analogen Tagung können weder durch animierte virtuelle 3D-Räume noch durch Chats ersetzt werden. Also haben wir das Konzept der virtuellen Göttinger Tagung auf das fokussiert, was real wie virtuell das Herzstück einer Konferenz ist, nämlich die Vermittlung von Spitzenwissenschaft, und dabei versucht, uns an den Bedürfnissen und Erwartungen der teilnehmenden Wissenschaftler zu orientieren.

Die Göttinger Tagung 2021 wird mit Blick auf das Vortragsprogamm deshalb in zwei Phasen aufgeteilt. Nämlich einerseits in eine rezeptive (Anschauen der Beiträge) und andererseits eine aktive (Videokonferenzen) Phase, die zeitlich voneinander entkoppelt sind. In Phase 1 werden alle Beiträge als vorab aufgezeichnete Videos und Präsentationen online zur Verfügung gestellt. Phase 2 ist die Diskussionszeit; diese beginnt zwei Wochen nach der Online-Bereitstellung und geht über anderthalb Wochen (22.–30. März 2021). In diesem Zeitraum werden täglich Video-Konferenzen für interessierte Teilnehmer mit den Sprechern der Hauptvorträge, Symposien und Workshops vorzugsweise am Nachmittag und Abend angeboten. Die Tageszeit wurde so gewählt, um eine Kollision mit der Arbeit im Labor zu vermeiden. Dieses Vorgehen hat den Vorteil, dass

(a) die Teilnehmer bereits im Vorfeld die Vorträge zu einem selbst gewählten Zeitpunkt anschauen können, bei Bedarf auch mehrmals. Fragen und Diskussionsansätze für die Videokonferenz können in Ruhe ausgearbeitet werden und
(b) die Sprecher werden nur dann bei der Videokonferenz anwesend sein müssen, wenn es auch verbindliche Anmeldungen für die Diskussionsrunden gibt. Es kommt somit nicht zu der Situation, die sich in den letzten Monaten oft bei den Chats nach Live-Vorträgen zeigte, wenn sich Sprecher vergeblich für eventuelle Anfragen bereit halten mussten. Nach Phase 2 werden die Vorträge weiterhin online zugänglich sein.

Poster werden ebenfalls über mehrere Monate zur Verfügung stehen. Jedes Poster bekommt einen kurzen „elevator pitch" und auch hier werden Diskussionszeiträume individuell und zeitlich flexibel vereinbart.

Wie für bisherige Göttinger Tagungen wird es auch 2021 Stipendien geben, und zwar zur Deckung der Registrierungsgebühr. Diese wird erheblich gesenkt, Studenten können bereits ab 45 Euro teilnehmen.

Wir sind uns bewusst, dass wir einen neuen Versuch wagen, mit einer schwierigen Situation umzugehen. Ob dieser gelingt und genügend Interesse auf sich zieht, zeigt sich allerdings erst, wenn eine kritische Teilnehmerzahl

erreicht wird. Die Mitgliederbefragung zur Teilnahme an einem virtuellen Göttinger Meeting 2021 hat ein eher ambivalentes Bild ergeben. 21% der Befragten haben geantwortet, was uns sehr freut. Von ihnen hatten sich 243 Mitglieder (53%) für eine virtuelle Tagung ausgesprochen, 212 Mitglieder (47%) dagegen.

Deshalb unser Aufruf: Gestalten Sie die anstehende virtuelle Göttinger Tagung der Neurowissenschaftlichen Gesellschaft durch ihre Beiträge in Form von Vorträgen, Postern und Diskussionen mit, und geben Sie ihr die Chance, auch in diesem Format die aktuelle neurowissenschaftliche Forschung in Deutschland darzustellen und zu repräsentieren. Die Deadline für die Anmeldung zur Teilnahme mit eigenem Beitrag ist Montag, der 14. Dezember 2020. Gegenüber einer analogen, sehr eng strukturierten Konferenz hat ein virtuelles Tagungsprogramm tatsächlich den Vorteil, dass es weder räumliche noch zeitliche Begrenzungen gibt, und somit nun oft vermisster Raum für weitere Aktivitäten entstehen kann – technische Tutorials, Job Interviews, Workshops und ähnliches. Anregungen und Ideen hierzu sind willkommen.

