ZMNH

Center for
Molecular
Neurobiology
Hamburg

Research Report
2006 - 2009

Universitätsklinikum
Hamburg-Eppendorf

Cover Image: Nuclear Hoechst 33342 staining of a coronal brain slice from a six days-old rat. The staining and the corresponding picture were done by Christoph Janiesch, BMBF/DFG Emmy-Noether Research Group "Developmental Neurophysiology" *Prof. Dr. Ileana Hanganu-Opatz*

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With special thanks to Eva-Maria Suciu and Hans-Martin Ziethen

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Welcome Address of the Dean and Director of the University Medical Center Hamburg-Eppendorf

Since its inception in 1987 the ZMNH has rapidly developed into a focal point of excellence in basic biomedical neuroscience research. Today, the institute is internationally regarded as world-class institution in the field of neurobiology and is considered as one of the premier research centers in Germany. The ZMNH has been the catalyst of a multitude of interdisciplinary projects and has substantially advanced neuroscience at the highest level. The combination of rich output of seminal scientific work published in the most renowned international peer reviewed journals, and the numerous scientific awards bestowed upon the researchers of the ZMNH, attest to the institute's international reputation. The impressive performance of the ZMNH is a reflection of exemplary cooperation between basic and clinical aims. Its research concerns fundamental questions of molecular neurobiology with a special emphasis on understanding the molecular mechanisms of synaptic transmission and plasticity in health and disease. In many cases, research results at the ZMNH are swiftly applied to diagnostic and therapeutic problems in medicine and human genetics. This process is greatly facilitated by the generation and analysis of transgenic and "knock-out" mouse lines as indispensable models

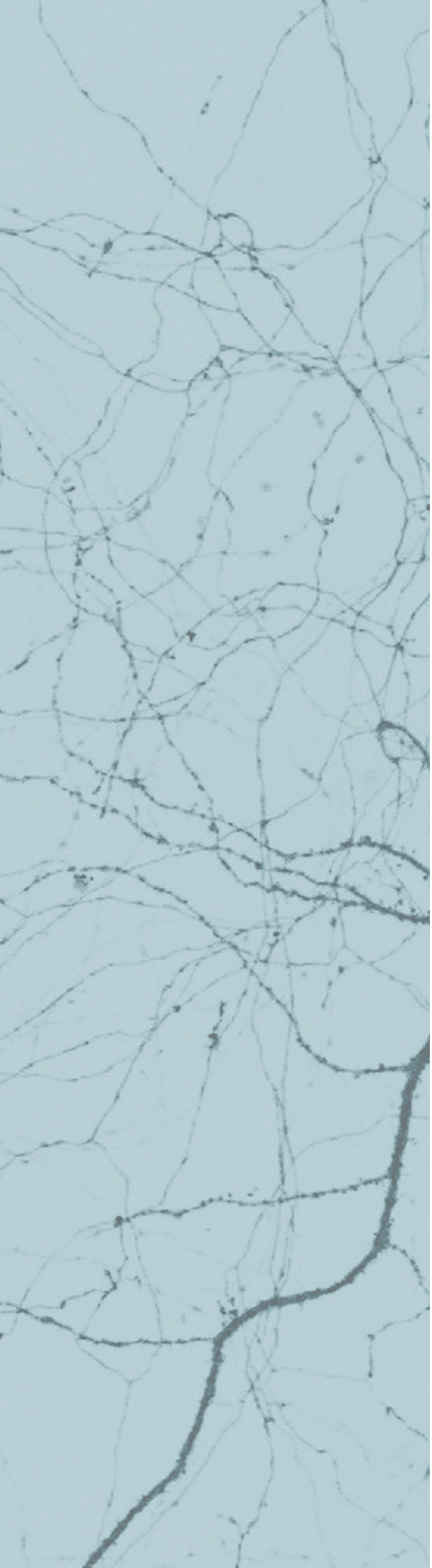
for the study of the molecular and cell biological basis of the diseases of the nervous system. The study of mutant mouse strains generated at the ZMNH has led to identification of novel disease genes and has greatly helped in understanding and elucidating the pathophysiology of human nervous system disorders. As such, the ZMNH has significantly contributed on various levels to improvements in diagnosis and therapy of human disease.

Currently, the ZMNH is undergoing a phase new recruitments. This process was initiated by imminent changes in leadership of the four original institutes. Such phases bear great potential for a successful future. With Dr. Martin and Dr. Kuhl, two preeminent scientists have recently been recruited to the ZMNH. Dr. Martin's research projects aim at understanding the pathogenesis of multiple sclerosis, developing novel animal models for this disease, and the implementation of therapeutic approaches. Dr. Kuhl's interest centers on learning and memory. Dr. Kuhl has conducted pioneering work on the contribution of activity-induced gene transcription to the consolidation of memories and paved the way for a better understanding of a variety of devastating diseases which disturb memory formation. Most recently Dr. Kneussel was recruited as new department head at the ZMNH. Dr. Kneussel is internationally renowned for his work on cellular processes of synaptic plasticity and memory through studies of intracellular cytoskeleton-based transport. The strategic hiring not only by the ZMNH but by the entire UKE in this area of neurobiological research over the last several years has allowed us to gather an impressive number of basic and clinical scientists who are working on different aspects of this endeavor, ranging from systems analysis to molecular neurobiology.

We take pride in fostering basic discovery research at the ZMNH of our University Hospital. Over the years, the ZMNH has served as model that has been copied at other places in Germany. The center's success is also owed to an external scientific advisory board which has the important function of evaluating the performance of the ZMNH at regular intervals and advising the Dean of the Faculty of Medicine as well as the Head of the University Hospital on matters of strategic importance. As a result, the University Medical Center Hamburg-Eppendorf is fully committed to conserving the model character of the ZMNH. We are aware that success comes at a cost and are devoted to further strengthening the ZMNH as highly supportive research environment, with minimal administrative burden and teaching obligations, and endowed with generous personnel and material support. We firmly believe in the value of the principles on which the ZMNH is based and wish the Center success in our common endeavour to understand and fight the debilitating diseases of the brain.

Uwe Koch, M.D., Ph.D.
Dean UKE

Jörg F. Debatin, M.D.
Director UKE



Director's Message

Men ought to know that from nothing else but the brain come joys, delights, laughter and sports, and sorrows, grieves, despondency, and lamentations. And by this, in an especial manner, we acquire wisdom and knowledge, and see and hear and know what are foul and what are fair, what are bad and what are good, what are sweet and what are unsavory… and by the same organ we become mad and delirious, and fears and terrors assail us... All these things we endure from the brain when it is not healthy… In these ways I am of the opinion that the brain exercises the greatest power in the man.

-Hippocrates, Fourth Century B.C.

The ZMNH is a research center of the Faculty of Medicine at the University Hospital Eppendorf (UKE). The mission of the ZMNH is to conduct basic research into the molecular mechanisms of synaptic transmission of the brain in health and disease. To understand the brain both as the organ of mental function and as a target of disease we will need to understand fundamental brain mechanisms in molecular and cellular detail. Such an understanding is a prerequisite for the development for new therapeutics to combat the neurological and mental illnesses that ever-increasingly affect both our young and aged population. The ZMNH has a strong foundation of basic discovery research which always has been the engine that drives the development of new and better therapies of debilitating diseases of the nervous system. In addition to research, the ZMNH engages in graduate training and carries out a two-year graduate course program in Molecular Biology.

As a step towards achieving this mission, I am delighted to report that the ZMNH has attracted world-renowned neuroscientists. The Center comprises five institutes which are headed by full professors. In addition, the Center provides

space and infrastructure for several internally and externally funded independent research groups, including Emmy-Noether and BMBF-funded groups, and for a scientific team headed by a Heisenberg Professor. Although working in different animal models and using a wide range of different techniques, all these scientific groups focus on understanding fundamental mechanisms that control brain functions. The Institute for Molecular Neurogenetics (Matthias Kneussel) has been recently established and aims to understand cellular processes of synaptic plasticity by studying intracellular cytoskeleton-based transport, synaptic turnover and anchoring of neurotransmitter receptors. My Institute for Molecular and Cellular Cognition (Dietmar Kuhl) was instantiated in 2009 and is taking an integrative approach to the studies of learning and memory building on expertise in genetics, biochemistry, molecular and cellular physiology, and behavioral analysis. The Institute for Neuroimmunology and Clinical Multiple Sclerosis Research (Roland Martin) studies molecular mechanisms of immunopathogenesis of multiple sclerosis and tries to implement the results in novel therapies for this disease. Research at the Institute for Neural Signal Transduction (Olaf Pongs) is focused on structural and functional studies of ion channels, in particular functional and dysfunctional potassium channels, and associated diseases of the nervous system. The Institute for Biosynthesis of Neural Structures (Melitta Schachner Camartin) is concerned with the function of neural recognition molecules in development of the nervous system, regeneration after brain lesions and processes mediating the induction and maintenance of synaptic plasticity in the adult brain. We have also enticed exceptional young scientists to strengthen the Center as independent Research Group leaders. These smaller research groups enjoy the accessibility to all developed expertise and infrastructure and bring in turn new themes and techniques. The Research Group for Neuroimmunology (Manuel Friese) is interested in pathomechanistic research of neuroimmunological disorders and, in particular, multiple sclerosis. Work of the Research Group for Neuronal Networks in Developing Brain (Ileana L. Hanganu-Opatz) centers on the mechanisms of cortical "wiring" during early development by analyzing the role of electrical activity in brain maturation. The major goal of the Research Group for Experimental Neuropediatrics (Dirk Isbrandt) is to understand the mechanisms of epileptogenesis and neuronal synchronization in hyperexcitable neuronal networks caused by dysfunctional ion channels. The group Synaptic Protein Networks (Hans-Christian Kornau) aims to understand synaptic physiology by unraveling novel interactions of G-protein coupled receptors and ion channels. The Research Group for Development and Maintenance of the Nervous System (Edgar Kramer) is interested in the function of cell surface receptor signaling in the nervous system during development and aging. Research at the ZMNH is supported by central scientific service units for bioanalytics (Sabine Hoffmeister-Ullerich), morphology (Michaela Schweizer), systems biology and protein-protein interactions (Christian Schulze), and by a facility for transgenic animals (Irm Hermans-Borgmeyer). These facilities are led by highly competent and experienced scientists, who are administratively associated with individual institutes but provide services to the entire Center. Also available is an in-house bioinstrumentation unit (Torsten Renz, Fritz Kutschera) that has proven indispensable for the development and maintenance of scientific instrumentation, and an in-house data processing and server unit (Hans-Martin Ziethen) which autonomously manages the entire data transfer and storage needs of the ZMNH. Furthermore, a largely independent administration led by Katja

Husen ensures efficient and flexible administrative support. Formerly responsible for the administration of the Center was Jürgen Dralle whom I would like to thank for his sedulous work.

The ZMNH is currently undergoing a generational turnover which allows its current and future investigators to decisively shape the scientific, conceptual and organizational directions for the next decade. A major change that has already been instituted has been to integrate a larger number of junior research group leaders into the ZMNH. On the directorial level, Roland Martin was the first of the 'new guard'. He has been endowed with a Hertie Professorship which in the long term will be integrated into the regular personnel and support structures of the ZMNH. Matthias Kneussel was a ZMNH Research Group leader and has most recently joined the board of directors and I relocated my Department from the FU Berlin to the ZMNH in May 2009. Another director position is currently in the process of negotiation and an additional opening will present itself when Olaf Pongs retires in 2011. In these decisive steps and future strategic planning we will draw on the expertise of our external scientific advisory board.

I could not be more pleased that at the Center I found colleagues that share my interest in bridging the gap between molecules and mind, and trying to understand the brain in health and disease at many levels utilizing multidisciplinary approaches and new ways of integrating insights from many areas of technical expertise. I feel privileged to work with such a distinguished group of researchers and have complete faith that the ZMNH will act as a research accelerator and a focal point for innovation at the University Medical Center. I am more optimistic than ever that research at the ZMNH, in collaboration with our colleagues on the University Hospital campus, significantly advances our understanding of the brain and helps to insightfully and effectively treat the avalanche of neurological and mental ill-health disorders that account for almost half of the disease burden of our community. I invite you to peruse this booklet and share our pride in our accomplishments and our optimism for the future.

Dietmar Kuhl, Ph.D.
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Structure of the ZMNH

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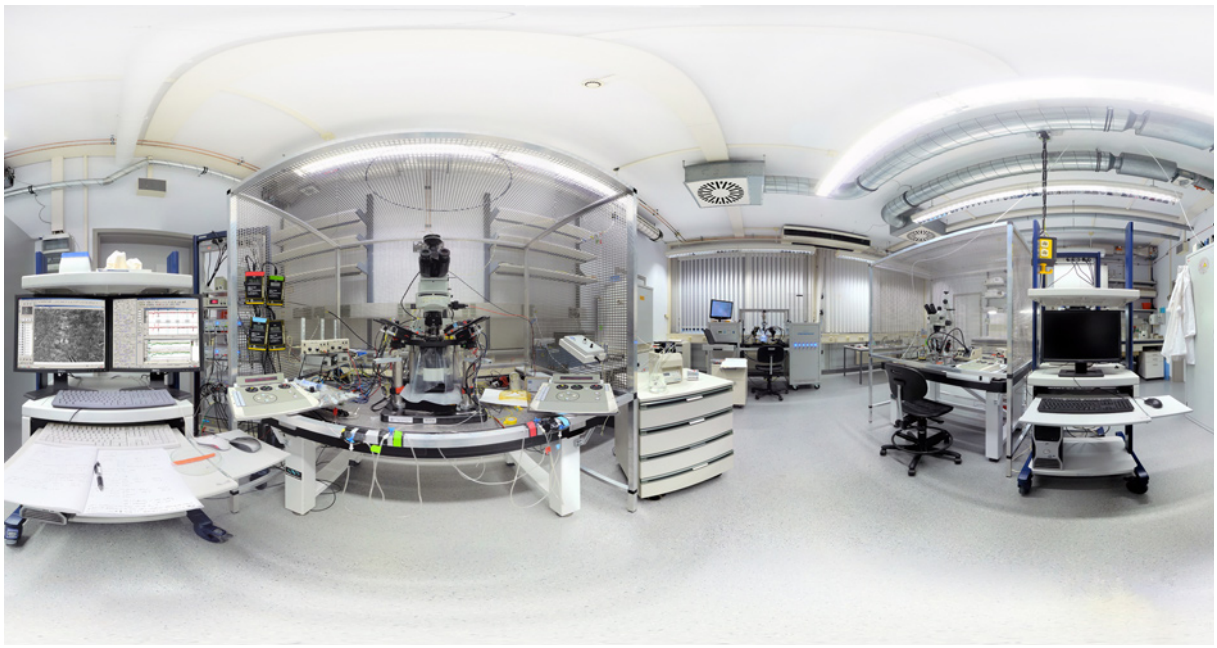


Institute for Molecular and Cellular Cognition (IMCC)

Dietmar Kuhl

Learning about activity-dependent genes, synaptic plasticity and the persistence of memories

Memory binds our mental world together and allows us to have continuity in our lives. Much of what we know about the outside world and about ourselves we have learned. To a good measure we are who we are because of what we have learned and remember. Conversely, the loss of memory, as can be seen in many diseases, leads to the loss of our live history and of our immediate self. Scientists of the IMCC are taking an integrative approach to the studies of learning and memory building on their expertise in mouse genetics, biochemistry, molecular and cellular physiology, and behavioral analysis. Several of the activity-regulated genes first identified in our laboratory code for proteins that can directly modify the physiology of neurons. Our research moves from the identification of activity-regulated genes that are induced during learning to the analysis of synaptic plasticity in the brain and wants to assess which consequences they convey on the behavior of animals and their capability to learn and store information. We bring to these problems a multi-disciplinary approach that includes (i) genomic and proteomic approaches, (ii) reverse genetic approaches in the animal and primary neuronal cultures, (iii) electrophysiological recordings from hippocampal and cortical neurons *in vivo* and *in vitro*, and (iv) analysis of acquisition and consolidation of memory traces using behavioral learning tasks. We anticipate that this analysis will provide insights into how expression of genes that are activated in coordinated biochemical pathways may contribute to the formation of synaptic plasticity. In as much as the identified genes bear the potential to act as direct effectors of neuronal physiology, they become promising targets for the therapeutic intervention of the devastating diseases that disturb synaptic plasticity and memory.

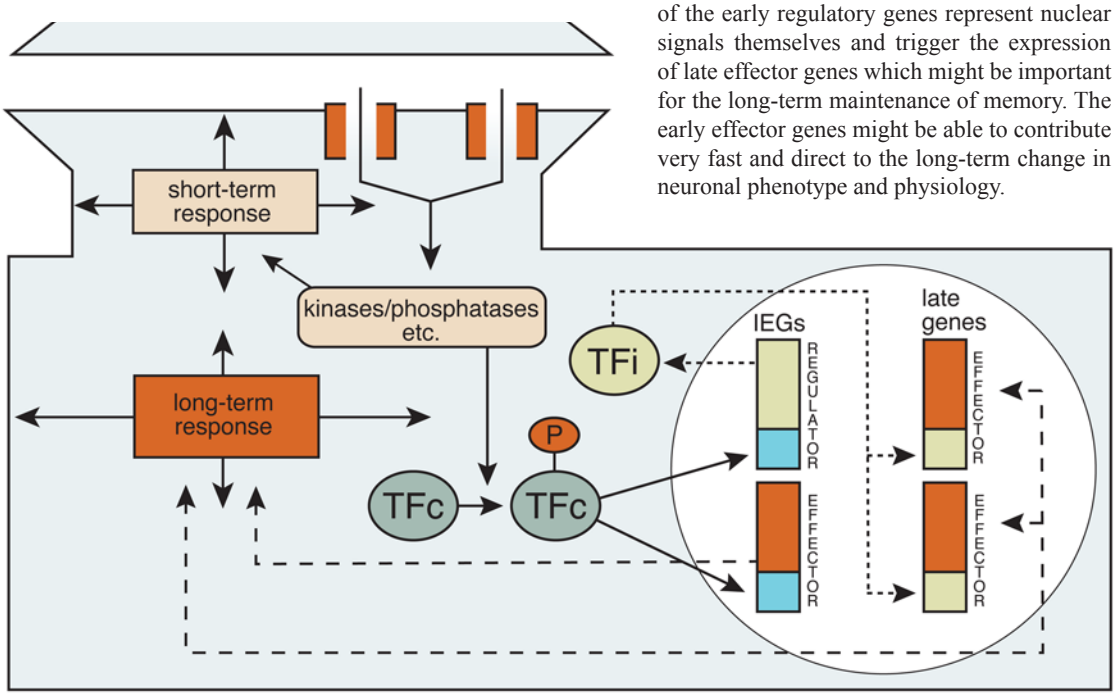
1. Profiling of the activity regulated transcriptome

Guido Hermey, Daniel Mensching, Andre Matzke

Neurons have the capacity to undergo activity-dependent changes in their molecular composition and structure in order to adjust their synaptic strength. Such synaptic plasticity underlies learning and memory and dysfunctions of synaptic plasticity contribute to brain diseases such as epileptogenesis, responses to ischemia, drug addiction, and neuropsychiatric disorders. Since enduring forms of synaptic plasticity, like long-term potentiation (LTP) and long-term memory require activity-dependent gene induction that is important in defining neuronal connectivity in the brain, it is anticipated that many forms of mental disabilities, including neurodegenerative processes and cognitive disturbances will be understood as cortical or limbic cognates of disturbed activity-dependent gene transcription.

We therefore have focussed much attention on the identification and functional characterization of the specific genes that are induced by patterned synaptic activity. In the past we used differential screening and subtractive cloning strategies to identify the first activity-regulated genes (e.g. Nature (1993) *361*, 453-457; Proc. Natl. Acad. Sci. USA (1995) *92*, 5734-5738; EMBO J. (1999) *18*, 3359-3369 and EMBO J. *18*, *20*, 5528-5539) More recently we have been using Affymetrix GeneChip technology to monitor changes in mRNA expression on a whole-genome scale in an unbiased way by comparing gene-expression before and at several time points after neuronal stimulation. This allowed us in a first step to establish a comprehensive library of activity-regulated genes. These genes are then searched for common elements, such as being present in

Activity-regulated gene expression is required for the formation and consolidation of long-term memory
A common extracellular signal, a transmitter released by a presynaptic neuron, acts on the postsynaptic neuron to initiate separate memory processes with different durations. Short-term memory has a time course of minutes to hours and involves covalent modification of preexisting proteins. The duration of these covalent modifications determines the duration of the short term response. Unlike these covalent modification mechanisms, acquisition of long-term memory that lasts for more than one day depends on the induction of new proteins. The gene products of the early regulatory genes represent nuclear signals themselves and trigger the expression of late effector genes which might be important for the long-term maintenance of memory. The early effector genes might be able to contribute very fast and direct to the long-term change in neuronal phenotype and physiology.



the same pathway, sharing interactions or functions, or having similar DNA-motifs in their promoter region. In this way we can identify pathways of genes involved in synaptic plasticity and transcription factors mediating the activity-dependent expression programs. Moreover, by comparing knock-out animals with wild-type animals, or by using time-resolved measurements we are now beginning to unveil causal upstream-downstream relations between these genes. As these techniques allow us to discover novel pathways that so far have been elusive, we begin to understand the neuron specific genomic response to synaptic activity.

2. Analysis of specific activity-regulated genes in the physiology and pathophysiology of synaptic plasticity

2.1. SorCS1

Guido Hermey

SorCS1 belongs to the Vps10p-Domain receptor family, which defines a group of recently identified receptors binding trophic factors (Cell. Mol. Life Sci. (2009) *66*, 2677-2689). Recent reports relate SorCS1 to the genetically complex disorders of Alzheimer's disease and type 2 diabetes. We previously identified SorCS1 and the highly homologous SorCS3 as activity-regulated genes at a time, when the cellular roles of SorCS1were largely unknown (J. Neurochem. (2004) *88,* 1470- 1476).We studied the general function of the receptor, identified platelet derived growth factor-BB (PDGF-BB) as ligand, and described the proteolytic processing of SorCS1 (Hermey *et al*., Biochem J, (2006) *395*, 285-293).

Furthermore, we identified different splice variants encoding receptors with identical extracellular and transmembrane moieties but different cytoplasmic domains and established that two of these variants convey endocytosis and targeting of ligands to lysosomes, whereas the other variants of SorCS1 are not mediating trafficking events but may act as co-receptors modulating signal transduction processes (Traffic (2008) *9*, 980-994). SorCS1 is localized to hippocampal dendrites and we currently extend our studies to define SorCS1 function at the postsynapse.

2.2. Sgk1

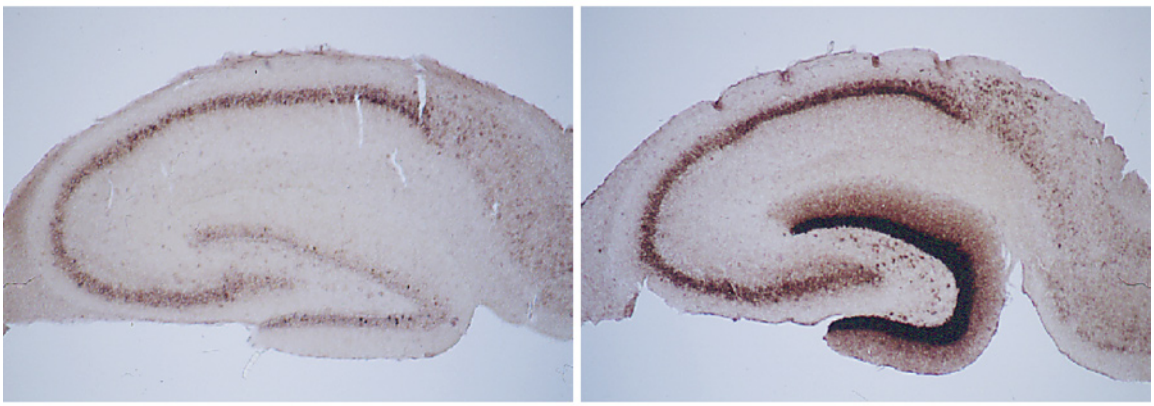
Claudia Mahlke

Using subtractive cloning strategies we identified the serum and glucocorticoid-inducible kinase sgk 1 as an activity-regulated gene in the brain. Sgk1 has a very short half-life and has been implicated in a variety of cellular functions (Biochem J. (2006), *399*, 69-76). Based on these observations we generated a complete knockout of this kinase. Together with the laboratory of Florian Lang (University of Tübingen) we observed that sgk1 knock-out animals exhibit a number of non neuronal deficits, including defects in renal function (e.g. J. Clin. Invest. (2002) *110*, 1263-1268; J. Clin. Invest. (2007) *117*, 773-783) and mast cell activation (J. Immunology (2009) *183*, 4395-4402). In the brain of wild-type animals we find that activity-dependent induction of sgk1 occurs in two different cell types and is regulated by two independent mechanisms. In oligodendrocytes induction is dependent on glucocorticoid release, whereas in dentate granule neurons transcriptional activation is independent of glucocorticoids but strictly dependent on synaptic activity. Our initial behavioral studies using complete knockout animals reveal strongly reduced locomotor and exploratory activity. To study whether the behavioral phenotype is related to motivational deficits or based on a dysfunctional motor-system we generated conditional sgk1 ko-mice. These mice will allow us to analyze the consequences of cell-type specific deletions of sgk1 in neurons and oligodendrocytes.

2.3.Arc/Arg3.1

Xiaosong Mao, Jerome Gruhlich, Lars Binkle, Jakob Gutzmann, Guido Hermey, Claudia Mahlke, Tiemo Marquarding, Ora Ohana

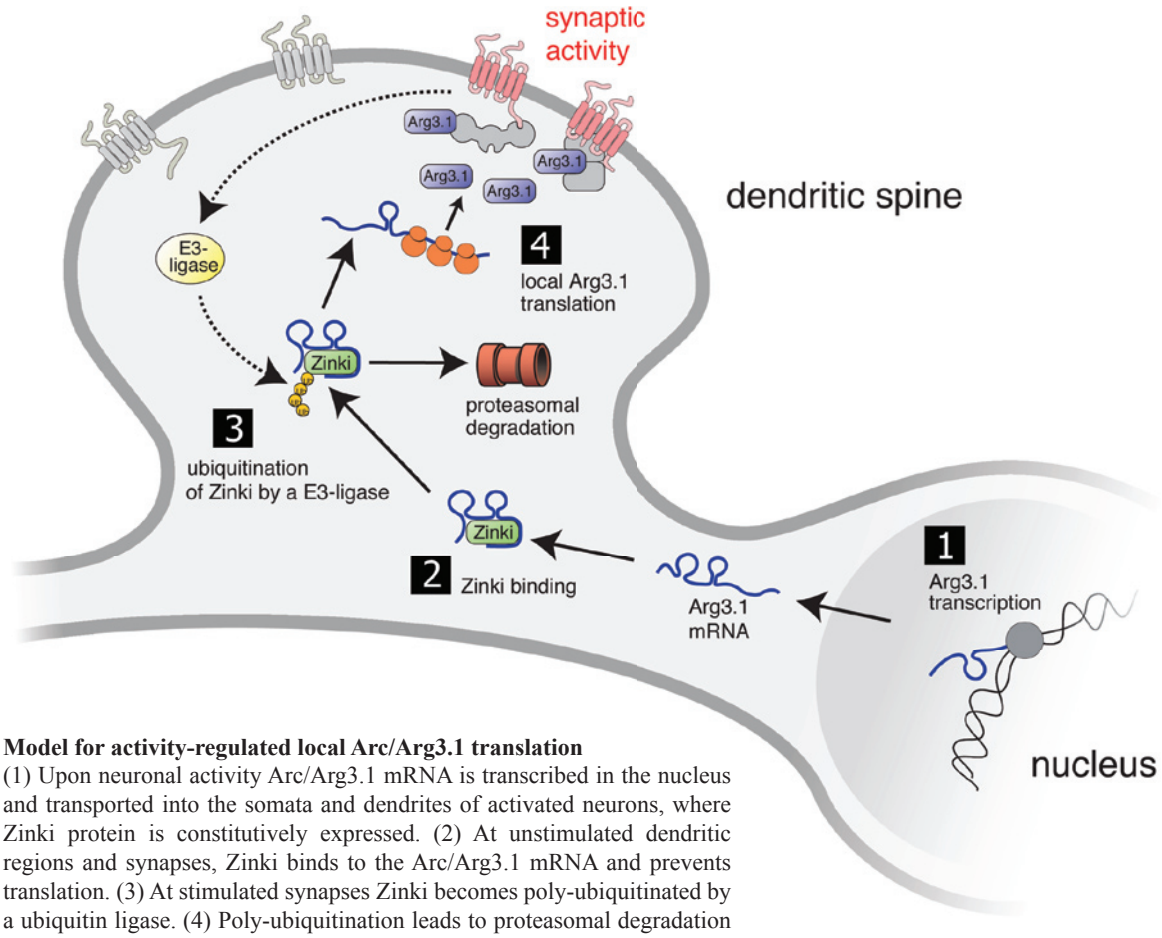
Previous experiments established a strong link between gene expression and physiological and pathological neuronal plasticity, however, it remains an open question how transcriptional activation taking place in the nucleus can selectively modify stimulated synaptic sites in the distant dendritic compartment of the neuron. Such selective modifications of synapses that have experienced coincident activity are required by



Arc/Arg3.1 mRNA is rapidly distributed to dendrites of activated neurons
Non-isotopical in situ hybridization of a non-stimulated and stimulated hippocampus. Arc/Arg3.1 transcript levels are very low before stimulation (left). Following synaptic activity Arc/Arg3.1 mRNA dramatically increases in the granule layer which contains the somata of the granule cells. Most remarkably, a very unusual localization of the transcripts is observed in the molecular layer, which contains the dendrites of the granule cells.

the Hebbian rule and might be a prerequisite for input specificity of LTP. The analysis of the immediate early gene Arc/Arg3.1 which we identified and characterized might guide our thinking and provide insights into this problem. Most strikingly, following LTP-producing stimulation Arc/Arg3.1 mRNA is localized to the dendrites of neurons that received patterned synaptic activity. (Proc. Natl. Acad. Sci. USA (1995) *92*, 5734-5738).