Prof. Dr. med. Albert C. Ludolph
Präsident

Prof. Dr. Christine Rose
Vize-Präsidentin

NEU auf dasGehirn.info

Mit dem Themenschwerpunkt **Gestörter Stoffwechsel** konnte im September ein weiteres wichtiges Thema dem Portfolio von dasGehirn.info hinzugefügt werden. Dabei handelt es sich um eine Partnerschaft mit der Schering Stiftung.

Hierfür wurden die folgenden Beiträge erstellt:

Das Gehirn isst mit: Kalorien sind überlebenswichtig. Entsprechend eingespielt ist die Zusammenarbeit zwischen Darm und Gehirn, die unsere natürlichen Ernährungsratgeber sind. Doch moderne Lebensmittel können sie in die Irre führen.

Wenn Hunger und Genuss aus dem Gleichgewicht geraten: Die krankhafte Fettleibigkeit (Adipositas) ist weltweit auf dem Vormarsch. Verstehen lässt sich dieses Phänomen nur mit einem Blick in das Gehirn.

Zu süßes Blut: Menschen mit Diabetes mellitus haben einen zu hohen Blutzuckerspiegel, weil sie zu wenig Insulin produzieren oder das Hormon seine Wirkung nicht entfalten kann. Experten vermuten Zusammenhänge zwischen Diabetes und neurodegenerativen Erkrankungen.

Adipositas und Diabetes 2 haben sich zu Volkskrankheiten entwickelt. Welche Rolle das Gehirn dabei spielt – und wie beide Krankheiten auf das Gehirn zurückwirken – erforscht der Endokrinologe und Genetiker Jens Brüning am Max-Planck-Institut für Stoffwechselforschung. In dem Videointerview **Gehirn, Diabetes, Adipositas** gibt er Auskunft über den gestörten Stoffwechsel.

Das Schwerpunktthema **Sucht** in Themenpartnerschaft mit dem TRR 265, mit dem dasGehirn.info im Mai 2020 online gegangen ist, wurde um den Text **Die Neurobiologie der Sucht** ergänzt: Wer an einer Suchterkrankung leidet, dessen Gehirn hat sich verändert. Sein Belohnungssystem folgt nicht mehr dem evolutionär durchaus sinnvollen Zweck, sondern wurde zweckentfremdet: Es hat die Sucht gelernt.

In der Rubrik **Neues aus der Wissenschaft** macht dasGehirn.info Anfang September auf die folgenden **Pressemeldungen** aus den Instituten aufmerksam:

- Maßgeschneidertes Molekül knüpft Nervenkontakte | Deutsches Zentrum für Neurodegenerative Erkrankungen e. V. (DZNE) (31.08.2020)
- Menschen mit erhöhtem Alzheimerrisiko haben Defizite beim Navigieren | Ruhr-Universität Bochum (31.08.2020)
- Großzügig teilen, länger leben | Max-Planck-Institut für demografische Forschung (01.09.2020)
- Zeit und Hirngröße – von Mäusen und Menschen | Max-Planck-Institut für Molekulare Zellbiologie und Genetik (03.09.2020)
- Corona und die Folgen für die Psyche: ein differenzierter Blick ist nötig | Zentralinstitut für Seelische Gesundheit (07.09.2020)
- Verklumpte Proteine lassen Blutgefäße des Gehirns versteifen | Deutsches Zentrum für Neurodegenerative Erkrankungen e. V. (DZNE), Hertie Institute für klinische Hirnforschung (11.09.2020)
- Risiko-Gen wirkt sich frühzeitig auf das Gehirn aus | Deutsches Zentrum für Neurodegenerative Erkrankungen e. V. (DZNE), Ruhr-Universität Bochum (15.09.2020).

Möchten Sie eine Pressemeldung an "dasGehirn.info" weitergeben, wenden Sie sich bitte an Arvid Leyh (E-Mail: a.leyh@dasgehirn.info).

Methodenkursprogramm 2021 der NWG

Comparative Anatomy and Pathology of the Rodent and Human Brain

March 1–3, 2021
Venue: Section Clinical Neuroanatomy, Neurology, Center for Biomedical Research (ZBF), Helmholtzstr. 8/1, 89081 Ulm
Topics: Overview of the anatomy of the rodent and human brain and spinal cord; hands-on-lab sessions for introduction into neuroanatomical techniques to study the human brain; pathological neuroanatomy of neurodegenerative disorders including but not limited to Alzheimer's disease, Parkinson's disease and Amyotrophic Lateral Sclerosis
Organisation and registration: Prof. Dr. Deniz Yilmazer-Hanke, Tel. (office): +49 (0)731 500 63157, (lab): +49 (0)731 500 63158, E-Mail: deniz.yilmazer-hanke@uni-ulm.de
Pathoanatomy of the Human Central Nervous System
Registration deadline: February 19, 2021

Pathoanatomy of the Human Central Nervous System

March 4–5, 2021
Venue: Section Clinical Neuroanatomy, Neurology, Center for Biomedical Research (ZBF), Helmholtzstr. 8/1, 89081 Ulm
Topics: Introduction to neuroanatomical techniques to study the neuroanatomy of the human brain including hands-on laboratory sessions; pathological anatomy, histology and histopathology of the human brain and spinal cord in neurodegenerative diseases; staging of pathological changes in Alzheimer's and Parkinson's disease and Amyotrophic Lateral Sclerosis
Organisation and registration: Prof. Dr. Deniz Yilmazer-Hanke, Tel. (office): +49 (0)731 500 63157, (lab): +49 (0)731 500 63158, E-Mail: deniz.yilmazer-hanke@uni-ulm.de
Behavioral Testing in Rodents: from Cognition, Motor Function, Emotion, and Anxiety to Pain
Registration deadline: February 19, 2021

Behavioral Testing in Rodents: from Cognition, Motor Function, Emotion, and Anxiety to Pain

March 18–19, 2021
Venue: Interdisciplinary Neurobehavioral Core INBC, University of Heidelberg, INF 515; 69120 Heidelberg
Topics: Behavioral testing in rodents: from cognition, motor function, emotion, anxiety to pain. A hands-on course.
Organisation and registration: Dr. Claudia Pitzer, Tel.: +49 (0)6221 1858504, E-Mail: Claudia.Pitzer@pharma.uni-Heidelberg.de
Cellular Models of Neurodegenerative Diseases
Registration deadline: March 4, 2021

Cellular Models of Neurodegenerative Diseases

May 2021
Venue: Sektion für Translationale Neurodegeneration, Klinik für Neurologie, Universitätsmedizin Rostock, Gehlsheimer Strasse 20, 18147 Rostock
Topics: Isolation of fibroblasts, production and culture of patient-derived IPS cells (including principles of CRISPR/CAS9), differentiation of IPS cells to neurons and glial cells, Live Cell Imaging of different cell organelles using baculovirus
Organisation and registration: Prof. Dr. Dr. Andreas Hermann, Frau Bianca Hartung; Sektion für Translationale Neurodegeneration "Albrecht Kossel"; Klinik für Neurologie, Rostock, Tel.: +49 (0)381 494-9541: E-Mail: sektionsleiter.akos@med.uni-rostock.de
Registration deadline: March, 2021

Tübingen Systems Neuroscience Symposium 2021

June 17–18, 2021
Venue: MEG-Zentrum der Universität Tübingen, Otfried-Müller-Straße 47, 72072 Tübingen
Topics: The 2020 Tübingen Systems Neuroscience Symposium brings together leading international researchers in the field of systems neuroscience. Topics range from neurophysiological testing in animals to functional imaging in humans (MEG, EEG, fMRI). One focus of the symposium is the presentation of state of the art methods. The talks target students and researchers with profound previous knowledge.
Organisation and registration: Prof. Dr. Christoph Braun, Tel: 07071 29 87706, Fax: 07071 29 5706, E-Mail: christoph.braun@uni-tuebingen.de
4th Modelling Symposium: Introducing Deep Neural Networks
Registration deadline: April 30, 2021

4th Modelling Symposium: Introducing Deep Neural Networks

July 26–30, 2021
Venue: Otto-von-Guericke University Magdeburg, G28 (R 027), 39106 Magdeburg (may change to different location in Magdeburg)
Topics: A hands-on course about applied deep learning covering: machine learning basics, common building blocks, design patterns and architectures (e.g. CNNs, RNNs, attention mechanisms etc.), common applications including image, audio and text processing, optimisation and regularization techniques, introspection and model diagnosis, model compression and transfer learning, best practices and general workflows (https://www.noesseltlab.org/events-presentations/4th-modellingsymposium/)
Organisation and registration: Dr. Felix Ball, OvG Universität Magdeburg, Institut für Psychologie – Biologische Psychologie, E-Mail: events.biopsych@ovgu.de
Transcranial Brain Stimulation in Research and Clinic: Best Practice
Registration deadline: March 31, 2021