To our knowledge Arc/Arg3.1 is the only example of a gene whose RNA occurs in the dendrites and whose targeted delivery is regulated by synaptic activity. Consequently, Arc/Arg3.1 mRNA may be locally translated at activated synapses and may have a key role in synapse specific modifications during plastic events in the brain. We find that Arc/Arg3.1 protein is associated with the NMDA-receptor complex in the postsynaptic density. This association is not seen in animals that lack PSD95/SAP90. Moreover, Arc/Arg3.1 regulates AMPA receptor trafficking by binding to proteins of the endocytic machinery. To get a more complete understanding of the post-synaptic protein networks Arc/Arg3.1 interacts with, we generated TAP-tagged animals and conducted Y2H screens. Mice in which we have disrupted the Arc/Arg3.1 gene show altered synaptic plasticity and disturbed homeostatic scaling. These observations correspond to severe deficits in hippocampus-dependent and -independent cognitive tasks, which require the consolidation of newly encoded memories (Neuron (2006) *52*, 437-444; Neuron (2006) *52*, 445-459; Neuron (2006) *52*, 475-484); Neuron (2008) 59, 70-83). Using patch-clamp experiments, we are further studying the role of Arc/Arg3.1 in homeostatic regulation of synaptic gain in *in vitro* and animal models of epilepsy. Using stereotactic injection of Cre-expressing recombinant adeno-associated



Model for activity-regulated local Arc/Arg3.1 translation
(1) Upon neuronal activity Arc/Arg3.1 mRNA is transcribed in the nucleus and transported into the somata and dendrites of activated neurons, where Zinki protein is constitutively expressed. (2) At unstimulated dendritic regions and synapses, Zinki binds to the Arc/Arg3.1 mRNA and prevents translation. (3) At stimulated synapses Zinki becomes poly-ubiquitinated by a ubiquitin ligase. (4) Poly-ubiquitination leads to proteasomal degradation of Zinki protein and subsequent release of the Arc/Arg3.1 mRNA from translational repression.

virus into specific cortical regions of conditional Arc/Arg3.1 knockout animals, we examine the role of Arc/Arg3.1 in cortical sensory processing and the formation of remote memories. We have generated phage artificial chromosomes harboring specific mutations in Arc/Arg3.1 and crossed these transgenes into the ko-background. These tools allow us to ask which aspects of Arc/Arg3.1 function are rescued in these animals.

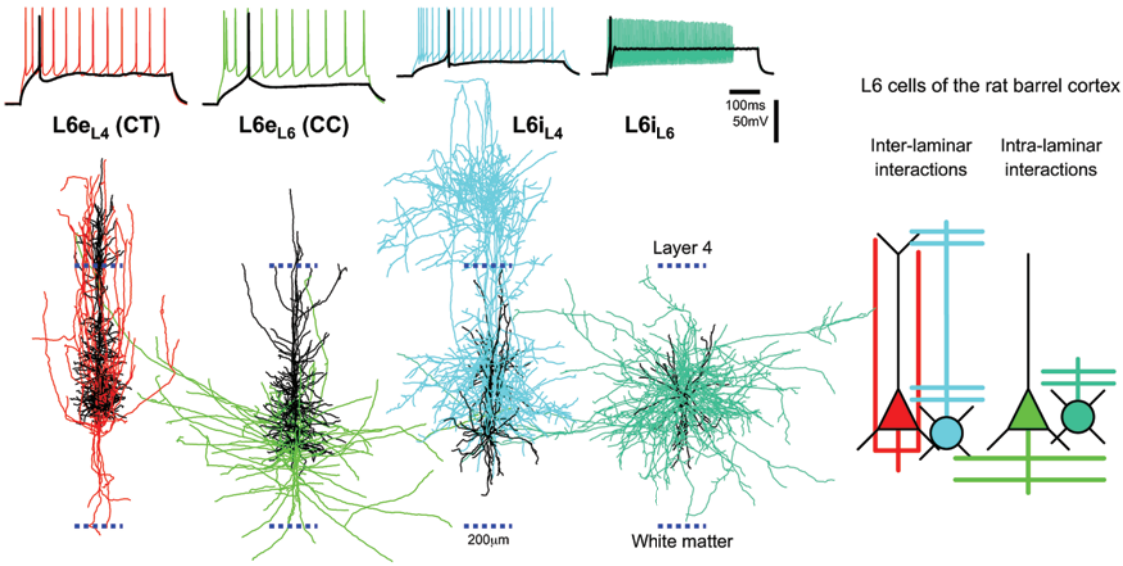
While Arc/Arg3.1 is essential for the consolidation of synaptic plasticity and memories, little is known about the mechanisms that govern Arc/Arg3.1 mRNA transport into dendrites. To address this issue, we developed the Tri-Hybrid-Method for the *in vivo* reconstruction of specific RNA-protein interactions. Using this technique in a genetic screen we identified several clones that specifically interact with Arc/Arg3.1 mRNA but not with perikaryal-control RNAs. One of these proteins we named Zinki because it contains a domain of repeated zinc fingers required for the specific binding to Arc/Arg3.1 mRNA. Moreover, Zinki can act as translational repressor of Arc/Arg3.1 *in vitro* and *in vivo*. Upon synaptic activity Zinki becomes a substrate for proteosomal degradation. Following dendritic lamina specific stimulation, Zinki vacates those dendritic regions in which translation of the Arc/Arg3.1 mRNA is enhanced indicating that Zinki controls translation of the Arc/Arg3.1 mRNA in this compartment. We are currently using biochemical, transgenic, and knockout approaches to determine how activity-dependent ubiquitylation and proteasomal degradation of Zinki might contribute to synaptic input specificity.

3. Physiology and pathophysiology of cortical plasticity

3.1. Functional subcircuits within L6 of the somatosensory cortex

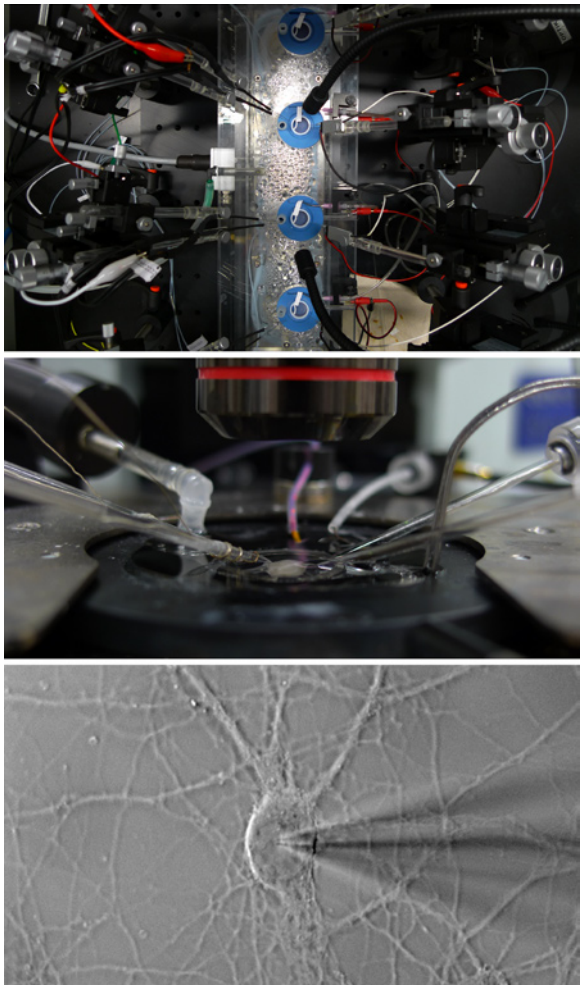
Ora Ohana, Pratap Kumar

Neurons within cortical layer 6 provide at once numerous feedback connections to the thalamus and to the thalamic-recipient L4, yet the properties of this cortical loop and its' role remain unknown. Using methods of *in vivo* dye tracing and *in vitro* patch clamp recordings we have revealed and described the existence of 2 parallel subcircuits in L6; one that projects to L4 and at the same time to the thalamus (the corticothalamic neurons, TC) and a second which connects heavily within the



Subcircuit-specific excitatory and inhibitory neurons in L6
Examples of the two basic excitatory and inhibitory cell types in L6 (left), a schematic representation of each class (middle), and the spiking responses associated with each (right). Specific types of both excitatory and inhibitory neurons in L6 are seen to target L4. Unique sub-circuits can therefore be identified in L6, one involved in interactions with superficial laminae and the other restricted to infragranular interactions.

cortex (corticocortical, CC) and L6 but avoids L4 and the subcortical structures (J Neurophysiol. (2008) *100*, 1909-1922). Neurons in these subcircuits differ in their morphologies (reflecting their different afferent and efferent connections) and in their biophysical properties and thereby allow for differential information processing within each subcircuit. We continued exploring this hypothesis following two approaches: 1. By simultaneously recording from synaptically connected TC and CC neurons which revealed that while neurons of these subcircuits do interconnect, their synaptic properties strongly depend on the target neuron. 2. By performing laser-induced uncaging of glutamate to map the connectivity patterns of CT and CC neurons to- and from- L4. In the future, we plan to continue mapping the L6-L4 subcircuitry and how it is modulated by neuronal activity and sensory input. We plan to perform *in vivo* patch clamp recordings from identified CT and CC neurons in order to map their synaptic responses during real-time sensory processing.



Patch clamp electrophysiology

3.2. Tinnitus aurium – Animal models to investigate the generation of an auditory phantom percept

Claudia Mahlke, Florian Theden

Subjective tinnitus is a sound sensation that cannot be attributed to external sounds and is therefore regarded as an auditory phantom percept. In animals tinnitus can be induced experimentally by ototoxic drugs, like salicylate, or by noise trauma, both of which result in a reduction of auditory input from the periphery. Using such treatments we previously observed a pathological activation of the auditory cortex in response to the auditory input. We propose that this cortical activation is generated by compensatory mechanisms within the central nervous system and can be regarded as the neuronal correlate for tinnitus. Another crucial factor in tinnitus generation is ongoing stress, which activates the limbic system, namely the amygdala, and triggers plasticity-related changes in the auditory cortex. These long-term plastic changes might result in the stabilization of the auditory cortical activity causing the chronic tinnitus perception (Neuroreport (2006) *17*, 1487-1491). Recently we established an animal model to investigate acute versus chronic tinnitus. We here use high intensity impulse noise to induce tinnitus and the 2-deoxy-D-[14C]-glucose (2-DG) method to screen for neuronal activity within the auditory and limbic system of the gerbil (Meriones unguiculatus). Currently, we are using this model in cooperation with Merz Pharmaceuticals, Frankfurt to evaluate the mode of action of neramexane, an uncompetitive NMDA-receptor antagonist, in the pharmacological treatment of tinnitus aurium.

4. Future perspectives

The several findings described above open up new avenues and pave the way to investigate mechanisms of plasticity underlying addiction, or

when disturbed are the cause of mental diseases, psychiatric disorders or play roles in tinnitus, epileptogenesis and ischemia. The main focus of our research, however, will remain on the analysis of learning and memory. Much progress has been made, within discrete levels of analysis, characterizing biophysical, molecular and cellular adaptations associated with plasticity and cognitive functions. However, it has proven difficult to integrate these findings and translate the specific knowledge at each level into an understanding of information processing and storage. A long-term goal of our research is to elucidate how mental functions emerge from specific changes at molecular levels. We see the use of mouse genetics as an important means of building bridges between molecular biology and systems neurobiology and between systems neurobiology and behavior. This provides the rationale for an integrated approach to follow the flow of information from excitatory events in the dendrite through neural networks in behaving animals. We hope in this way to extract some of the fundamental rules that govern dendritic information processing in the activity-driven refinement of networks that underlies learning and memory.

Support

The work in our laboratory is supported by grants from the Bundesministerium für Bildung und Forschung, the Deutsche Forschungsgemeinschaft, NeuroCure (cluster of excellence of the German Federal Government), Neurodapt (cluster of excellence of the State Government of Hamburg), and Merz Pharmaceuticals.

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Institute for Neuroimmunology and Clinical Multiple Sclerosis Research (inims)

Roland Martin

Since its inception the inims has been building up expertise, methodologies and infrastructural aspects that are required to conduct research with a clear focus on a complex human disease, multiple sclerosis (MS). Different from all other ZMNH institutes inims is also directly involved in patient care, which is one of the most important aspects, if it wants to fulfil its mission. Our main goals are the following:

1. To develop a better understanding of the etiology and pathogenesis of MS.

2. To develop efficacious and well-tolerated treatments for all courses and stages of MS.

3. To provide state-of-the art care to MS patients.

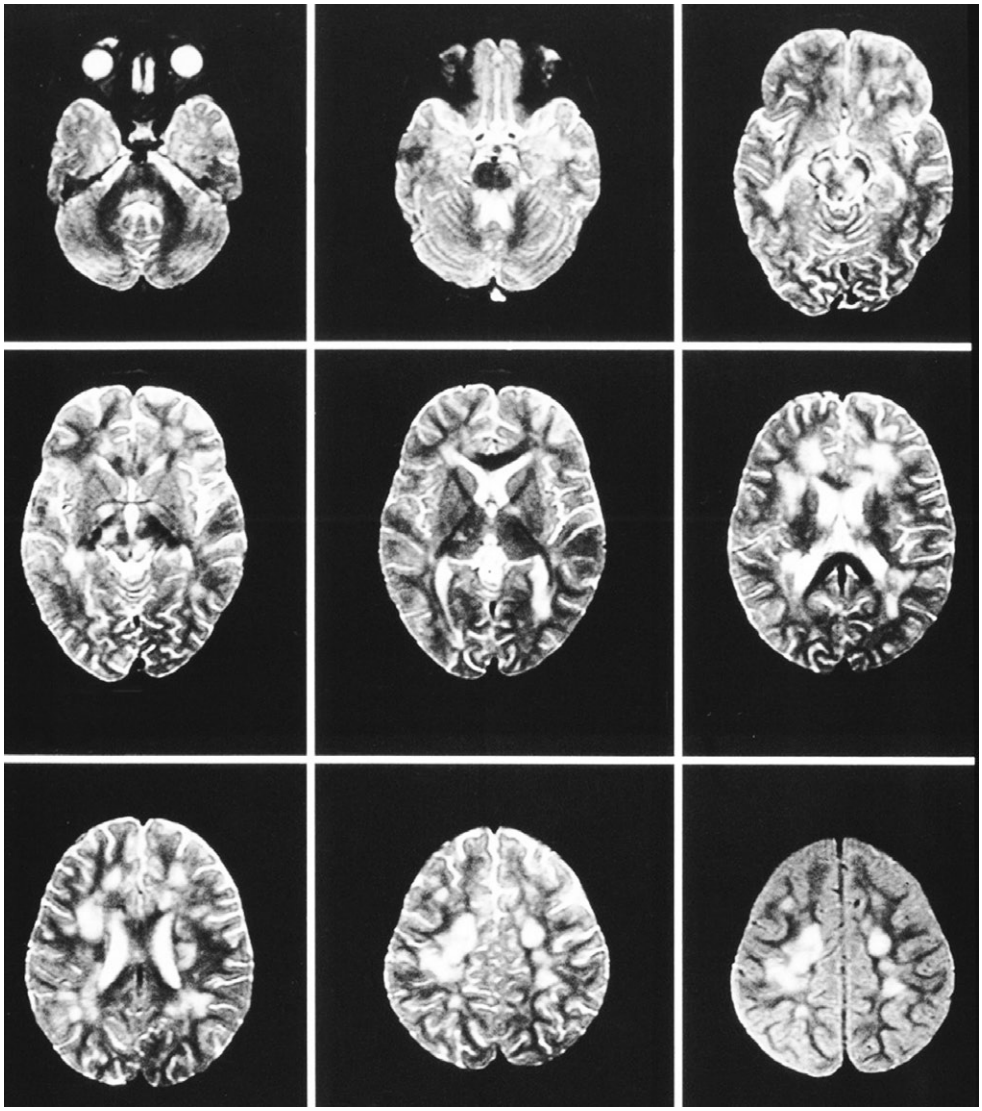
As stated already providing patient care and having access to MS patients is essential, and our research cannot be pursued without patients, who are highly motivated, come for diagnostic visits, participate in clinical trials and are willing to give us biosamples for research. The involvement of patients and pursuit of medical activities creates opportunities and problems at the same time. Seeing and discussing patients, who are afflicted with the medical condition, that the institute works on, is stimulating and motivating not only for medical and nursing staff, but also for scientists at the laboratory. At the same time, problems arise from the inherent diversity of the group and much broader spectrum of scientific questions, when compared to a "pure" basic science institute with a focus on a specific biological process or molecule. Inims has to spread its resources much thinner by sustaining a clinical team, a laboratory group, which has to be accordingly smaller, and build and maintain a demanding biobank, and furthermore it requires steady attention and work to maintain a scientific dialogue and mutual understanding of the scientific and clinial aspects among the members of the group, who have very diverse backgrounds, e.g. from research nurses to biochemists.

Despite these challenges, the inims has taken great strides during the last three years to establish its basic, translational and clinical research program and further improve MS patient care. We have profited in many respects from the excellent, open and stimulating ZMNH environment, but also begun fruitful interactions and specific projects both within the ZMNH, but similarly with groups on the UKE campus and beyond.

Rather than describing below the projects of individual groups or grants in detail, the inims activities will be broken into basic-, translational-, and clinical research, and the participating individuals will be mentioned in these sections. The Emmy Noether DFG junior group of Dr. M. Friese is physically located within the inims and participates in a number of the inims activities, but due to its junior group status and independence it will appear separately in the ZMNH report.

Basic research

Phenotype and functional characterization of Th17 cells in the peripheral blood and cerebrospinal fluid (CSF) of healthy individuals and MS patients (V. Brucklacher-Waldert, R. Martin, E. Tolosa, *et al*.). Th17 cells are a specific subset of CD4+ T cells that has gained interest in autoimmune diseases during the last few years and is still relatively poorly characterized when compared with other T helper cells. The main findings of



Acute MS: Magnetic resonance imaging shows solitary or multiple lesions within the CNS

this project have so far been the demonstration of a specific surface marker on Th17 cells, i.e. membrane-bound IL-17, and a relative increase of this population during the evolution of MS (V. Brucklacher-Waldert, K. Steinbach, E. Tolosa, *et al*.). This project has been supported by the Gemeinnützige Hertie Foundation.

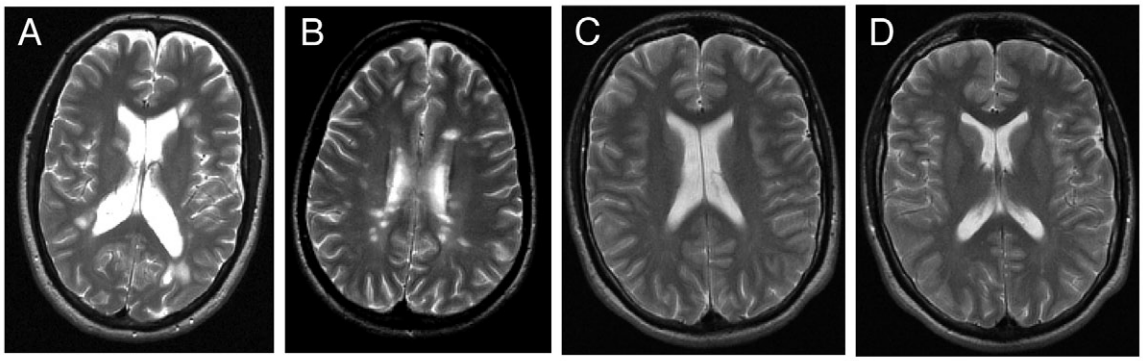
MS lesions are characterized by inflammation, demyelination and axonal damage. In order to gain better insight into the relatively poor recovery from inflammatory damage in MS lesions, we begun to examine the role of myelin-associated inhibitory factors of axonal damage (Nogo and the Nogo receptor complex) in the MS model experimental autoimmune encephalitis (EAE) both by antibody blocking experiments, but also in knockout systems of the various Nogo-NogoR components (K. Steinbach, R. Martin; in collaboration with M. Reindl and C. Bandtlow, University of Innsbruck, and Schwab, ETH Zürich). Of particular interest have been the respective roles of Nogo and its ligands in both the central nervous system (CNS) and the immune system. These experiments are ongoing and in many aspects not clear at present. This project has been supported by the Gemeinnützige Hertie Foundation.

Characterizing the functional involvement of MS risk haplotypes, genes and SNPs in disease etiology is another important goal. During the last two years, we have focused on SNPs of the cytokine receptors for interleukin-2 (IL2RA) and interleukin-7 (IL7RA), which have recently been identified in whole genome association studies as important genetic risk factors for MS (J. Jäger, S. Rösner, C. Schulze, R. Martin). IL-2 is the major autocrine growth factor of T lymphocytes, and IL-7 serves essential roles in generating and maintaining T cell homeostasis. Although their impact at the level of the individual gene is probably small, we speculate that they act in combination or even with other risk alleles in modulating apoptosis, cell growth and other important aspects of immune function in MS. Initially, we genotyped a large set of MS patients from our "Hamburg MS cohort" and controls for the presence of protective versus risk SNPs and are currently undertaking functional *in vitro* experiments as well as structural studies (in collaboration with S. Walsh, Center for Advanced Research in Biotechnology, University of Maryland, Rockville). This work is supported by a DFG grant.

Adaptive immune responses have been the center of attention in MS research for a long time, and relatively less is known about innate immunity. Among innate immune mechanisms neutrophils play very important roles in the defense against extracellular pathogens, particularly fungi and bacteria, and a number of evidences point at their importance also in autoimmune diseases and MS. One reason for the relative lack of knowledge about neutrophils and their functions are the methodological difficulties in handling and examining them. For this reason, a group headed by M. Sospedra and including K. Tillack, S. Yousef and M. Nägele have systematically established a host of assays and methods to examine all aspects of neutrophil phenotypes and function and their potential role in shaping Th17 responses. Currently, one focus is on the role of estrogen in regulating an enzyme, PAD4, which is involved in the citrullination of histones and gene regulation in neutrophils and other cells in general. The differential expression of proteins in neutrophils is examined by proteomics methods in collaboration with H. Schlüter, Institute for Clinical Chemistry, UKE. This work is supported by a DFG grant.

In collaboration with A. Zander and C. Lange (Department of Bone Marrow Transplantation, UKE) we investigate the functional properties of bone marrow-derived mesenchymal stem cells (MSCs) with respect to migration, immunomodulation and immune privilege in the EAE model (P. Abramowski, K. Steinbach, S. Schippling, R. Martin). MSCs are already in clinical use in osteogenesis imperfecta in children and in acute graft-versus-host disease, and there is a world-wide effort by investigators in the MS field to examine their therapeutic usefulness in MS with respect to blocking CNS inflammation and providing an environment that is conducive to tissue repair. Inims is part of these international efforts, and together with A. Zander's group we have transplanted 4 patients during the last two years with autologous and allogeneic human MSCs with the objective of assessing its tolerability and safety first. In the EAE model, the focus is on examining the relative advantages of autologous versus allogeneic cells with respect to cell migration and survival and the mechanisms of action of MSCs. Inims is part of the EU FP7 initiative CellThera, headed by A. Radbruch (Deutsches Rheumaforschungszentrum, Berlin), and which will focus on cell therapies such as hematopoietic stem cell transplants, MSC transfer and tolerizing cell therapies, which are all performed or will be performed at ETIMS.

Also in collaboration with A. Zander and his group, A. Lutterotti, I. Jelcic, C. Schulze R. Martin and others examine the differences in gene expression, methylation status and miRNA expression of CD34+ hematopoietic stem cells from MS patients and controls. The major underlying question is whether differences in e.g. gene expression are already in these uncommitted pluripotent precursor cells, before the respective hematopoietic cell repertoires are shaped during the evolution of the disease. This project is pursued in collaboration with R. Saccardi (University of Florence), the European Bone Marrow Transplantation Group (EBMT) and P. Muraro (Imperial College, London) and will be continued under the EU FP7 CellThera project.



A: MRI of MS patient with periventricular lesions. For comparison see **C:** healthy control
B: MRI of MS patient with periventricular lesions (different level in the brain). For comparison see **D:** healthy control

Besides a complex genetic background environmental factors are considered relevant for the etiology of MS. I. Jelcic, a DFG fellow from 2006-2008, examined the etiological role of two different families of viruses, anelloviruses and among them particularly Torque Teno virus (TTV) and its variants, but also EBV, which is currently considered the most important environmental factor in MS. The background for the TTV project is that Sospedra *et al*. recently demonstrated that CSF-infiltrating CD4+ T cell clones, which were *in vivo* clonally expanded during MS relapses, preferentially recognized arginine-rich protein motifs, most notably of TTV. The TTV-related studies (establishing quantitative PCR methods to measure viral load for all anellovirus family members in children and adults with MS and controls, in brain tissue) have been pursued in collaboration with E. de Villiers and H. zur Hausen, DKFZ, Heidelberg, where I. Jelcic trained, and also with J. Gärtner and W. Brück, Departments of Pediatrics and Neuropathology, University of Göttingen. The main finding thus far is that TTV viral load can be reliably determined with the new qPCR technique and that TTV prevalence is much higher in children with MS when compared to age-matched controls and that it steadily declines with age during adulthood. The EBV-related work was focused on the cross-reactivity of EBV EBNA-specific T cells with myelin autoantigens and has been pursued with J. Lünemann and C. Münz, formerly Rockefeller University, now Department of Experimental Immunology, University of Zürich.

Translational research

The most important and visible translational project has been the "Establish Tolerance in MS" (ETIMS) first-in-man, investigator-iniated phase I and II trial, which we prepare for since opening of the institute. ETIMS will be the first tolerization trial that employs autologous, peptide-coupled, fixed antigen-presenting cells as the therapeutic principle. We will use seven myelin peptides, that Martin *et al*. have previously shown to be target of high avidity myelin-specific T cells in MS, and these peptides will be chemically crosslinked with autologous peripheral blood mononuclear cells. The ETIMS study has been supported in its preclinical stages by the "innovative therapies" funding mechanism of the BMBF, and will also be supported by this mechanism during the clinical testing. The following investigators are involved in ETIMS: A. Lutterotti, who has been a Humboldt fellow at inims for two years and is now back at the University of Innsbruck, M. Sospedra, K. Stürner, C. Schulze, S. Reinhardt, S. Yousef (all inims), A. Sputtek (Department of Transfusion Medicine, UKE), M. Daumer (Sylvia Lawry Center for MS Research, SLCMSR, Munich), and S.D. Miller (Northwestern University, Chicago; the investigator, who pioneered this approach during the last 20 years in various animal models). Besides mechanistic studies, we performed all preclinical work including animal toxicity, establishing standard operating procedures for all steps of the production of the ETIMS product, and generated all the regulatory documentation for the involved

agencies, the Paul Ehrlich Institut (PEI), the local authorities in Hamburg, and the Ethics committee. After three years of preparation, we have approval from the Ethics committee and the local authorities, and the final approval by the PEI is still pending after the second submission of the project.

A number of other projects explore both the mechanisms of action and the therapeutic use of natural substances and small molecule drugs including a standardized extract from the resin of the incense tree Boswellia serrata. Many of the Boswellia acids (BA) contained in this extract have potent anti-inflammatory activities by specifically inhibiting neutrophils and key neutrophil proteins such as cathepsin G, 5-lipoxygenase and microsomal prostaglandin E synthase. BA extracts have been part of the traditional eastern medicine for virtually thousands of years, and their antiinflammatory activities have already been explored in clinical trials of various medical conditions (osteoarthritis, M. Crohn, and others). We will start a phase IIa, two center, proof-of-concept clinical trial beginning 2010 and perform a series of mechanistic studies along this trial. The involved investigators are K. Stürner, C. Heesen, M. Sospedra, S. Yousef, R. Martin, F. Paul (NeuroCure, Charité, Berlin), O. Werz (Department of Pharmacology, University Tübingen), and Medeon Pharmaceuticals (Berlin). The trial will be funded by the Biopharma grant of the BMBF.

Further projects examine the neuroprotective effects of hydroxytyrosol, a compound of olive oil, olive waste-water and olive leaves, in neuronal cell lines and primary neurons and its potential usefulness as a novel therapy for MS (K. Chakroun, J. Reynault, R. Martin; in collaboration with Puleva Biotech, Granada, Spain, and C. Espejo, Hospital Val D'Hebron, Barcelona). P. Abramowski in collaboration with A. Zander and F. Ayuk (Department of Bone Marrow Transplantation) has been examining the therapeutic potential in the EAE model and the molecular mechanisms of action on immune cells of edelfosine, an ether phospho-lipid, that induces apoptosis in proliferating cells, particularly CD4+ T cells.

We hope to test each of the above approaches also in clinical trials.

Together with A. Zander, but also other investigators in Europe (P. Muraro, R. Saccardi; see above), we (M. Sospedra, S. Yousef, R. Martin) investigate the reconstitution of the immune repertoire after autologous hematopoietic stem cell transplants (aHSCT) in cases with severe forms of MS. This projects continues activities from R. Martin's lab at NIH, where it was shown that the T cell repertoire is completely renewed after aHSCT. However, important questions about the composition of the specificity repertoire of T- and B cells, the replicative senescence and homeostatic proliferation of newly emerging immune cells and many other basic research issues remain to be examined. Furthermore, the ideal patient population, type of conditioning regimen and others are also open on the clinical side, and we aim to address these in the next years within the CellThera context of the EU FP7 project. In the last three years, we have transplanted four patients on an exploratory basis here in Hamburg and currently join the ASTIMS (autologous stem cell transplant in MS) trial, run by EBMT as a controlled, multi-center randomized trial in Europe.

As part of an EU-supported training grant, we continue to explore the mechanism of action of anti-CD25 monoclonal antibody treatment in MS patients (A. Cuapio, E. Tolosa, R. Martin). In a small number of MS patients, who receive compassionate use treatment with anti-CD25 therapy after failing approved treatments, we examine the saturation of CD25 and the expansion of CD56bright NK cells, which Martin *et al*. previously identified as the main mechanism of action of this therapy.

Finally, an evidently important translational project examines the specificity and function of CD4+ T cells against JC virus, a small DNA virus, that leads to latent and inapparent infection in about 85% of the population. Under conditions of immunocompromise such as AIDS, cancer treatment or certain immunosuppressive treatments JCV infection may lead to progressive multifocal leucoencephalopathy (PML), an

often fatal infection of oligodendrocytes. In the MS context PML has rapidly gained importance because it occurs in a small fraction of patients treated with natalizumab (27 cases so far), a highly effective and recently approved monoclonal antibody that targets the adhesion molecule VLA4. Natalizumab primarily inhibits T cell and immune cell migration from the periphery into the CNS and as a result also the occurrence of new CNS inflammatory lesions. The reason for the development of PML in natalizumab-treated patients is not clear, but one hypothesis is the reduced immune surveillance of the CNS due to the block of cell migration. Astonishingly, relatively little has been known on the physiological immune mechanisms that keep JCV infection in latency and well controlled. Since PML is frequent in AIDS patients when CD4+ T cell counts drop to low levels, we have begun to map the CD4+ T cell response against all open reading frames of JCV with overlapping peptides (I. Jelcic, L. Aly, M. Sospedra, R. Martin; in collaboration with R. Girones, University of Barcelona, T. Weber, Marien Krankenhaus, Hamburg) in MS patients and controls, before and on natalizumab treatment and in MS PML cases. So far, we have identified at least two reasons that lead to low CD4+ immune responses against JCV, and we hope to validate these findings further. This work is of very high importance for MS patients, since natalizumab, which is by far the most efficacious treatment of MS until now, may be taken off the market, unless low-responders to JCV or patients at risk of PML can be identified.

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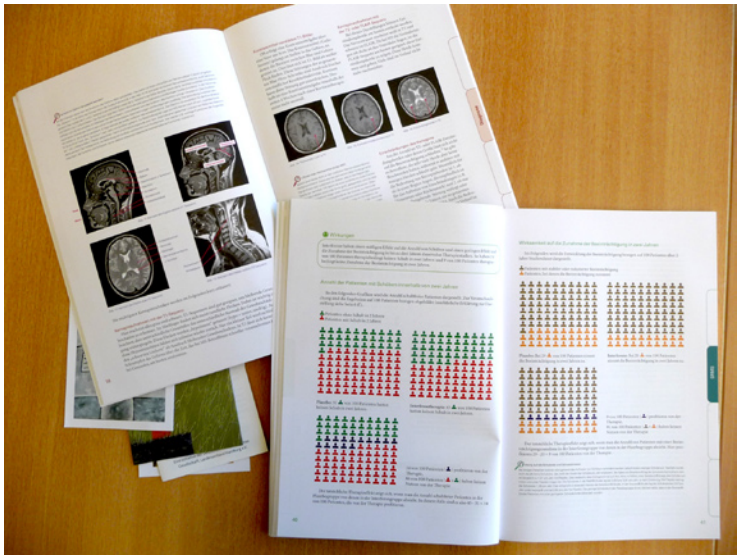
Clinical research

Projects supervised by R. Martin

We continue to develop clinical aspects of aHSCT- and MSC transplantation (see above) (with S. Schippling, C. Heesen and other members of the clinical team). An investigator-initiated small scale, mechanistic study, which shall examine if MSCs migrate into fresh inflammatory and/or "old" and quiescent MS CNS lesions is in preparation.



Evidence-based information for patient education concerning diagnosis, prognosis, early therapy and immunotherapy in MS

Development and continuation of a teaching programme for MS patients on immunotherapy (J. Kasper).

Development of a study protocol to examine the responsiveness of patient-based outcomes. Supported by a grant of the Gemeinnützige Hertie Stiftung.

Development of risk knowledge inventories for all forms of MS based on data from a patient survey (I. Backhus). Furthermore, an inventory is being developed that will query patients about factors determining therapy decisions according to the concepts of "planned behavior".

In close collaboration with the Department of Neuroradiology (B. Holst, M. Bester and J. Fiehler) and funded by the recently started Kompetenznetzwerk MS of the BMBF, we (with L. Winkler, C. Heesen, S. Schippling) examine the heterogeneity of MS as visualized by a variety of magnetic resonance imaging (MRI) measures. The major goal of this project is to phenotype patients according to their quantity and quality of CNS inflammation and respectively focal and global tissue destruction. This information will be of high importance for identifying ideal patient groups for clinical trials, for linking it with clinical and treatment outcomes and for correlating biological data (gene expression profiling, proteomics, targeted immune studies) with MRI-based phenotypes.

Projects supervised by C. Heesen

One project pursues further evidence-based medicine-related questions in MS that aim at involving patients as early as possible into decision making and informing them in a structured way about current evidence regarding the efficacy, side effects and mechanisms of existing MS treatments (with N. Schäffler, K. Fischer; supported by an unconditional grant of Merck & Serono).

A recently concluded study examined the occurrence of conditioned immunosuppression under mitoxantrone therapy (MIMIK), the first investigator-initiated study that fulfilled AMG guidelines. This pilot project should clarify if classical conditiong can be used to induce immunosuppression during mitoxantrone treatment (G. Mina).

Conclusion of studies that assess disturbances in the hypothalamic-pituitary-adrenal axis in depressive and not depressed MS patients (S. Krüger).

Projects supervised by S. Schippling

Assessment whether perturbations in transcallosal inhibitory systems occur very early during the course of MS by complementary methods including transcranial magnetic stimulation (TMS), MRI diffusion tensor imaging, and in the future also magnetoencephalography (MEG; in collaboration with A. Engel, Institute for Physiology, UKE). The working hypothesis is that structural damage in the corpus callosum occurs very early in MS evolution and their reliable identification by the above techniques may serve as a an important readout for longitudinal follow-up in patients with clinically isolated syndromes (CIS), the first manifestations of MS (S. Großmann).

Along similar lines, it has been shown that damage of the retinal fiber layer of the eye occurs early during MS and can be used as a parameter to follow CNS tissue atrophy. These alterations can be visualized and measured reliably by optical coherence tomography (OCT), and we therefore have begun to examine the use of OCT during the course of MS for various purposes, e.g. as a sensitive outcome in phase II clinial trials with neuroprotective approaches (in collaboration with A. Hassenstein, Department of Ophthalmology, UKE). We already use OCT for this question in an investigator-initiated three center phase II trial that examines the neuroprotective properties of erythropoietin (EPO) in patients following optic neuritis (PI: R. Diem, University of Homburg, Saar).

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Booklets, brochures and flyers for patient education concerning diagnosis, prognosis, early therapy and immunotherapy in MS

Patient care-related activities

In this area, we expanded the diagnostic and therapeutic activities of the MS outpatient clinic and particularly the MS day hospital. Currently, all aspects of diagnostic workup, differential diagnosis, and patient counseling as well as the full range of approved, exploratory and meaningful off-label therapies are being offered. These range from symptomatic treatment to hematopoietic stem cell- and MSC transplants. A large part of the clinical care is meanwhile financed through income from patient care, particularly from the day hospital. Last year, we saw overall 3000 patients with approximately 300 2-3 day visits in the MS day hospital. In about two thirds of cases a CIS and in one third a clinically definite MS according to McDonald criteria was diagnosed. Together with clinical information and a comprehensive biobanking program that collects cells, serum, CSF, DNA, RNA, and urine, we are generating a systematically followed "Hamburg MS cohort". Our focus is on being involved in patient decisions and second-opinion provision with subsequent transfer of patients back to their local neurologist, while at the same time trying to follow closely patients with first diagnosis, those not on treatment, or cases of special interest for our research activities. We scaled back activities regarding long-term management of chronically ill MS patients, in whom the disease course is relatively stable and does not require important new treatment decisions. This step is intended to free resources for clinical research and the development and conduct of investigator-initiated inims trials. We continue to participate in industry-sponsored phase II and –III trials, though at a moderate level. This shall allow us to gain experience with upcoming treatment, but at the same time leave patient resources for our own studies. The investigator-initiated studies, Suniphenon (testing epigallocatechin-gallate, an ingredient of green tea as neuroprotective agent;

PI F. Paul, NeuroCure, Charité, Berlin) and Vision Protect (EPO, see above), are currently being conducted, and a number of own investigator-iniated trials are about to start (see above; www.ms-netz-hamburg.de). Together with K.P. Wandinger, C. Heesen, and members from the Departments of Neurology, Dermatology, Nephrology and Ophthalmology, we have installed an interdisciplinary case conference for clinical-neuroimmunological-rheumatological issues.

Support

The institute has been supported by the Gemeinnützige Hertie-Stiftung, grants of the Deutsche Forschungsgemeinschaft (DFG), Bundesministerium für Bildung und Forschung (BMBF), Alexander von Humboldt Stiftung, Emmy Noether Research Group, National Multiple Sclerosis Society, United Europeans for Development of Pharmacogenomics in Multiple Sclerosis (UEPHA MS/EU FP7)

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* during part of the periodgr

Institute for Neural Signal Transduction

Olaf Pongs

A fundamental property of neurons is the generation and propagation of electrical signals. They are generated by a regulated flow of ions across the neural membrane upon excitation. In this process voltage-dependent ion channels have key roles in generating and propagating the action potential. In response to a change in membrane voltage, the channels gate a pore, a process, which ensures a regulated and selective passage of ions across the plasma membrane. Our work focuses on the structure and physiology of voltage-gated potassium channels, in particular those associated with heritable human diseases. Here two channels stand out: KCNQ (Kv7) channels and the TRPM4 channel. Mutations in Kv7 channel genes are associated with various forms of channelopathies, for example a benign form of juvenile epilepsy (BNFC, see report of D. Isbrandt) and arrhythmogenic cardiac diseases. By characterizing ion channel dysfunction resulting from gene mutations we aim to get insight into the pathophysiological bases of channellopathies.

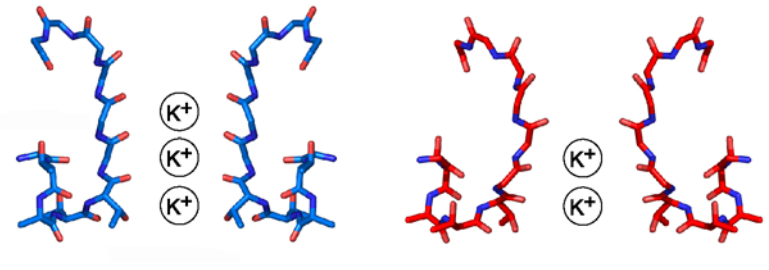


Figure 1: Conformation of selectivity filter in the activated (blue) and inactivated (red) state of the KcsA-Kv1.3 K^+ channel in a lipidic environment.

Atomic Resolution of Conformatonal Changes Correlated with K^+ Channel Gating

Sönke Hornig, Alexander Prokofyev, Phanindra Velisetty

K^+ channel gating is choreographed by a complex interplay between external stimuli, K^+ concentration and lipidic environment. In order to delineate stimulus, K^+-, and blocker-effects on channel structure and function in a membrane setting, we collaborated with the group of Marc Baldus at the Max-Planck Institute for Biophysical Chemistry (now at the University of Utrecht) and combined electrophysiological experiments on a chimeric KcsA-Kv1.3 channel with solid-state NMR spectroscopical investigations. The quality of the spectroscopical data allows deriving at a structure of the K^+ channel backbone and, importantly, to obtain high-resolution structural data of important parts of the K^+ channel such as the selectivity filter (see Fig. 1), the gating hinge and the activation gate. Our results show that stimulus-induced activation is correlated with protonation of glutamate residues at or near the activation gate. Moreover, K^+ and channel blockers distinctly affect the open-probability of both the inactivation gate comprising the selectivity filter of the channel, and the activation gate. The results indicate that the two gates are coupled and that effects of the permeant K^+ ion on the inactivation gate modulate activation gate opening. The data suggest a mechanism for controlling coordinated and sequential opening and closing of activation and inactivation gate in the K^+-channel pore and offer a close look at the conformational changes involved.

Non-Obligatory Gating in Voltage-Dependent K^+ Channel

Dörte Clausen, Lijuan Ma, Iris Ohmert, Vitya Vardanyan*

Through the combination of structural, electrophysiological, and biophysical data, we are beginning to decipher the principles of the electromechanical coupling mechanism by which voltage-dependent ion channels, for example the voltage-gated *Shaker* K^+ (Kv) channel couple conformational changes in voltage-sensor domains to mechanical pore opening and closing. The *Shaker* Kv channel commands a strong coupling device which procures that channel activation follows an obligatory gating mechanism. That is the channel's pore opens only after voltage-sensor activation. In contrast, voltage-dependent ion channel activation may also involve voltage-independent reaction pathways, and follow a non-obligatory gating mechanism. That is the conduction pathway can open either before or after voltage-sensor activation. In fact, non-obligatory gating may have preceded obligatory gating during voltage-gated ion channel evolution.

To further our understanding on the device, which couples voltage sensor actions to pore gating, we investigated the non-obligatory gating in the Kv7.1 channel in collaboration with B. Attali (University of Tel Aviv). We focussed our study on the pore domain combining systematic scan mutagenesis, double-mutant cycle analysis, and electrophysiological recordings to analyse mutational effects of 240 pore mutations on K^+ channel gating behaviour. Based on a cyclic four-state allosteric gating model we developed a novel quantitative approach to determine a coupling constant which defines the ratio of equilibrium constants for voltage-independent and voltage-dependent gating transitions. The approach provides criteria to demonstrate coupling-sensitive sites within a Kv channel where mutations specifically affect the strength of the electro-mechanical coupling device. The important qualitative point of our analysis is the principal observation that the size of the coupling constant describes the choreography of channel gating. Thus, we derived at a coherent simple gating model for obligatory and non-obligatory channel gating.

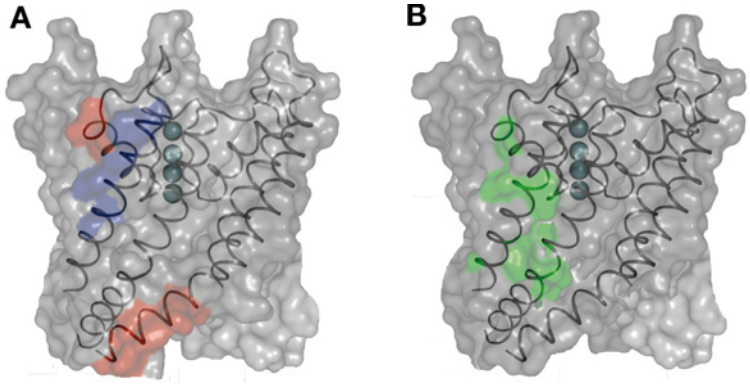


Figure 2: (A) Surface representation of the interfaces between the voltage sensor and the pore of the *Shaker* Kv channel in the structure of the paddle chimera (PDB ID: 2R9R). Contact surfaces corresponding to the interfaces between S1 and the pore helix and the S4-S5 linker and the S6 helix are colored light red. Contact surface between S4 and S5 helices is colored light blue. (B) Surface representation of coupling-sensitive Kv7.1 residues in the structure of the paddle chimera colored in green. Part of S4-S5 linker and inner S6 helix has been removed in one subunit in order to better visualize clustering of Kv7.1 coupling-sensitive residues within the pore domain.

Our mutagenesis data indicates remarkably little overlap between gating-sensitive interfaces in the *Shaker* Kv channel and coupling-sensitive areas in the Kv7.1 channel. Mapping coupling-sensitive Kv7.1 residues onto the structure of the voltage-dependent Kv1.2 channel reveals that coupling-sensitive sites are essentially clustered to a confined region in between activation gate and the surface of the pore domain which occupies only a small area at the surface of the pore domain (see Fig. 2). We conclude that extent and kind of the interface making contact between voltage-sensor domains and pore domain are critical for the coupling strength between conformational changes in the voltage sensor and Kv channel pore gating properties.

Cardiac Diseases and Hypertension

Alf Beckmann, Frederik Flenner, Martin Kruse, Nina Kursawe, Güven Kurtbay*, Iris Ohmert, Gregor Sachse, Yu Wu*

We analyzed mutations in four families afflicted with a dominantly inherited bundle-branch disorder known as isolated cardiac conduction disease (ICCD) and, respectively, progressive familial heart block type I (PFHBI) in collaboration with P. Bouvagnet (University of Lyon), V. Corfield and P. Brink at the University of Stellenbosh, and E. Schulze-Bahr (University of Münster). We showed that members of the families carry a mutated TRPM4 gene. TRPM4-mRNA is highly expressed in the conduction system, e.g. Purkinje fibres. Whole-cell patch-clamp recordings of HEK293-cells transiently transfected with mutant TRPM4 channel showed for each of the four mutants a significant gain-of-function associated with a two- to sevenfold increase in TRPM4 current density in comparison to wild type. Further analysis showed that this correlated with increased surface density of mutant TRPM4 channels. Remarkably, the mutations attenuate de-SUMOylation of the TRPM4 channel which is apparently required for effective dynein-dependent endocytosis of the TRPM4 channel. The biophysical properties of the TRPM4 channel are reminiscent of the notorious Ca^{2+}-activated non-selective cation (CAN) channel. The CAN channel plays an important role in regulating cardiac conduction, for example action potential propagation in the His-Purkinje fibre system. We propose that enhanced cardiac TRPM4-channel activity slows or even blunts cardiac conduction because of its effects on membrane depolarization and Ca^{2+} influx. In a separate study we analyze the regulation of blood pressure in hypertensive BKβ1 knock-out mice. At the cellular and tissue level the phenotype is rescued by specific transgenic expression of BKβ1 subunits in smooth-muscle cells. Characterization at the systemic level is ongoing.

NCS-1 Physiology

Joanna Hermainski, Malte Stockebrand

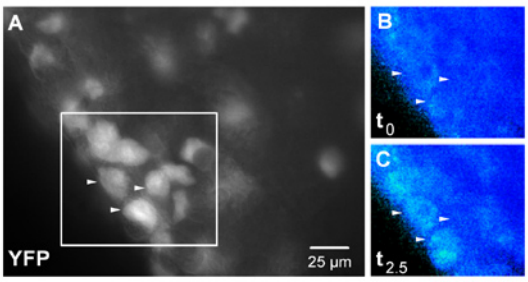
Neuronal calcium sensor-1 (NCS-1) has attracted much attention, because it may function as a calcium-sensor to modulate synaptic activity and secretion. Yet unambiguous identification of NCS-1 targets is still elusive. We have approached this problem by immunopurification of a high molecular weight NCS-1 – protein complex isolated from a mouse strain expressing GFP-tagged NCS-1. Subsequent mass-spectroscopical analysis and biochemical binding studies showed that NCS-1 associates with proteins involved in vesicle trafficking. The implications of these results for NCS-1 function are, however, still unclear. In a second approach, we have generated NCS-1 knock-out mice and have begun to study their phenotype. The mice exhibit no obvious gross abnormalities. As we detected a prominent and specific expression of NCS-1 in α-cells of islets, we have recently focused on characterizing physiological properties of NCS-1 -/- α-cells in comparison to wild-type. The results showed that NCS-1 -/- α-cells have a reduced response to glutamate. Based on the results we hypothesize that NCS-1 may affect glutamate receptor activity, possibly by modulating its vesicular turn over.

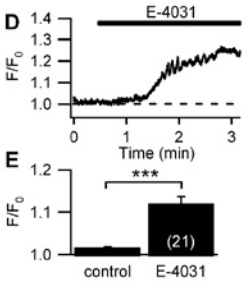
Erg K^+ Channel Physiology

Crenguta Dinu, Wiebke Hirdes, Dragos Niculescu, Jürgen R. Schwarz

Our group investigates the function of ion channels and neuropeptide receptors in neurons and neuroendocrine cells using electrophysiology, calcium imaging, immunocytochemistry and molecular biology. Presently we are analyzing the function of ether-à-go-go-related gene (erg) K channels in gonadotropes of the anterior pituitary, mitral cells of the olfactory bulb and Purkinje neurons of the cerebellum. In addition, we study the function of erg channels in cell lines of lung cancer cells. In gonadotropes and in neurons where erg channels are expressed, erg K channels mediate a window current which tends to shift the resting membrane potential towards more negative potentials. Because of the slow

Figure 3: Blockage of erg channels increases $[Ca^{2+}]_i$ in mouse gonadotropes
(A) Section of a pituitary slice with fluorescent gonadotropes. Boxed area is shown in (B) and (C). White arrowheads point to the same gonadotropes in (A), (B) and (C). Images of fura-2 fluorescence before (B) and during (C) application of E-4031. (D) time course of E-4031-induced increase in F/F0 in a gonadotrope. (E) mean ± SEM of F/F0 of all analyzed gonadotropes before (control) and 60 to 180s after onset of E-4031 application (n = 21). ***, level of significance, $P < 0.001$.



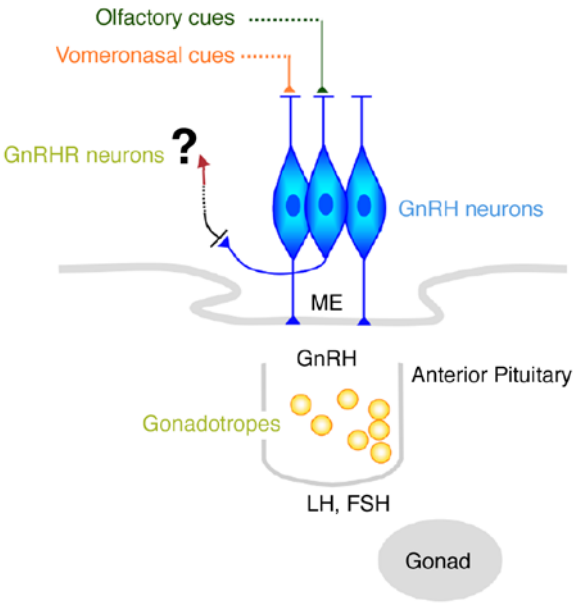


erg channel activation and deactivation kinetics erg currents accumulate during repetitive firing of action potentials and thereby induce accommodation. In gonadotropes of mice expressing YFP (Wen *et al*., 2008) blockage of erg channels by E-4031 increases $[Ca^{2+}]_i$. GnRH also reduces erg currents, due to a depolarizing shift of the activation curve and a reduction of the maximal erg current. In gonadotropes the erg current is presumably needed to stabilize a negative resting potential to preserve a low resting $[Ca^{2+}]_i$.

Olfaction and GnRH neural circuitry

*Nergiz, Avcu *, Zahara Alim, Ulrich Boehm, Dagmar Drexler, Devesh Kumar, Soumya Kusumakshi, Oliver Mai, Christian Mayer, Annette Marquardt, Shuping Wen*

The olfactory system detects odorants that elicit odor perceptions as well as pheromones that stimulate instinctive behaviors or hormonal changes. Odor perception and the memories it can evoke involve higher cortical processing areas and neural circuits capable of the plasticity needed for learning. In contrast, the stereotyped behaviors and physiological responses induced by pheromones imply that "hard-wired" neural circuits mediate these effects in the central nervous system. Elucidating the design and neuronal composition of these and other behavioral circuits stands as a challenge to comprehending the mechanisms underlying behavior.
Pheromone effects on reproductive behavior and physiology have been linked to gonadotropin releasing hormone (GnRH), a peptide produced by a subset of neurons in the hypothalamus. In addition to regulating gonadotropin release from the pituitary, GnRH stimulates reproductive behaviors in both male and female mammals via neural circuits in the hypothalamus. To analyze the neural circuitry of GnRH neurons in mice, we have used genetic transneuronal tracing and identified neurons directly presynaptic and postsynaptic to GnRH neurons in the brain's reproductive neural circuitry. Postsynaptic neurons are found in brain areas involved in sexual behavior, raising the possibility that the GnRH peptide itself might be released locally within the CNS and act on downstream neurons expressing the GnRH receptor (GnRHR). To selectively study this aspect of GnRH neural circuits, we developed a binary genetic mouse model and visualized GnRHR neurons with single cell resolution for the first time (Fig.4).



Our experiments are aimed at characterizing GnRHR neurons in live cell preparations, at analyzing their neural circuitry, and at manipulating them in the behaving animal with light-activatable channels. This should set the stage to analyze how pheromone-triggered effects on reproductive behavior and physiology are elicited in the mouse brain.

Support

The work in our institute was supported by grants of Deutsche Forschungsgemeinschaft including Forschergruppen For 604 and For 885, EU, Fonds der Chemischen Industrie, GIF, GIP, NIH, PROCOP, BMBF.

Selected Publications

Kruse M., Schulze-Bahr E., Corfield V., Beckmann A., Stallmeyer B., Kurtbay G., Ohmert I., Schulze-Bahr E., Brink P., and Pongs O. (2009) Impaired endocytosis of the ion channel TRPM4 is associated with human progressive familial heart block type I. J Clin Invest. *119*, 2737–2744.

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Christian Schauer *

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Institute for Biosynthesis of Neural Structures

Melitta Schachner Camartin

1. The L1 family of neural cell adhesion molecules

Yifang Cui, Daria Guseva*, Igor Jakovcevski, Nicole Karl, Ralf Kleene, Isabel Köhlitz*, Annika Lieberoth*, Gabriele Loers, Iris Oezen, Norman Rusche, Thomas Tilling, Gerrit Wolters**

The L1-like molecules are multifunctional molecules that are present in overlapping and distinct subpopulations of neurons at different stages of development and have been implicated in neuronal migration, neurite extension and fasciculation, myelination in the peripheral nervous system, and synaptic plasticity. L1 family members have been characterized in respect to structure-function relationships of the different domains and to the molecular interactions with their binding partners. Like other cell adhesion molecules, like NCAM, L1 and the close homolog of L1 (CHL1) are proteolytically processed by different proteases. This processing is required for neurite outgrowth.

In the lesioned adult central nervous system of rodents, application of recombinant L1 (L1-Fc) by osmotic infusion into the contused spinal cord enhances recovery of locomotor functions. Furthermore, recombinant monoclonal L1 antibody Fab fragments have been produced and showed *in vitro* the ability to stimulate L1 and trigger thereby the functions of L1. Mice treated with these antibody fragments also showed enhanced recovery of locomotor functions after lesion of the spinal cord.

Understanding the molecular mechanisms of cell recognition mediated by the Ig-superfamily of cell adhesion molecules requires a detailed knowledge of the molecule‘s structure. To this end, the extracellular domains of the cell adhesion molecules L1 and P0 are recombinantly produced and will be used for crystallization and structure analysis.

2. Neural recognition molecules and signal transduction

Claas Cassens, JuMi Chung*, Carina Figge*, Sabrina Jabs*, Gunjan Joshi, Ralf Kleene, Gabriele Loers, Tatjana Makhina*, Ingo Meier*, Ekaterina Mikautadze, Mounir Mzoughi*, Daniel Novak, Elisa Ramser*, Nils Tappenbeck*, Thomas Theis, Thomas Tilling, Ann-Kathrin Tranziska*, Gerrit Wolters*, Meifang Xiao*

The identification and characterization of intracellular signaling cascades activated by homophilic (self binding) or heterophilic (binding to other molecules) interactions of cell-adhesion molecules such as L1, CHL1 or the neural cell adhesion molecule NCAM are of central importance for the understanding of adhesion molecule-mediated neuritogenesis and growth cone repulsion. Previously we showed that surface localization of G protein inwardly rectifying K^+ channels (Kir3 channels) is controlled by NCAM and could explain how cell adhesion molecules are involved in the regulation of neural activity. In the meantime, we have identified proteins interacting with NCAM and Kir3, and are investigating the possible role of these interacting partners in regulating Kir channel surface localization and NCAM-mediated neurite outgrowth.

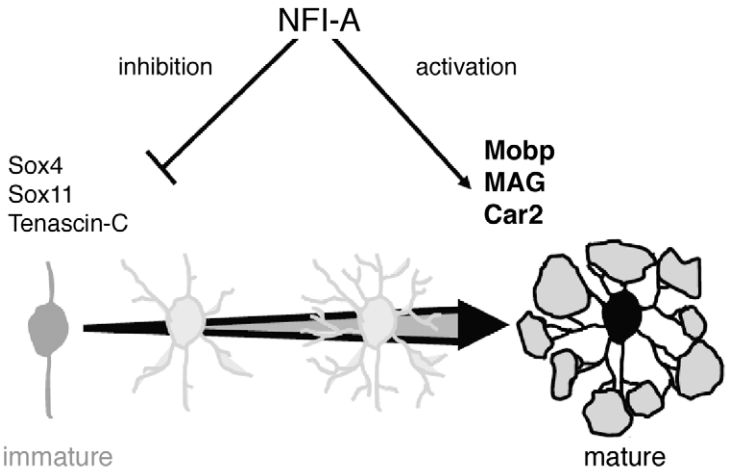
Hints on signal transduction mechanisms downstream of L1 have been gained by yeast-two hybrid and immunoprecipitation studies and by examining mice ectopically expressing L1 in astrocytes (GFAP-L1 mice). We are investigating

several of these "candidate proteins" with regard to their involvement in either regulation of L1 expression or L1-triggered signaling pathways. A novel finding indicates that glyceraldehyde-3-phosphate dehydrogenase is an extracellular binding partner for L1. L1 autophosphorylation and GAPDH-dependent phosphorylation of L1 are novel mechanism in regulating L1 signal transduction.

By detailed analyses of several intracellular signaling cascades, we could show that substrate L1-triggered neuritogenesis and neuroprotection depended on distinct but also overlapping signal transduction pathways and on the expression of L1 at the neuronal cell surface.

Control of inside-out signaling via neural cell recognition molecules also takes place at the transcriptional level. We could show that the site-specific transcription factor nuclear factor I-A (NFI-A) represses L1 transcription. Furthermore, results of our microarray analysis of mRNA expression in *Nfia*$^{-/-}$ *vs. Nfia*$^{lewis/+}$ mouse brains at two developmental stages strongly imply NFI-A in brain maturation, particularly in gliogenesis as shown below for oligodendrocytes (Fig. 1). L1, absent from mature astrocytes and oligodendrocytes, is also likely to be suppressed by NFI-A in glial cells. Notably, putative NFI-A targets identified in our study include the cell recognition molecules MAG, tenascin-C, CD24, and AMOG, further suggesting that NFI-A is a crucial factor in the control of cell recognition molecule expression.

Figure 1: Hypothetical model illustrating how NFI-A could promote oligodendrocyte differentiation. For reasons of clarity, only selected genes are indicated (Wong *et al*., 2007).



3. Prion protein and amyloid precursor protein as recognition molecules

Ute Bork, Vasudharani Devanathan, Nevena Djogo, Ralf Kleene, Iryna Leshchyns'ka*, Gabriele Loers, Nina Stemmer*, Vladimir Sytnyk**

The cellular form of prion protein (PrPc) is a glycosylphosphatidylinositol (GPI) anchored neural cell adhesion molecule involved in neurite outgrowth, neuronal survival, and synaptic function. Conversion of PrPc to an abnormal conformer (PrPSc) is a central event in the pathogenesis of prion diseases, such as Creutzfeldt-Jakob disease in humans, bovine spongiform encephalopathy in cattle and scrapie in sheep.

We identified the α2/β2-Na^+/K^+-ATPase and showed that this astroglial ATPase interacts directly with the cell adhesion molecule basigin. In cultured astrocytes, PrP is involved in regulating lactate transport via the astroglial monocarboxylate transporter 1 (MCT1) and in conjunction with α2/β2-ATPase and basigin. Lactate transport via MCT1 is glutamate dependent and regulated by glutamate receptor 2 (GluR2)-containing AMPA receptors with which PrP interacts. The functional interplay between PrP, GluR2, α2/β2-ATPase, basigin, and MCT1 in regulating lactate transport of astrocytes may be functional in the metabolic cross talk between astrocytes and neurons, most likely under stress.

Amyloid precursor protein (APP) and amyloid beta-peptide (Aβ) have been implicated in a variety of physiological and pathological processes underlying nervous system functions. We have identified ATP synthase subunit α as a binding partner of the extracellular domain of APP and Aβ. APP and

ATP synthase colocalize at the cell surface of cultured hippocampal neurons and astrocytes. ATP synthase subunit α reaches the cell surface via the secretory pathway and is N-glycosylated during this process. The extracellular domain of APP and Aβ partially inhibit the extracellular generation of ATP by the ATP synthase complex. Aβ impair both short- and long-term potentiation via a mechanism that could be suppressed by blockade of GABAergic transmission. These observations indicate that APP and Aβ regulate extracellular ATP levels in the brain, thus suggesting a novel mechanism in Aβ-mediated Alzheimer's disease pathology.

4. Carbohydrates and the fine tuning of cell interactions

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We are engaged in studies on different glycans that are carried by partially overlapping sets of glycoproteins, many of which have been shown to be neural recognition molecules, like CD24 which function depends mainly if not exclusively on glycans. The analysis of the composition of O- and N-glycans of CD24 revealed interesting functionally relevant glycostructures.

Some of these neural recognition molecules, e.g. L1, MAG, NCAM and basigin, are capable to bind distinct carbohydrate structures, thus functioning as lectins. For example, the adhesion molecules TAG-1 and Contactin which show sequence homologies with fucose-specific lectins, mediate Lewis^x-dependent CD24-induced effects on neurite outgrowth while L1 bind to α2,3-linked sialic acid present on CD24. Thus, L1, TAG-1 and Contactin function as lectin-like neuronal receptors. Their cis-interactions with neighboring adhesion molecules, e.g. Caspr1 and Caspr2, and with their triggered signal transduction pathways elicit cell type-specific promotion or inhibition of neurite outgrowth induced by glial CD24 in a glycan-dependent trans-interaction.

We focus our studies on several functionally important glycans, e.g. HNK-1 carbohydrate, oligomannosidic carbohydrates, α-2,8-linked polysialic acid (PSA), the LewisX epitope and α-2,3-linked sialic acid and their receptors. *In vitro* assays showed that these glycans are involved in cell adhesion and migration, outgrowth of neuritic and astrocytic processes as well as in synapse formation and synaptic plasticity. For HNK-1, oligomannosidic carbohydrates and PSA new receptors could be identified and the functional effects of their interactions are now under investigation.

We have identified carbohydrate peptide-mimetics and are using these as surrogate carbohydrates to trigger or block cell interactions: they are more easily obtained in large amounts than many structurally complex carbohydrates and can be manufactured as better binding ligands with higher metabolic stability. The HNK-1 peptide-mimetics we identified are able to enhance motoneuron growth and survival *in vitro* and locomotor recovery after peripheral nerve injury in mice. PSA peptide-mimetics stimulate process formation and proliferation of Schwann cells and outgrowth of neurons *in vitro* and locomotor recovery after peripheral nerve and spinal cord injury in mice. Additionally, we are searching for organic carbohydrate mimetics, which could be even more useful for therapy. Furthermore, we are producing anti-carbohydrate antibody fragments in E. coli for crystallization studies. For these antibody fragments will be crystallized together with the carbohydrates they bind to or the carbohydrate mimetics. These experiments will help to develop improved carbohydrate mimetics for therapeutical use.

The HNK-1 carbohydrate is a well-characterized example of a protein- and lipid-linked oligosaccharide. This epitope is regulated in its expression independently of the protein backbone, is phylogenetically conserved, and is functional in cell-cell and, particularly, cell-substrate interactions. Interestingly, the sulfate group is essential for most of the functions contributed by this epitope.

The enzyme transferring the sulfate group to the oligosaccharide backbone, the HNK-1 sulfotransferase (HNK-1 ST, Chst10), has previously been cloned and the HNK-1 ST knockout mutant has been generated and characterized. Based on the homology to the HNK-1 sulfotransferase we and others have identified and cloned six more members of this enzyme family. The sulfotransferases GalNAc-4ST1 (Chst8) and GalNAc-4ST2 (Chst9) have been shown to synthesize sulfated beta1-4-linked GalNAc found on the GGnM epitope characteristic of glycopeptide hormones of the pituitary and to add sulfate to non-terminal beta1-4-linked GalNAc found on chondroitin and dermatan. The sulfotransferases C4ST1 (Chst11), C4ST2 (Chst12), C4ST3 (Chst13) and D4ST1 (Chst14) confer sulfate to beta1-4-linked GalNAc on chondroitin and dermatan. While homozygous ablation of the GalNAc-4ST1 does not show any gross anatomical and morphological differences, C4ST1 deletion causes perinatal lethality and only some D4ST1 homozygous KO mice survive until adulthood, but show reduced body size and differences in brain morphology and CNS regeneration compared to the wild-type littermates.

5. Regeneration in the nervous system

Yifang Cui, Daria Guseva*,*
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Andrey Irintchev, Ali Mehanna*,*
Sandra Nickel

Peripheral neurons can regenerate their axons after nerve injury and reinnervate peripheral targets. Despite the robust regenerative potential, the clinical outcome of nerve repair is often disappointing. Regrowth of severed axons to improper targets is considered a major reason for poor functional recovery. A valuable paradigm which allows studies on accuracy of pathway finding and molecular mechanisms determining reinnervation selectivity is the femoral nerve model in rodents. We developed and validated a novel video-based motion analysis approach, the single-frame motion analysis, for objective numerical evaluation of quadriceps muscle function which proved to be reliable and sensitive in several studies of femoral nerve lesion.
We had previously identified a molecular cue potentially related to preferential motor reinnervation. HNK-1 epitope was found to be associated with myelin profiles of motor axons, but not of sensory axons, both in intact and regenerating femoral nerves of mice. Another glycan involved in nerve regeneration is PSA. We tested the possibility to promote functional recovery after nerve lesion by application of exogenous HNK-1 or PSA carbohydrate in the form of synthetic peptides that functionally mimic the carbohydrates. For these glycomimetics we demonstrated that they enhance recovery after femoral nerve lesion by different mechanisms. Furthermore, application of a novel cyclic HNK-1 glycomimetic enhanced functional recovery *in vivo* not only in rodents but also in non-primate monkeys. Our results indicate the potential of HNK-1 and PSA mimetics as therapeutic agents promoting motor recovery after peripheral nerve injury.

In contrast to the peripheral nervous system, in the central nervous system paucity of permissive molecules and abundance of inhibitory molecules prevent axons from successful regeneration and, thus, contribute to the failure of functional recovery. We also designed a motion analysis approach allowing numerical assessment of motor functions after spinal cord injury. We tested this approach on TNR-/- and CHL1-/- mice and their wild-type littermates and found that motor function after injury improved more in TNR-/- and CHL1-/- mice than in respective wild-type mice. Morphological analyses revealed that TNR restricts functional recovery by limiting posttraumatic remodeling of synapses around motoneuronal cell bodies where TNR is normally expressed in perineuronal nets. On the other hand, CHL1 is a glial scar component that restricts posttraumatic axonal growth and remodeling of spinal circuits by homophilic binding mechanisms. Using an adeno-associated viral (AAV) vector, we overexpressed the regeneration-promoting cell adhesion molecule L1 in both neurons and glia in the lesioned spinal cord of adult mice. L1 overexpression led to improved functional recovery associated with enhanced reinnervation of the lumbar spinal cord. The expression of the neurite outgrowth-inhibitory chondroitin sulphate proteoglycan NG2 was decreased in AAV-L1-treated spinal cords, along

with reduction of the reactive astroglial marker GFAP. Thus, AAV-mediated L1 overexpression appears to be a potent means to favourably modify the local environment in the injured spinal cord and promote regeneration. As a new mode to stimulate L1 functions a L1 triggering antibody and Fab fragments obtained from this antibody are currently applied to the lesioned spinal cord and recovery is evaluated.

Furthermore, we are now evaluating the effects of the carbohydrates LewisX, HNK-1, PSA and of chondroitin sulfates on regeneration by using mice deficient for the enzymes synthesizing these carbohydrates or by addition of peptide mimetics for these glycans to the lesioned spinal cord. We applied PSA and HNK-1 mimetic peptides by subdural infusions with an osmotic pump immediately after injury. Our results suggest that PSA mimetic peptide could be an efficient therapeutic tool improving, by augmenting plasticity, functional recovery when applied during the acute phase of spinal cord injury.

6. Immunoglobulin superfamily (IgSF) molecules and their roles in organization of the pre- and post-synaptic machinery

Aksana Andreyeva, Vsevolod Bodrikov*, Yana Chernyshova*, Babett Baraniec*, Doreen Westphal*, Aparna Shetty*, Vasudharani Devanathan*, Dmytro Puchkov*, Nan Tian, Shen Li, Ute Eicke-Kohlmorgen, Iryna Leshchyns'ka*, Vladimir Sytnyk**

Cell adhesion molecules expressed at the neuronal surface membrane are now recognized as important regulators of synapse formation. In our work we have identified the immunoglobulin superfamily cell adhesion molecule close homologue of L1 (CHL1) as a novel synaptic component accumulating in the presynaptic membrane of the central nervous system synapses. We have shown that CHL1 interacts via its intracellular domain with clathrin-uncoating ATPase Hsc70. CHL1 recruits Hsc70 to the presynaptic membrane thereby promoting the uncoating of clathrin-coated synaptic vesicles, an important process which is required to maintain an efficient synaptic transmission. In contrast to CHL1, the largest isoform of the neural cell adhesion molecule, NCAM180, accumulates in the postsynaptic membrane of glutamatergic synapses. Our research shows that NCAM180 promotes assembly of the spectrin scaffold within postsynaptic densities. NCAM180/spectrin synaptic scaffold recruits proteins, which are essential for memory formation, such as the N-methyl-d-aspartate (NMDA) receptor and Ca^{2+}/calmodulin-dependent protein kinase II α (CaMKIIα). Neurons, in which the NCAM180/spectrin scaffold was disrupted, were unable to recruit and activate CaMKIIα in synapses. Levels of the activated CaMKIIa were dramatically reduced in NCAM deficient mice. Thus, our observations on the novel functions of CHL1 and NCAM180 provide first molecular details of the mechanisms by which mutations in NCAM and CHL1 genes can lead to psychological abnormalities in humans.

7. Recognition molecules and synaptic plasticity

Olena Bukalo, Alexander Dityatev*, Galina Dityateva*, Gaga Kochlamazashvili*, Eka Lepsverdize*, Giorgi Papashvilli*, Oleg Senkov*, Luminita Stoenica**

Our major interest is to understand how recognition molecules modulate synaptic transmission and plasticity. We previously found that mice deficient in the cell adhesion molecules NCAM and CHL1 and extracellular matrix glycoproteins tenascin-C and tenascin-R mutants show reduced long-term potentiation (LTP) in the CA1 region of the hippocampus. Analysis of the underlying mechanisms revealed that impairments in LTP in tenascin-C deficient mice are related to a deficit in L-type Ca^{2+} voltage-dependent channel (L-VDCC)-dependent signaling, whereas tenascin-R-deficient mutants exhibit a deficit in perisomatic inhibition in CA1, which triggers a metaplastic increase in the threshold for induction of LTP. In contrast, in juvenile CHL1-deficient mice LTP is impaired due to elevated perisomatic inhibition. Also a fragment of amyloid precursor protein, the amyloid beta-peptide, may inhibit LTP via elevation of GABAergic inhibition through interaction with the ATP synthase. Both tenascin-R and tenascin-C deficient mice

have increased power of gamma-oscillations in the cortex and hippocampus. Tenascin-R deficient mice also show a reduced rate of kindling, which correlates with increased expression of astroglial secreted protein S100B in the dentate gyrus. The role of NCAM in CA1 LTP and contextual fear conditioning is mediated by the glycan polysialic acid (PSA), which inhibits NR2B-containing NMDA receptors and restrains activation of p38. Also other glycans associated with recognition molecules have important neuronal functions. The HNK-1 carbohydrate carried by tenascin-R interacts with the GABAB receptor and reduces its activity. Enzymatic removal of chondroitin sulfates impairs LTP in CA1, disperses several components of the extracellular matrix surrounding perisomatic inhibitory interneurons in so-called perineuronal nets and increases interneuronal excitability. Another glycan of perineuronal nets, hyaluronic acid, supports activity of L-VDCCs and L-VDCC-dependent forms of LTP. In summary, our data demonstrate that recognition molecules and associated glycans have numerous synaptic functions in adult brains, some of which are implemented through their interactions with transmitter receptors and channels.

8. Neural recognition molecules and behaviour

Laetitia Fellini, Mira Jakovcevski*, Gila Jung*, Ia Lapadze*, Fabio Morellini*, Ian Belle Stein**

Our research was characterised by the use of an ethological perspective to understand how (ultimate causes) and why (proximate causes) determined behavioural responses are used by mice, and thereby to interpret the mechanisms underlying altered behaviours in transgenic mice deficient for specific molecules of interest. In terms of behavioural functions, our studies have been particularly focussed to coping strategies and cognition. For instance, we have observed that high levels of trait anxiety correlate with enhanced sensitivity of the stress system and with elevated hippocampal levels of glucocorticoid receptors. Noteworthy, this study also reported, for the first time, that conspicuous differences exist within an inbred strain like the C57BL/6 mouse, thus indicating how epigenetically determined individuality is an important factor that is often underestimated. In the context of cognitive functions, we have shown that mice are capable to extrapolate geometric information from asymmetric environments and that they use prevalently this information for navigation. Aiming at cognitive tasks relevant for the ecology of the house mouse, we have established new hippocampus-dependent paradigms in which the mouse performance can be interpreted in terms of cognitive abilities and it is not influenced by factors such as sensory-motor function, motivation or anxiety. By means of these tasks specifically designed for the house mouse, we could show that expression of the snoRNA host gene GAS5 in the hippocampus does not regulate, as previously suggested, spatial learning and memory, but affects novelty-induced behaviours and is regulated by activation of the stress response.

9. Stem cells and neural transplantation

Christian Bernreuther, Shan Bian, Yifang Cui*, Gunnar Hargus*, Iris Oezen, Janinne Sylvie Schmid*, Yuliya Tereshchenko*, Jinchong Xu*

Neurodegenerative diseases and spinal cord injury are insults to the central nervous system leading to neuronal loss and degeneration of axons. Since current treatments cannot replace degenerated neurons, research on alternative therapeutic approaches need to be pursued. In this context, the transplantation of genetically modified stem cells into lesioned brain areas of patients is a possible alternative. We have generated a murine embryonic stem cells constitutively expressing the neural cell adhesion molecule L1 or the extracellular matrix molecule tenascin-R at all stages of neural differentiation to investigate their effects on stem cell proliferation, migration, differentiation, cell death, and ability to influence drug-induced rotation behavior in an animal model of Huntington's disease. L1 overexpression resulted in an increased yield of GABAergic neurons and enhanced migration of embryonic stem cell-derived neural precursor cells into the lesioned striatum. Mice grafted with L1-transfected cells showed recovery in rotation behavior after trans-

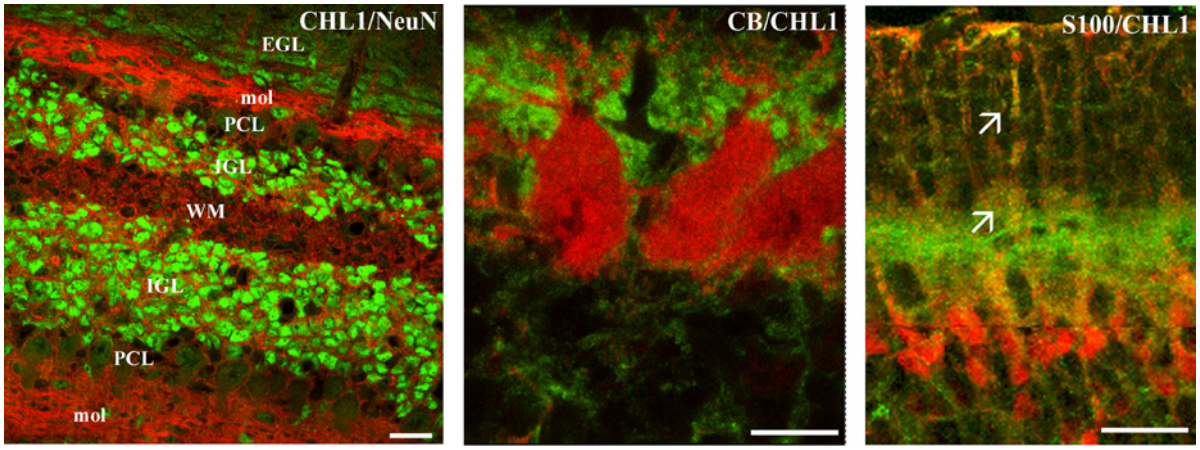

Figure 2: Double-immunofluorescence for CHL1 and cell-type specific markers in P7 mouse cerebellum. Left pannel: CHL1 (red) and NeuN (green) in the cerebellar cortex at P7. Middle panel: Calbindin+ Purkinje cells (red) do not express CHL1 (green) at P7. Right pannel: $S100^{+}$ Bergmann glia cells (red) express CHL1 (green) in their radial processes (arrows). EGL - external granule layer; PCL - Purkinje cell layer; WM - prospective white matter; mol - molecular layer. Scale bar: 20 µm; middle panel: 10 µm.

plantation compared with mice that had received non-transfected cells, thus demonstrating for the first time that a recognition molecule is capable of improving functional recovery in a syngeneic transplantation paradigm. Furthermore, a new differentiation protocol was established allowing the transplantation of embryonic stem cell-derived aggregates of neurons and radial glial cells leading to enhanced survival of graft-derived neurons with decreased tumour formation after transplantation into a mouse model of Huntington's disease. Furthermore, transplantation of L1 overexpressing embryonic stem cells that had been subjected to this protocol led to enhanced survival of graft-derived dopaminergic neurons after transplantation into with enhanced functional recovery.

Transplantation of embryonic stem cells stably transfected to overexpress the extracellular matrix molecule tenascin-R, which is expressed by striatal GABAergic neurons, into a mouse model of Huntington's disease, led to increased generation of GABAergic neurons and decreased numbers of astrocytes and attracted host-derived neuroblasts from the rostral migratory stream thus promoting stem cell-mediated recruitment of host-derived newborn neurons within the grafted area.

These findings encourage combinatorial approaches in stem cell therapy of neurodegenerative diseases.

10. Structural substrates of brain dysfunctions caused by mutations of recognition molecules

Alexander Dityatev, Andrey Irintchev*, Igor Jakovcevski, Fabio Morellini*, Yuliya Tereshchenko**

We have studied the effects of deletions of genes encoding cell recognition molecules on brain structures and tried to correlate morphological changes to behavioral and electrophysiological alterations caused by these mutations.

Using CHL1-/- mice we addressed the question of how a gene mutation can influence the postnatal fate of inhibitory interneurons, since they are born with abnormally high numbers of parvalbumin-positive (PV^{+}) hippocampal interneurons, and develop a deficit during adulthood compared with wild-type littermates. We found that region-specific aberrant synaptic connectivity resulting from the CHL1 ablation led to enhanced synaptic elimination during brain maturation, microgliosis,

cytokine over-production and loss of susceptible interneurons. Interneuron loss, in turn, causes reduced inhibition and functional deficits like impaired synaptic plasticity.

How the balance between excitation and inhibition controls fundamental aspects of the hippocampal function was also investigated in TNR-/- mice, which have increased ratio of inhibitory to excitatory neurons in the dentate gyrus, accompanied by GABAA receptor-dependent impairment of synaptic plasticity and enhancement of activity-dependent changes in excitability. TNR-/- mice also showed faster reversal learning than wild-type littermates in the water maze and olfactory learning tests, and enhanced working memory in the spontaneous alternation and win-shift tests. Our data demonstrate that modification of the extracellular matrix by ablation of TNR leads to a new structural and functional design of the dentate gyrus, with enhanced GABAergic innervation, i.e. enhanced ratio of inhibitory to excitatory cells, and altered plasticity, supportive for working memory and reversal learning.

The HNK-1 carbohydrate is detectable in perineuronal nets around inhibitory neurons in the hippocampus and neocortex. To address the functional contribution of HNK-1 to interneuron function in the adult brain, we recorded EEG in freely moving mice deficient for HNK-1 sulfotransferase (ST-/- mice) and in wild-type littermates. While ST-/- mice displayed normal theta oscillations, both cortical and hippocampal oscillations within the beta range were enhanced, and gamma oscillations showed an opposite trend. Morphological analysis revealed a decreased density of PV^+ interneurons in the hippocampal CA3 subfield of ST-/- mice, which may contribute to the observed changes in networks oscillations. These findings reveal alterations in ST-/- mice that differ from EEG abnormalities of mice deficient in the HNK-1 carrier molecule TNR.

We also found that constitutive ablation of CHL1 in mice caused a significant loss of Purkinje and granule cells in the 2-month-old cerebellum. Purkinje cell loss occurred before the first postnatal week and was associated with enhanced apoptosis, presumably as a consequence of CHL1 deficiency in afferent axons. Granule cell deficiency in adult CHL1-/- mice appeared to result from decreased precursor cell proliferation after the first postnatal week. Our results indicate that CHL1 promotes Purkinje and granule cell survival and granule cell migration during cerebellar development.

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Cell Biochemistry and Clinical Neurobiology

Dietmar Richter: Founding Director of the ZMNH (retirement 2005)

Due to my retirement in 2005 the Institute for Cell Biochemistry and Clinical Neurobiology was closed down. The help of the directorate of the Center for Molecular Neurobiology by providing space for the continuation of my scientific activities is greatly acknowledged. Most of the ongoing research was carried out in collaboration with my former co-workers Stefan Kindler and Hans-Jürgen Kreienkamp, now group leaders at the Institute for Human Genetics, University Medical Center Hamburg-Eppendorf. Research has been focused on how nerve cells manage to respond to external and internal signals in order to maintain and regulate their cellular architecture. As shown earlier signal transduction processes involving neurotransmitter receptors are mediated by a series of defined protein-protein interactions. We have identified specific interacting proteins for the C-terminal, intracellular regions of each subtype of G-protein coupled somatostatin receptors (SSTR1-5). Some interacting partners, such as the PDZ domain protein PIST, have a function in membrane targeting of SSTR3 and SSTR5. Others such as the tight junction protein MUPP1 or the postsynaptic scaffold proteins PSD-95 (interacting with SSTR4) or SSTRIP/shank (interacting with SSTR2) link the receptors to large signaling complexes, such as the postsynaptic density (PSD) in excitatory synapses of the central nervous system.

We have also continued our work on the structure and function of the members of the shank protein family which represents master scaffold proteins of the PSD and appears to play a central role in neuronal morphogenesis and synaptogenesis. We have shown that shank proteins interact directly or indirectly with neurotransmitter receptors, actin binding proteins and other prominent postsynaptic scaffold proteins such as PSD-95. More recently, we have studied the pathogenesis of the fragile X-mental retardation syndrome (FXS). To date, the molecular events leading from the loss of the fragile X mental retardation protein (FMRP) to the diverse devastating symptoms of FXS, including cognitive impairment and autism, are still poorly understood. Much research on the cellular causes for FXS has been focused on synaptic dysfunction. In brain neurons of wild-type mice, FMRP is found in the proximity of synapses where it primarily represses translation of mRNAs at postsynaptic sites. This translational block can be abolished via stimulation of metabotropic glutamate receptors (mGluRs). Thus, lack of FMRP leads to excessive mGluR-dependent protein synthesis at synapses which may be the major cause for synaptic malformation and dysfunction. Indeed, in FMRP deficient mice several FXS symptoms can be corrected by a reduction of neuronal mGluR levels.

While mGluRs act *upstream* of FMRP in the signal cascade regulating local synaptic protein synthesis, the *downstream* components involved in FXS pathogenesis are less well described. Recently, we reported that the PSD is altered in FMRP deficient mice. We could show that several mRNAs encoding components of the PSD are *in vivo* associated with FMRP. Via this interaction FMRP controls dendritic mRNA translation and postsynaptic protein levels, but not local transcript stability. In particular, FMRP was shown to repress the translation of shank 1-mRNA in an mGluR-sensitive manner by binding to the 3' untranslated region (3'UTR). Thus, our data suggest that the mGluR/FMRP pathway controls shank 1 levels in the PSD. In agreement with this idea is the finding by Durand *et al*., 2007, that mutations in the gene encoding shank 3 are associated with autism spectrum disorders and mental retardation. Based on our hypothesis that elevated shank 1 levels in PSDs represent key molecular events of the FXS pathology we presently try to reduce synaptic shank 1 levels in FMRP deficient mice (*Fmr1-/-*) by genetic manipulation followed by analyzing various structural, molecular and behavioral parameters of the mutant mice. We expect that similar to a reduced concentration of mGluR5 (Dölen *et al*., 2007), an *upstream* component of the FMRP-dependent synaptic protein synthesis pathway, the geneti-

cally induced reduction of postsynaptic levels of shank 1, a *downstream* signaling molecule, will correct at least some of the pathogenic alterations observed in *Fmr1-/-* mice. Thus, shank 1 may emerge as an attractive novel drug target for the treatment of FXS patients.

We also extened our previous work on allatostatin receptors in invertebrates, a G-protein coupled receptor (GPCR) family initially identified by a reverse pharmacological approach in Drosophila melanogaster (Birgül *et al*., 1999). Recently, we described allatostatin receptors from Periplaneta americana and Aedes aegypti, all are structurally related to vertebrate galanin/somatostatin/opioid receptors. Expression studies revealed that allatostatin receptors are widely expressed in adult insect tissues and in early larval instars. The spatial expression supports the known pleiotropic activity of allatostatins and a role as a paracrine effector.

Support

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Book

Richter, D. and Tiedge, H. (2006-2009). Results and Problems in Cell Differentiation. Vol. 41-49 (Heidelberg: Springer Press).

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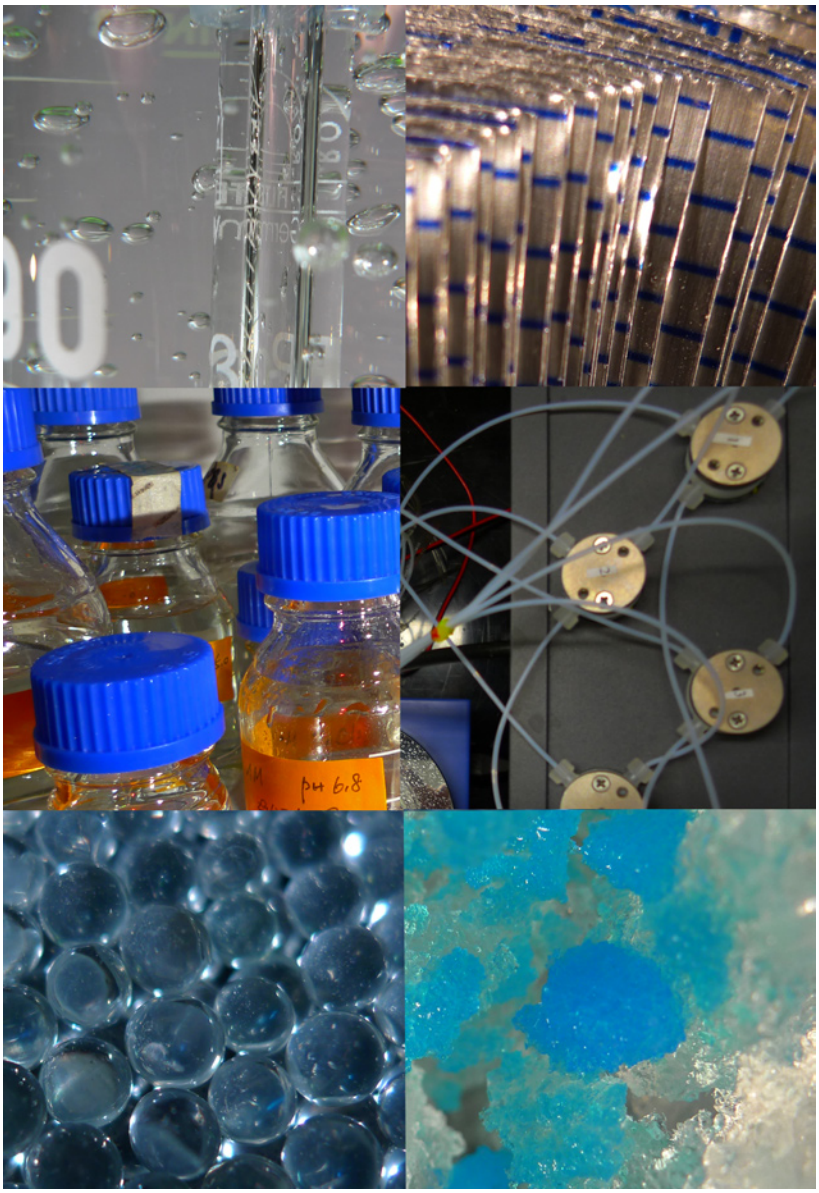
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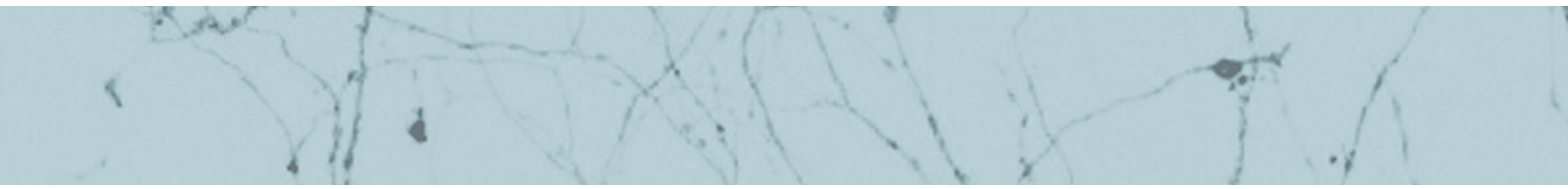

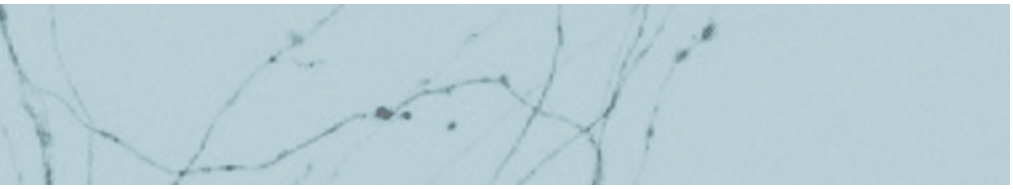

Research Groups

Neuroimmunology

Manuel A. Friese

We are interested in pathomechanistic research of neuroimmunological disorders and here in particular multiple sclerosis (MS).

MS is an autoimmune disease with high population prevalence, which is governed by multiple genetic and environmental factors. It is clinically reflected by neurological symptoms including motor dysfunction, hyperreflexia, spasticity, ataxia, visual and sensory impairment, bladder and bowel disturbances and fatigue. The disease is characterized by a permissive immune system that fails to impose tolerance to a number of self-antigens which reside within the central nervous system (CNS) with the pathological correlate consisting of immune cell infiltration in the CNS, demyelination, axonal and neuronal degeneration. As in other autoimmune diseases it is speculated that multiple checkpoints need to be bypassed, in which genetic predisposition cooperates with acquired defects in other cells prompting a reaction overriding limiting mechanisms.

Our work has two specific objections concerning the pathogenesis of MS, (1) the mechanisms of deregulation of autoreactive $CD8^+$ T cells and (2) the consequences of chronic CNS inflammation for neuronal integrity (neurodegeneration).

Autoreactive $CD8^+$ T cells in multiple sclerosis

Cytotoxic $CD8^+$ T cells have been considered to be deregulated in MS and cooperate with the $CD4^+$ T cells in their attack on the CNS. Indeed, $CD8^+$ T cells are the most frequent T cell subset found in acute and chronic MS lesions and $CD8^+$ T cells have now been shown to mediate disease in certain animal models of MS (experimental autoimmune encephalomyelitis; EAE), while others report a protective effect of $CD8^+$ T cells in different EAE models and MS. It is presently unclear, why $CD8^+$ T cells show sometimes a cytotoxic and at other times a protective phenotype in EAE mice and MS patients. Additionally, there is evidence that the MHC class I allele HLA-A3 predisposes independently to MS, while HLA-A2 protects. However, we understand remarkably little about the functional roles of HLA molecules in the aetiology and pathogenesis of MS. It is possible that certain HLA alleles do not properly eliminate autoreactive T cells during thymic negative selection. We have studied these mechanisms using humanized mice transgenic for HLA-A3 and an MS patient's HLA-A3-restricted T cell receptor (TCR), recognizing an epitope from a major myelin protein. This leads to MS-like disease in these transgenic mice. Interestingly, an additional HLA-A2 transgene was strikingly protective, leading to the complete absence of disease. Mechanistically, HLA-A2 greatly reduced not only the number of pathogenic TCR-positive $CD8^+$ T cells but also their peptide-responsiveness and cytokine secretion. These reductions were due to HLA-A2–mediated negative selection in the thymus and, in the periphery, to down-regulation of the transgenic TCR and dilution by a second TCR α-chain from the host mice.

These findings suggest that $CD8^+$ T cells can be crucial in the aetiology and pathogenesis of MS. Having characterized pathogenic $CD8^+$ T cells in this humanised animal model, we are now taking

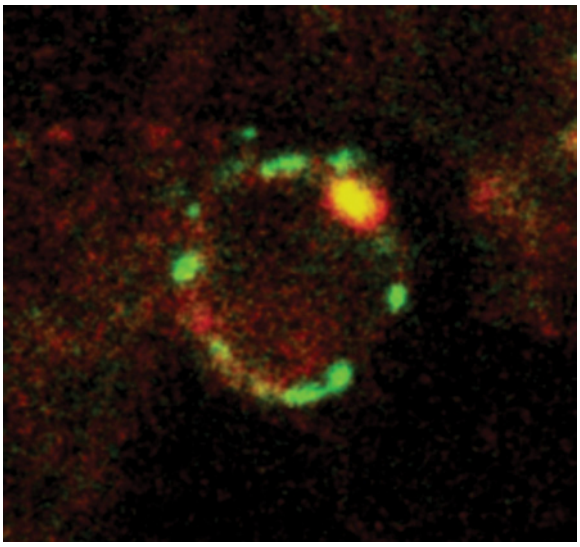

Figure 1. Polarisation of cytotoxic granules in myelin-specific $CD8^+$ T cells infiltrating the CNS. CD107a (green), A3/PLP45–53-tetramer (red). (Friese *et al*. (2008) Nature medicine *14*, 1227-1235.)

these results back into humans to characterize the phenotype and differentiation of pathogenic $CD8^+$ T cells in MS patients and the role of co-stimulatory molecules for antigen-dependent activation. $CD8^+$ T cells are cloned from the cerebrospinal fluid and their pathogenicity tested *ex vivo* and in novel transgenic animal models.

Neurodegeneration in multiple sclerosis

Although inflammation and primary demyelination are the most characteristic features of CNS lesions in MS, axonal/neuronal degeneration correlates best with clinical deficits in patients. It has been suggested that the inflammatory insult in MS leads to axonal degeneration by causing neuronal mitochondrial dysfunction, energy failure and alteration of ion exchange mechanisms. Indeed, it has been demonstrated that mitochondrial dysfunction with reduced ATP production is present in MS lesions, supporting the existence of energy failure. The final pathway of axonal degeneration would then be mediated by Na^+ and Ca^{2+} influxes which activate proteases and disrupt the cytoskeleton. However, the mechanisms and ion channels involved in this influx of cations are only partly described and their pathophysiological functions not known. We are interested how different ion channel families might contribute to inflammatory-induced neurodegeneration; e.g. we recently described the neuronally expressed proton-gated acid-sensing ion channel 1 (ASIC1), which is permeable to Na^+ and Ca^{2+} to contribute to axonal degeneration, by sensing a lowering in pH in inflammatory CNS lesions.

Currently we are investigating the role of other ion channel families for their pathological activation under chronic inflammatory conditions by using knock-out technology. Ion channels can be easily modified in their function by small compounds, making them particularly attractive as drug targets for rapid translation into clinical studies for the benefit of MS patients.

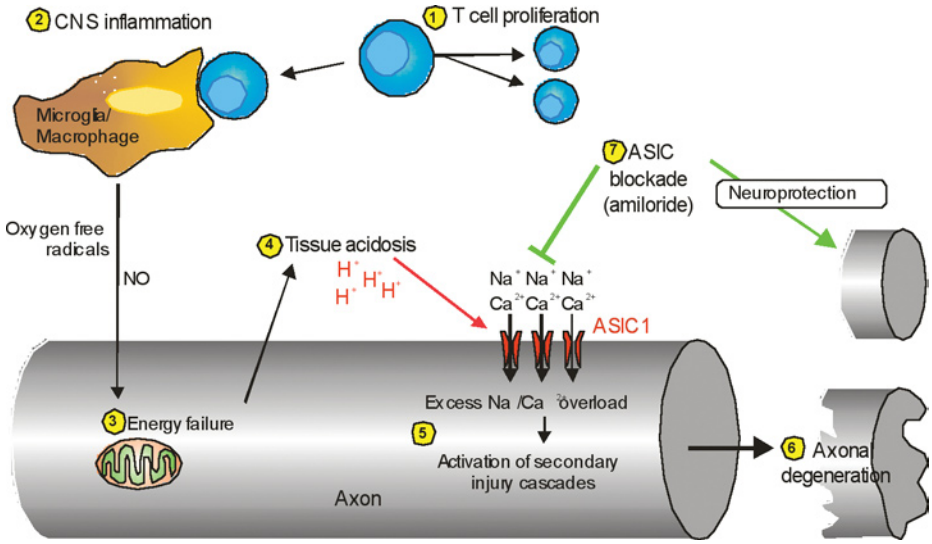

Figure 2. Schematic demonstration of the mechanism of action for ASIC1 in axonal degeneration. (1) T cell activation and proliferation increase the population of auto-reactive T cells within the CNS and drive the inflammatory response. (2) Autoimmune CNS inflammation then initiates a cascade with subsequent release of toxic mediators, including nitric oxide and oxygen free radicals. (3) Energy failure ensues due to nitric oxide induced mitochondrial dysfunction causing (4) tissue acidosis. The increased concentration of protons (acidosis) leads to activation of ASIC1 expressed along axons, dendrites or neuronal cell bodies. (5) Opening of ASIC1 leads to influx and accumulation of injurious Na^+ and Ca^{2+} ions. Activation of secondary injury cascades, such as proteases, causes breakdown of the axonal cytoskeleton leading to (6) axonal degeneration and development of non-remitting clinical deficits. (7) Pharmacological blockade of ASIC by amiloride and other related compounds acts through neuroprotective mechanisms with potential for amelioration of CNS inflammatory disease.
(Friese *et al*. (2007) Nature medicine *13*, 1483-1489.)

The aspect of neurodegeneration in MS has only recently been the focus of drug development. Interestingly, the licensed anti-hypertensive drug amiloride is a blocker of the ASIC1 channel and effective in inhibiting neurodegeneration in the animal model of MS. This off-target effect could be beneficial for MS patients and will be tested in a clinical trial.

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* equal contribution

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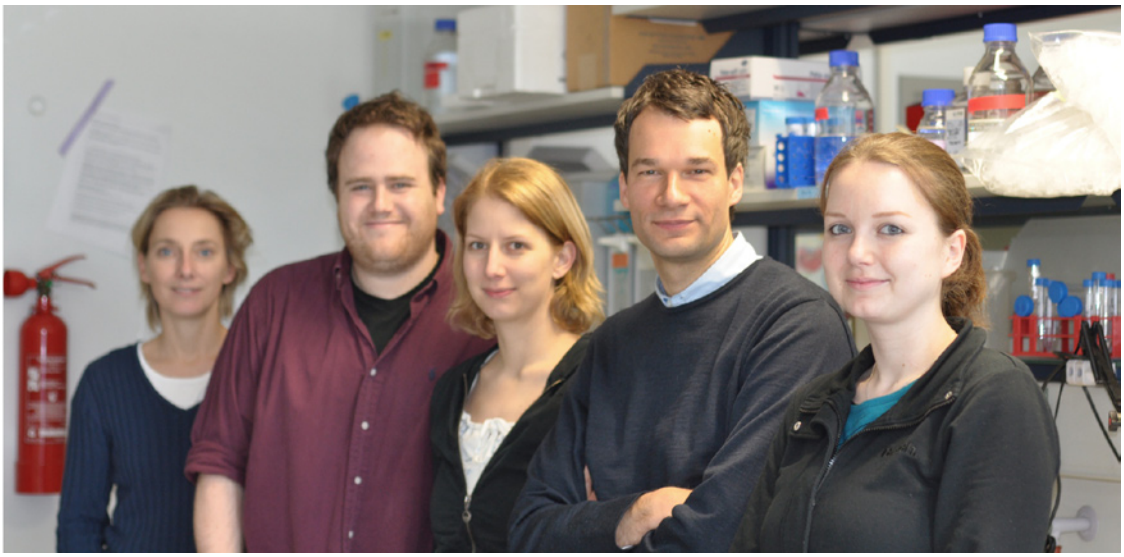

BMBF/Emmy-Noether Team: Developmental Neurophysiology

Ileana Hanganu-Opatz

Binding of neuronal assemblies by synchronizing their activity patterns in oscillatory rhythms enables higher brain abilities such as attention, perception and memory. The relevance of synchronized binding of neural assemblies is exemplary illustrated in the case of prefrontal cortex (PFC). Considered as the "psychic" cortex par excellence due to its involvement in gating of memory and attention, the PFC is part of a complex network including both hippocampus and thalamus as well as neuromodulatory subcortical nuclei. The function of adult PFC relies on transient coupling and decoupling of oscillating neuronal populations.

Oscillatory activity starts to synchronize neuronal populations already during early development, but its patterns and role differ remarkably from the adult ones. In the developing sensory systems of both humans and rodents the intermittent oscillatory patterns of activity act as a template for the correct formation of cortical maps underlying complex sensory functions. Whether similar mechanisms account for the formation of frontal-subcortical networks responsible for executive and mnemonic functions is widely unknown. Our research focuses on the properties, the mechanisms of generation/modulation and the function of early patterns of activity synchronizing the developing PFC-hippocampo-subcortical networks. By combining *in vivo* and *in vitro* extra- and intracellular recordings with immunohistochemistry and behavioral analysis of neonatal and young rodents we currently address following questions:

- Does the development of PFC and of frontal-subcortical networks follow similar activity-dependent mechanisms as the sensory systems?
- How do coordinated patterns of electrical activity trigger/modulate the maturation of PFC-hippocampo-subcortical networks and how do they contribute to the acquirement of executive and mnemonic abilities?
- Do abnormal patterns of activity during early development correlate with an impaired functional state of developing networks underlying behavioral deficits in neuropsychiatric disorders?

1. Development of oscillatory activity patterns within the PFC-hippocampo-subcortical networks

Our previous investigations performed at INSERM Marseille and at Johannes Gutenberg-University Mainz from 2006 to the end of 2008 led to the conclusion that coordinated patterns of electrical activity facilitate the development of cortical maps in sensory cortices even before the environment shapes the connectivity during the critical period. Endogenous activation of sensory periphery that occurs before the maturation of perception abilities reliably synchronizes the cortical networks in a template of later emerging cortical maps (Fig. 1) (Dupont *et al*., 2006; Hanganu *et al*., 2006; Yang *et al*., 2009). We characterized the early intermittent patterns of oscillatory activity in the rodent visual and barrel cortex and identified their main triggers as follows: (i) perception-independent activation

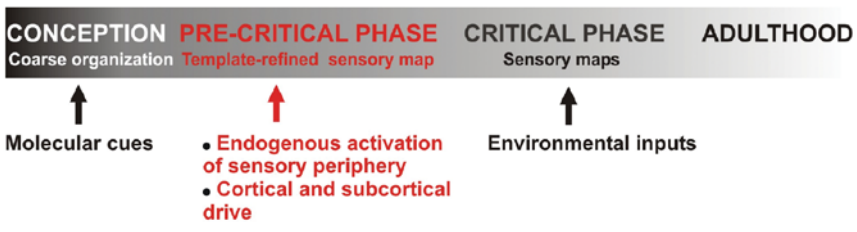

Figure 1. Formation of neuronal networks relies on genetic information and on experience-dependent and –independent electrical activity.

of sensory periphery, e.g. spontaneous retinal waves traveling over the light-insensitive retina, passive whisker activation before the maturation of whisking ability (Hanganu *et al*., 2006; Yang *et al*., 2009); (ii) neuromodulatory innervation from subcortical nuclei, e.g. cholinergic drive from basal forebrain acting on cortical muscarinic ACh receptors (mAChR) (Hanganu *et al*., 2007; Hanganu *et al*., 2009); (iii) intracortical connectivity involving gap junctional coupling and tonic GABA depolarization (Dupont *et al*., 2006; Hanganu *et al*., 2009).

The common mechanisms by which coordinated activity shapes the maturation of primary sensory cortices and sensory networks mirror the general principles of function shared by the majority of sensory systems. However, these principles are not applicable to association cortices and neuronal networks underlying cognitive abilities. To shed light onto the activity-dependent mechanisms governing the early maturation of association cortices, like the PFC, and of corresponding networks we firstly characterized their early patterns of coordinated activity. For this we developed an extracellular recording technique by which both network oscillations and individual spiking of neurons in the anterior cingulate (Cg) and pre/infralimbic (PL/IL) regions of PFC can be monitored *in vivo* in neonatal and young rodents. Similar to the sensory cortices, the PFC shows intermittent oscillatory events during the first 9 postnatal days and switches to continuous theta-band rhythm overlapped with short gamma episodes from P10 on. However, the early PFC oscillations show different properties as well as spatial and temporal dynamics when compared to the events in sensory cortices

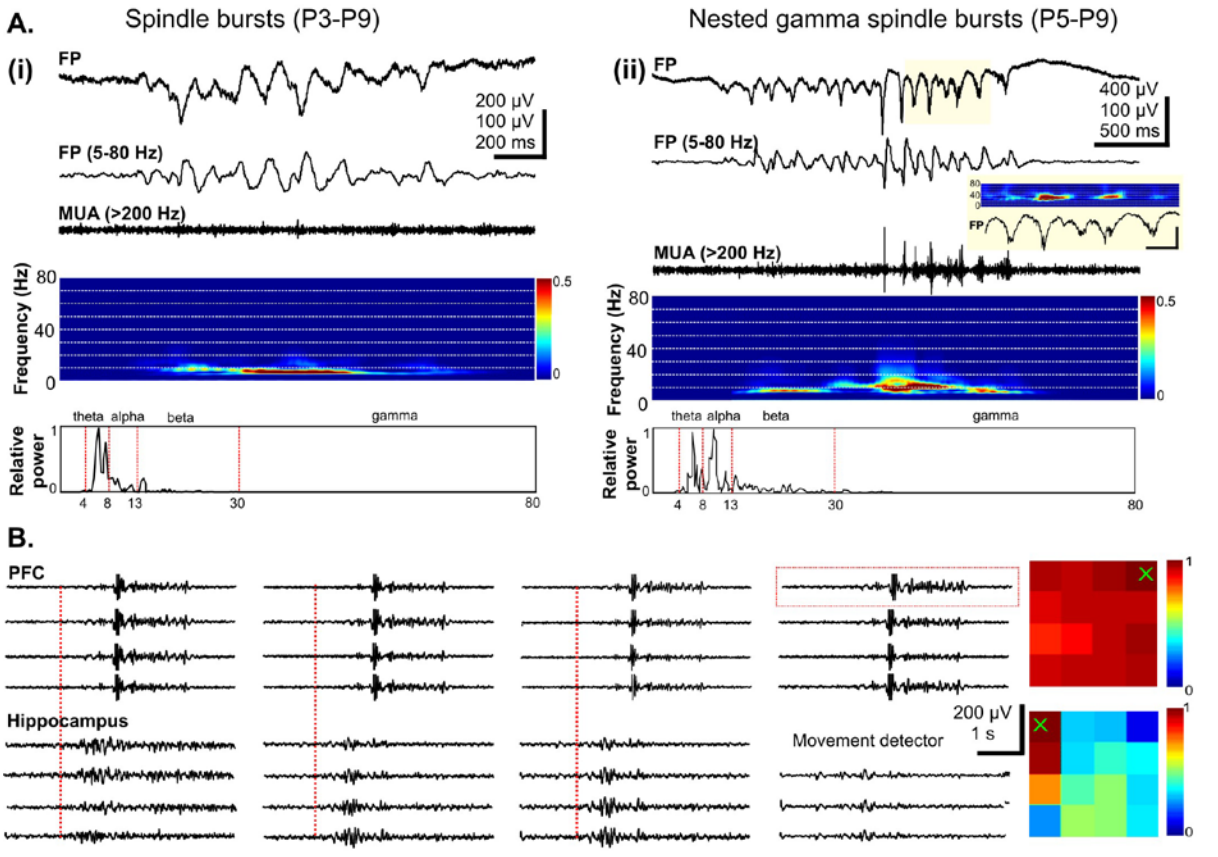

Figure 2. Patterns of oscillatory activity in the developing PFC and their temporal correlation with the hippocampal activity. A. Field potential recordings of SB (i) and NGS (ii) accompanied by the corresponding color-coded wavelet spectrum. Inset, nested gamma episodes at larger time scale. B. FP recordings from PFC and hippocampus of a P7 rat using 4x4-electrodes. Right, color-coded cross-correlation maps for PFC and hippocampus.

(Fig. 2A). Starting with the postnatal day (P) 3 spindle bursts (SB) oscillating principally in alpha (7-12 Hz) frequency band occur predominantly in the Cg. The unique pattern of activity in the immature PFC, the nested gamma spindle burst (NGS) accompanies the SB from P5 on. These oscillations of ~8 Hz in frequency including periodically short gamma episodes synchronize predominantly the PL/IL. As indicated by the current source density analysis as well as by spike sorting and phase correlation algorithms the SB and NGS involve different neuronal networks for their generation. Whereas the SB are most likely the result of networks within Cg, the NGS involve local networks in PL/IL, the neurons of which fire synchronously with the nested gamma episodes.

The adult PFC is strongly controlled by the hippocampal drive when accomplishing executive and mnemonic tasks. The mechanisms governing the maturation of prefrontal-hippocampal interactions and the corresponding development of underlying executive abilities remain largely unknown. In a first attempt to identify the role of hippocampal activity for the PFC oscillations, we monitored the ingrowth of hippocampal projections into PFC and we characterized the early oscillations in the intermediate-ventral hippocampus as main source of PFC innervation. Moreover we correlated the temporal patterns of oscillatory activity in PFC and hippocampus (Fig. 2B). The preliminary results indicate that intermittent theta oscillations in hippocampus shortly precede and probably drive the PFC NGS. A more comprehensive phase-correlation analysis is meant to decide on the role of hippocampal activity for the development of PFC networks.

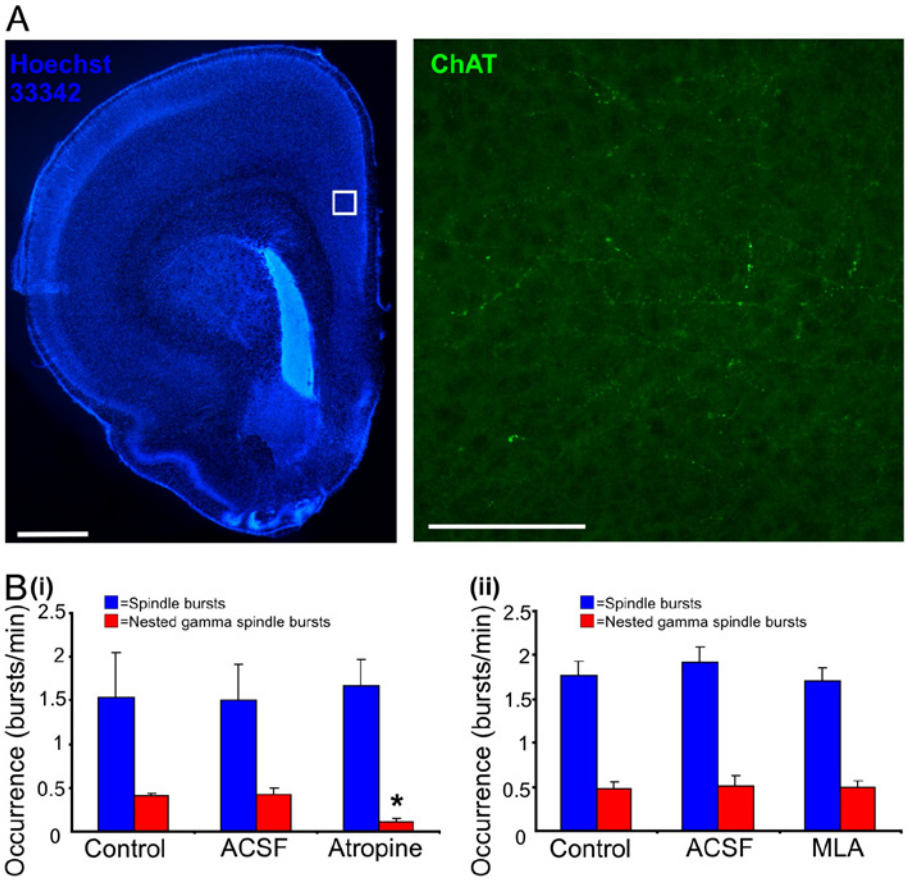

Figure 3. Role of cholinergic innervation for the oscillatory activity of developing rat PFC. A. Nuclear Hoechst 33342 staining (blue) combined with ChAT staining (green) of a 50 µm-thick coronal slice of a P6 rat. B. Effects of mAChR and nAChR blockade respectively, on the occurrence of spindle bursts (blue) and nested gamma spindle bursts (red). The mAChR antagonist atropine (n=5 pups) and the nAChR antagonist mecamylamine (MLA, n=5 pups) were applied locally over the PFC of P7-8 rats.

2. Subcortical control of early oscillatory activity within the PFC-hippocampal network

Cholinergic innervation from basal forebrain modulates the activity of the adult PFC and the frontal-subcortical networks during mnemonic and executive tasks. We currently address the question whether this subcortical drive plays a similar role during development and whether it can modulate the early activity within the PFC-hippocampal network. The ability of cholinergic drive to facilitate already by birth the generation of oscillatory activity in primary sensory cortices has been already demonstrated (Hanganu *et al*., 2007; Hanganu *et al*., 2009). As showed by choline acetyltransferase (ChAT) immunohostochemistry the cholinergic projections accumulate in the PFC towards the end of the first postnatal week (Fig. 3A). To assess their function for the generation of PFC oscillatory activity, we used two experimental approaches. Firstly, we blocked either the mAChR or the nicotinic acetylcholine receptors (nAChR) *in vivo* by intracortical application of the antagonist atropine or mecamylamine according to a previously developed protocol (Hanganu *et al*., 2007). Whereas blockade of mAChR modified the properties of SB and decreased significantly the occurrence of NGS, manipulation of nAChR had no major effects on the early patterns of PFC oscillatory activity (Fig. 3B).

Secondly, chronic and progressive lesion of cholinergic neurons in the basal forebrain using 192 IgG saporin led to reduction of PFC activity. These results indicate that mAChR-mediated cholinergic drive from the basal forebrain shapes the oscillatory patterns of PFC.

3. Abnormal patterns of early oscillatory activity underlying impairment of developing frontal-subcortical networks and of cognitive abilities

Early impairment of the neuronal network including the PFC, the hippocampus and several subcortical nuclei has been hypothesized to cause cognitive and behavioral deficits later in life. The mechanisms underlying this impairment are still unknown. In collaboration with the group of Dr. Carina Mallard at the Perinatal Center of Gotheburg University we currently develop a rodent model of mild hypoxia-ischemia that aims to mimic the cognitive impairment at school age of children experiencing a mild peri/neonatal ischemic episode. The patterns of electrical activity within PFC-hippocampal network of ischemic rodents will be characterized simultaneously with their behavior (ultrasound vocalization, attention/working memory tasks) over the entire developmental period.

Support

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Yang*, J.W., Hanganu-Opatz*, I.L., Sun, J.J., Luhmann, H.J. (2009) Three patterns of oscillatory activity differentially synchronize developing neocortical networks *in vivo*. J. Neurosci. *29,* 9011-9025.

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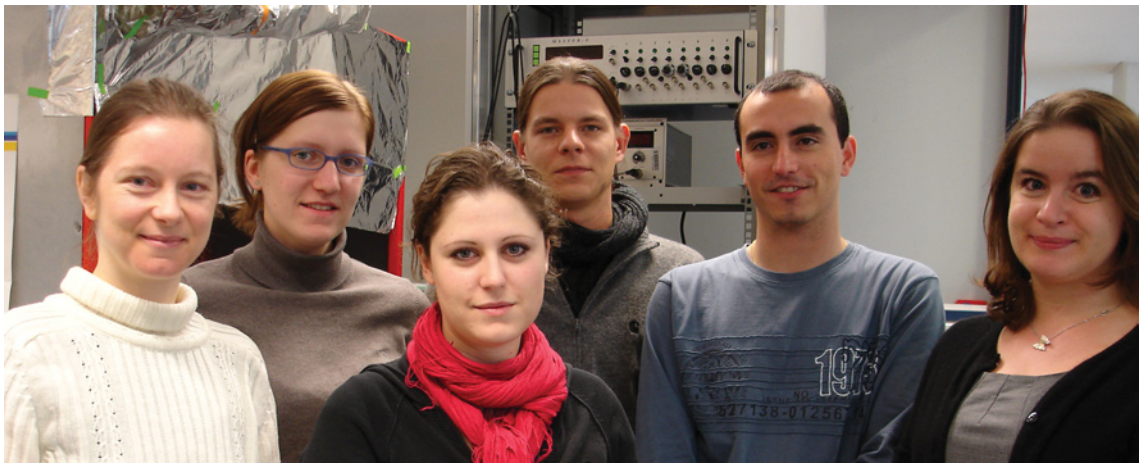

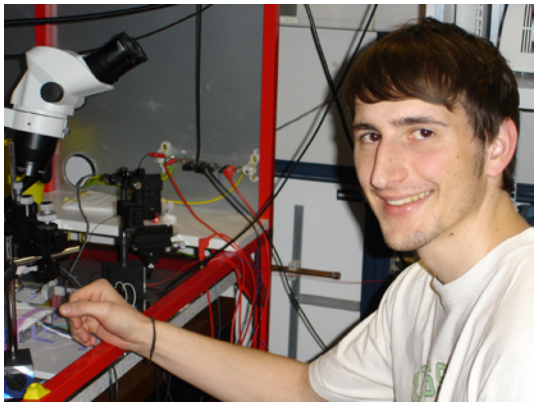

DFG-Heisenberg Team for Experimental Neuropediatrics

Dirk Isbrandt

The major goal of our research is to understand the mechanisms of epileptogenesis and neuronal synchronization in hyperexcitable neuronal networks to develop new strategies for the prevention and cure of neonatal and childhood epilepsies. To this end, we use our previously generated mouse lines in which the activity of Kv7/KCNQ channels (mediating the M current) or HCN channels (mediating the h current (I_h)) is under control of the Tet-Off system to investigate the changes at molecular, cellular, and systemic levels and to develop treatment strategies. Our ENP team is now affiliated with both the Center for Molecular Neurobiology (ZMNH) and the Center for Obstetrics and Pediatrics of the University Medical Center Hamburg-Eppendorf to promote translational research.

Since the award of a DFG-Heisenberg (W3) Professorship to Dirk Isbrandt in January 2009, the structure of the laboratory has changed. Our laboratory, which was previously located in the Institute for Neural Signal Transduction, is now on the third floor of the ZMNH.

Pathophysiology of ion channelopathies

In the last years, we have studied the molecular causes of human diseases and their pathophysiological consequences by identifying and characterizing mutations in ion channel genes and analyzing mutant ion channel subunits to uncover novel pathophysiological mechanisms (e.g., Isbrandt *et al*., 2002; Schulze-Bahr *et al*., 2003; Choe *et al*., 2006). Our team has also increasingly focused on generating conditional transgenic mouse lines to analyze the systemic effects of channel dysfunction in complex excitable organs such as brain and heart *in vivo*. Two examples are given below:

In one of our transgenic mouse lines, the activities of M channels in brain can be selectively suppressed by conditional expression of a dominant-negative subunit in a cell type/region-specific and reversible manner (Peters *et al*., 2005). This strategy allowed us to distinguish secondary changes induced by suppression of ion channel activity during brain development from changes caused by the acute loss of physiological ion channel function in adult animals. As a result of our studies, we were able to identify a postnatal developmental period critical to phenotype severity in M channel-deficient transgenic mice. Our findings not only suggest a new role for these ion channels in postnatal network development, but will also help us understand the maturation of neuronal networks in early childhood. Most importantly, this period defines a critical time window for therapeutic intervention because pharmacological treatment during this time — in contrast to treatment during other periods — prevents morphological and behavioral alterations observed in untreated KCNQ/M-channel-deficient mice (Le *et al*., SFN 2008).

To study the physiological and pathophysiological roles of HCN channels in cardiac pacemaking and to investigate directly the role of cAMP-dependent regulation of HCN channels in sinus node activity, we generated mice with heart-specific and inducible expression of the human HCN4 mutation (573X) that abolishes the cAMP-dependent regulation of HCN channels *in vitro* (Schulze-Bahr *et al*., 2003). Our data demonstrate that cAMP-mediated regulation of I_f determines basal and maximal heart rates. In addition, we identified the pathophysiological mechanism of hHCN4-573X-linked SAN dysfunction in humans (Alig *et al*., 2009).

Subthreshold ion channels in epileptogenesis and neuronal synchronization

In cooperation with our collaborators in the Divisions of Neonatology and Neuropediatrics at the UKE, we aim at translating our findings from basic research into improved clinical management of neonatal and childhood epilepsies. Using the mouse lines generated by our team in which the activity of Kv7/KCNQ channels (mediating

the M current) or HCN channels (mediating I_h) is under control of the Tet-Off system, we investigate the changes at molecular, cellular, and systemic levels to be able to test compounds for their therapeutic efficacy in the postnatal period. As the treatment options in neonatal epilepsy are limited, there is a great need and potential for the development of early therapeutic and preventive strategies in neonatal and childhood epilepsies. Our data have already provided a "proof-of-principle" for the identification of a novel therapeutic strategy by the pharmacological prevention of M channel deficiency-associated morphological and behavioral abnormalities (hyperactivity, stereotypic behavior, epilepsy; Le *et al*., SFN 2008).

Our team combines a broad range of scientific and methodological expertise that includes molecular and cellular biology, *in vitro* electrophysiology, *in vivo* electrophysiology, and behavioral neuroscience, allowing us to implement a multi-level experimental strategy and, in particular, to perform chronic multi-electrode depth recordings in freely moving mutant mice for the characterization of hippocampal and cortical network activities during behavioral tasks or during seizures. These techniques are especially important to our second major research focus, the transgenic suppression of subthreshold-activating ion channels (i.e., I_M, I_h, I_A) in principle neurons and interneurons for the investigation of their importance to learning, plasticity, and behavioral performance. Our aim here is to induce specific changes in the intrinsic membrane properties of these cells, and to analyze how these alterations translate into changes in extracellular current oscillations and unit activity in the cortex and hippocampus.

Pathophysiology of energy metabolism in creatine deficiency disorders

For many years, our group has studied the pathophysiological consequences of disturbed energy metabolism caused by disorders of creatine metabolism, which are a group of recently identified, severely disabling diseases that affect creatine synthesis or transport. The first neurological symptoms in affected patients usually appear in early childhood and include developmental arrest, mental retardation, ataxia, and epilepsy. To be able to study GAMT and AGAT deficiencies, two severe creatine synthesis defects, in a systematic manner, we generated knockout mouse models for both diseases by targeted gene deletion. These mice display the biochemical features that are seen in human creatine deficiencies. Depending on when creatine deficiency is induced or reversed (by removing creatine from or adding it to the diet), our mice display alterations in energy metabolism (changes in body weight and body composition) and behavioral deficits. As our creatine-deficient mice lack the cellular energy buffer provided by the creatine kinase/creatine phosphate system, they are valuable models for studying the consequences of disturbed cellular energy metabolism in excitable and energy-demanding tissues such as heart, skeletal muscle, and brain. These mice are also particularly useful to investigate the susceptibility of neurons toward epilepsy- or ischemia/stroke-induced brain injuries with specific consideration of altered cellular energy homeostasis.

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Protein Trafficking and Synapse Formation

Matthias Kneussel

Neuronal transport is a fundamental process to target organelles, proteins and mRNAs to their sites of action. The polar and excitable nature of neuronal cells requires the selective trafficking of various molecules to either axons or dendrites as well as to subcellular regions that differ in electrical input and synaptic strength. Increased synaptic transport has been shown to promote learning and higher order memory consolidation. In contrast, interference with mRNA transport, a prerequisite for local protein synthesis, is thought to participate in the mental retardation phenotype of fragile-X-syndrome. Severe blockade of protein transport furthermore induces intracellular aggregates, thereby participating to the pathology of neurodegeneration, including Alzheimer`s disease.

Our research group applies molecular biology, live cell video microscopy, mouse genetics and behavioural analysis to address the following biological problems:

- Identification and characterization of motor-cargo complexes that shuttle molecules to and from synaptic contacts.
- Analysis of cellular mechanisms that i.) tag synapses as target sites for protein delivery and ii.) regulate intracellular cytoskeleton-based traffic.
- Study of neurotransmitter receptor turnover between intracellular vesicle compartments and the synaptic surface membrane.

1. Molecular motors: the driving force for intracellular transport

A number of motor-cargo complexes have been identified in neurons to transfer molecules to their functional destinations. Most proteins are synthesized in the cell body, however function at peripheral sites, for instance at the plasma membrane. Active transport employs cytoskeletal elements as rails, with microtubules mediating long-distance and actin filaments mediating short-distance cargo delivery (Fig. 1). The number of mammalian genes encoding motor proteins is limited (<50), however several thousand cargoes

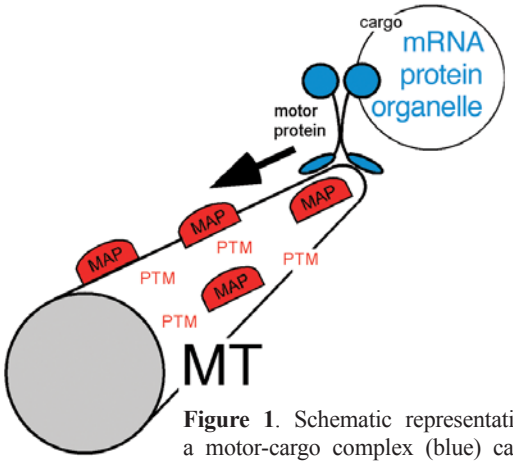


Figure 1. Schematic representation of a motor-cargo complex (blue) carrying mRNA, protein or organelles as cargo. The complex moves along (arrow) a microtubule (MT). Microtubule-associated proteins (MAPs) and tubulin posttranslational modifications (PTMs) (red), regulate transport parameters.

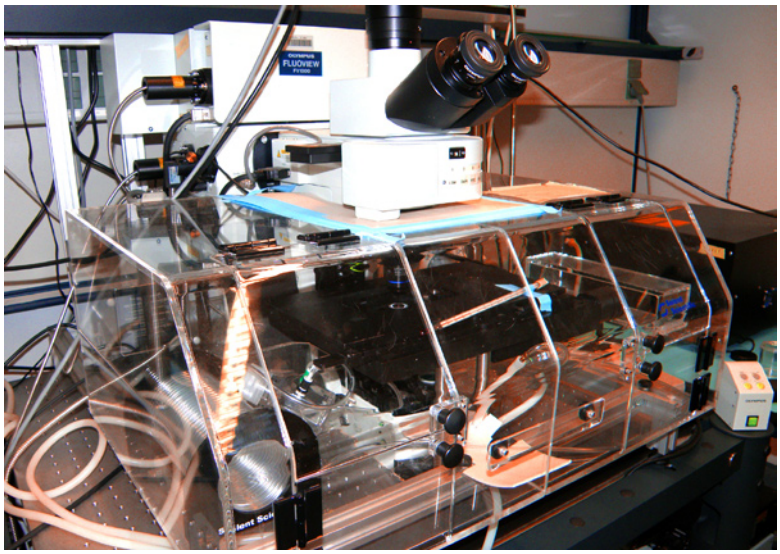
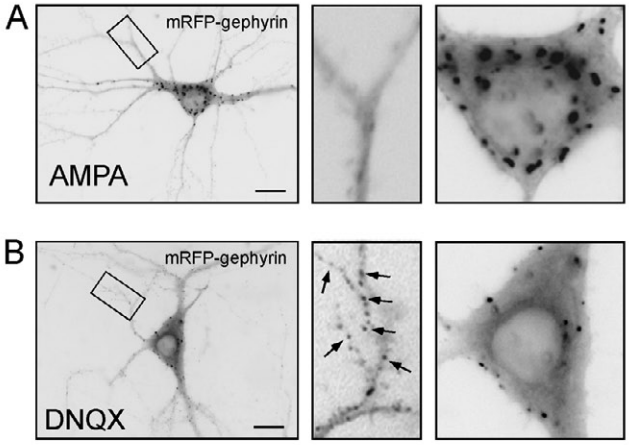
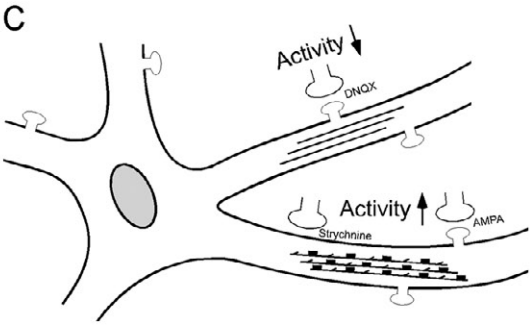


Figure 2. (A) Application of AMPA, which activates AMPA-type glutamate receptors, leads to a transport block of a model fusion protein (mRFP-gephyrin) into peripheral neurites. (B) In contrast, blockade of AMPA-type glutamate receptors reverses this effect. (C) Model. Increased activity increases tubulin polyglutamylation and MAP2 binding to microtubules (black squares) and represents a negative signal for cargo delivery (modified from Maas *et al*, Proc. Natl. Acad. Sci. USA, 2009).





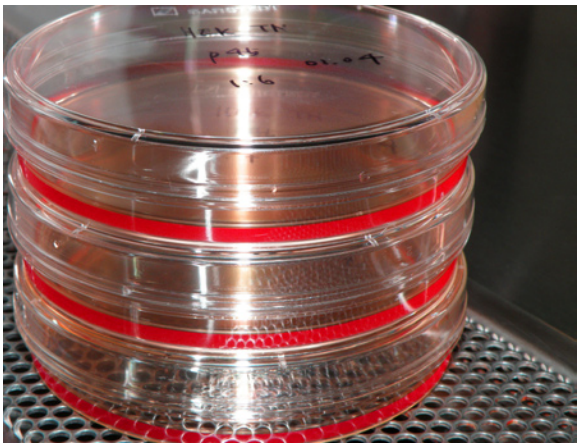
are to be transported throughout a neuron. The transport machinery therefore employs adaptor proteins, which connect individual motors with their actual cargo, thereby mediating specificity of transport. Using ATP as their driving force, kinesin family motors (KIFs) mediate transport of synaptic proteins in microtubule plus-end directions mainly toward the plasma membrane. In contrast microtubule minus-end directed dynein complexes participate in internalization processes and shuttle synaptic cargo to recycling endosomes or organelles of degradation.

We identified and characterized motor-cargo complexes that drive neurotransmitter receptors (AMPA-, $GABA_A$- and glycine receptors), as well as cell adhesion molecules (neuroligins) to and from synaptic sites (Loebrich *et al*. 2006; Maas *et al*., 2006; Maas *et al*., 2009; unpublished data). Interestingly, our and other data revealed that the cargo adaptor proteins, which couple motors to their cargoes, represent the same set of molecules, previously known to anchor plasma membrane receptors at the underlying cytoskeleton scaffold (known as the postsynaptic density).

Understanding the complexity of motor-cargo interactions, will be a prerequisite to selectively manipulate neuronal transport routes in order to design specific treatments against the pathology of cellular traffic jam, known to cause neurodegeneration.

2. Regulation of intracellular transport

Transport regulation can occur at the level of the motor-cargo complex or at the level of the track along which motors move. The differential use of specific cargo adaptors has been shown to regulate whether the same motor moves either into axons or dendrites (Nobutaka Hirokawa laboratory, 2003). Other regulatory components of transport include microtubule-associated proteins (MAPs) that are thought to regulate the accessibility of tracks for motor movement. Recent data have shown that posttranslational modifications (PTMs) of α- and β-tubulin (acetylation, detyrosination, polyglutamylation etc.) determine the nature of the microtubule surface, thereby regulating the binding of MAPs and motors to microtubules (Fig. 1).



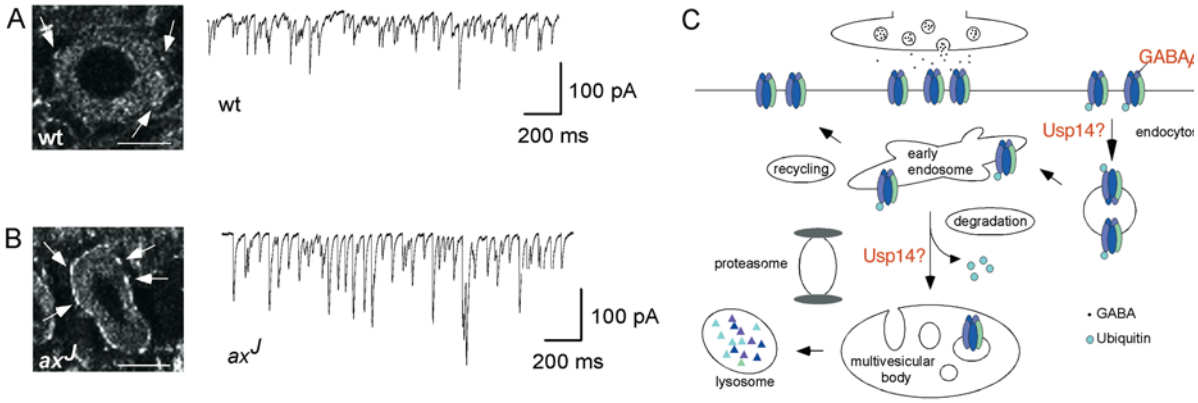


Figure 3. AxJ mice, represented through a genetic knockdown of the ubiquitin-specific protease USP14, display increased levels of surface membrane $GABA_A$ receptors in neurons (B), as compared to wildtype (wt) littermates (A). The immunochemistry data are verified through analysis of mIPSCs from cerebellar brain slices. (C) Model. The currently available data suggest a role of USP14 in cleaving ubiquitin from $GABA_A$ receptors prior to their entry into multivesicular bodies (modified from Lappe-Siefke *et al*, PLOS Genetics, 2009).

Our laboratory has shown that tubulin PTMs undergo regulation in a synaptic activity-dependent manner. According to these results, increased synaptic activity promotes microtubule polyglutamylation with negative consequences for synaptic transport, whereas reduced synaptic activity or functional blockade of the respective polyglutamylase enzyme, reverses these effects. This generates a functional feedback loop, suggesting that transport could be directed to synaptic sites according to local activity changes (Fig. 2).

3. Receptor turnover at synapses

In addition to molecular motors, which mediate the driving force of receptor turnover at synapses, ubiquitinylation represents a molecular signal that labels specific proteins to enter endocytic and/or degradation pathways. Mono-ubiquitinlyation has been identified as a marker for the internalization of surface membrane proteins. The ubiquitin-specific protease USP14 represents a proteolytic enzyme that cleaves ubiquitin labels from target proteins.

Our data have shown that the genetic knockdown of USP14, known to cause an ataxia phenotype in mice, leads to increased levels of α1 subunit-containing $GABA_A$ receptors at synaptic sites (Fig. 3) (Lappe-Siefke *et al*., 2009). Genetic crossings that lead to overexpressed USP14 on the background of the axJ knockdown mouse, rescue this phenotype. This indicates that USP14 acts as an important factor in a synaptic receptor turnover pathway. The currently available data suggest that ubiquitin cleavage might be a prerequisite for $GABA_A$ receptor entry into multivesicular bodies that act upstream of lysosomes (Fig. 3).

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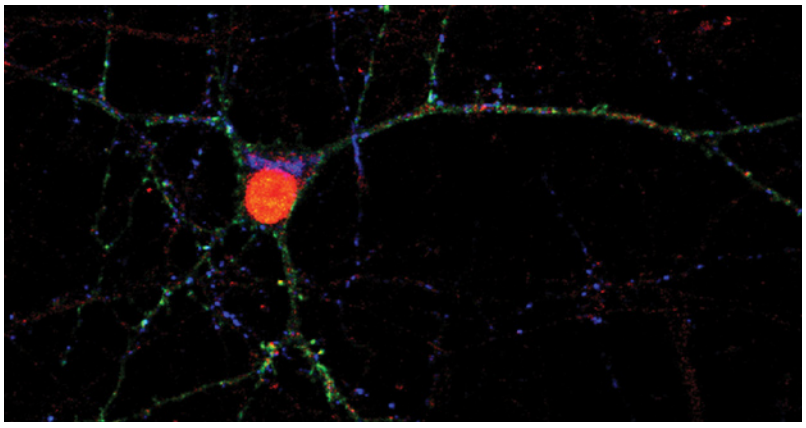

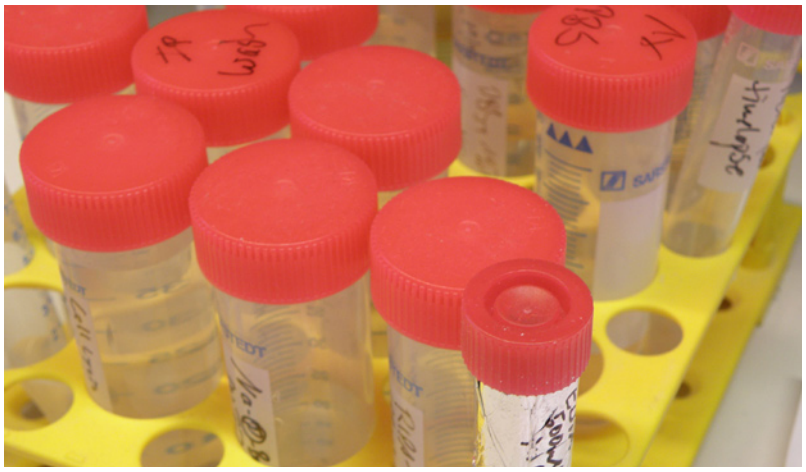

Synaptic Protein Networks

Hans-Christian Kornau

Neuronal communication, fundamental for all aspects of brain function, occurs at specialized cell-to-cell contact sites termed synapses. Here, a chemical message is released from the presynaptic terminal to transmit information by activating receptors in the postsynaptic membrane. Excitatory synapses employ glutamate as neurotransmitter, whereas inhibitory synapses use γ-aminobutyric acid (GABA), and they target different subcellular postsynaptic structures (Fig. 1). Defects in the balance between excitatory and inhibitory neurotransmission contribute to a variety of neurological diseases. The strength of individual synapses is adjusted in response to specific activity patterns, which is thought to be an important element of memory storage.

The function of synapses relies mainly on the particular proteins involved, their numbers and physical interactions. Synapses contain macromolecular protein complexes to accomplish proper signal perception and integration, e.g. the postsynaptic density of excitatory synapses. Therefore, our understanding of the molecular mechanisms at central synapses builds on identifying physical interactions of synaptic proteins. We search for novel constituents of neuronal receptors using both genetic screens and biochemical purifications with a focus on G-protein-mediated pathways modulating synaptic transmission. To

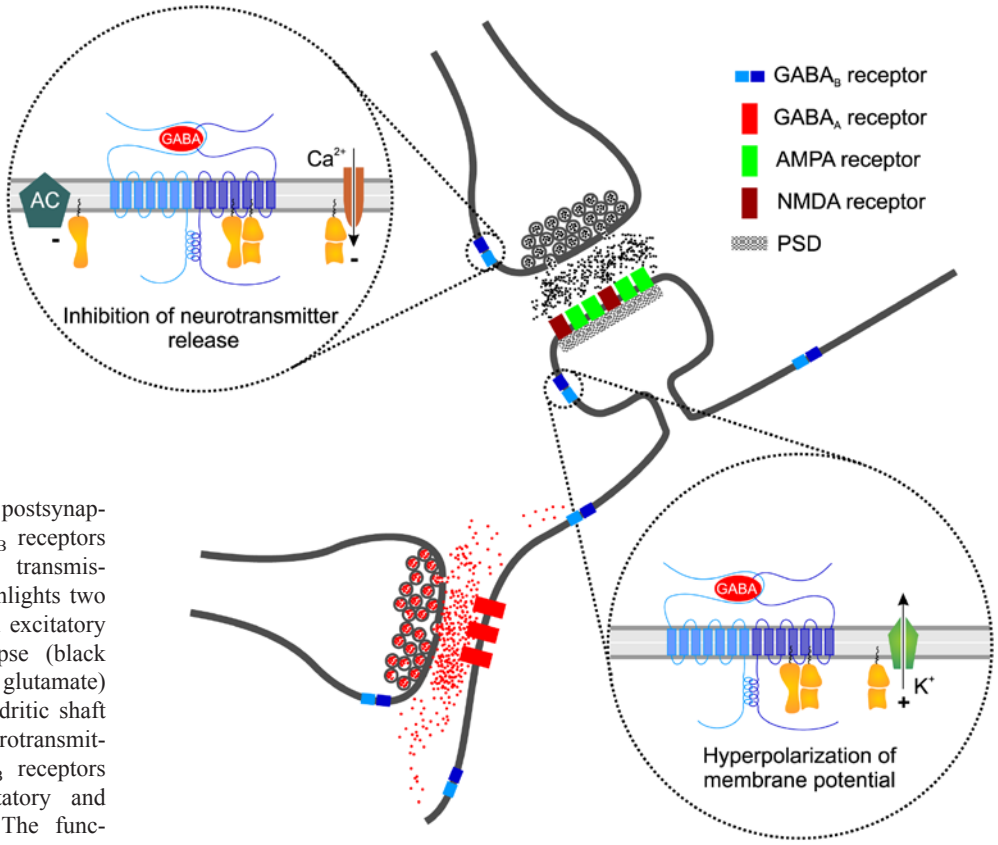

Figure 1. Pre- and postsynaptic actions of $GABA_B$ receptors modulate synaptic transmission. The scheme highlights two forms of synapses, an excitatory dendritic spine synapse (black neurotransmitter, glutamate) and an inhibitory dendritic shaft synapse (red neurotransmitter, GABA). $GABA_B$ receptors modulate both excitatory and inhibitory synapses. The functional consequences of $GABA_B$ receptor activation are indicated in the enlargements. Presynaptic $GABA_B$ receptor signaling affects the release of neurotransmitter, mainly via inhibition of voltage-gated Ca^{2+} channels. Postsynaptically, $GABA_B$ receptor activation induces slow inhibitory postsynaptic currents through G-protein-activated inwardly rectifying K^+ channels. AC, adenylate cyclase. PSD, postsynaptic density.

comprehend the functional complexity of synaptic signal transduction, we apply techniques ranging from *in vitro* measurements of enzyme kinetics and cell-culture-based assays to mouse genetics and electrophysiology.

1. Protein-protein interactions of $GABA_B$ receptor subunits

$GABA_B$ receptors are G-protein-coupled receptors that mediate slow inhibitory effects of the neurotransmitter GABA on synaptic transmission in the mammalian brain. They modulate transmitter release and the postsynaptic membrane potential at different types of synapses (Fig. 1). $GABA_B$ receptors function exclusively as heterodimers of the seven-transmembrane domain proteins $GABA_{B1}$, which contains the ligand-binding site, and $GABA_{B2}$, which mediates coupling to a heterotrimeric G-protein. The two $GABA_B$ receptor subunits associate in the endoplasmic reticulum, in part through a C-terminal coiled-coil interaction, and this assembly is essential for the trafficking of the functional receptor to the plasma membrane. N-terminal isoforms of $GABA_{B1}$ underlie the main $GABA_B$ receptor subtypes that convey separate functions at pre- and postsynaptic sites and are thought to contain a differential set of associated proteins.

We set out to identify molecular constituents of the modulatory $GABA_B$ receptor. At first we used genetic screens, either based on the plasma membrane recruitment of activated ras (ras recruitment system) or on the complementation of β-lactamase activity by two protein fragments, to detect direct binding partners of $GABA_B$ receptors and found a novel coiled-coil interaction of the $GABA_{B1}$ subunit. This interaction appears to precede $GABA_B$ receptor assembly at specific sites of the endoplasmic reticulum. To gain further insight into the protein complex around $GABA_{B1}$, we generated transgenic mice that facilitate biochemical isolation of $GABA_B$ receptors. These mice express, in addition to their endogenous subunits, $GABA_{B1}$ isoforms that contain tags for tandem affinity purification (Fig. 2). The transgenic subunits assemble with $GABA_{B2}$, indicative of their participation in functional $GABA_B$ receptors. Purification of $GABA_{B1}$ complexes from brain membrane preparations of these mice followed by mass spectrometry analysis revealed two novel constituents of the $GABA_B$ receptor, a chaperone and a trafficking factor for $GABA_{B2}$. It appears that the expression of functional $GABA_B$ receptors is tightly regulated and that the receptor's maturation involves a series of protein interactions of both subunits.

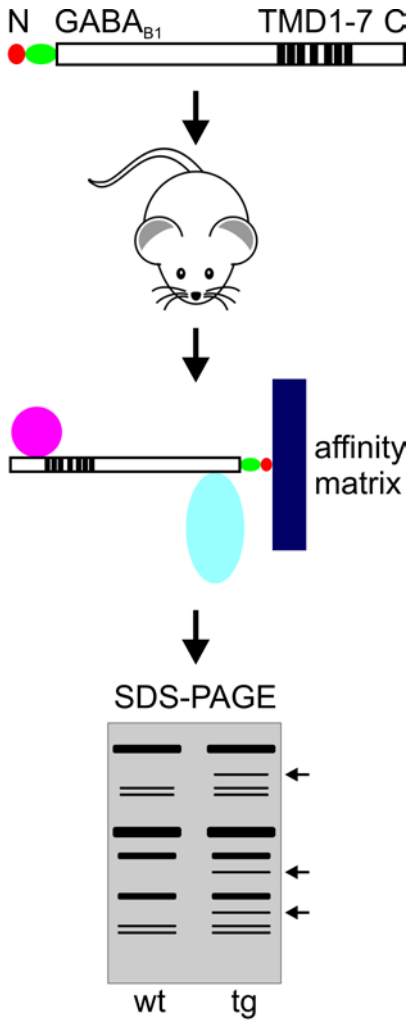

Figure 2. Strategy for the identification of $GABA_B$ receptor constituents. $GABA_{B1}$ isoforms equipped with two affinity purification tags (red and green) are expressed in transgenic mice. $GABA_B$ receptor complexes isolated from brain preparations of transgenic (tg) and wild-type (wt) control mice by tandem affinity purification are separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Protein bands enriched specifically in the transgenic sample (arrows) are excised from the gel and analyzed by mass spectrometry. TMD, transmembrane domain.

2. AMPA receptor endocytosis and long-term synaptic depression

AMPA receptors are glutamate-gated ion channels that mediate the majority of fast excitatory neurotransmission in the brain (Fig. 1). In accord, their number in the postsynaptic membrane and their conductive properties determine the strength of an individual synapse. Cellular models of synaptic plasticity referred to as long-term potentiation (LTP) and long-term depression (LTD) reflect activity-dependent changes of the synaptic strength. It has turned out that regulated synaptic insertion and removal of AMPA receptors underlie these bidirectional changes. AMPA receptors laterally diffuse into and out of the synaptic region and they undergo continuous vesicular cycling between intracellular and plasma membrane locations. However, upon induction of LTD, an additional regulated removal of AMPA receptors from the synaptic plasma membrane reduces the number of AMPA receptors available for transmission. Given that LTD and LTP support learning and memory processes in a variety of neuronal connections, the molecular mechanisms that regulate the induced changes in synaptic AMPA receptor number are of pivotal interest. These involve the activation of other receptors by glutamate, mainly NMDA receptors and metabotropic glutamate receptors, as well as intracellular enzymes, particularly kinases and phosphatases, and culminate in the generation of AMPA receptor-containing transport vesicles. Intriguingly, subunit-specific protein-protein interactions of the divergent intracellular C-terminal regions of AMPA receptors are thought to govern their local exo- and endocytosis. Although several AMPA receptor-interacting proteins have been discovered, our insight into the molecular mechanisms that control these vesicular transport pathways has remained incomplete.

Within the intracellular C-terminal region of the rat AMPA receptor subunit GluA2, two motifs (single letter amino acid sequences KRMKVAKNPQ and YKEGYNVYG) have gained attention due to their importance for regulated endocytosis of the receptor during LTD. In fact, peptides derived from these sequences block LTD in various regions of the rodent brain and have therefore become valuable tools to analyze the importance of LTD in a given brain area for the animal's behaviour. The first motif mediates an interaction with the clathrin adaptor protein complex AP2, an important step in clathrin coat assembly. No direct function has yet been described for the other motif; it is therefore just termed 3Y based on the presence of three tyrosine residues. We found that the 3Y motif can interact with a postsynaptic regulator of the coat-recruitment GTPase Arf6. Our data suggest that GluA2, depending on both the phosphorylation state and ligand-binding, engages this enzyme for targeted internalization of surface AMPA receptors in response to LTD-inducing stimuli. Thus, our findings introduce a current-independent form of AMPA receptor signaling and provide insight into the mechanisms controlling LTD.

Publications

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Kornau, H.-C. (2006). $GABA_B$ receptors and synaptic modulation. Cell Tissue Res. *326*, 517-533.

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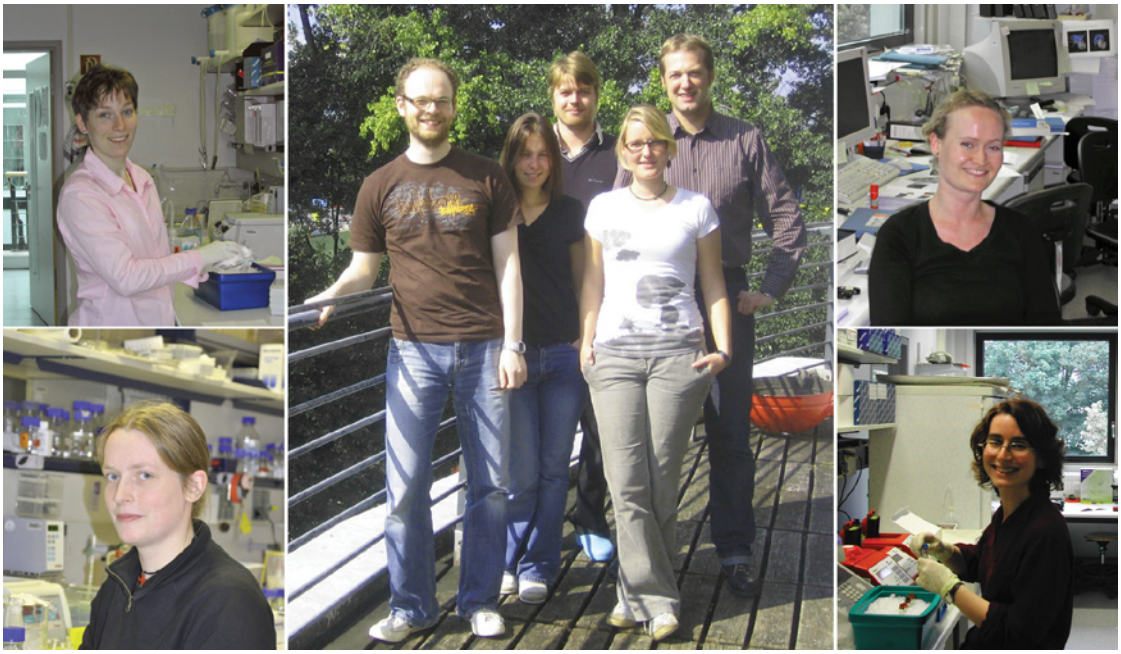


Development and Maintenance of the Nervous System

Edgar Kramer

During the establishment and maintenance of complex cellular networks such as the nervous system, participating cells need to communicate constantly with each other. Cell surface receptors of the involved neurons and glial cells need to receive signals from the surrounding environment that allows a coordinated assembly of these cells into the highly organized nervous system with its close contact to all other parts of the organism. Cell surface receptors are not only required during development but are also indispensable to preserve the integrity of the nervous system and to ensure its proper function. Alterations in cell surface receptor signaling have been implicated in the pathogenesis of neurodegenerative disorders such as Parkinson's disease, motor neuron diseases, and Alzheimer's disease.

Our research group focuses on investigating the function of the receptor tyrosine kinase Ret in the nervous system, its cross talk with other cell surface receptors and how disruption in Ret receptor signaling might lead to pathological alterations. To study this on a molecular and cellular level as well as in the intact animal, we use diverse experimental approaches such as molecular biological techniques, mouse genetics, live and in vivo imaging as well as behavioral and electrophysiological experiments. Currently, we address the function of Ret in motor and dopaminergic neurons.

1. Ret function in dopaminergic neurons

Dopaminergic neurons of the central nervous system are essential for mental and physical health and pathological alterations can cause diseases such as Parkinson's disease (PD) and drug addiction. It is a key challenge to find substances to prevent or reverse the physiological changes leading to PD and drug dependence.

Inexplicably, dopaminergic neurons of the substantia nigra (SN) innervating the striatum die prematurely in PD patients. *In vitro* and pharmacological studies suggest that Ret and its ligands may play an important role in the survival and proper function of dopaminergic neurons. Consistently, our in vivo studies of mice lacking the Ret receptor have shown that Ret is indeed required in aging mice to specifically maintain nigrostriatal dopaminergic neurons and their innervation in the striatum (Kramer *et al*., 2007). Although polymorphisms in the ret gene are not associated with PD (Lücking *et al*., 2008), reduced Ret signaling can be considered as a

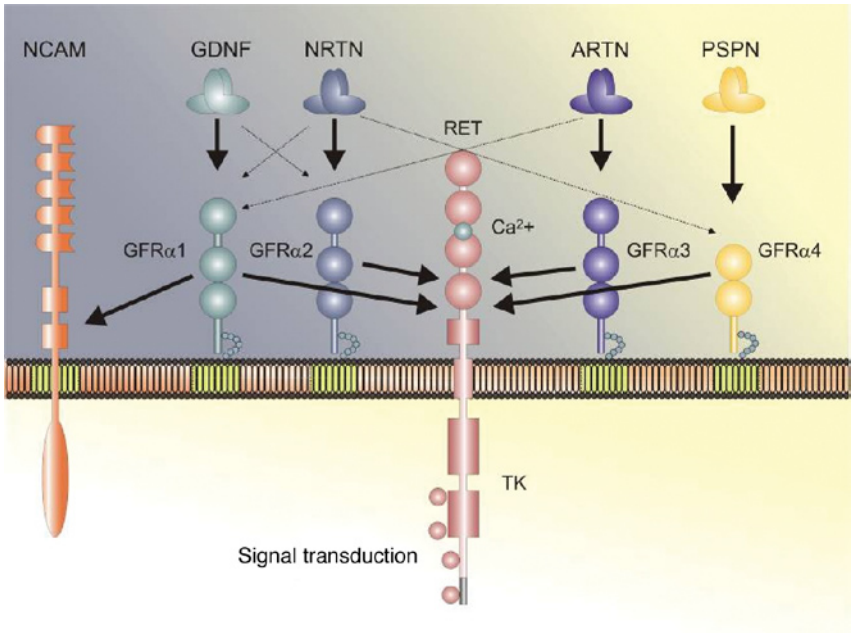
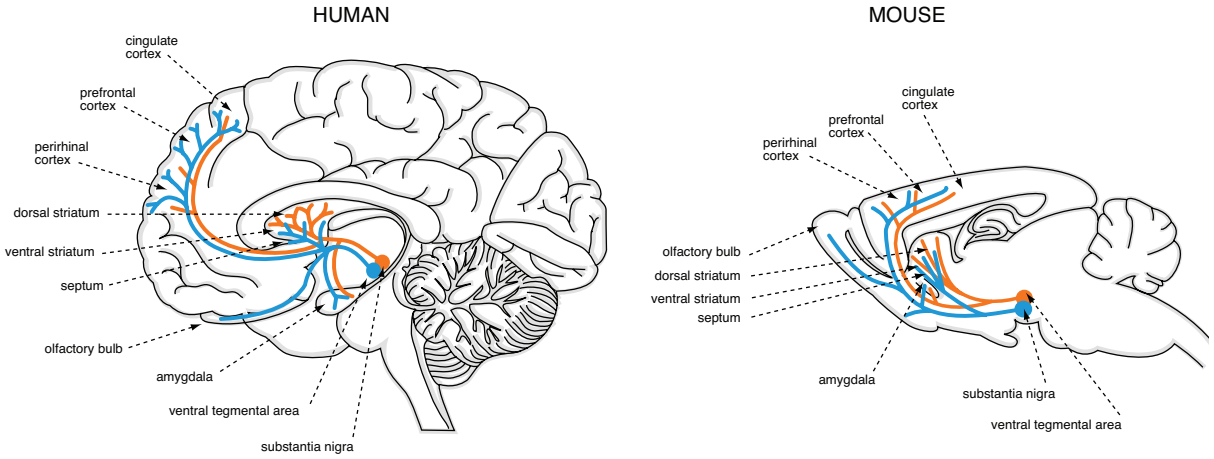


Figure 1. Glial cell line-derived neurotrophic factor family members (GDNF, NRTN, ARTN, PSPN) bind to the GDNF family receptor (GFR) α1 to α4 and can stimulate together the canonical receptor Ret or the alternative Receptor NCAM. Ret and NCAM can activate multiple signaling cascades through their intracellular domains leading to a variety of different responses in the nervous system depending on the cell type, context and region.

Figure 2. Schematic representation of the midbrain dopaminergic system in the human and mouse brain. The cell bodies of the dopaminergic neurons are located in the SN and VTA and they project with their axons into the striatum, the cortex and other brain areas. While dopaminergic neurons of the SN are especially vulnerable in Parkinson's disease patients, dopaminergic neurons of the VTA are altered in drug addicts.



secondary consequence in the etiology of this multifactorial disease. Furthermore, we showed that Ret is required for the regeneration of the dopaminergic innervation of the striatum after toxic insults (Kowsky *et al.*, 2007). Taken together, this data on Ret signaling is of high interest for the ongoing clinical trials using Ret ligands to treat PD patients.

In drug addicts, the physiology of dopaminergic neurons of the VTA - next to the Substantia nigra - is dramatically altered. GDNF treatment attenuates the same biochemical and behavioral processes observed in rodents upon exposure to abusive drugs. We found that Ret deficient mice respond significantly different to those drugs than wild type mice.

Understanding the precise role of Ret indopaminergic neurons of the SN and the VTA is a prerequisite for manipulating the Ret signaling pathway for the benefit of PD patients and drug addicts.

2. Ret function in motor neurons

In motor neurons, Ret and its ligand GDNF can affect axon outgrowth, muscle innervation, survival and the identity of different motor neuron pools. We could show that Ret is also involved in axon guidance of motor neurons innervating the flexor muscles in the hindlimb (Kramer *et al.*, 2006). While Ret is enriched in the dorsal peroneal nerve, its ligand GDNF is expressed in a territory just dorsal to the sciatic plexus suggesting that Ret/GDNF signaling has an important role in the pathfinding of peroneal nerve axons. Indeed, analysis of hindlimb motor innervation in GDNF and Ret knockout mice revealed a dorsal/ventral pathfinding defect. Recently, we could visualize

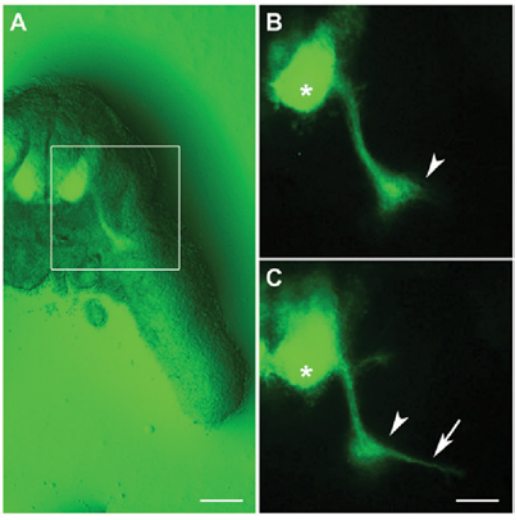


Figure 3. Spinal nerves innervate the hind limb in organotypic slices prepared from E11.5 days post conception HB9:GFP embryos. A: Stereomicroscopic image of the slice after 2 hr in culture. The frame indicates the enlarged region. B,C: Enlarged epifluorescent images of the framed area photographed after 2 hr (B) or 24 hr (C) in culture. Limb innervating motor neurons are located in the lateral motor column (LMC, asterisks) of the spinal cord. LMC axons separate (arrowheads) at the base of the limb into a dorsal trajectory (peroneal nerve, arrow) and a ventral trajectory (tibial nerve, not shown). Scale bars = 200 µm in A and 100 µm in B, C.

the axon guidance defect also in 3D using ultramicroscopy (Becker, *et al*., 2008; Jährling *et al*., 2008). Dorsal-fated axons are redirected into the ventral limb and join the tibial nerve trajectory. This phenotype was also observed after nervous system specific ablation of Ret confirming the significant role of Ret in regulating dorsal/ventral axon guidance in motor neurons. The Ret loss-of-function phenotype is enhanced in the absence of receptor tyrosine kinase EphA4 and vice versa, suggesting that the GDNF/Ret and ephrinA/EphA4 signaling pathways act in parallel. Thus, Ret and EphA4 mediate attractive and repulsive responses, respectively, to reinforce the precision of the same binary choice in motor axon guidance (Kramer *et al*., 2006).

Investigating the signaling events downstream of the Ret receptor and its crosstalk with EphA4 during axon guidance will improve our understanding of this fundamental process and the development of therapies to treat spinal nerve injuries.

Selected Publications

Deininger K, Eder M, Kramer ER, Zieglgänsberger W, Dodt H-U, Dornmair K, Colicelli J, Klein R (2008). The Rab5 guanylate exchange factor Rin1 links internalization of activated EphA4 to synaptic plasticity. Proc. Natl. Acad. Sci. USA 105:12539-12544.

Becker K, Jaehrling N, Kramer ER, Schnorrer F, Dodt H-U (2008). Ultramicroscopy: 3D reconstruction of large microscopical specimens. J. Biophoton. 1: 36-42.

Kowsky S*, Poeppelmeyer C*, Kramer ER*, Falkenburger BH, Klein R, Schulz JB (2007). Ret does not modulate MPTP toxicity but is required for regeneration. Proc. Natl. Acad. Sci. USA 104: 20049-20054.

Kramer ER, Aron L, Ramaker GMJ, Seitz S, Zhuang X, Beyer K, Smidt MP and Klein R (2007). Absence of Ret signaling in mice causes progressive and late degeneration of the nigrostriatal system. PLoS Biol. 5: 616-628.

Kramer ER*, Knott L*, Su F, Dessaud E, Krull C, Helmbacher F, and Klein R (2006). Cooperation between GDNF/Ret and ephrinA/EphA4 Signals for Motor-Axon Pathway Selection in the Limb. Neuron 50: 35-47.

* contributed equally

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Praveen Meka
Technician: Barbara Merz
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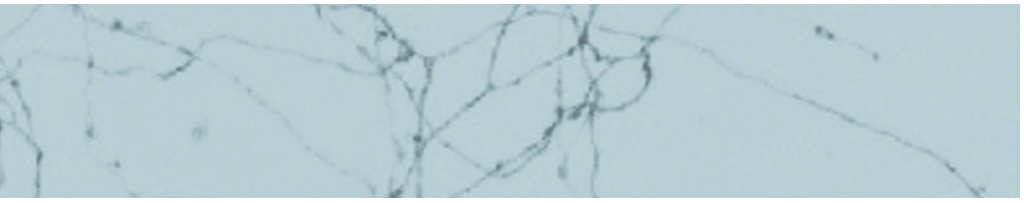
Institute boat outing



Service Groups

Bioanalytics

Sabine Hoffmeister-Ullerich

The DNA-sequencing facility of the ZMNH was established in October 1995. Automated DNA-sequencing started with an ABI Prism 373 DNA sequencer which was replaced by an ABI Prism 377 DNA sequencer in May 1996 to enable faster gel runs with higher throughputs. The latter was then up-graded in June 1999 from 64 to 96 gel lanes per run.

The biochemical concept underlying the above mentioned DNA-sequencers can be deduced from the chain-termination method developed by Sanger and coworkers in the late seventies. This method uses radioisotope labels in order to detect DNA-fragments, whereas the automated sequencers give preference to fluorescence-based detection. Presently an improved set of fluorescence dyes (big dye) is used which greatly reduces the notorious weak G after A pattern characteristics of its predecessor. The ABI Prism 377 sequencer enables a reading-length of about 450 bases after a gel run time of only 4 hours, whereas the number of bases which can be read after 10 hours amounts to about 750 bases.

From January 2007 until September 2009 approximately 40,000 sequence analyses were performed. Starting from December 2009 the ABI Prism 377 DNA sequencer will be replaced by an ABI 3100 AVANT four capillary instrument.

Publication:

Hoffmeister-Ullerich, S. (2007). Hydra - ancient model with modern outfit. Cell. Mol. Life Sci. *64*, 3012-3016.

Structure of the Group

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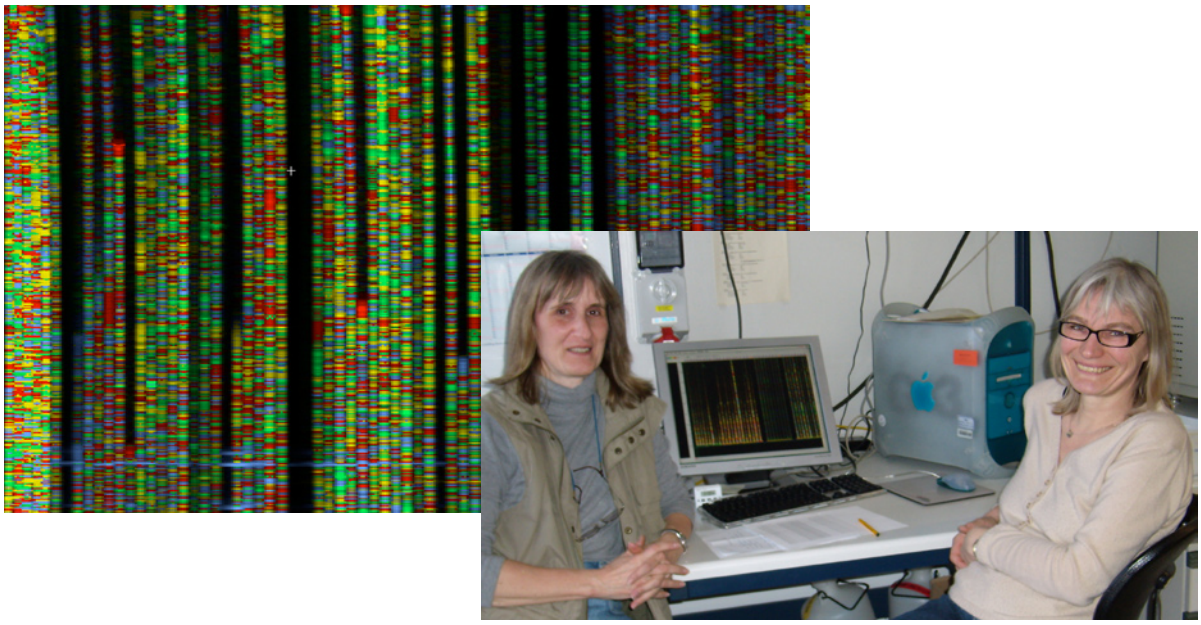
*during part of the reported period



Morphology

Michaela Schweizer

As a part of the general services of the ZMNH the Morphology Unit performs investigations of neurobiological questions and supports scientists in many areas of microscopy. The histological characterisation of genetically engineered animals has become a major approach in our facility. We investigate the histopathology of selected organs or tissues of interest to study the effect of genetic modifications. We give advice on morphological questions, teach and train researchers in the application of microscopical techniques. We introduce and establish new techniques and guarantee efficient use of the respective equipment.

1. Offered services

- Performance of light- and electron microscopical investigations
- Advice and practical instruction in the application of histochemical techniques
- Instruction of researchers in operation of microscopes and accessories
- Introduction of useful new (immuno-) histochemical techniques and/or equipment

2. Techniques

- Morphological studies of many kinds of tissues with light-, confocal laser scanning-, or transmission electron microscopy
- Patho-histological analysis of the whole body of transgenic mice
- Histo- (cyto) chemical staining procedures
- Immunohisto- (cyto) chemistry
- In situ hybridisation
- Pre- and postembeeding immunogold labelling techniques

We prepare cell and tissue samples for scientific histological and (immuno-)histochemical light and fluorescence microscopy. All preparation steps, (including fixation, sectioning with vibratome, cryotome or microtome, staining, mounting etc.) are performed by the group. The Morphology Unit has at its disposal both conventional and fluorescence microscopes (Zeiss Axiophot), as well as two confocal scanning laser microscopes in inverted (Leica SP2) and in upright configuration (Olympus Fluoview 1000).

We localise m-RNA expression routinely by hybridisation of radioactively labelled cRNA-probes to cryo-sections of fresh-frozen tissues or to cultured cells. The hybridisation signals are shown autoradiographically using high resolution X-ray films or application of photographic emulsion followed by light microscopy.

We process cells and tissues for conventional transmission electron microscopy (Zeiss 902) and offer immunolocalisation of gene products applying pre- and postembedding protocols. We take care to preserve both, antigenicity and structural integrity. All results are documented in high resolution digital images.

Publications

Lappe-Siefke, C., Loebrich,.S., Hevers, W., Waidmann, O.B., Schweizer, M., Fehr, S., Fritschy, J.M., Dikic, I., Eilers, J., Wilson, S.M., and Kneussel, M. (2009). The ataxia (axJ) mutation causes abnormal GABAA receptor turnover in mice. PLoS Genet. *5*(*9*), e1000631.

Wartosch, L., Fuhrmann, J.C., Schweizer, M., Stauber, T., Jentsch, T.J. (2009). Lysosomal degradation of endocytosed proteins depends on the chloride transport protein ClC-7. FASEB J. *23,* 4056-4068.

Krebs, C., Hammig, I., Sadaghiani, S., Steinmetz, O.M., Meyer-Schwesinger, C., Fehr, S., Stahl, R.A,, Garrelds, I.M., Danser, A.H., van Goor, H., Contrepas, A., Nguyen, G., and Wenzel, U. (2007). Antihypertensive therapy upregulates renin and (pro)renin receptor in the clipped kidney of Goldblatt hypertensive rats. Kidney Int. *72*, 725-730.

Tagnaouti, N., Loebrich, S., Heisler, F., Pechmann, Y., Fehr, S., De Arcangelis, A., Georges-Labouesse, E., Adams, J.C., and Kneussel, M. (2007). Neuronal expression of muskelin in the rodent central nervous system. BMC Neurosci. *8*, 28.

Blaesse, P., Guillemin, J., Schindler, J., Schweizer, M., Delpire, E., Khirug, L., Friauf, E., and Nothwang, H., (2006). Oligomerization of KCC2 correlates with development of inhibitory neurotransmission. J. Neurosci. *26*, 10407-10419.

Structure of the Group

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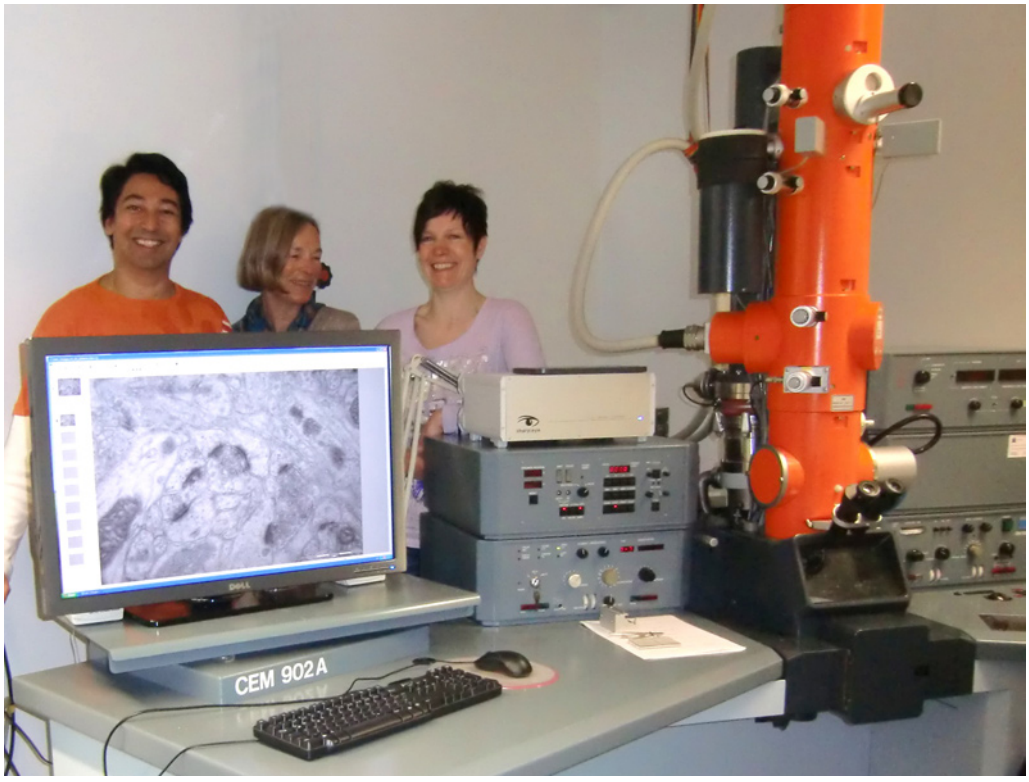
*during part of the reported period



Systems Biology and Protein-Protein Interaction

Christian Schulze

The service group was recently renamed to describe more precisely its main working area and to indicate that the mass spectrometry service is discontinued. A technique for analysing protein-protein interaction is kept. The surface plasmon resonance (SPR) biosensor (Biacore 3000) allows functional characterization of molecular interactions. In favourable cases, numerical evaluation of the binding data can be used to derive reaction rates and binding constants. The application area of a surface plasmon resonance (SPR) biosensor is not limited to the investigation of protein-protein interaction but mainly used for this purpose (Mehanna *et al*., 2009). In certain cases it is possible to recover the minute amounts of material that bound to the immobilized ligand from the sensor chip and continue with proteomics based analyte identification (Makhina *et al*., 2009).

The other steadily evolving branch of the service group is systems biology which attempts to achieve a holistic picture of biological systems to reach a deeper understanding of cellular processes beyond binary molecular interactions. Here, gene regulation by transcription factors is a major topic. Transcription factors recognize short nucleotide motifs in the upstream region of a gene and by that participate in activation or repression of genes. Using motif information binding sites can be predicted and functional analysis of putative target genes allows to construct subsets of regulated genes which are then compared to gene sets describing already known signaling pathways and regulatory networks. Microarray experiments describing manipulation of selected transcription factors are a reasonable starting point for this type of analysis (Wong *et al*., 2007). A complementary concept would start from publicly available or newly defined motif descriptions trying to define a set of putative target genes. This *in silico* approach can easily take into account the contribution of single nucleotide polymorphisms to aberrant direct or indirect gene regulation finally correlating regulatory systems dynamic and phenotypic occurrence.

Publications

Makhina, T., Loers, G., Schulze, C., Ueberle, B., Schachner, M. and Kleene, R. (2009). Extracellular GAPDH binds to L1 and enhances neurite outgrowth. Mol. Cell. Neurosci. 41, 206-218.

Mehanna, A., Mishra, B., Kurschat, N., Schulze, C., Bian, S., Loers, G., Irintchev, A. and Schachner, M. (2009). Polysialic acid glycomimetics promote myelination and functional recovery after peripheral nerve injury in mice. Brain 132, 1449-1462.

Wong, Y.W., Schulze, C., Streichert, T., Gronostajski, R.M., Schachner, M. and Tilling, T. (2007). Gene expression analysis of nuclear factor I-A deficient mice indicates delayed brain maturation. Genome Biol. 8, R72.

Structure of the Group

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Transgenic Mouse Facility

Irm Hermans-Borgmeyer

The transgenic mouse facility supports scientists of the ZMNH and the UKE in all aspects of transgenic mouse production.

The injection laboratory is equipped with two injection set ups, one for pronucleus and one for ES cell injection. In 2009 state of the art micromanipulators replaced the old ones.

Forster mothers recovering from surgery

The cell culture laboratory serves to provide ES cells and mouse embryonic fibroblasts. Although ES cell culture is not a service of the facility scientist may perform their gene targeting experiments there under supervision. Since 2008 a second sterile flow hood makes it possible to grow cells from outside sources (e.g. International Gene Trap Consortium) in parallel to those of the facility.

The molecular biology laboratory is used for genotyping and DNA preparation. Since 2008 additional lab space is available used for PCR reactions and preparation of mouse tissues.

In 2006 a former storage room of the central mouse facility at the ZMNH was reconstructed and equipped with two IVC racks, a changing station and a sterilisator, housing vasectomized males and forster mothers. All mice are now generated on SPF level and can be transferred to any mouse containment of the ZMNH and the UKE.

We offer as service:

- Help with the design of experiments
- The generation of transgenic mice by pronucleus injection of DNA constructs into one cell stage mouse embryos
- The injection of recombinant mouse ES cells into mouse blastocysts
- ES cells and mouse embryonic fibroblasts for ES cell culture
- Supervision of gene targeting experiments carried out in the cell culture laboratory of the facility
- Mouse lines of general interest (Cre-, Flip-Deleter and reporter mice)
- Help with the preparation of DNA for pronucleus injection and electroporation into ES cells
- Help with the analysis of recombinant ES cell clones as well as of the generated mouse lines
- Embryo transfer of mouse lines carrying more than one transgene

In addition the facility offers training courses to animal care takers and participates in the organization of the FELASA B-course established at the UKE since 2009.

Publications

Nielsen, M.S., Keat, S.J., Hamati, J.W., Madsen, P., Gutzmann, J.J., Engelsberg, A., Pedersen, K.M., Gustafsen, C., Nykjaer, A., Gliemann, J., Hermans-Borgmeyer, I., Kuhl, D., Petersen, C.M., and Hermey, G. (2008). Different motifs regulate trafficking of SorCS1 isoforms. Traffic 9, 980-994.

Hentschke, M., Wiemann, M., Hentschke, S., Kurth, I., Hermans-Borgmeyer, I., Seidenbecher, T., Jentsch, T.J., Gal, A., and Hübner, C.A. (2006). Mice with a targeted disruption of the Cl-/HCO3- exchanger AE3 display a reduced seizure threshold. Mol. Cell. Biol. 26, 182-191.

Urny, J., Hermans-Borgmeyer, I., and Schaller, H.C. (2006). Cell-surface expression of a new splice variant of the mouse signal peptide peptidase. Biochim. Biophys. Acta.. 1759, 159-165.

Structure of the Group

Group leader: Irm Hermans-Borgmeyer
Technicans:
Sarah Homann (since August 2007)
Tina Koppelmann (until March 2007)
Ivonne Deutschmann (July 2007 - February 2009)
SHK:
Sascha Pridat
Nils Zimmermann

Sarah Homann plug checking in the SPF room U33 of the facility

IT Service and Development

Hans-Martin Ziethen

The IT service group is responsible for the network, intranet, internet, E-mail and the support of about 500 clients of the center. Additionally, the operation of the central servers, data storage and protection are among our responsibilities. One part of our expertise is the integration and adaptation of the Open Source technology and the development of customized applications.

IT infrastructure and service

During the reporting period we have implemented a storage area network (SAN) and several RAID systems to extend the storage capacities of our servers to 30 TB and the backup capacity to 80 TB. The network bandwidth has been upgraded to Gigabit Ethernet and the mail server has been exchanged for a high availability E-mail system. Due to the continuous implementation of additional network services like LDAP, VPN, SFTP, content management, forum etc. the number of central servers has been enlarged by a factor of two to about 40 systems. By introducing an IT helpdesk we could improve our service. At the moment we are processing about 100 documented support requests per month.

Software development

Not only the infrastructure but also the software development tasks have increased. This includes the development of tools and programs for the analysis and conversion of neurobiological data, the optimization and redesign of cluster algorithms for spike sorting, the creation of management software and the design and realization of the INIMS biobanking project.

We offer as service:

- Engineering of special server and hardware configurations
- Conceptual design, planning and implementation of network and server infrastructures
- Development of software in the field of scientific computing, numerical mathematics, image processing and web applications
- Programming in Java, C/C++, PHP, Perl, MATLAB and Igor Pro
- Procurement of hard- and software equipment
- Web design and development

Structure of the Group

Group leader: Hans-Martin Ziethen
IT specialists:
Siegfried Koloschin
Stefan Rattai
Apprentices:
Laura Glau
(mathematical technical software developer, since September 2008)
Patrick Glomb (specialist systems, since August 2008)
Jan Christoph Meier (specialist application development, until January 2008)
Florian Osses (specialist systems, until July 2008)

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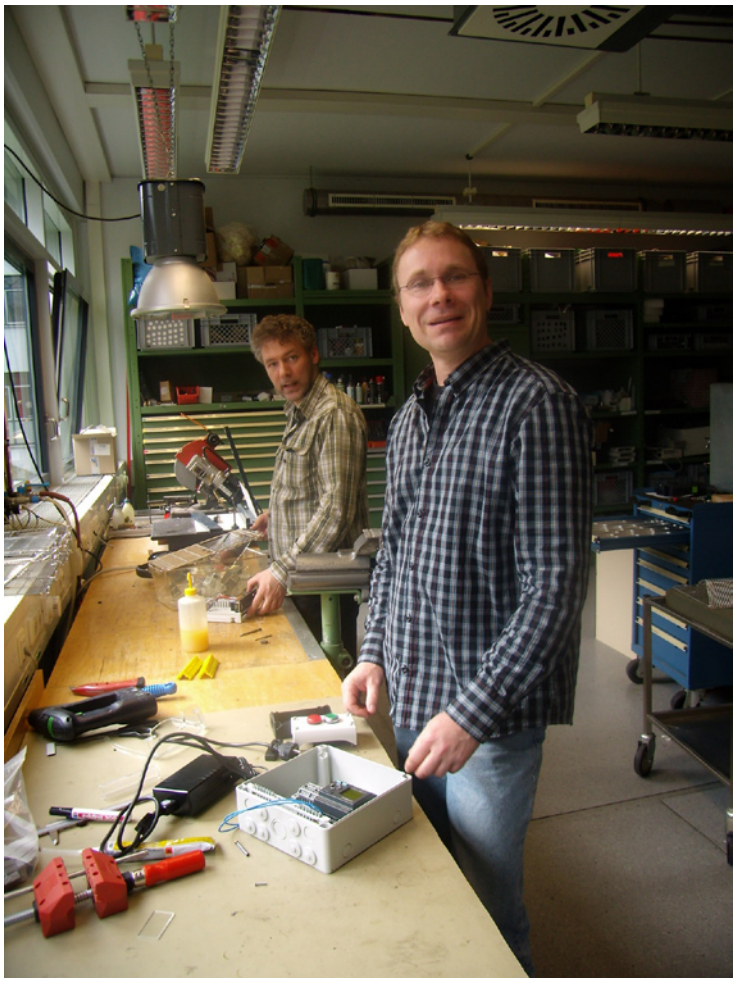
Scientific Workshop and Bioinstrumentation Group

Torsten Renz and Fritz Kutschera

The scientific workshop has been of central importance for supporting and accelerating research at the Center.

Innovative scientific research often requires the use of specific equipment and instruments that are either not commercially available or not suited for the specific research purpose. To be able to develop and manufacture these instruments/devices and to meet the high quality and versatility demands, the scientific workshop is equipped with state-of-the-art mechanic precision devices. Our expertise acquired over many years allows the processing and use of almost any raw material, such as plastics, acryl, epoxy resin, wood, and metals.



If no devices or instruments are available for a specific experiment, we often design, construct, manufacture and test new devices that are tailored to the needs specified by the scientists. Among these instruments are, for example, amplifiers, data loggers, pressure regulators, specific filling level indicators, gas sensor control devices, custom-made chambers for microscopic examination, step-down units for electrophysiological setups, or impulse generators, to name only a few.

The daily work of scientists in the fields of molecular and cell biology is often made easier by our expertise and dedication to details that do make a difference. Examples here include the manufacturing or adaptation of specimen holders, electrical and mechanical adapters, optical devices, PCR adapters, electrophoresis gel combs, etc. The workshop helps scientists to assemble and test scientific equipment, such as electrophysiological instruments which usually consist of several devices, or to eliminate interference and noise. Most recent examples are *in vivo* und *in vitro* electrophysiology setups used by the research teams of Hanganu-Opatz, Kuhl, and Isbrandt.

A wide range of behavioral experiments conducted in the ZMNH, mainly with mice, is made possible by the construction of test arenas and test devices (such as open field arenas, test chambers, walls, water maze components) that are uniquely tailored to the experimental requirements.

It is the ideal combination of scientific creativity and technical know-how that helps to develop and

implement innovative experimental approaches. The close proximity of the laboratories to the workshop ensures effective communication and collaboration with the scientists. This smooth exchange of information and ideas is indispensable to the daily work of researchers and has resulted in numerous scientific publications over the last years.

The most important fields of work include:

- Development and construction of scientific instruments
- Configuration or adaptation of instruments and devices for specific scientific and experimental purposes
- Assembly of components for experimental setups, troubleshooting of mechanical or electronic systems
- Providing advice to scientists of the ZMNH and planning in collaboration with them modifications of the structure of the building
- Overseeing an extensive range of equipment/ systems and the building's infrastructure (except of the IT network)
- Coordination of the maintenance or repair of equipment/systems with the subsidiaries of the University Medical Center Eppendorf (KME/ KFE) and coordination or conduct of important safety checks

Structure of the Group

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Glass Washing Facility

Mr. Thorairajoo, Ms. Heins, Mr. Lubrich

Teaching

The ASMB (Aufbaustudium Molekularbiologie) was founded in 1984 by Professor Dr. G. Koch at the UKE and was then permantly established at the ZMNH to promote in a multi-disciplinary approach the ability for scientific thinking and working. Thus, it may represent the first graduate school founded at a German University. The program presents molecular biology within a broader context of the basic sciences and biomedicine. Fundamental and clinical aspects are explored and are integrated with relevant areas of other disciplines.

The course is of particular value to those who plan a career in academic biomedical research or industry. It harnesses the high quality expertise existing within the biomedical sciences and elsewhere within the University of Hamburg.

The program is taught at the ZMNH in collaboration with the Faculty of Medicine and is

- interdisciplinary
- focused on research
- oriented towards practical aspects

Students are expected to have a graduation in a physiological, biological or other science, or a medical qualification.

Academic content

The ASMB is a 4-semester`s course and is taught entirely in English. In parallel to lectures, seminars and practical courses students have to perform a project study which can be done as part of a PhD/MD thesis or independent of that.
At the beginning of the first semester the students present in a short talk their research project. Lectures and seminars teach background and techniques in molecular biology dealing primarily with nucleic acids. The second semester focuses on cell biology and proteomics. At the end of the second and beginning of the third semester students are requested to give a progress report about their project study. The third semester covering neurobiology and immunology is taught in collaboration with the Bernhard-Nocht-Institute. Topics are presented primarily as “Fresh from the bench” lectures which give students the opportunity to discuss ongoing research projects with the lecturer and to develop ideas how to address scientific problems. In the fourth semester mechanisms of inherited diseases are discussed. At the end of each semester two practical courses held in groups of not more than 4 students have to be successfully passed. At the end of the fourth semester students present the results of their project study and write a report in the style of a “letter to Nature”. Successful students are awarded with a certificate.

Head of Commission:

Prof. Dr. M. Schachner Camertin
(until 2008)

Prof. Dr. Dietmar Kuhl
(since 2008)

Lecturers:

Institute directors of the ZMNH
and their co-workers

Heads of the research groups

Heads of the service groups

Lecturers from other institutes:

PD Dr. Minka Breloer,
Bernhard-Nocht-Institute

PD Dr. Fritz Buck
Institute for Clinical Chemistry,
UKE

Dr. Adam Grundhoff
Heinrich-Pette-Institute

Prof. Dr. Thomas Eschenhagen,
Institute for Clinical Pharamcology,
UKE

Prof. Dr. Bernhard Fleischer,
Bernhard-Nocht-Institute

Lecturers from other institutes (cont.):

PD. Dr. Thomas Jacobs,
Bernhard-Nocht-Institute

PD Dr. Hans-Jürgen Kreienkamp,
Institute for Human Genetics, UKE

Dr. Benjamin Otto,
Institute for Clinical Chemistry, UKE

Dr. Thomas Streichert,
Institute for Clinical Chemistry, UKE

Dr. Eva Tolosa,
Institute for Immunology, UKE

Professor Dr. Dietmar Kuhl in discussion with students of the first term during a seminar break.

Administration

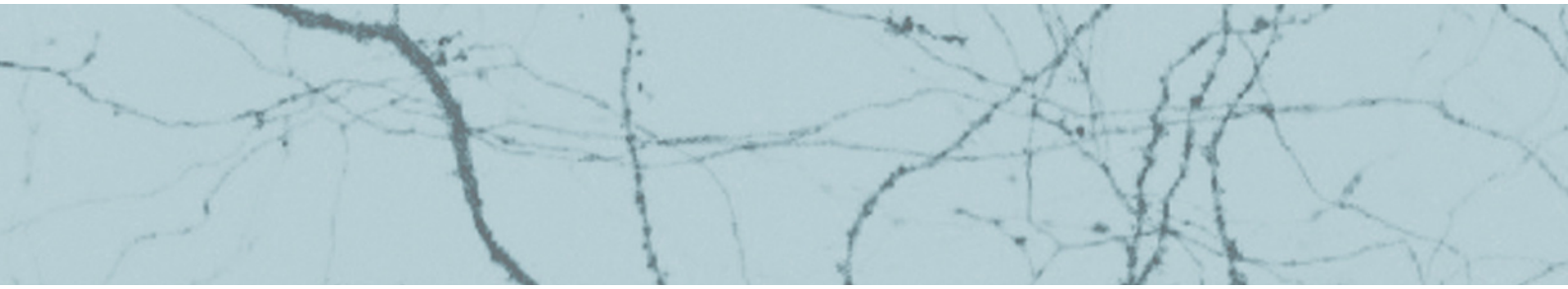
Managing director: Katja Husen

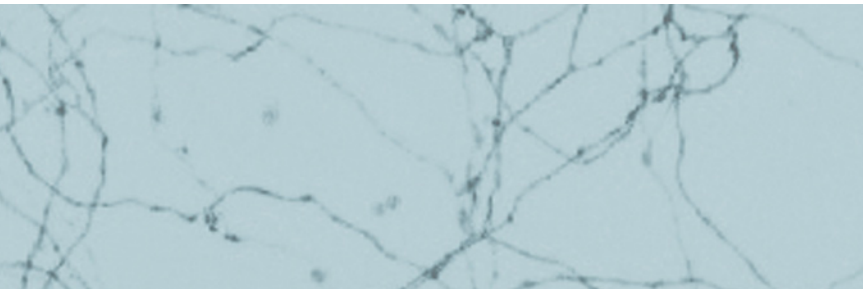
Personnel manager: Rolf Maronde

Financial manager and coworkers:
Uwe Csizmadia--Barth
Herma Dörnbrack
Heike Pehlke

ZMNH Secretary: Eva-Maria Suciu

Ms. Suciu, Mr. Maronde, Ms. Pehlke, Mr. Csizmadia, Ms. Husen. (Missing: Ms. Dörnbrack)





Publications
Theses
Awards

Publications by ZMNH Scientists 2006 - 2009

Dietmar Kuhl: Institute for Molecular and Cellular Cognition
(established in October 2008)

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Medical Dissertations

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Dissertations (PhD thesis)

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Diplomas

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Dietmar Richter: Guest Group

Dissertations (PhD theses)

Kerstin Berhörster (2006). Die Funktion des Insulinrezeptorsubstratproteins von 53 kDa für Dendriten und Synapsen in der Maus. University of Hamburg.

John Jia En Chua (2006). Translational regulation of mRNAs encoding SAPAP3 in Rattus norvegicus. University of Hamburg.

Katrin Falley (2007). Lokale Proteinbiosynthese von Shank1 in neuronalen Dendriten der Ratte (Rattus norvegicus, Berkenhout 1769). University of Hamburg.

Janin Schütt (2009). Das Fragile X Syndrom: Molekulare Veränderungen der postsynaptischen Dichte in der Maus (Mus musculus L.). University of Hamburg.

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Inga Schapitz (2006). Zelluläre Funktion von Ataxin-10. University of Hamburg.

DFG Heisenberg Research Group: "Experimental Neuropediatrics"

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Alig , Jaqueline (2009) Rolle der cAMP-Sensitivität der HCN-Kanäle für die Herzfrequenzregulation in der adulten Maus (Mus musculus, Linnaeus 1758). University of Hamburg.

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Bachelor

Eichler, Ronny (2009). Pharmacological treatment of Kv7/M-current-deficient mice. Lausitz University of Applied Sciences.

Research Group "Protein Trafficking and Synapse Formation"

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Research Group "Synaptic Protein Networks"

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Diploma

Rathgeber, Louisa (2008). Determination and manipulation of the Arf6 activity in cultured hippocampal neurons of *Rattus norvegicus*. University of Konstanz.

Awards and Honors 2006-2009

Institute for Neural Signal Transduction

Prof. Dr. Olaf Pongs

Gay Lussac-Humboldt-Forschungspreis 2007

Dr. med. Chi-un Choe
(AG Isbrandt)

Hans-Dietrich Herrmann-Promotionspreis für Molekulare Medizin 2008

Prize awarded by the Medical Faculty of the UMC Hamburg-Eppendorf for the best doctoral thesis 2008

Dietmar Richter: Guest Scientist

Dr. Krishna H. Zivraj

Gebhard Koch-Promotionspreis für Zellbiochemie und Neurobiologie 2006

BMBF/DFG Emmy-Noether Research Group: "Developmental Neurophysiology"

Prof. Dr. Ileana Hanganu-Opatz

DFG Emmy Noether Fellowship 2008

Du Bois Reymond-Award of the German Society of Physiology 2008

Award "Independent Research Groups in Neuroscience" of the Federal Ministry of Education and Research (BMBF) 2008

DFG Emmy-Noether Research Group: "Neuroimmunology"

Dr. Manuel A. Friese

DFG Emmy Noether Fellowship 2008

Helmut Bauer Prize for Multiple Sclerosis Research 2008 awarded by the Medical Faculty of the University of Göttingen and Biogen Idec.

DFG Heisenberg Research Group: "Experimental Neuropediatrics"

Dr. Jacqueline Alig

Prize awarded by the Medical Faculty of the UMC Hamburg-Eppendorf for the best doctoral thesis 2009

Prof. Dr. Dirk Isbrandt

DFG-Heisenberg Professorship 2008

Award of a 'Directeur de Recherche' (DR2, equivalent to associate professor) position by INSERM (Paris, France) 2007

Award of the international 'AVENIR' funding by INSERM (Paris, France) 2007

Dr. Martini Research Prize of the Dr. Martini Foundation Hamburg 2006

Research group "Protein Trafficking and Synapse Formation"

PD Dr. Matthias Kneussel

Award of the Chica and Heinz Schaller Foundation, Heidelberg, 2006

Dr. Christoph Maas

Gebhard Koch-Promotionspreis 2007