Transcranial Brain Stimulation in Research and Clinic: Best Practice

September 8–10, 2021
Venue: Klinik für Klinische Neurologie, Universitätsmedizin Göttingen, Robert-Koch-Strasse 40, 37075 Göttingen
Topics: transcranial magnetic-, direct current - alternating current and random noise stimulation, theoretical background of the stimulation, animal models, modelling of current flow in the brain, research and clinical applications; neuronavigation, neuronal oscillations, cognition, ethical aspects of transcranial stimulation
Organisation and registration: apl. Prof. Andrea Antal, Tel.: +49 (0)551 398461, E-Mail: AAntal@gwdg.de
Imaging Techniques in Neuroscience
Registration deadline: September 1, 2021

Imaging Techniques in Neuroscience

September 20–24, 2021
Venue: Leibniz Institute for Neurobiology (LIN), Brenneckestraße 6, 39118 Magdeburg
Topics: Hands-on introduction into advanced imaging techniques to study neuronal function: super-resolution STED, lightsheet microscopy (including clearing methods), 2photon microscopy, calcium imaging, FLIM/FRET, label-free metabolic imaging (NADH/FAD), image analysis

Organisation: Leibniz Institute for Neurobiology, Combinatorial NeuroImaging Core Facility (CNI)
Registration: Torsten Stöter, Combinatorial NeuroImaging Core Facility (CNI), Leibniz Institute for Neurobiology, Tel.: 0391 6263 92171, E-Mail: cni-reg@lin-magdeburg.de
Details unter https://nwg-info.de/aktivitaeten/kurse_workshops/2021
Registration deadline: July 31, 2021

Wissenschaftlicher Koordinator: Prof. Dr. Hans Werner Müller, Labor für Molekulare Neurobiologie, Neurologische Klinik, Universitätsklinikum Düsseldorf, Moorenstr. 5, D-40225 Düsseldorf, E-Mail: HansWerner.Mueller@uni-duesseldorf.de

Neueintritte

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

Shih-pi Ku, Dr. (Magdeburg)
Nicole Kucharowski (Bonn)
Daniela Mauceri, Dr. (Heidelberg)
Julian Rottschäfer (Göttingen)
Debora Rabea Tibbe (Hamburg)
Daniel Woike (Hamburg)
Alina Zacher (Magdeburg)

Der Mitgliedsstand zum 1. Oktober 2020 beträgt 2.192 Mitglieder.

Ausblick

Thomas Wachtler et al.
Building a community for data management in neuroscience: The NFDI-Neuro consortium

Michael Hanke et al.
Research data management in neuroimaging

Hansjörg Scherberger et al.
Rigour and efficiency in handling complex neurophysiological data

Otto Witte et al.
Needs and challenges for data management in clinical neuroscience

Maryanne Martone
Open and FAIR neuroscience

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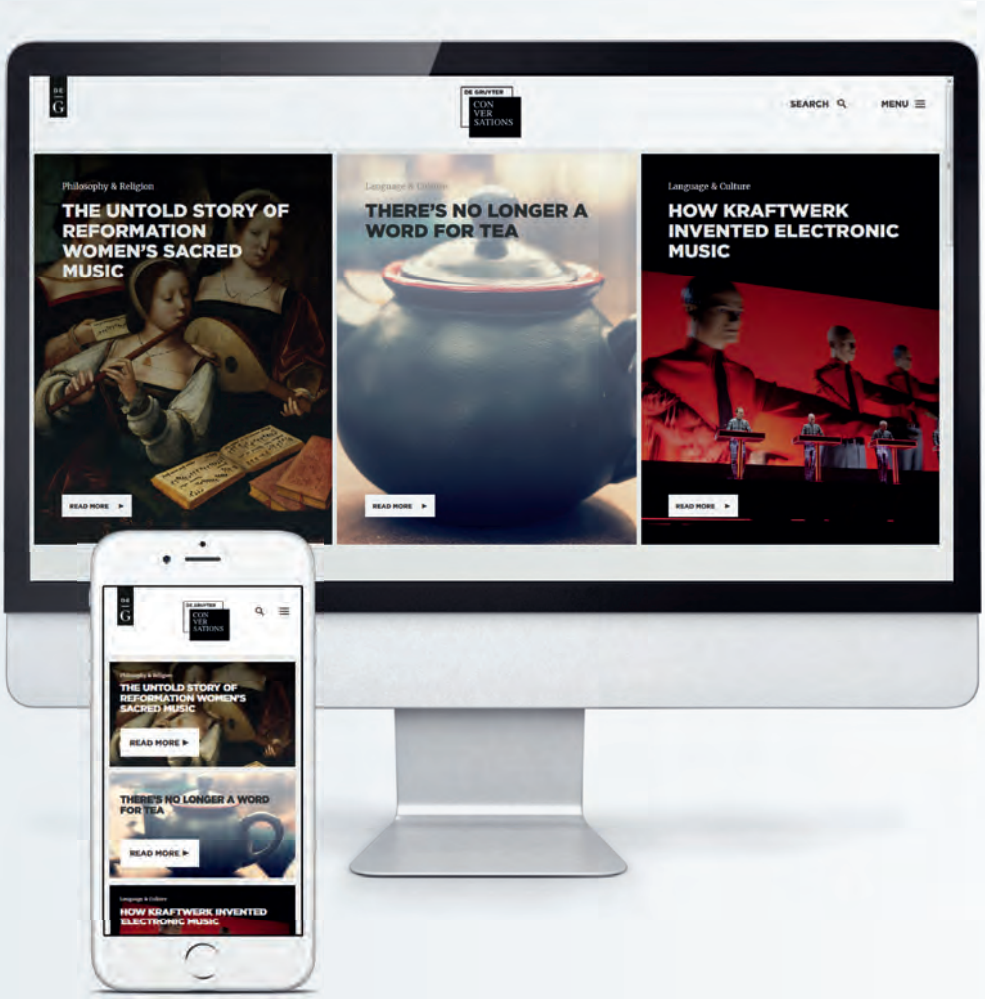

degruyter.com/conversations

Neurowissenschaftliche Gesellschaft e.V. (NWG)

- Beitrittserklärung -

Hiermit erkläre ich meinen Beitritt zur Neurowissenschaftlichen Gesellschaft e.V. (NWG).

Eintrag in das Mitgliederverzeichnis:

Name

Vorname

Titel

Dienstadresse

Universität/Institut/Firma

Straße

PLZ/Ort | Land

Telefon/Email

Privatadresse

Straße

PLZ/Ort

Telefon

Rechte und Pflichten der Mitgliedschaft siehe Satzung (nwg-info.de/de/ueber_uns/satzung). Mit meiner Unterschrift bestätige ich, dass ich die Satzung sowie die Datenschutzrichtlinie (nwg-info.de/de/datenschutz) zur Kenntnis genommen habe und diese anerkenne.

Datum/Unterschrift

Ich unterstütze den Antrag auf Beitritt zur NWG e.V.

Datum/Unterschrift des Mitglieds | Datum/Unterschrift des Mitglieds

Bitte senden Sie Ihren Antrag an die Geschäftsstelle der NWG:

Stefanie Korthals
Neurowissenschaftliche Gesellschaft e.V.
MDC
Robert-Rössle-Str. 10
13092 Berlin
Email: korthals@mdc-berlin.de
Tel.: +49 30 9406 3127

Ich optiere für folgende 2 Sektionen:

- ☐ Computational Neuroscience
- ☐ Entwicklungsneurobiologie/Neurogenetik
- ☐ junge NWG (jNWG)
- ☐ Klinische Neurowisschenschaften
- ☐ Kognitive Neurowissenschaften
- ☐ Molekulare Neurobiologie
- ☐ Neuropharmakologie und -toxikologie
- ☐ Systemneurobiologie
- ☐ Verhaltensneurowissenschaften
- ☐ Zelluläre Neurobiologie

Ich bin Student ☐ ja ☐ nein
(Bescheinigung anbei)
Ich bin ☐ weiblich ☐ männlich ☐ divers

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Unterschrift:

Kontoinhaber:

Anschrift: