NEUROSCIENCE WINTER CONFERENCE

16th International

Sölden Austria
April 8 -April 12 2014

Hotel Das Central

Final Scientific Program

Time schedule of keynote lectures and symposia
List of poster presentations
List of participants
Abstracts speakers
Abstracts posters

Program Committee:

Tobias Bonhoeffer
Nils Brose
Alois Saria
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Organizer:
brainplatform.net e.U.

Conference Chair:
Alois Saria, Austria

Contributors:

- Austrian Neuroscience Association
- Das Central
- Lundbeck Austria GmbH
- International Society for Neurochemistry
- Andor Technology Ltd.
- Tocris Bioscience
- Novus Biologicals

Exhibitors:

- Abcam plc

Tuesday
April 8

15:00 - 16:30 Registration

16:30 - 17:00 Welcome Cocktail

17:00 - 19:00 Symposium 1

Rapid cross-talk between synaptic receptors: A key path of synaptic plasticity
Chair: **Dmitri Rusakov (UK)**

Dmitri Rusakov (UK) A sub-millisecond mGluR-NMDAR dialog triggering LTP
Julie Perroy (France) Cross-talk between glutamate receptors
Antoine Triller (France) Control of synaptic strength by gypherin
Tobias Böckers (Germany) Autistic-like behaviours linked to the ProSAP/Shank function

Wednesday
April 9 Morning

08:15 – 09:00 Keynote Lecture 1

Herwig Baier (Germany) Retinal ganglion cell diversity creates separate visual processing channels that are tailored to behavioral output

09:00 - 11:00 Symposium 2

Functions of lyso-phosphatidic acid (LPA) signaling pathways in the CNS
Chair: **Robert Nitsch (Germany)**

Orly Reiner (Israel) Novel activities of autotaxin (ATX) in neuronal progenitors of the cerebral cortex
Johannes Vogt (Germany) Control of early neuronal activity by synaptic phospholipids governs connectivity and memory
Andrew J. Morris (USA) Role of lysophosphatidic acid in traumatic brain injury
Robert Nitsch (Germany) Altered synaptic lipid signaling affects cortical information processing involved in psychiatric disorders

11:00 - 11:20 Coffee Break

11:20 - 13:20 Symposium 3

Multivariate and genome-wide approaches in imaging genetics
Chair: **Gunter Schumann (UK)**

Jianfeng Feng (UK) Brain-wide association study of resting state activity identifies functional links associated with psychiatric disorders
Jean-Luc Martinot (France) Imaging brain microstructure in mood disorders
Sylvane Desrivières (UK) Transcriptional control of neural development: Impact on brain structure and cognition
Gunter Schumann (UK) Genome-wide methylation analysis of monozygotic twins identifies association of protein phosphatase, Mg2+/Mn2+ dependent, 1G (*PPM1G*) hypermethylation with alcohol use disorder and measures of impulsiveness

Wednesday
April 9 Afternoon

13:20 - 15:20 Symposium 4

Glutamatergic transmission in schizophrenia
Chair: **Robert Schwarcz (USA)**

Christine Konradi (USA) Hippocampal interneurons and mitochondrial abnormalities in psychotic disorders
Joseph Coyle (USA) Serine racemase knockout mice: A mouse model of NMDA receptor hypofunction
Robert Schwarcz (USA) The role of endogenous kynurenic acid in hippocampal function and dysfunction
W. Wolfgang Fleischhacker (Austria) Glutamatergic agents in the management of the symptoms of schizophrenia

Wednesday
April 9 Evening

16:00 – 18:00 Symposium 5

Learning-related dynamics in neuronal circuits in vivo
Chairs: **Sonja Hofer (Switzerland)** and **Mark Hübener (Germany)**

Adi Mizrahi (Israel) Structural and functional plasticity of adult born neurons in the mouse olfactory bulb
Florian Engert (USA) Neural circuits underlying operant learning in larval zebrafish
Tobias Rose (Germany) Neurons in visual cortex retain a memory of their inputs after monocular deprivation
Sonja Hofer (Switzerland) Neural dynamics in visual cortex during learning

18:00 - 18:20 Coffee Break

18:20 - 19:05 Special Guest Lecture

Petra Schwille (Germany) Divide and conquer - synthetic biology of cell division

19:05 – 20:30 Poster Session

Thursday
April 10 Morning

08:15 - 09:00 Keynote Lecture 2

Zach Mainen (Portugal) Neural circuits for spontaneous action timing in the frontal cortex

09:00 - 11:00 Symposium 6

Nanophysiology of presynaptic Ca^{2+} signaling
Chair: **Tobias Moser (Germany)**

Erwin Neher (Germany) Ca^{++}-handling and superpriming at the Calyx of Held
Jakob Neef (Germany) Number, topography and coupling to release of Ca^{2+} channels at hair cell active zones
Tomoyuki Takahashi (Japan) Perimeter Ca^{2+} channel coupling to transmitter release at developing calyces of Held
Annalisa Scimemi (USA) Number and organization of Ca^{2+} channels in the active zone of Schaffer collateral synapses
Peter Jonas (Austria) Loose coupling between Ca^{2+} channels and release sensors enables presynaptic plasticity at a cortical glutamatergic synapse

11:00 - 11:20 Coffee Break

11:20 - 13:20 Symposium 7

ISN Symposium: Mitochondrial dysfunction in Parkinson's disease: Failure of protein quality control
Chairs: **Jörg Schulz (Germany)** and **Philipp Kahle (Germany)**

Richard J. Youle (USA) Role of PINK1 and Parkin on mitochondrial QC *in vitro* and *in vivo*
Philipp Kahle (Germany) UBE2N, UBE2L3 and UBE2D2/3 ubiquitin-conjugating enzymes are essential for parkin-dependent mitophagy
Konstanze Winklhofer (Germany) Parkin maintains mitochondrial integrity via linear ubiquitination of NEMO
Aaron Voigt (Germany) TRAP1, a new player in Parkinson's disease

13:20 - 15:20 Symposium 8

NeuroBioengineering: New strategies targeting diverse neural cells for regenerative neurobiology
Chairs: **Philip Beart (Australia)** and **Eva Sykova (Czech Republic)**

Eva Sykova (Czech Republic) Stem cells and biomaterials for treatment of CNS diseases
Sarka Kubinova (Czech Republic) Advanced methods for nervous tissue engineering
Sue Barnett (UK) Can scaffold design affect CNS myelination?
Philip Beart (Australia) Therapeutic potential of bioengineering strategies targeting astrocytes

Thursday
April 10 Evening

16:00 – 18:00 Symposium 9

Ca^{2+} signaling and synaptic transmission
Chairs: **Markus Missler (Germany)** and **Martin Heine (Germany)**

Ralf Schneggenburger (Switzerland) Mechanisms of ultrafast transmitter release at CNS synapses
Martin Heine (Germany) Calcium channel mobility as a variable of synaptic transmission
Gerald W. Zamponi (Canada) Regulation of calcium channels by ubiquitination
Markus Missler (Germany) Calcium channels and neurotransmitter receptors as target molecules of α–neurexin-based complexes

18:00 – 18:20 Coffee Break

18:20 – 20:20 Symposium 10

Coupling excocytotic neurotransmitter release to endocytic membrane retrieval
Chair: **Volker Haucke (Germany)**

Stephan J. Sigrist (Germany) Shedding light on active zone structure and function
Jürgen Klingauf (Germany) Coupling of exo- and compensatory endocytosis
Ling-Gang Wu (USA) Post-fusion structural changes and their roles in exocytosis and endocytosis
Volker Haucke (Germany) Mechanisms of pre-synaptic membrane retrieval and synaptic vesicle reformation

Friday
April 11 Morning

08:15 – 09:00 Keynote Lecture 3

Christopher Harvey (USA) Neuronal circuit dynamics during navigation-based decision tasks

09:00 – 11:00 Symposium 11

From circuits to behavior: Sensorimotor interactions in neural processing
Chair: **Georg Keller (Switzerland)**

Bence Ölveczky (USA) Motor cortex independent skill execution
Adam Kampff (Portugal) Moving with cortex: New techniques for studying behaviours that require motor cortex
Dinu Albeanu (USA) Understanding the roles of cortico-bulbar feedback in encoding odor identity

11:00 - 11:20 Coffee Break

11:20 - 13:20 Symposium 12

Multiple Sclerosis: New vistas
Chair: **Frauke Zipp (Germany)**

Ralf Gold (Germany) New therapies
Bernhard Hemmer (Germany) New antigens
Heinz Wiendl (Germany) New cellular pathways
Frauke Zipp (Germany) Novel mechanisms and targets in Multiple Sclerosis

Friday
April 11 Evening

16:00 - 16:45 Keynote Lecture 4

Bill Hansson (Germany) Coding good and bad odors in the *Drosophila* olfactory system

16:45 – 17:05 Coffee Break

17:05 - 19:05 Symposium 13

Cellular trafficking in the brain
Chair: **Matthijs Verhage (The Netherlands)**

Angus Silver (UK) Vesicle mobility and supply at a central excitatory synapse
Matthias Kneussel (Germany) Transport and trafficking of neuronal proteins underlying synaptic plasticity
Frederic Saudou (France) Huntingtin: Linking energy supply to axonal transport and neurotrophin signaling
Matthijs Verhage (The Netherlands) Trafficking and fusion of dense core vesicles in mammalian CNS neurons

19:30 Gala Dinner (free for Das Central hotel residents, others book at registration desk for 50,- € until Thursday evening)

Saturday
April 12 Morning

08:15 – 09:00 Keynote Lecture 5

Ole Kiehn (Sweden) Neural circuits for controlling rhythmic movements

09:00 – 09:30 Coffee Break

09:30 – 11:30 Symposium 14

Tracing neural processing hierarchies in auditory function, from synapse to perception
Chairs: **Jan Schnupp (UK)** and **Yale Cohen (USA)**

Benedikt Grothe (Germany) GABAergic mechanisms underpin sub-millisecond precision in mammalian binaural hearing
Kasia Bieszczad (USA) A mnemonic function of primary auditory cortical remodeling to predict the significance of sound
Jan Schnupp (UK) How the brain creates and encodes pitch and timbre percepts for complex sounds
Micah Murray (Switzerland) The speed of sound in the human brain

11:30 End of meeting and departure

Wednesday
April 9
19:05 - 20:30
Poster Session

1. Mitochondrial contributions to neuronal autophagy: Links to energetics and mitophagy?
Shin YS, Britto JM, Ryall JG, Higgins GC, Devenish RJ, Nagley P and Beart PM

2. Response to trauma and abuse presenting with overactivity, impulsivity and distractibility that is not secondary to severe ADHD symptoms but the fight and flight response, implications for treatments
Klaus Martin Beckmann

3. Visual speech gestures modulate efferent auditory system
Aravind Namasivayam, Dinaay Sharma, Wing Yiu Stephanie Wong, Dimitra Chaldi, Pascal van Lieshout

4. Left-right asymmetry is required for the habenulae to respond to both visual and olfactory stimuli
Elena Dreosti, Nuria Vendrell Llopis, Matthias Carl, Emre Yaksi and Stephen W. Wilson

5. Can GAP-43 be an early marker of neuronal stress? In vivo imaging and immunofluorescence study of GAP-43 after ischemic brain lesion
S. Gajovic, I. Bohacek, D. Gorup, T. Milicevic, J. Kriz

6. Tissue oxygen measurement for patients with brain injury
R. Gal, M. Slezak, M. Smrcka, A. Mrlian, M. Colonova

7. Distribution and roles of the Onecut transcription factors in spinal dorsal interneurons
K.U. Kabayiza, G. Masgutova, V. Rucchin and F. Clotman

8. Activation of cannabinoid receptor 1 induces ramification in primary microglia cells through activation of the signaling cascade PKCε-Src/Fyn-Raf-ERK1/2
Aikaterini A. Kalargyrou and Dimitra Mangoura

9. LH stimulation could potentiate the effect of inefective dose of morphine and induce morphine sensitization
Sara Karimi, Abbas Haghparast, Mahtash Baniardalan, Sara Sadeghi, Alireza Omranifard

10. Contrast normalization in cat primary visual cortex
Andreas Keller, Nuno Maçarico da Costa, Kevan A. C. Martin

11. Cav1.4 IT mouse as model for vision impairment in human congenital stationary night blindness type 2
Dagmar Knoflach, Verena Burtscher, Gerald J. Obermair, Martin Glösmann, Mathias Seeliger, Amy Lee, Klaus Schicker and Alexandra Koschak

12. Network dynamics in resting state EEG of youths with Autism Spectrum Disorder
Bates, E., Seitzman, B., Coppers, K., and Malaia, E.

13. Cortical plasticity following perceptual learning
Ido Maor and Adi Mizrahi

14. Effects of loud noise exposure on sound processing in the mouse primary auditory cortex
Ondřej Novák, Ondřej Zelenka, Tomáš Hromádka, and Josef Syka

15. Study of proteins associated with epileptic seizures in primary hippocampal cultures under basal and stimulated conditions
Austin O'Reilly

16. Role of K_V channels in activity-dependent biphasic changes of Schaffer collateral fiber volleys
Benjamin Owen

17. Modulating synaptic function through neurexophilin/alpha-neurexin complex formation
Astrid Rohlmann

18. Activation of GABA A receptors of medial prefrontal cortex produces anxiolytic-like response
Jalal Solati, Ramin Hajikhani, Gunther H. Mol l, Oliver Kratz, Yulia Golub

19. Prion protein facilitates synaptic vesicle release by enhancing release probability
Susan W. Robinson, Marie L. Nugent, David Dinsdale and Joern R. Steinert

20. Disruption of the circadian system in patients with neuropsychiatric disorders
Sumová A., Nováková M., Sládek M., Nevšímalová S., Praško J.

21. Lateralization of language function in epilepsy patients: An event-related potential (ERP) study of a word/pseudoword task
Karin Trimmel, Ekaterina Pataraia, Gerald Lindinger, Marlene Weberberger, Judith Ifkovits, Eduard Auff, and Michael Trimmel

22. Neuronal expression of complex gangliosides is necessary for the maintenance of axon and axo-glial junction integritiy
R. McGonigal, D. Yao, J.A. Barrie, H.J. Willison

23. Ca^{2+}-binding Calmyrin 2 functions in endocytosis in hippocampal neurons
Potrzebowska K, Błazejczyk M., Jaworski J., Kuźnicki J., Hoogenraad C.C., Wojda U

List of Participants

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Voigt	Aaron	Germany
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Wiendl	Heinz	Germany
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Winklhofer	Konstanze	Germany
Wintersteller	Elisabeth	Austria
Wojda	Urszula	Poland
Wu	Ling-Gang	USA
Youle	Richard	USA
Zamponi	Gerald	Canada
Zelenka	Ondrej	Czech Republic
Zipp	Frauke	Germany

Abstracts Speakers

Abstracts are listed alphabetically according to presenting author

Missing abstracts have not been forwarded to the meeting secretariat.

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Understanding the roles of cortico-bulbar feedback in encoding odor identity

Gonzalo H. Otazu, Hong Goo Chae & **Dinu F. Albeanu**

Sensory circuits integrate inputs from the environment, as well as feedback signals from higher brain regions. The interplay of feed-forward and feedback signals has been proposed to be fundamental for processing behaviorally relevant information related to expectation, reward and attention, during learning and memory recall. Though rich glutamatergic cortical feedback projections innervate inhibitory interneurons in all olfactory bulb layers, to date their specific dynamics and contribution to olfactory processing remains unknown.

We have directly recorded the activity of cortico-bulbar axonal boutons using multiphoton calcium imaging of GCaMP5 and GCaMP6 signals in awake head-fixed mice. Feedback fibers showed rich, locally diverse and brief (<1s) spontaneous activity in ~60% of the imaged boutons.
Odor presentation triggered responses in only 55% of the boutons. Individual boutons were sparsely activated across odors, resulting in both enhancement (~45%), as well as suppression (~55%) compared to the baseline activity. Strikingly, we observed roughly two types of bouton responses, suggestive of distinct piriform cortex output channels. ~40% of the imaged boutons showed purely enhanced responses, while ~55% of boutons were consistently suppressed by odors. Only ~5% responded through both enhancement and suppression to different odors. Interestingly ~50% of responses outlasted odor presentation by several seconds, and ~15% of responses were triggered after termination of the odor (OFF responses). These observations suggest that transient odor input can trigger long lasting activity (suppression or enhancement) which may further impact bulbar dynamics. This long lasting activity may originate in the bulb itself, or may result from local inhibitory interactions in the piriform cortex. To distinguish between these possibilities, we are currently combining cortical feedback imaging to varying odor pulse durations with pharmacological blocking of intra-cortical interactions.

The enhanced and suppressed bouton responses to a particular odor appeared clustered in spatial domains. However, pairwise analysis of simultaneously imaged boutons revealed functional local diversity across our panel of 30 odors. No spatial organization was apparent in bouton responses across odors within the imaged field of view (<150 µm).

To directly determine the effect of cortical feedback on the dynamics of the OB output, we are suppressing piriform cortex activity using pharmacological and optogenetic methods, in conjunction with simultaneous monitoring of granule and mitral cell activity via multiphoton microscopy. Preliminary results show that suppressing cortico-feedback bi-directionally alters spontaneous activity and decreases the responsiveness of granule cells to odors and across concentrations, diminishing both enhanced and suppressed responses.

We propose that cortical feedback modulates the dynamics of OB output such as to sharpen odor responses and maintain diverse neuronal representations across different stimuli. We are currently testing this hypothesis in behaving mice engaged in odor discrimination tasks.

Retinal ganglion cell diversity creates separate visual processing channels that are tailored to behavioral output

Herwig Baier

Max Planck Institute of Neurobiology, Department Genes – Circuits – Behavior, 82152 Martinsried, Germany

Retinal representations of the external world are transmitted to the brain via the axons of retinal ganglion cells (RGCs). Distinct RGC types can be defined using physiological, genetic and morphological criteria. There is a lack of information regarding the cell type-specificity of RGC projections to the retinorecipient brain regions. Also, the precise function of many of these areas is unknown.

We have constructed a comprehensive map of the connectivity between RGCs and retinorecipient areas of the larval zebrafish. Unbiased sparse genetic labeling, in conjunction with in vivo imaging, allowed us to reconstruct the projection patterns of >400 individual RGCs. Most visual areas do not receive dedicated retinal input, but rather are comprised predominantly of collateral branches formed by axons that terminate in the optic tectum, the largest target region. Overall, we have identified more than 75 RGC types based on the combination of axonal targets and dendrite stratification patterns. This number far exceeds current estimates of RGC diversity derived from work in other vertebrates.

Using optogenetic perturbations, laser ablations and GCaMP6 imaging, we functionally annotated several visual areas. While the tectum is important for localizing objects and transforming this information into a directed motor response, subtectal areas appear to be tailored to specific behaviors, such as prey capture, collision avoidance, optokinetic and optomotor responses or circadian photo-entrainment. Frequently, dendrite morphologies of RGCs predict functional properties of their target AFs, demonstrating the power of our combined anatomical, optogenetic and optophysiological approach for the dissection of visual processing channels.

Can scaffold design affect CNS myelination?

Susan C Barnett, Peter Donoghue and Mathis Riehle*

Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, 120 University Place, Glasgow G12 8TA, UK
*Centre for Cell Engineering, Institute of Molecular, Cell and Systems Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK

Spinal cord injury (SCI) is a major cause of persistent disability. The repair of spinal cord injury (SCI) currently favours a combinatorial approach incorporating several factors, including exogenous cell transplantation and biocompatible scaffolds. Cell transplantation is a promising therapeutic approach to fill the damaged injury site and encourage axonal outgrowth to fill the gap. A range of neural/stem and engineered cells have provided tested evidence that this is a plausible approach in animal models of SCI. However, although cell transplants can provide a environment conducive to axonal regeneration, the resulting axonal growth is poorly organised and this approach will not be possible where physical disruption of the spinal cord is extensive. Moreover, as growth of human cells in culture can be slow and it is technically challanging to grow large cell numbers necessary to fill a human lesion, it may be necessary to use artifical scaffolds seeded with glial cells to bridge the gap and promote repair. Using an established myelinating culture system of dissociated spinal cord rat cells, recapitulating many of the features of the intact spinal cord, including myelination we examined the ability of these mixed cell cultures to interact and myelinate axons on various artificial scaffolds, including PCL and a novel super-macroporous polymer scaffold. Our long term aim is to validate the scaffolds using our cultures that mimic the intact CNS environment prior to using animal models of SCI.

Therapeutic potential of bioengineering strategies targeting astrocytes

Beart PM

Florey Institute of Neuroscience and Mental Health, University of Melbourne, VIC 3052, Australia

Astrocytes are a target in regenerative neurobiology because in injury their phenotype arbitrates brain integrity and subsequent reconstruction. We found treatment of 2D astrocytes with ROCK inhibitors produced stellation, less actin stress fibres and increased G-actin. Here the astrocytic transcriptome revealed alterations to extracellular matrix (ECM) and a cytotrophic phenotype exhibiting elevated expression of EAAT2, BDNF and anti-oxidant genes. Since bioengineered scaffolds provide cues for cellular organization and in concert with manipulation of surface chemistry can replicate components of ECM, we explored abilities of 3D scaffolds to direct astrocytes into cytotrophic phenotypes. Murine astrocytes (PND 1.5, subcultured 10 *div*) survived, proliferated and migrated into poly-Ɛ-caprolactone (PCL) scaffolds adopting 3D morphologies: on randomly aligned PCL scaffolds cells grew as circular colonies extending processes deep within fibres, whereas astrocytes on aligned scaffolds exhibited rectangular colonies with processes following the direction of fibre alignment but also penetrating the scaffold. At a further 12 *div* astrocytes displayed stellated morphology, reduced GFAP expression, increased G-actin and a cytotrophic gene profile matching that described above. Here ROCK inhibitors appeared to promote migration, as shown by the novel marker Ahnak, and especially on aligned scaffolds. Based upon our transcriptomic analyses of ECM in 2D astrocytes treated with ROCK inhibitors, we synthesized a novel random scaffold featuring an immobilized sugar-moiety. Morphometric analyses of immunocytochemical data (GFAP, Ahnak) indicated astrocytes expanded less than on 3D PCL scaffolds suggesting a further shift to a cytotrophic phenotype. Our work illustrates the therapeutic potential of bioengineered 3D electrospun scaffolds that direct astrocytes into phenotypes supporting brain repair.

A mnemonic function of primary auditory cortical remodeling to predict the significance of sound

Kasia Bieszczad

Center for the Neurobiology of Learning and Memory, University of California, Irvine
Wissenschaftskolleg zu Berlin

In addition to its neural code for the features of sound, the auditory cortex is known to be a substrate for auditory associative learning and memory, i.e., auditory cognition. Most extensively studied is the representation of tonal frequency in the primary auditory cortex (A1), in which frequency-specific representational physiological plasticity develops when both animal and human subjects learn the behavioral relevance of sound-frequency. First discovered in studies of associative learning, local shifts in receptive fields for tonal frequency can transform the global representation of frequency in the tonotopic A1 map to enlarge the representation of a tone-frequency signal (i.e., a conditioned cue). The amount of cortical increase in the signal's representational area is a direct function of the level of its acquired behavioral importance. A possible reason for signal-specific area gains in A1 to be so graded by signal importance may be to strengthen memory for more important signals, which would enable linkage of environmental stimuli to their significant outcomes, and permit adaptive motor outputs. Rodent models of auditory-cued reward learning and memory indicate that greater learning-induced gains in A1 area are significantly positively correlated with stronger memory. Furthermore, area gains that survive interfering episodes of associative learning predict the recovery of the prior auditory-cued behavior. In addition, artificial induction of primary auditory cortical remodeling of frequency-representation (by brain stimulation) appears to induce *de novo* frequency-specific behavioral memory. Therefore, the amount of representational area of a signal-frequency in A1 appears to enable memory formation to link auditory cognition to action. A discussion of a framework for investigation of primary auditory cortical plasticity will serve as a summary highlighting the need for identification of factors (critical for the induction of neuroplasticity), forms (that neurophysiological plasticity takes), and functions (for adaptive behaviors), including the dynamics thereof over time and experience.

Autistic-like behaviours linked to the ProSAP/Shank function

Tobias Böckers

Anatomy and Cell Biology, Ulm University

Scaffold proteins, like the Shank family of multidomain proteins, are abundant and essential components of the postsynaptic density (PSD). They play a major role in many synaptic functions including the trafficking, anchoring and clustering of glutamate receptors and adhesion molecules. Moreover, they link postsynaptic receptors with their downstream signaling proteins and regulate the dynamics of cytoskeletal structures. By definition, PSD scaffold proteins do not have intrinsic enzymatic activities but are formed by modular and specific domains deputed to form large protein networks. Given that scaffold proteins are central components of PSD architecture it is not surprising that deletion or mutations in their human genes cause severe neuropsychiatric disorders including autism, mental retardation and schizophrenia. Thus, their dynamic organization and regulation are directly correlated with the essential structure of the PSD and the normal physiology of neuronal synapses. Over the last years several Shank mutations have been described that are causative for autism spectrum disorders (ASDs) as well as schizophrenia. Shank mouse models, as well as neurons derived from iPS cells of affected patients are used to find mechanisms that could explain the pathophysiology of ASD.

Serine racemase knock out mice: A mouse model of NMDA receptor hypofunction

Joseph T. Coyle, MD (joseph_coyle@hms.harvard.edu), Darrick Balu, PhD (balu1030@gmail.com), Mathew Puhl, PhD (mpuhl@mclean.harvard.edu), Michael Benneyworth (mbenneyworth42@gmail.com) and Shunsuki Takagi, MD (stff1950@gmail.com).

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Recent results from genome wide association studies and from copy number variants associated with schizophrenia (SCZ) have implicated genes encoding proteins involved in glutamatergic neurotransmission. This includes serine racemase (SR), the enzyme responsible for the synthesis of D-serine, the co-agonist at NMDA receptors (R), which is concentrated in the cortico-limbic regions. Mice with null mutations of SR have less than 10% of the wild-type (WT) levels of D-serine in their cortex and hippocampus. They exhibit many of the pathologic stigmata of SCZ including impaired working memory, increased ventricular volume and decreased cortical and hippocampal volume. Quantitative morphometry of Golgi stained pyramidal neurons in frontal and primary sensory cortex revealed reduced dendritic complexity, length and spine density similar to schizophrenia. Analysis of the hippocampus showed decreases in mediators of neuronal plasticity including BDNF, phospho-TrkB, phospho-Akt, phospho-CREB, phospho-mTOR, and miR132 similar to schizophrenia. Consistent with impaired contextual memory, long-term potentiation (LTP) was also attenuated in SR-/- mice. Treatment of SR-/- mice for 3 weeks with 300mg/Kg/day of D-serine normalized D-serine levels in cortex. D-serine reversed contextual memory and LTP deficits and restored the neuroplasticity mediators to WT levels, suggesting that pharmacologic enhancement NMDAR might resolve the cognitive deficits and negative symptoms of schizophrenia..

To understand better the localization of SR and D-serine, we used a combination of quantitative genetic and immunologic methods. SR expression was blocked in a cell specific fashion using mice expressing Cre in astrocytes with a GFAP promoter and in forebrain glutamatergic neurons with CAMKIIα promoter. Cortical D-serine levels were unaffected in the astrocyte SR-/- and were reduced by only 30% in the glutamatergic SR-/-. Less than 15% of SR was expressed in astrocytes and ~65% in glutamatergic neurons. Using the SR-/- mouse as a control for immuno-specificity, we found that neither SR nor D-serine was expressed in the astrocytes in the adult brain, but virtually all SR and D-serine was localized to Gabaergic and glutamatergic neurons in neocortex.

JT Coyle, MD has consulted with AbbVie and EnVivo within the last 2 years and holds a patent on the clinical use of D-serine that is owned by Massachusetts General Hospital. D. Balu, M. Puhl, M. Benneyworth and S. Tagaki report no conflicts of interest. This research was supported by grants from the N.I.H.

Transcriptional control of neural development: Impact on brain structure and cognition

Sylvane Desrivières

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Spatiotemporal alterations in brain structure and function occur over a lifetime, a plasticity that is particularly important in childhood and adolescence. Neuroimaging studies have enabled the demonstration that heritable, region- and age- specific variations in structure occur in the brain in patterns that appear to follow cognitive and functional maturation. At the cellular level, such changes during adolescence are consistent with known cellular maturational alterations, such as the changes in synaptic density and intra-cortical myelination occurring during this developmental stage. Thus, the age-related changes in heritability noted above may be linked to the timing of the expression of given set of genes involved in specific stages of neural development. Yet, although genetic factors are thought to be important, little is known about genes accounting for inter-individual differences in brain structure and cognition.

I will describe two of our recent studies using a combination of transcriptional profiling in human neural progenitor cells and human brains and imaging genetics to investigate the genetic and neural basis of this brain plasticity. These analyses that have identified the synaptic cell adhesion glycoprotein-encoding gene *NPTN* as a gene linking brain structure and cognition and uncovered antagonistic actions of *MYT1L* and *MEF2* transcription factors, support a potential role for regional synaptic dysfunctions in forms of intellectual deficits.

Neural circuits underlying operant learning in larval zebrafish

Drew Robson, Jennifer Li, Alex Schier, **Florian Engert**

MCB, Harvard University

During operant conditioning, animals learn to respond to stimuli with actions that lead to favorable outcomes. Recordings in mammals have uncovered neural signals that link stimulus, action, and outcome, but a limited number of recording sites have hindered the comprehensive discovery of learning signals. Here, we use brain-wide functional imaging in larval zebrafish to screen more than 100,000 neurons while animals learn to terminate a heat stimulus with a directional tail movement. We identify neurons comprising two major classes: class 1 consists of action-selective neurons that encode the direction of heat-evoked tail movements seconds before and after their execution. Class 2 consists of neurons that encode outcome prediction and prediction error. This class includes both positive relief prediction signals that are enhanced by learning, and negative relief prediction signals that are suppressed by learning. These positive and negative relief prediction signals not only have opposing patterns of activity but are also found on opposing sides of the habenula. Strikingly, both outcome-predictive and action-selective signals are correlated with natural variability in learning performance across animals. This study provides the first comprehensive survey of the neural dynamics during learning, suggests that lateralized neuronal activity contributes to operant conditioning, and raises novel hypotheses about the roles of action-selective and outcome-prediction neurons.

Brain-wide association study of resting state activity identifies functional links associated with psychiatric disorders

Jianfeng Feng

Warwick University and Fudan University

There are many papers published in the literature which reported abnormal resting state networks associated with different mental disorders, but many results are hardly repeatable in another dataset. It requires us not only to increase our samples but also to develop more stable statistical approaches to identify abnormal circuits. In this talk, we will review some of our recent successful endeavours along these lines with examples mainly on schizophrenia (autism and ADHD).

With a total number of around 600 samples in schizophrenia from USA, UK and China, we identified that a large abnormal circuit with the hub at thalamus using our brain-wide association study (BWAS) approach. In general, the links between the thalamus and sensory part are enhanced, but with the frontal part are reduced. Using the illness duration as a control variable, we found that the thalamus gradually deteriorates as the illness duration progresses. However, using Nottingham data where structure variables are available, it is shown that the longer the illness duration, the more difficult to separate healthy people from schizophrenia, possibly implying the compensation mechanism in our brain.

Functional network is dynamic and contains rich information. After a study on exploring the meanings of these oscillations, we introduce a new statistical approach based upon functional analysis, together with our BWAS, to reliably identify abnormal circuits in moderate sample sizes.

Glutamatergic agents in the management of the symptoms of schizophrenia

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Based on the hypothesized involvement of glutamatergic pathways in the pathophysiology of schizophrenia the past decade has seen a number of attempts to capitalize on this knowledge in treatment trials. Various routes have been explored: These have included ampakines, compounds targeting metabotropic glutamate receptors and molecules modulating NMDA receptors, either directly via the glycine side or indirectly by blocking the reuptake of glycine. Early studies with both glycine and D-serine used as adjunctive treatments to antipsychotics have reported promising results but some larger replication efforts have not been successful. Enhancing the availability of glycine at the relevant receptor side of the NMDA receptor via the inhibition of glycine reuptake was first successfully explored with sarcosine, a number of independent randomized controlled trials have produced favorable results. More recently, bitopertin has been studied extensively. After a positive phase II study, in which this compound showed a positive signal with regard to the improvement of negative symptoms in an observed cases analysis, two large scale phase III replication studies have yielded discouraging results. The compound did not differentiate from placebo in any respect. Nevertheless, clinical trials of glutamatergic compounds are ongoing.

Coding good and bad odors in the *Drosophila* olfactory system

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How are attractive and repellent odors coded in the *Drosophila* peripheral and central nervous system? Does e.g. the glomerular topography of the insect antennal lobe have any significance? We know that male insects optimized to detect conspecific intersexual pheromones have a specific part of the antenna and the lobe devoted to this task, but we know very little regarding the importance of specific sensory neurons and antennal lobe regions beyond this. Besides sex attractants, brains have to decide whether and how to respond to detected stimuli based on, often, complex sensory input. The vinegar fly *Drosophila melanogaster* evaluates potential food sources largely based on olfactory cues. We performed a comprehensive behavioral screen using *Drosophila* and established the innate valence of 110 odors. By observing neuronal activation patterns evoked by behaviorally positive and negative odors from the antenna to the antennal lobe, we could identify aversive-specific projection neuron activation patterns in the glomerular array of the lobe (Knaden et al., 2012). The representation of odor valence is thus formed already at the output level of the antennal lobe. Secondly, we studied coding of one specific odor, geosmin, emitted by harmful microbes. By using a large array of methods, including a novel paradigm to test for behavioral responses, we could show that a single neural line from the periphery and past the antennal lobe delivers the message regarding the presence of geosmin. Activation of this line is necessary and sufficient for a very strong aversive behavior to be elicited by geosmin (Stensmyr et al., 2012). Thirdly, we studied another ecologically labeled line involved in egg laying. Flies strongly prefer to lay their eggs on citrus fruit. This preference is mediated by a specific receptor tuned to citrus-specific odors. Interestingly, activation of this line only elictits oviposition, not attraction (Dweck et al., 2013)
In all we studied how odors involved in food attraction – and avoidance – are coded in the *Drosophila* peripheral and central nervous system. The results have an important bearing on our understanding of food choice in these insects.

These studies were financed by the Max Planck Society.

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Neuronal circuit dynamics during navigation-based decision tasks

Christopher Harvey

The posterior parietal cortex (PPC) has an important role in many cognitive behaviors; however, the neuronal circuit dynamics underlying PPC function are not well understood. We have studied circuit activity dynamics in the PPC of mice during navigation-based choice tasks using a combination of a virtual reality system and two-photon microscopy. We find that during working memory tasks the PPC activity dynamics are best characterized as choice-specific sequences of neuronal activation, rather than long-lived stable states, implemented using anatomically intermingled microcircuits. I will also discuss on-going work using imaging methods and new memory and decision tasks that examine in more depth the dynamics in the mouse PPC.

Mechanisms of pre-synaptic membrane retrieval and synaptic vesicle reformation

Volker Haucke

Neurotransmission depends on the local endocytic recycling of synaptic vesicles (SVs) at nerve terminals. During sustained activity efficient endocytic membrane retrieval is required to keep membrane surface area constant and SVs need to be reformed to replenish the SV pool. The mechanisms involved in membrane retrieval and SV reformation have been debated controversially over more than four decades with evidence being presented for rapid kiss-and-run exo-endocytosis, ultrafast clathrin-independent endocytosis (CIE), slow bulk endocytosis, and clathrin-mediated endocytosis (CME).

In my talk I will present evidence that that hippocampal synapses mainly use dynamin 1/3-mediated CIE to retrieve SV membranes, though clathrin/ AP-2-mediated endocytosis contributes to presynaptic membrane retrieval during low-frequency stimulation. Depletion of clathrin or knockout of AP-2 results in defects in SV reformation and an accumulation of endosome-like vacuoles, indicating that clathrin/ AP-2 mediate SV reformation mainly form internal endosomal structures rather than the plasma membrane. These experimental results together with theoretical modelling provide a conceptual framework for how synapses capitalize on CIE and clathrin/ AP-2 to maintain excitability over a broad range of stimulation frequencies.

Calcium channel mobility as a variable of synaptic transmission

Romy Schneider, Johannes Kohl, Eric Hosy, Ulrich Thomas, Andreas Voigt,
Martin Heine

Presynaptic activity and short term plasticity depend strongly on the action of presynaptic high voltage activated calcium channels (VDCC). Particular their coupling to the ready releasable pool of synaptic vesicles as well as their kinetic properties has been identified to modulate synaptic transmission. Calcium channels themselves are composed of a pore forming alpha1-subunit, an intracellular beta-subunit and a mainly extracellular located alpha2delta-subunit. In order to directly investigate the localisation and dynamic of P/Q- and N-type calcium channels in the presynaptic membrane we use single particle tracking methods to visualise the organisation of calcium channels within the presynaptic membrane. Tracking of alpha1-subunits and the alpha2delta1-subunit showed strong differences in the surface dynamics of both proteins.
The channel forming subunit of P/Q- and N-type calcium channels are confined within the presynaptic membrane, whereas the alpha2delta1-subunit does only transiently stop within the presynaptic membrane. Changing synaptic activity strongly reduces the mobility of the alpha2delta1-subunit, but not the local dynamic of alpha1-subunits. Manipulating the cytosolic concentration of calcium by introducing exogenous calcium buffers as EGTA or BAPTA immobilises the majority of alpha1-subunits. We suggest that the local dynamics of alpha1-subunits does influence the coupling distance between the sensor for vesicular transmitter release and will have impact in the local current cooperativity and hence the size of the intracellular calcium domain. Whereas the transient association of alpha2delta subunits with the alpha1-subunit influences the kinetic properties of single channels and might thus impact in the calcium domain. Based on these observations we suggest that the dynamic organisation of calcium channel subunits modulate short term synaptic plasticity within hippocampal synapses.

New antigens

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Multiple sclerosis (MS) is a chronic disease of the CNS that is characterized by inflammation, demyelination and axonal injury. Although the etiology of MS is still unknown, many findings point toward a central role for the immune system in the pathogenesis of MS. It is widely accepted that the adapted immune response is crucial for initiating and perpetuating disease. While many studies have demonstrated a highly focused T- and B cell response in the CNS of MS patients, the target antigens of the these cells are still largely uncertain. With the development of new technologies to search for molecular targets of T cells, B cells and in particular antibodies, first candidate autoantigens in MS and its variants have been identified. Knowledge on the targets of the adapted immune response is essential for the development of specific immune therapies in MS.

Neural dynamics in visual cortex during learning

Sonja Hofer

How we perceive and interpret sensory stimuli is strongly dependent on our internal state, our past experiences and expectations about the stimulus. Sensory processing in a behaving animal therefore relies on the integration of internal top-down information with feed-forward sensory input. Making new experiences and learning new associations is expected to change how sensory signals are modulated by top-down input. We use the mouse visual cortex as a model system to study how visual information is processed in awake animals dependent on their behavioral state and how stimulus representations change during visually-guided learning. To address these questions we are following the activity neuronal populations while mice learn to perform a visual discrimination task using two-photon imaging of genetic calcium indicators. In my talk I will outline the changes we find in the representation of a visual stimulus in primary visual cortex as it becomes behaviorally relevant to the animal.

Loose coupling between Ca^{2+} channels and release sensors enables presynaptic plasticity at a cortical glutamatergic synapse

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The coupling between presynaptic Ca^{2+} channels and release sensors is a key factor that determines the efficacy and speed of synaptic transmission. Classical work at the young calyx of Held suggested that the coupling between Ca^{2+} channels and release sensors is loose, with a coupling distance of ~100 nm. However, more recent work indicated that the coupling is much tighter in several cortical synapses, especially inhibitory synapses, with a coupling distance of 10–20 nm. Furthermore, it has been reported that loose coupling at the calyx is converted into tight coupling during development, implying that loose coupling might be a purely developmental phenomenon. To examine whether any synapse in the mature CNS makes use of loose coupling, we studied the hippocampal mossy fiber–CA3 pyramidal neuron synapse. To probe the coupling distance, we made paired recordings between presynaptic mossy fiber boutons and postsynaptic CA3 pyramidal neurons, infusing the fast Ca^{2+} chelator BAPTA or the slow chelator EGTA into presynaptic sites. We found that coupling at this synapse was surprisingly loose, with a mean coupling distance of ~80 nm. Furthermore, washout experiments (bouton-attached recordings followed by whole-bouton recordings without chelators) suggested that endogenous Ca^{2+} buffers had similar effects as exogenous chelators, reducing transmitter release probability. Conversely, wash-in experiments (bouton-attached recordings followed by whole-bouton recordings with defined concentrations of BAPTA or EGTA) suggested that the endogenous buffers had properties comparable to 0.2 mM BAPTA. Finally, modeling indicated that saturation of the endogenous buffers contributed to facilitation of release, a hallmark property of this synapse. For both reduction of release probability and buffer saturation, loose channel–sensor coupling was a critical requirement. Thus, loose coupling enables presynaptic plasticity at a mature cortical glutamatergic synapse.

UBE2N, UBE2L3 and UBE2D2/3 ubiquitin-conjugating enzymes are essential for parkin-dependent mitophagy

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Depolarized mitochondria are degraded via mitophagy in a process that depends on the Parkinson's disease gene products PINK1 and Parkin. This is accompanied by ubiquitination of several mitochondrial substrates. The roles of E2 ubiquitin-conjugating enzymes (UBE2) in mitophagy are poorly understood. Here we investigate a set of UBE2 enzymes that may regulate Parkin-mediated mitophagy. Knockdown of the E2 enzymes UBE2N, UBE2L3 or UBE2D2/3 significantly reduced autophagic clearance of depolarized mitochondria. However, this did not interfere with mitochondrial PINK1 stabilization and Parkin translocation. UBE2N knockdown prevented specifically K63-linked ubiquitination at mitochondrial sites. Nevertheless, poly-ubiquitin and p62 were still found on mitochondria after individual UBE2 knockdown. Knockdown of all three UBE2s together significantly reduced the mitochondrial poly-ubiquitinylation and p62 recruitment. Moreover, reduced ubiquitination of mitofusins, mitochondrial import receptor subunits TOM20 and TOM70, the voltage-dependent anion channel protein 1, and of Parkin was observed in cells silenced for all three UBE2s. The Parkin active site mutant C431S failed to ubiquitinate these mitochondrial substrates even in the presence of UBE2s. We conclude that UBE2N, UBE2L3 and UBE2D2/3 synergistically contribute to Parkin-mediated mitophagy.

Moving with cortex:
New techniques for studying behaviours that require motor cortex

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The role for motor cortex in the control and learning of behaviour remains unclear, particularly in lower mammals (e.g. rodents). I will first describe a behaviour assay that demonstrates that motor cortex is required to rapidly respond to changes in an environment. I will next discuss a new electrical recording method, based on 'active' silicon devices, which will facilitate detailed investigation of the changes in neural activity that underlies such fast behavioral adaptations. My talk will thus have two parts:

Part I: We constructed a new behavioural assay with which we can manipulate the "predictability" of the environment. The assay consists of a series of obstacles coupled to a motorized brake that allows independent control of the rotational stability for each obstacle. During training, animals first learned to negotiate the assay with all of the obstacles stable, and performance was comparable between lesion and sham animals. Following one week in this environment, the center two steps were covertly released (i.e. made free to rotate upon contact). Upon *first* encountering the new, unexpected state of the center obstacles, animals without motor cortex failed to respond to the change. In contrast, control animals either rapidly produced compensatory behaviors or immediately begin exploring the altered obstacle. This finding highlights a novel role of motor cortex in the preparation and control of fast responses to unexpected change.

Part II: Advanced microfabrication techniques (CMOS), which are standard in the semiconductor industry, permit the integration of *active* electronics directly into the shaft of a silicon probe. Such "active neural probes" are not limited by the number of connection wires that can be fit across the probe surface and can thus monitor thousands of distinct electrode sites. I will discuss our ongoing efforts to develop and characterize these active devices, as well as their potential for investigating the changes in neural activity that occur during our behavioural studies.

Neural circuits for controlling rhythmic movements

Ole Kiehn

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Rhythmic movements, like locomotion, are one of the many motor acts that the nervous systems controls. Locomotion circuits are to a large degree controlled by neuronal circuits in the spinal cord itself. These circuits are at the core of generating locomotion and understanding the operational organization of these circuits are key to understand how locomotion is generated. In this talk, I will discuss findings from our lab that have revealed the role of designated populations of neurons that serve key functions in the locomotor network, including dual neuronal populations that secure left-right alternation at different speeds of locomotion, and excitatory interneuron networks with distinct molecular identity that are engaged in rhythm- and pattern-generation. I will also discuss the use of optogenetics approaches in transgenic mice models as a tool for functional probing of spinal locomotor networks. Our experiments provide fundamental insight to the principal mode of operation of large-scale mammalian motor circuit.

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Coupling of exo- and compensatory endocytosis

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Fusion of synaptic vesicles (SVs) during fast synaptic transmission is mediated by SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) complex assembly formed by coil-coiling of three members of the protein family: synaptobrevin 2 (syb2) and the presynaptic membrane SNAREs syntaxin-1A and SNAP-25. In order to maintain neurotransmission exocytosed SV components need to be retrieved from the surface by compensatory endocytosis. Clathrin-mediated endocytosis (CME) is thought to be the predominant mechanism of SV recycling. However, it might be too slow for fast SV recycling. Therefore, it was suggested that a pre-sorted and pre-assembled pool of SV proteins on the presynaptic membrane might support a first wave of fast CME. We monitored the temporal dynamics of such a 'readily retrievable pool' of SV proteins in hippocampal neurons using a novel probe, CypHer 5, a new cyanine dye-based pH-sensitive exogenous marker, coupled to antibodies against luminal domains of SV proteins. This way we could for the first time demonstrate the preferential recruitment of a surface pool of SV proteins upon stimulated endocytosis. Using fluorescence nanoscopy (isoSTED, FPALM, an STORM) of labeled SV proteins we could resolve the spatial distribution of the surface pool at the periactive zone and identify early steps of its formation. Dimerisation of the vesicular SNARE syb2 and cross-linking by the second most abundant SV protein Synaptophysin appear to be important for sequestering syb2 in nanodomains; this way efficiently clearing the release site from SV constituents and preventing cis-SNARE complex formation, which would otherwise result in short-term depression.

Transport and trafficking of neuronal proteins underlying synaptic plasticity

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The delivery, removal, and recycling of synaptic neurotransmitter receptors involves post-Golgi transport through molecular motors along microtubules and actin filaments. In addition, at the cell surface, receptors exchange between synaptic and extrasynaptic positions via membrane diffusion. Using $GABA_A$ receptor ($GABA_AR$) subunits as baits, we identified two receptor-binding proteins that regulate $GABA_AR$ transport and receptor trafficking. $GABA_AR$ α1-containing receptors bind to muskelin, which mediates association with myosin VI or dynein motor complexes in subsequent steps of $GABA_AR$ endocytosis. Inhibition of either transport route selectively interferes with receptor internalization or degradation. Muskelin KO mice display depletion of both transport steps and a high-frequency ripple oscillation phenotype.
$GABA_AR$ α5-containing receptors concentrate extrasynaptically through radixin (Rdx)-mediated anchorage at the actin cytoskeleton. Rdx regulates adjustable plasma membrane receptor pools in the control of synaptic receptor density. Rdx gene knockout impairs reversal learning and short-term memory in mice.
Our data suggest that trafficking factors are critical mediators in the regulation of neurotransmitter receptor density underlying synaptic plasticity, learning and memory.

Hippocampal interneurons and mitochondrial abnormalities in psychotic disorders

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Background: Interneurons of the human hippocampus are crucial for the tonic and phasic inhibition of neighboring neurons and essential for cognitive processing. Two particular interneuron populations of the hippocampus, characterized by the expression of somatostatin and parvalbumin, were examined in post mortem tissue from patients with schizophrenia and bipolar disorder. In an independent set of specimens from patients with bipolar disorder, we found a large decrease in mRNA expression levels of proteins coding for the respiratory chain. In my talk I will address the connection between interneuron function and abnormal mitochondrial respiration.

Methods and Results: Somatostatin- and parvalbumin positive interneurons were stained with antibodies specific for either peptide and cells counted throughout whole hippocampal specimens at 5 mm intervals. Both neuronal populations were reduced in schizophrenia as well as in bipolar disorder. Independently, a gene expression microarray analysis of fresh-frozen hippocampi from bipolar patients showed a downregulation of mRNAs coding for mitochondrial respiratory chain proteins. This altered molecular profile was also observed in glucose-deprived primary lymphocytes of BPD patients, suggesting an abnormal cellular response to bioenergetic deprivation. In humans as well as rodents a significant correlation was observed between mRNA expression levels of mitochondrial respiratory chain proteins and somatostatin- or parvalbumin, supporting the hypothesis that interneurons are particularly dependent on mitochondrial respiration and vulnerable to mitochondrial stress.

Conclusions: Hippocampal interneurons may play a crucial role in psychotic disorders such as schizophrenia and bipolar disorder. Their strong link to mitochondria suggests a vital dependence on optimal mitochondrial function. Minor mitochondrial pathologies that may become evident during increased bioenergetic demands would be particularly detrimental to certain interneuron populations rendering them dysfunctional and leading to their demise.

Advanced methods for nervous tissue engineering

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The transplantation of stem cells is currently under study as a therapeutic approach for spinal cord injury, where the number of transplanted cells that reach the lesion is one of the critical parameters how to achieve significant therapeutic benefit. To promote the homing of transplanted cells to the site of injury, a magnetic targeting strategy can be used to deliver magnetic nanoparticle-loaded cells to specific *in vivo* locations. In this study, intrathecally transplanted cells labeled with superparamagnetic iron oxide nanoparticles (SPION) were guided by a magnetic field and successfully targeted near the lesion site in the rat spinal cord. We show how the magnetic implant affects the distribution and kinetics of the transplanted cells in the spinal cord after intrathecal implantation. To increase targeting efficiency, we propose magnetic systems that produce spatially modulated stray fields. Such magnetic systems with tunable geometric parameters may provide the additional level of control needed to enhance the efficiency of stem cell delivery in spinal cord injury. We also show that fast stem cell targeting can be achieved by using a non-invasive magnetic system consisting of two cylindrical magnets with the poles facing each other that produce a magnetic flux, allowing the focusing of SPION-labeled cells in a lesion site. Moreover, the proposed magnetic delivery system can be attractive for clinical application development.

Supported by grants GACR P304/11/0731, P304/12/1370, 14-14961S.

Neural circuits for spontaneous action timing in the frontal cortex

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Adaptive behavior requires not only choosing what to do but also choosing when. Action timing is particularly important for acts that are self-initiated rather than triggered by an event in the environment. What neural circuits are ultimately responsible for the initiation of such “spontaneous” or “self-paced” decisions? In my laboratory, we are studying this issue with a particular interest in the role of the frontal cortex. To do so, we train rats or mice to perform a task asking them to wait a random delay before leaving to harvest a large reward but allowing them to leave at any time and settle for a small reward. We then use simultaneous neural recordings and manipulations to attempt to predict and influence leaving times. We found using pharmacological inactivation that both premotor cortex and the medial prefrontal cortex are important for performance of this task. Neural recordings showed that the precise timing of waiting could be predicted only by neural activity in the premotor cortex. These premotor neurons showed two interesting patterns. One population slowly ramped to a threshold just before leaving. The other population displayed more transient firing that was triggered at different moments in the trial and that fluctuated from trial-to-trial in a manner predictive of future waiting times. Together, these two populations strongly resembled, respectively, the output and input of a “neural integrator”, as has been hypothesized as a mechanism of decision-making. A simple but quantitative model confirmed that the magnitudes of inter-neuronal correlations and neural-behavioral correlations were consistent with this hypothesis. By helping to elucidate the neural circuit mechanisms understanding spontaneous action timing these studies might ultimately shed light on the origin of self-control and perhaps even such thorny issues as the nature of self and free will.

Calcium channels and neurotransmitter receptors as target molecules of α-neurexin-based complexes

Markus Missler

Neurexins (Nrxns) are predominantly presynaptic transmembrane molecules linked to neuropsychiatric disorders such as autism. They are encoded by three genes, each expressing two major isoforms (α-Nrxn/β-Nrxn) that differ in extracellular sequences. α-Nrxn variants occur in most neurons, and play essential roles in transmission at excitatory and inhibitory terminals. Previous data indicated that α-Nrxns link voltage-activated Ca^{2+}-channels to synaptic vesicle release because deletion of α-Nrxn in mice led to reduced spontaneous and evoked transmission, impaired Ca^{2+}-currents and unresponsiveness to blockers. More recently, $GABA_A$ (R)receptors, $GABA_BR$, AMPAR and NMDAR have been identified as additional target molecules of Nrxn or Nrxn-ligand complexes by our laboratory and other groups. This talk will outline some of the relevant data with emphasis on unpublished results. We observed that overexpression of ectopic neurexophilin/α-Nrxns can recruit functional $GABA_AR$, $GABA_BR$ and NMDAR to transgenic synapses where they affect important properties of short-term plasticity. We also obtained novel data on the cross-talk with Ca^{2+}-channels by studying the impact of Nrxns on electrophysiological properties of $Ca_V2.2$ channels in relation to three different α2δ subunits. Co-expression of α-Nrxn, but not of β-Nrxn or SynCAM, impaired the maximal current density and steady state inactivation of Ca^{2+}-channels containing the α2δ3 isoform, whereas α-Nrxn had no effect in presence of α2δ1 and α2δ2 subunits. Although the exact mechanisms of how Nrxns interfere with Ca^{2+}-channel or receptor function is unclear, we found evidence that surface mobility of Nrxn is a key component in the process, and is regulated by co-expression with neurexophilins.

Structural and functional plasticity of adult born neurons in the mouse olfactory bulb

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The mammalian the olfactory bulb (OB) maintains a continuous inflow of new neurons to its circuitry throughout adulthood. The role of these newborn neurons in sensory processing or the bulbs' function remains unknown. We use *in vivo* imaging and electrophysiology to study the structure and function of these neurons. I will discuss the development and plasticity of adult-born interneurons during their development as well as that of their resident counterparts. We used two-photon imaging of single neurons to probe their morphology and two-photon targeted patch to study their physiology in high spatiotemporal resolution. Our data shows that odor responsiveness reaches a peak early during neuronal development, which then recedes at maturity. Sensory enrichment during development enhances the selectivity of adult-born neurons after maturation, without affecting neighboring resident neurons. Thus, in the OB circuit, adult-born neurons functionally integrate into the circuit, where they acquire distinct response profiles in an experience-dependent manner. We speculate that the constant flow of these sensitive neurons into the circuit provides it with a mechanism of long-term plasticity, wherein new neurons mature to encode odor information based on past demands.

Role of lysophosphatidic acid in traumatic brain injury

Andrew J. Morris

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My laboratory uses genetic, pharmacological and advanced mass spectrometry-based analytical approaches to investigate the involvement of bioactive lysophospholipids in human disease processes. Although our work to date has focused primarily on cardiovascular disease a growing body of evidence identifies a role for one of these lipids, lysophosphatidic acid (LPA) in the diagnosis and treatment of traumatic brain injury (TBI) which is a disruption of brain function resulting from a blow or jolt to the head or a penetrating head injury. We have profiled LPA molecular species and defined the mechanisms responsible for the synthesis and inactivation of LPA in human and rodent cerebrospinal fluid. I will present data from clinical studies in TBI patients identify a potential role for dynamic changes in CSF LPA in the onset of and recovery from neurotrauma. Studies using LPA directed antibodies in rodent models indicate that neutralization of LPA signaling improves TBI outcomes. Taken together, these data support the idea that LPA is a potential diagnostic marker and therapeutic target in TBI.

The speed of sound in the human brain

Prof. Micah M. Murray

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This talk will provide an overview our efforts to detail the spatio-temporal brain dynamics by which sounds of environmental objects are recognized. These efforts have been particularly facilitated by parallel developments in EEG signal analyses that on the one hand render EEG a true brain imaging technique and on the other hand allow for single-trial classification in individual subjects and patients. In a series of studies in healthy adults, we have delineated a temporal hierarchy over which environmental sounds are discriminated. The levels of discrimination include: general categorization as well as the identification of specific exemplars, conspecific vocalization discrimination, and associating sounds with their actions. We have likewise shown that sound object representations are subject to learning-induced plasticity across timescales from seconds to days and also as a function of the consistency in the spatial location of the sound across exposures. Application of single-trial classification techniques in both healthy subjects and clinical populations, including coma, are revealing links between specific phases of the abovementioned temporal hierarchy, conscious awareness, and perceptual decision-making. Collectively, our findings are revealing the multiple and dynamic representations of sound objects.

Number, topography and coupling to release of Ca^{2+} channels at hair cell active zones

Jakob Neef

The ribbon-type synapses between auditory inner hair cells and spiral ganglion neurons encode acoustic information with remarkable precision, reliability, and dynamics. Understanding the nanoanatomy and -physiology of the active zone (AZ) at the nanometer scale is crucial to elucidate the mechanisms employed by the cell to accomplish this challenging task. Our morphological efforts towards the topography of Ca^{2+} channels and readily releasable vesicles at the AZ comprise STED imaging of immunolabeled Ca^{2+} channels and AZ scaffolds and high resolution EM tomography.

We have utilized laser scanning and spinning disk confocal as well as STED Ca^{2+} imaging in order to quantify the number and distribution of Ca^{2+} channels at the AZ. Specifically, we counted the number of Ca^{2+} channels by optical fluctuation analysis and by quantifying the reduction of whole-cell Ca^{2+} current during imaging-controlled abolition of Ca^{2+} influx at a single AZ. Both methods converged on a range of approximately 20-250 channels per AZ. STED Ca^{2+} imaging revealed presynaptic Ca^{2+} nanodomains often also assuming the stripe-like shape that we found for the Ca^{2+} channel cluster. These approaches allow us to improve the accuracy of our biophysical modelling and thus promise to further our understanding of AZ Ca^{2+} signalling. They are also instrumental for our parallel efforts to decipher functional coupling of Ca^{2+} influx and exocytosis. There, experimental and theoretical work indicates that the release of a given fusion competent vesicle is controlled by few Ca^{2+} channels within nanometer meter proximity (Ca^{2+} nanodomain control of release).

Ca^{++}-handling and superpriming at the Calyx of Held

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Short-term plasticity is highly modulated by second messengers, such as Ca^{++} and diacylglycerol (DAG). At the Calyx of Held, there is pronounced heterogeneity between individual synapses, some showing moderate to strong depression during stimulus trains of 100 Hz or 200 Hz, others displaying a sequence of facilitation and depression. After application of phorbol ester, mimicking the effect of DAG, EPSCs are enlarged and strong depression only is observed. Lee et al. 2013 (PNAS 110, 15079) showed that application of DAG accelerates a process, which they termed 'superpriming' a slow transition of release-ready vesicles from a 'normally' primed state to a faster, 'superprimed' one. Some evidence will be shown, which suggests that the heterogeneity between synapses is due to different degrees of 'superpriming' at rest.

In order to distinguish between DAG-mediated effects and Ca^{++}-mediated ones, a precise quantitative description of Ca^{++}-buffers is necessary. Some data will be shown, which characterize such properties under conditions of both whole-cell recording and minimally perturbed cytoplasm.

Altered synaptic lipid signaling affects cortical information processing involved in psychiatric disorders

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Loss of the neuron-specific plasticity related gene 1 (PRG-1), which regulates synaptic phospholipid signaling, leads to neuronal hyperexcitability via increased glutamate release at the synapse. Using EEG-recordings in a human population-based cohort, we detected a balance shift between excitatory and inhibitory transmission (E/I-balance) in human mono-allelic carriers of a PRG-1 mutation (mutPRG-1) and impaired sensory gating, known to be an endophenotype of psychiatric diseases such as schizophrenia. mutPRG-1 leads to an arginine (R) to threonine (T) exchange at position 345 of the amino acid chain and affects approximately 3.5 million European and 1.5 million US citizens. Biochemical and electrophysiological studies showed that this mutation leads to a loss-of-PRG-1-function at the glutamatergic synapse due to its inability to control synaptic LPA-levels via a cellular uptake mechanism. Analysis of PRG-1+/- mice, which are an animal correlate of PRG-1+/mut carriers, revealed an altered cortical network function and behavior, indicative for psychiatric disorders. This could be reversed by modulation of phospholipid signaling via pharmacological inhibition of the LPA-synthesizing molecule autotaxin. Therapeutic intervention into bioactive lipid signaling might thus be a promising strategy to interfere with glutamate-dependent symptoms in psychiatric disorders such as schizophrenia.

Motor cortex independent skill execution

Bence P. Ölveczky

Motor skills - spatiotemporally precise motor patterns acquired through practice - underlie much of what we do, from playing instruments and sports to tying our shoelaces. Motor cortex is widely believed to be integral to skill execution, but this consensus was largely formed by studying tasks requiring fine digit control or constrained precision movements. Such studies could conflate motor cortex's established function as a controller of dexterous movements with a putative role in storing and generating learned motor sequences. To disambiguate these aspects, we trained rats to press a lever in a precise and reproducible temporal sequence – a difficult to master, but not necessarily dexterous, task. The complex motor sequences that emerged after weeks and months of training showed many of the hallmarks of human motor skills, being idiosyncratic, spatiotemporally precise, and stably maintained. Remarkably, motor cortex lesions had very little effect on the learned behaviors, which were expressed in their distinct pre-lesion form on the first day of post-lesion training. In contrast, animals trained to generate dexterous precision movements showed lasting impairments post-lesion. Our results reveal a previously unappreciated capacity of sub-cortical motor circuits to autonomously generate complex learned motor sequences and suggest that motor cortex's role in skill execution depends more on the nature of the movements than on the complexity of the underlying sequence.

Cross-talk between glutamate receptors

Julie Perroy

Although the notion of receptosome slowly becomes incontrovertible to understand the function of a receptor, not much is known about the versatility of protein-protein interactions and its functional consequences. Previous results indicate that group I mGlu receptors are indeed engaged in intricate and dynamic interactions with scaffold complexes that control not only mGlu receptor-own functions but also its cross-talk with other receptors. Our main objective is to identify where and when the integrity of the glutamate receptosome is disrupted in neurons, and to elucidate the functional significance of this phenomenon during long-term synaptic plasticity both in neurons and *in vivo,* studying associated behaviors. Defects in scaffold remodeling can lead to cognitive deficiencies, rescuing protein-protein interactions within the glutamate receptosome will restore adequate plasticity.

Novel activities of ATX in neuronal progenitors of the cerebral cortex

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The intricate formation of the cerebral cortex requires a well-coordinated series of events, which are regulated at the level of cell-autonomous and non-cell autonomous mechanisms. Neuronal progenitors proliferate in well-defined niches, at the ventricular zone and the subventricular zone. Whereas cell-autonomous mechanisms that regulate cortical development are well-studied, the non cell-autonomous mechanisms remain poorly understood. A non-biased screen allowed us to identify ATX as a non cell-autonomous regulator of neural stem cell proliferation. We will present our data defining novel activities of ATX in regulation of different populations of progenitors in the cerebral cortex.

Neurons in visual cortex retain a memory of their inputs after monocular deprivation

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A classic example of experience-dependent cortical plasticity is the shift in ocular dominance (OD) after monocular deprivation (MD). However, up to now, changes in cortical responsiveness after MD have largely been studied on the population level and experiments were mostly performed acutely. It therefore has remained unclear how changes in tuning and response strength of individual neurons give rise to global OD changes. In particular, it is unclear if population OD shifts are realized by changing the tuning of individual neurons (instructive plasticity), or by selective recruitment or silencing of distinct populations of cells (permissive plasticity). Moreover, it is unknown if and how single cells recover from their OD shift, given that MD is accompanied by the formation of long-term stable new spine synapses.
We perform chronic two photon imaging of cellular structure and function after viral cotransduction with a genetically encoded Ca^{2+} indicator together with a bright structural marker (AAV1/2-mRuby2-P2A-GCaMP6s). In adult mice, OD of the same excitatory L2/3 neurons imaged repeatedly over months is largely stable and tuning variance is not correlated with the equally stable absolute response amplitude. The change in OD after MD is achieved by a variable combination of increased open eye responses and decreased responses to the deprived eye, and is realized largely as instructive tuning change on the single cell level. Even though cellular OD-shifts after MD can be pronounced, the majority of cells faithfully return to their pre-deprivation OD after ~3 weeks of recovery. Therefore, L2/3 pyramidal neurons retain a clear memory of their baseline OD even after prominent experience-dependent plasticity. We currently assess how these reversible cellular OD shifts relate to functional and structural synaptic changes by chronic dual color imaging of dendritic spines of sparsely transfected neurons.

A sub-millisecond mGluR-NMDAR dialog triggering LTP

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Synaptic currents inside the narrow synaptic cleft could influence diffusion of electrically charged glutamate molecules. We find that in cerebellar synapses formed on electrically compact granule cells a single postsynaptic action potential transiently retards escape of released glutamate. This retardation boosts activation of perisynaptic group I metabotropic glutamate receptors (mGluRs). The latter rapidly, on a millisecodn scale, facilitates local NMDA receptor currents. The underlying mechanism involves a Homer-containing protein scaffold, but not GPCR- or Ca^{2+} -dependent signaling. Through the mGluR-NMDAR interaction, the coincidence between a postsynaptic spike and glutamate release triggers a lasting enhancement of synaptic transmission altering the basic integrate-and-spike rules in the local circuitry.

Huntingtin: Linking energy supply to axonal transport and neurotrophin signaling

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Huntington's disease is caused by the abnormal polyglutamine expansion in the N-ter part of huntingtin (HTT), a large protein of 350kDa. Over the past years, we proposed that HTT acts a scaffold for the molecular motors and through this function, regulates the efficiency and directionality of vesicular transport along microtubules in neurons. This function is conserved in Drosophila. In particular, HTT controls the microtubule-based fast axonal transport (FAT) of neurotrophic factors such as BDNF. PolyQ expansion in HTT alters this function, leading to a decrease in neurotrophic support and death of striatal neurons. Interestingly, the defect in transport might not be restricted to axons but could also involve defects in the retrograde transport of TrkB in striatal dendrites.
In addition to the role of HTT in scaffolding the molecular motors both in cortical and striatal neurons, we found that HTT scaffolds GAPDH on vesicles and that vesicular GAPDH is necessary to propel vesicles in GAPDH deficient neurons. Here we will extend these findings and discuss how HTT by specifically localizing the glycolytic machinery on vesicles may supply constant energy for the transport of vesicles over long distances in axons. We will also discuss how this machinery is altered in disease situation.

Mechanisms of ultrafast transmitter release at CNS synapses

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Active zones create a microdomain of 100 - 200 nm length where docked vesicles (usually, 3 - 10) can closely interact with voltage-gated Ca^{2+} channels at distances of 10 - 50 nanometer. Such short diffusional distances are a pre-requisite for fast activation of Ca^{2+} sensors during fast transmission. However, the exact co-localization of Ca^{2+} channels and docked vesicles is unknown, and active zone organization and tightness of Ca^{2+} channel - vesicle coupling might vary from synapse to synapse.

Here, we investigated the tightness of Ca^{2+} channel - vesicle coupling, and its possible rearrangement during postnatal development at the calyx of Held synapse. Using paired pre- and postsynaptic recordings, the steepness between release and Ca^{2+} entry (called "Ca^{2+} cooperativity") was high at postnatal days 9 - 11 (~ 4.5). This agrees with previous studies and suggests release control by as many as 10 - 15 channels ("domain overlap"; Meinrenken et al., 2002). At P15 - P16, the oldest age amenable to paired recordings, the Ca^{2+} cooperativity was still high (~ 3.5), although the efficiency of small Ca^{2+} charge in triggering release was increased. To extend our study to adult ages, we made use of EGTA-AM. Surprisingly, EGTA-AM blocked EPSCs in an indistinguishable fashion between P8 - P10, and P70 - P100 (~ 80% block by 200 µM at both ages). These data suggest that the calyx of Held uses a domain overlap regime for ultrafast release control up to adulthood, different from other fast-releasing synapses (Bucurenciu et al. 2010; Schmidt et al. 2013). Thus, domain overlap control of release seems to be advantageous for the function of calyx synapses, maybe because it allows efficient modulation of release probability following changes in Ca^{2+} channel open probability. In conclusion, active zone organization for ultrafast release can differ between different types of CNS synapses.

How the brain creates and encodes pitch and timbre percepts for complex sounds

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The pitch of a sound is perhaps one of its most salient properties. It allows us to distinguish and recognize melodies, and its salience appears to be exploited by a strikingly wide variety of vertebrate life-forms, from song birds and “singing” hump-back whales all the way to human composers of advertising jingles. But physiologically pitch is a surprisingly complex phenomenon. Not all sounds evoke a clear pitch, but those which do have *periodic* waveforms. This periodicity constrains their frequency content. A sound wave can only be periodic if all its frequency components “conform to the common underlying period”, i.e. the spectra of periodic sounds are always composed of the harmonics (integer multiples) of a given fundamental frequency. But not all harmonics need be equally intense. For example, in vowel sounds, the periodicity, and hence the spacing of the harmonics, will determine the pitch, but resonances in the vocal tract emphasize certain frequency ranges (the “formant” frequencies) which determines their spectral timbre, and hence the identity of a vowel. Thus, the brain transforms physical aspects of the sound waveform and spectrum, namely periodicity and spectral envelope, into the “perceptual qualities” of pitch and spectral timbre. In this presentation I will summarize behavioural and electrophysiological work we carried on ferrets over the last eight years to investigate the neural encoding of these fundamental sound properties. These studies have demonstrated that the pitch and timbre of vowel sounds are not encoded by separate populations of cortical neurons, but are instead represented in an interdependent, distributed and “multiplexed” manner. Interestingly, this widely distributed representation encodes the qualities an animal reports to have heard in its behaviour more accurately than the actual physical sound parameters, which suggests that these cortical codes represent perception more accurately than physical sound.

Genome-wide methylation analysis of monozygotic twins identifies association of protein phosphatase, Mg2+/Mn2+ dependent, 1G *(PPM1G)* hypermethylation with alcohol use disorder and measures of impulsiveness

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The genetic component of alcohol use disorder (AUD) is substantial but monozygotic (MZ) twin-discordance indicates a role for non-heritable differences that could be mediated by epigenetics. Despite growing evidence associating epigenetics and psychiatric disorders, it is unclear how epigenetics, particularly DNA-methylation, relate to brain function and behavior. We carried out a genome-wide analysis of DNA-methylation of 18 MZ twin-pairs discordant for AUD and validated differentially-methylated regions (DMR). Hypermethylation in the 3'-protein-phosphatase-1G (*PPM1G)* gene locus was associated with AUD (pfp<$1x10^{-5}$). After validation we characterized this DMR using personality trait-assessment and functional-magnetic resonance in a sample of 499 adolescents. We found association of *PPM1G*-hypermethylation with early escalation of alcohol use and increased impulsiveness. We observed association of *PPM1G*-hypermethylation with increased BOLD-response in the right subthalamic nucleus during an impulsiveness task. Overall, we identified and characterized an epigenetic marker associated with AUD in adults and with neurobehavioral risk factors for AUD in adolescents.

The role of endogenous kynurenic acid in hippocampal function and dysfunction

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(USA)

Kynurenic acid (KYNA) is an astrocyte-derived tryptophan metabolite that antagonizes the α7 nicotinic acetylcholine receptor and, possibly, the glycine co-agonist site of the NMDA receptor at endogenous brain concentrations. As both receptors are intimately involved in brain development, synaptic plasticity and cognitive processes, KYNA elevations may impair cognitive functions. Moreover, since KYNA levels are significantly increased in the brain of individuals with schizophrenia (SZ), the metabolite may be causally involved in the pathophysiology of cognitive deficits seen in this disease. Studies in rats and mice have shown that fluctuations in KYNA in the endogenous, i.e. nanomolar, range *bi-directionally* modulate extracellular glutamate levels in the hippocampus. Applied locally by reverse dialysis, KYNA (100 nM) reduces extracellular glutamate in the hippocampus by ~40% of baseline levels within one hour. This effect, which is readily reversible upon discontinuation of the KYNA perfusion, is duplicated by systemic administration of the KYNA precursor kynurenine. Conversely, attenuation of KYNA neosynthesis by selective kynurenine aminotransferase II (KAT II) inhibition reduces extracellular KYNA (-30% of baseline values) and significantly increases extracellular glutamate levels (+80% of baseline values) in the rat hippocampus. These biochemical changes are associated, respectively, with impaired (more KYNA, less glutamate) and improved (less KYNA, more glutamate) performance in hippocampus-dependent cognitive tasks (passive avoidance, Morris water maze). Taken together with recent studies demonstrating long-term cognitive dysfunctions following perinatal kynurenine treatment in rats, these experiments suggest that a reduction in KYNA formation through KAT II inhibition constitutes an attractive new strategy for cognitive enhancement under physiological conditions and may be especially efficacious in the treatment of cognitive deficits in SZ.

Divide and conquer - Synthetic biology of cell division

Petra Schwille

In recent years, biophysics has accumulated an impressive selection of novel techniques to analyze biological systems with ultimate sensitivity and precision. Single molecule imaging, tracking and manipulation have enabled us to unravel biological phenomena with unprecedented analytical power, and to come closer to revealing fundamental features of biological self-organization. The power of physics has always been the reductionist approach, i.e. the possibility to define an appropriate subsystem simple enough to be quantitatively modeled and described, but complex enough to retain the essential features of its real counterpart. Transferring this approach into biology has so far been extremely challenging, because most “modern” biological systems usually comprise so many modules and elements, many of them still awaiting to be functionally resolved, that it is a risky task to define truly essential ones. Nevertheless, the strive for identifying minimal biological systems, particularly of subcellular structures or modules, has in the past years been very successful, and crucial *in vitro* experiments with reduced complexity can nowadays be performed, e.g., on reconstituted cytoskeleton and membrane systems. As a particularly exciting example for the power of minimal systems, self-organization of essential proteins of the bacterial cell division machinery could be shown in a simple assay, consisting of only two protein species, an energy source, and a membrane. In my talk, I will discuss some recent results of our work on membrane-based systems, using single molecule optics and biological reconstitution assays. I will further discuss the perspective of assembling a minimal system to reconstitute bacterial cell division.

Number and organization of Ca^{2+} channels in the active zone of Schaffer collateral synapses

Annalisa Scimemi

The relative distribution of calcium channels and neurotransmitter vesicles in the active zone has important functional implications for neurotransmitter release. It has been suggested that at many central synapses there is an abundance of calcium channels in the active zone and that specific molecules tether calcium channels in the active zone to neighboring neurotransmitter vesicles ready to undergo release. By using a combination of patch-clamp recordings and two-photon calcium imaging experiments in acute mouse hippocampal slices we show that, at CA3-CA1 hippocampal synapses, glutamate release from individual vesicles is typically initiated by opening of a single calcium channel. By using realistic 3D reaction-diffusion simulations, we examine how the release properties of the vesicles docked at the active zone vary depending on whether every vesicle is stereotypically coupled or randomly placed close to one calcium channel. The largest heterogeneity in the release properties of the vesicles is observed when the calcium channels are few and randomly distributed with respect to neurotransmitter vesicles docked at the active zone. Under these conditions, the release probability of the synapse approximates the release probability of the vesicle closest to a neighboring calcium channel. The calcium dependence of the paired-pulse facilitation measured in the electrophysiology experiments is best reproduced by the simulations when few calcium channels are randomly spread within the active zone. These findings suggest that different central synapses may have distinct functional organizations, and that the existence of molecular tethers may not be indicative of a stereotypic coupling efficiency of calcium channels and vesicles in the active zone of small central synapses.

Presynaptic spinophilin restricts neurexin signaling to ensure typical design of presynaptic active zones

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Bi-directional signaling mechanisms entailing Neurexin (Nrx) / Neuroligin (Nlg) family cell adhesion molecules are wild held responsible for coordinating assembly between presynaptic active zone and postsynaptic neurotransmitter receptors. The mechanisms cross-linking and adapting Nrx/Nlg signaling to the synaptic assembly program are actively investigated.

Previously, the presynaptic cytoplasmic scaffold protein Syd-1 was shown to promote Nrx-1/Nlg-1 signaling, and consequently active zone assembly at glutamatergic synapses of developing Drosophila neuromuscular terminals.

Here, we find that presynaptic scaffold protein Spinophilin (Spn) limits synaptic Nrx-1/Nlg-1–signaling, operating antagonistic to Syd-1. Screening through candidate proteins, we found that loss of presynaptic Spn provoked the formation of too many but too small active zone scaffolds, resulting in excessive spontaneous release and changed short-term plasticity of evoked release. Nrx-1/Syd-1 clusters increased at spn terminals, and heterozygosity for nrx, syd and nlg-1 effectively suppressed the spn phenotypes. Molecularly, the PDZ domain of Spinophilin strongly bound the Nrx C-terminus using highly conserved motifs, and Nrx-motility was suppressed in absence of Spn. Our analysis suggests that presynaptic Spn provides a molecular “buffer/sponge” which limits the entry of Nrx-1 into signaling complexes, where it could promote synapse assembly together with Syd-1.

Vesicle mobility and supply at a central excitatory synapse

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The speed at which sensory representations can be formed in the brain depends on the transmission characteristics of axons and synapses in the sensory pathways. Cerebellar mossy fiber synapses transmit rate-coded signals over a wide bandwidth by rapidly reloading vesicles from a large pool. Here we examine whether vesicle supply at these large central synapses could be mediated by diffusion, as proposed at ribbon-type synapses. Vesicle mobility was measured by performing FRAP in slices from Venus-VGLUT1 knock-in mice. Conventional analysis revealed a high apparent vesicle diffusion coefficient. 3D Monte Carlo reaction-diffusion simulations constrained by our vesicle and mitochondrial density measurements, predicted that vesicle mobility on the nanometer scale is 3-fold higher for larger spatial scales due to vesicle crowding, while simulations of the active zone predicted supply rates up to 1 kHz. Our results establish that vesicle mobility at this central synapse is comparable to Ribbon type synapses, and suggest that Brownian motion-based vesicles supply is substantially faster than reloading rates.

Funded by the Wellcome trust

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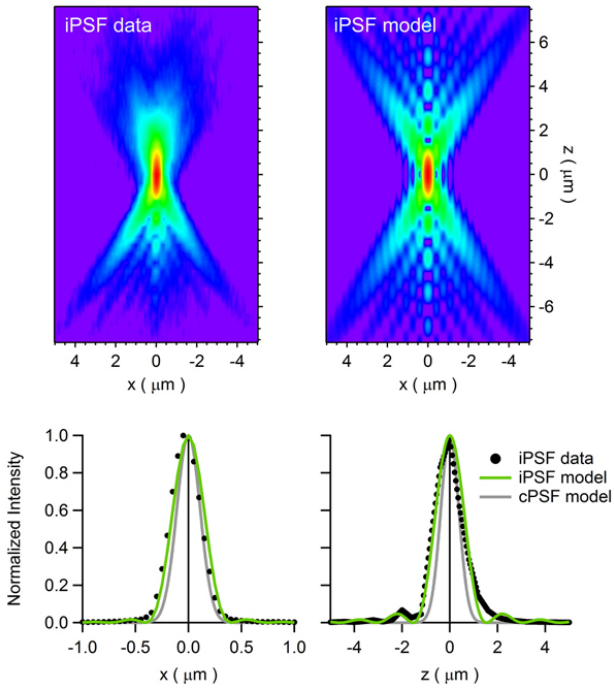

Figure 1. *X-Z profile of the illumination point-spread function (iPSF, top row; y=0) of our confocal microscope (data) and a fit to a theoretical function (model). Bottom row shows center cross sections (z=0 and x=0) and for comparison the confocal point-spread function (cPSF, gray) estimated from bead line scans.*

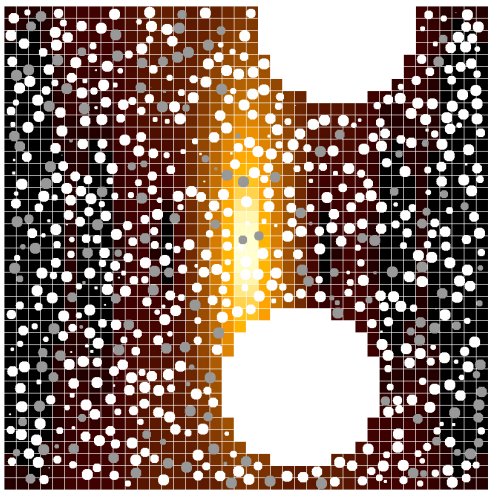

Figure 2. *X-Z cross section through one Monte Carlo simulation of a FRAP experiment inside a MF terminal. The simulation consisted of a 2 um cube with randomly placed mitochondria (large white regions defined as non-diffusible space) and randomly placed 50 nm fluorescent vesicles that were either mobile (white) or immobile (gray). The illumination PSF (yellow) drove the vesicle fluorescence bleaching reaction.*

Stem cells and biomaterials for treatment of CNS diseases

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Stem cells have been investigated for their therapeutic potential in spinal cord injury (SCI). We compared human mesenchymal stem cells (MSC) from bone marrow, a conditionally immortalized human stem cell line from fetal spinal cord (SPC-01) and human induced pluripotent stem cell-derived neural precursors (iPS-NPs) for their capacity to migrate towards lesion sites, differentiate and induce better regeneration. We used a balloon-induced compression lesion in rats, followed by the transplantation of MSC, SPC-01 or iPS-NPs labeled in culture with iron-oxide nanoparticles for MRI tracking. Electrophysiology was used to study the properties of stem cell-derived neurons in vitro. Animals were tested using the BBB (motor) and plantar (sensory) tests for up to 6 months after acute (7 days post-injury) or chronic (5 weeks post-injury) transplantation. Various biocompatible hydrogels (degradable and nondegradable), including those based on non-woven nanofibres, have been developed for bridging tissue defects and for use as 3D stem cell carriers. Animals with chronic injury were implanted with a PHPMA hydrogel seeded in vitro with cells. Ca^{2+} imaging on single SPC-01 cells revealed voltage-activated Ca^{2+} channels, typically observed in neurons. In vivo MRI proved that MSC, SPC-01 or iPS-NPs migrated into the lesion and survived for several months. Implanted animals showed functional improvement; MSC rarely differentiated into neurons, while SPC-01 or iNP-NP implantation resulted in greater improvement, and many implanted cells differentiated into motoneurons. Improved motor and sensory scores in chronic SCI were found after the implantation of biomaterials seeded with MSC or SPC-01. Recently, we also used hydrogels composed of decellularized porcine extracellular matrix (ECM), which can facilitate constructive remodelling of various tissues (Badylak et al., Acta biomaterialia 5:1-13, 2009). After implantation into SCI, we found considerable ingrowth of neurofilaments and blood vessels into this biological scaffold. The ECM biological scaffolds are therefore promising candidates for clinical use not only in the oesophagus, lower urinary tract, musles, tendons and myocardium, but even in spinal cord repair. A clinical trial using scaffolds seeded with stem cells is under consideration.

Supported by GACR 13-00939S, P304/12/G069 and 14-14961S

Trafficking and fusion of dense core vesicles in mammalian CNS neurons

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The regulated secretion of chemical signals in the brain occurs principally from two organelles, synaptic vesicles and dense core vesicles (DCVs). DCVs contain a diverse collection of cargo, including many neuropeptides that trigger a multitude of modulatory effects with quite robust impact, for instance on memory, mood, pain, appetite or social behavior. In addition, many other signals depend on DCVs, like trophic factors, but also signals that typically do not diffuse like guidance cues. Hence, it is beyond doubt that DCV signalling is a central factor in brain communication. However, many fundamental questions remain open on DCV trafficking and secretion. My lab has established new photonic approaches to quantitatively characterize DCV-trafficking and fusion of many cargo types in living mammalian CNS neurons with single vesicle resolution using dual reporter cargo constructs. We found that DCV secretion is quite different from other forms of regulated secretion. DCVs often do not stably dock before fusion, molecular factors essential for synaptic vesicle release are dispensable for DCV fusion and DCV fusion occurs with different kinetics at different cellular locations. The priming proteins Munc13 and CAPS are central factors in regulating both the kinetics and the location of DCV fusion events.

Control of early neuronal activity by synaptic phospholipids governs connectivity and memory

Johannes Vogt

Early neuronal activity is essential for proper brain connectivity. Already at the time when neurons start to form synaptic contacts, synchronized activity occurring over large groups of neurons influences developmental processes from correct formation of neuronal circuits up to transmitter specification. While the roles of spontaneously generated activity patterns begin to be understood, the molecular mechanisms synchronizing neuronal activity are largely unknown. Synaptic phospholipids modulate neuronal excitability in the juvenile brain suggesting an important role for bioactive lipids in the regulation of neuronal activity. Here we show that proper onset of early synchronous neuronal activity depends on intact synaptic bioactive lipid signaling and critically influences neuronal circuit formation. Entorhinal neuronal networks are essential for memory capabilities and develop several days prior to the input derived from external stimuli. During this period, synchronous activity occurs in a time-locked developmental fashion and alteration of synaptic bioactive lipid signaling induced a premature onset of synchronous activity. Premature onset of synchronized neuronal activity by only 2 days resulted in structural reduction of the entorhinal-hippocampal perforant path leading to memory deficits during adulthood. While it is widely accepted that delayed onset of neuronal activity can disturb proper brain development, here we show that premature synchronization of neuronal networks is equal detrimental. Our findings indicate that homeostatic regulation of neuronal activity – not too early and not too much – is of critical importance for proper brain development and describe an unexpected role of synaptic bioactive lipids in neuronal synchronization during early postnatal development.

TRAP1, a new player in Parkinson's disease

Aaron Voigt

Parkinson's disease (PD) is the second most common neurodegenerative disease. Despite extensive research efforts until today there neither is cure nor treatment to stop progression of the disease. Thus, understanding of the disease etiology is the key for a rational design of therapies. We show that the mitochondrial chaperone TRAP1 has a close connection to PD. First, overexpression of TRAP1 mitigates toxicity induced by a PD-associated mutant form of α-Synuclein *in vitro* and *in vivo*, whereas TRAP1 loss-of-function enhances α-Synuclein-induced toxicity. Moreover, TRAP1 is a substrate of the PD-linked kinase PINK1. PINK1 is protective against oxidative stress-induced cell death and this effect requires the phosphorylation of TRAP1 by PINK1. Furthermore, TRAP1 rescues PINK1 loss-of-function phenotypes and these rescuing effects demand TRAP1's ATPase activity and mitochondrial location. Interestingly, TRAP1 does not rescue phenotypes in case of Parkin deficiency and seems to act independently of PINK1/Parkin-mediated mitophagy. Altogether, these data strongly suggest that TRAP1 plays an important role in the eitiology of PD.

New cellular pathways

Heinz Wiendl

Department of Neurology, Muenster University

Multiple sclerosis is a complex CNS autoimmune disease with inflammatory neurodegenerative components. The interplay between the immune and the nervous system has elucidated and unrevealed a variety of new cellular pathways that have completed the earlier prevailing view of MS representing a mainly CD4 T helper cell mediated CNS disease. Most recently cytotoxic T cells, regulatory T cells and regulatory immune cells as well as a number of non-classical immune cells including natural killer cells, γδ T cells or other innate lymphoid cells have been demonstrated to be relevant in elegant animal models but also in humans with multiple scleroses, both as element involved in neural degeneration as well as important therapeutic goals for disease modification.
The talk will highlight the most recent advances in our understanding of natural killer cells in CNS immune regulation as well as the relevance of cytotoxic T cells in neuronal damage.

Parkin maintains mitochondrial integrity via linear ubiquitination of NEMO

A. Kathrin Müller-Rischart[1], Jörg Tatzelt[1,2], **Konstanze F. Winklhofer**[1,2]

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Mutations in the parkin gene, coding for an RBR-type E3 ubiquitin ligase, are linked to autosomal recessive Parkinson's disease. Parkin has a wide neuroprotective activity and prevents cell death in various stress paradigms. We recently observed that the pro-survival activity of parkin is lost in the absence of NEMO, the core regulatory component of the I-kappa B kinase complex which is essential for canonical NF-kappa B activation. It turned out that parkin is recruited to the linear ubiquitin assembly complex (LUBAC) under cellular stress to increase LUBAC activity and linear ubiquitination of NEMO. In a next step we established a link between parkin, NF-kappa B and mitochondria. Parkin can reduce cytochrome c release, and we found that this activity of parkin is also dependent on NEMO. Cytochrome c release is controlled by the GTPase OPA1, which is located at the inner mitochondrial membrane and prevents apoptotic cristae remodeling. We identified OPA1 as a novel NF-kappa B target gene that is transcriptionally upregulated via NF-kappa B-responsive promoter elements to maintain mitochondrial integrity and to protect from stress-induced cell death. Notably, in parkin-deficient cells linear ubiquitination of NEMO, activation of NF-kappa B, and upregulation of OPA1 is significantly reduced in response to TNFalpha stimulation, supporting the physiological relevance of parkin in regulating this anti-apoptotic pathway.

Post-fusion structural changes and their roles in exocytosis and endocytosis

Ling-Gang Wu

Vesicle fusion with the plasma membrane generates an Ω-shaped membrane profile. Its pore is thought to dilate until flattening (full-collapse), followed by classical endocytosis to retrieve vesicles. Alternatively, the pore may close (kiss-and-run), but the triggering mechanisms and its endocytic roles remain poorly understood. Here, using confocal and STED imaging of dense-core vesicles, we find that fusion-generated Ω-profiles may enlarge or shrink while maintaining vesicular membrane proteins. Closure of fusion-generated Ω-profiles, which produces various sizes of vesicles, is the dominant mechanism mediating rapid and slow endocytosis within ~1-30 s. Strong calcium influx triggers dynamin-mediated closure. Weak calcium influx does not promote closure, but facilitates the merging of Ω-profiles with the plasma membrane via shrinking rather than full-collapse. These results establish a model, termed Ω-exo-endocytosis, in which the fusion-generated Ω-profile may shrink to merge with the plasma membrane, change in size, or change in size then close in response to calcium, which is the main mechanism to retrieve dense-core vesicles.

Role of PINK1 and Parkin on mitochondrial QC *in vitro* and *in vivo*

Richard Youle

SNB, NINDS, NIH

Autosomal recessive mutations in PINK1 and Parkin can lead to parkinsonism. Genetic studies indicate that the mitochondrial kinase PINK1 and the RING-between-RING E3 ligase Parkin function in the same pathway. In concurrence, mechanistic studies show that PINK1 can recruit Parkin from the cytosol to the mitochondria and activate Parkin ubiquitination activity and Parkin-mediated mitophagy. How PINK1 activates Parkin ubiquitin ligase activity and how Parkin recognizes damaged mitochondria and mediates mitophagy remain unclear. We have identified a substrate of endogenous PINK1 kinase activity that can activate Parkin in cell free assays explaining how PINK1 activates and recruits Parkin. Recent work also indicates that Parkin activates Rab7 to mediate autophagosomal enulfment of damaged mitochondria. In vivo results in mouse models showing that endogenous Parkin can mitigate mitochondrial damage to prevent substantial nigral neuron loss will also be presented.

Regulation of calcium channels by ubiquitination

Gerald W. Zamponi

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Chronic pain is a severely debilitating condition that often reflects long-term sensitization of signal transduction in the afferent pain pathway. One target site to alleviate chronic pain is the dorsal root ganglion (DRG), which is the site of origin of both primary afferent axons and synaptic projections to the spinal dorsal horn. T-type (Cav3) calcium channels are the key ionic contributors that control both the excitability of DRG afferent axons and transmitter exocytosis at the dorsal horn synapses. Moreover, the Cav3.2 calcium channel isoform is known to exhibit aberrant upregulation in DRG cells following nerve injury and inflammation concomitant with development of pain hypersensitivity Our lab has discovered a novel mode of T-type channel regulation – the dynamic modification of channel trafficking to the membrane by ubiquitination and deubiquitination, a post-translational modification that regulates channel degradation. Specifically, we have shown that T-type channels are under the control of two enzymes - the ubiquitin ligase WWP1 and the de-ubiquitinase USP5 - whose competing activities determine the level of T-type channel ubiquitination and hence their density in the plasma membrane. We have shown that this process is dysregulated in both inflammatory and neuropathic pain. The common endpoint in both conditions is an upregulation of T-type channel membrane density that is expected to increase cell excitability and to enhance central synaptic transmission. Interfering with USP5 expression, or uncoupling USP5 from Cav3.2 channels *in vivo* by using TAT disruptor peptides results in potent analgesia and a reduction in synaptic communication in the dorsal horn. Altogether our data reveal a novel mechanism of pain sensitization and suggest new approaches for pain therapeutics.

Novel mechanisms and targets in Multiple sclerosis

Frauke Zipp

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Multiple sclerosis and its animal model, experimental autoimmune encephalomyelitis, are chronic, T-cell dependent diseases of the central nervous system (CNS). The reasons why autoimmune T-cell responses in the CNS tend to persist and/or relapse are, however, not clear. By applying intravital two-photon laser scanning microscopy, we demonstrate the gatekeeper function of CNS dendritic cells (DCs) in neuroinflammation. Within developing CNS lesions, CD11c-positive dendritic cells were found to preferentially interact with encephalitogenic interleukin (IL)-17A-EGFP/tdRFP double-positive T helper (T_H17) cells. Depleting CD11c-GFP cells markedly reduced disease severity and the survival of T_H17 in the CNS. Interaction of T_H17 cells with DCs, with the T_H17 cells in their state as high IL-17 and high GM-CSF producers, resulted in a strong increase in CNS DC numbers. In the inflamed CNS, DCs were organized in perivascular clusters, which were targeted by encephalitogenic T cells and critically determined their survival in the CNS. Our findings demonstrate a fundamental role of CNS DCs in the migration into and survival of T_H17 cells in the non-lymphoid organ CNS. In addition, our group and others have found that DC functions and their interaction with effector T_H17 cells provide novel targets for therapeutic concepts, which are currently in clinical testing.

Abstracts Posters

Abstracts are listed alphabetically according to presenting author

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Mitochondrial contributions to neuronal autophagy: Links to energetics and mitophagy?

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Dysfunctional mitochondria are recognized as a common theme amongst various neuropathologies. Recent genetic studies of Parkinson's disease have revealed mutations of PINK1 and parkin, which regulate degradation of damaged mitochondria through autophagy. Here we investigated neuronal auto-/mito-phagy in primary neurons. Primary cultures of cerebellar granule cells (Swiss mice) were exposed to insults targeting mitochondrial respiratory chain complexes I-V (rotenone, 3-nitropropionic acid, antimycin A, KCN and oligomycin respectively) to induce dysfunctional mitochondria. The extent of bioenergetic failure was determined by observing the level of ATP, depolarisation of mitochondrial membrane potential and decrease in oxygen consumption rate measured by the Seahorse XF24 extracellular flux analyzer. All stressors produced mitochondrial dysfunction as shown by concentration-, time-dependent decline in ATP over 4-24h ($n \geq 3$). Neurons with dysfunctional complex I, III and IV showed rapid loss of mitochondrial membrane potential and concentration-, time-dependent decrease in oxygen consumption rate over 4-24h ($n=5$). Neurons with dysfunctional complex II showed significant reduction (~80% reduction) in mitochondrial reserve capacity and oxygen consumption, showing the most significant damage to mitochondrial bioenergetics. Investigation of autophagy was followed by observing significant accumulation of puncta acidic vacuoles in cytoplasm after 4h of drug treatment ($p<0.05$) labelled by monodansylcadaverine. Immunofluorescent detection of PINK1 antibody revealed cytoplasmic translocation of PINK1 and immunoblotting for microtubule-associated protein 1 light chain 3 (LC3-I/II) showed overall increase in LC3-II bands 24h after inhibition of respiratory complexes, especially with complex I ($p<0.05$) and II inhibition ($p<0.01$; both $n=3$), suggesting the involvement of autophagic mechanisms with likely involvement of mitophagy.

Response to trauma and abuse presenting with overactivity, impulsivity and distractibility that is not secondary to severe ADHD symptoms but the fight and flight response, implications for treatments

Klaus Martin Beckmann

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ADHD and response to trauma on the surface can present similarly. Not all clients who present with ADHD symptoms may have the underlying neuropsychiatric disorder that responds to ADHD medication. This poster suggests that PTSD is a differential diagnosis to ADHD. PTSD that presents with ADHD symptoms will unlikely respond to ADHD medication.

Visual speech gestures modulate efferent auditory system

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Visual and auditory systems interact at both cortical and subcortical levels. Recent studies have demonstrated that visual speech gestures can modulate early auditory brainstem activity as evidenced by changes in amplitudes and latencies of evoked auditory brainstem responses (e.g. Musacchia, Sams, Nicol & Kraus, 2006). The present study builds on this work by testing 15 young healthy adults on whether visual speech stimuli evoke different responses in the auditory efferent system compared to visual non-speech stimuli. The descending cortical influences on medial olivocochlear (MOC) activity were indirectly assessed by examining the effects of contralateral suppression on Transient-evoked otoacoustic emissions (TEOAEs) under 3 conditions: (a) in the absence of any contralateral noise (Baseline or BL), (b) contralateral noise + observing facial speech gestures related to productions of vowels a and u (AU) and (c) contralateral noise + observing facial non-speech gestures related to smiling and frowning (SF). The results demonstrated that observing facial speech gestures (AU condition) yielded a significant increase in suppression ($p < 0.05$) relative to the BL and SF conditions, but this was only evident for 2 kHz, not 1 kHz TEOAE frequency. These results indicate that observing a speech gesture compared to a non-speech gesture may differentially trigger MOC activity, possibly to enhance peripheral neural encoding. This finding of audiovisual integration at a peripheral level is at odds with theories that suggest that these modalities are processed as unimodal sensory streams and only integrate at higher-order cortical structures (Massaro, 1998).

Left-right asymmetry is required for the habenulae to respond to both visual and olfactory stimuli

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The exquisite efficiency of many cognitive and behavioural processes is thought to be achieved by asymmetric processing of information in CNS circuits. However, it remains unclear how asymmetries are encoded within neural circuits, and how lateralization is important for normal brain function and, when altered, contributes to neurological deficits.

We have previously shown that the asymmetric epithalamic circuit of zebrafish, comprising the bilateral habenular nuclei and the asymmetric pineal complex, is one of the best models to study the formation of brain asymmetries and lateralized behaviours. We have now demonstrated that sensory responses are lateralized in the zebrafish habenulae. We find that in larval zebrafish, most habenular neurons that respond to light are on the left, whereas neurons that respond to odour stimuli are on the right.
In addition, we demonstrated that loss of brain asymmetry leads to loss of responsiveness to visual or olfactory inputs. Manipulations that reverse the direction of brain asymmetry reverse the functional properties of habenular neurons, whereas manipulations that generate symmetric double-left or double-right sided brains lead to loss of responsiveness to odour or light respectively.

Our results indicate that loss of brain lateralization has significant consequences upon sensory processing and circuit function. Various neurological conditions are associated with abnormalities in the lateralization of brain activity, but it is unclear if these abnormalities are a cause or consequence of disease. This study raises the possibility that defects in the establishment of brain lateralization could indeed be causative of cognitive or other symptoms of brain dysfunction.

Can GAP-43 be an early marker of neuronal stress? – In vivo imaging and immunofluorescence study of GAP-43 after ischemic brain lesion

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Growth Associated Protein 43 (GAP-43) is neuron specific phosphoprotein involved in neurites growth and plasticity-associated processes. It is present in the developing nervous system, but its expression remains silent in adult brain. The transgenic mouse model where luciferase was coupled to GAP-43 demonstrated through in vivo imaging strong up-regulation of GAP-43 was observed in adult neurons following transient cerebral ischemia.

In order to clarify the role of GAP-43 during acute phase after transient cerebral ischemia, Gap-43 fluc/gfp reporter mice were used. The ischemic brain lesion was induced by 1 hour transient middle cerebral artery occlusion (tMCAO). The spatial and temporal dynamics of the GAP-43 were monitored in vivo using biophotonic/bioluminescence signals. GAP-43 was significantly induced 24 hours after MCAO and remained upregulated during first 4 days of acute phase that were examined. To address the question whether GAP-43 was associated with early neuronal stress, VivoGlo™ Caspase-3/7 substrate was administered to Gap-43 fluc-gfp mice 1 and 4 days after tMCAO. Upon activation of caspase-3 or -7, DEVD peptide was intracellularly cleaved and then liberated, after which aminoluciferin reacted with luciferase and generated light.

This innovative application demonstrated an increase in bioluminescence signal following VivoGlo administration after tMCAO, suggesting a caspase-3 activity in the cells expressing the GAP-43 driven luciferase. Immunohistological analysis revealed localization of GAP-43 in NeuN positive cells - neurons. Furthermore, GAP-43 staining was co-localizing with cleaved-caspase-3 and ATF-3 markers, confirming that early GAP-43 induction was associated with the early neuronal stress/apoptosis response to transient ischemic injury.

Based on our findings, early induction of the GAP-43 signal in ischemic neurons may be associated with the initial, ischemia-induced neuronal stress.

Acknowledgments: This work was supported by EU FP7 project GlowBrain.

Tissue oxygen measurement for patients with brain injury

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Introduction: Multimodal monitoring is of vital importance in neurointensive care. In recent years, multimodal monitoring has gradually incorporated also the measurement of tissue oxygen in the brain tissue.

Materials and Methods: We present a study of 29 patients. All patients had severe brain injury (GCS≤7), 14 patients were treated with subdural haematoma, 4 with brain contusions, 5 with epidural bleeding and 6 patients were treated with diffuse axonal injury. All patients were initially implanted sensor for measurement of intracranial pressure (ICP), 14 patients were also monitored by a sensor for measuring $PbtO^2$. The data were recorded every hour. Patients were treated conservatively and surgically according to standard protocol of EBIC. Within the group, we analyzed the effect of different monitoring modalities and their combination in treatment strategy in these patients.

Results: Studied variables were in the following ranges (mean ± standard deviation): ICP 16.4 ± 5.8 mmHg, $PbtO^2$ 23.6 ± 6.3 mmHg, MAP 92.7 ± 11.4 mmHg. Statistical evaluation of the data (Pearson correlation coefficient) was found a high correlation between ICP and $PbtO^2$ with a correlation coefficient of 0.76. Total data was taken from 3 680 hours measurement, the shortest record includes data from 68 hours, the longest of 183 hours. The group achieved these results by medical GOS: good recovery - 6 patients, moderate disability - 5 patients, severe disability - 7, vegetative state-6patients,death-5patients.

Conclusions: Measurement of $PbtO^2$ provides continuous quantitative data which contributes to the correct management of treatment and important prognostic and pathophysiological data for the detection of secondary brain injuries. It was confirmed by a strong correlation between levels of $PbtO^2$ and ICP. More detailed assessment of the benefit measurement of tissue oxygen in the evaluation of outcome requires inclusion of a larger number of patients

Supported by grant from the Ministry of Health of the Czech Republic IGA MZČR n. NT12116-4.

Distribution and roles of the Onecut transcription factors in spinal dorsal interneurons

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Dorsal spinal cord interneurons are known to process, integrate and relay somatosensory information from peripheral sensory neurons to higher brains centres. Recent works demonstrated that some embryonic dorsal interneurons populations also migrate ventrally and participate in motor circuits. The correct establishment of regional neuronal identities during embryogenesis is necessary for proper formation of these neuronal circuitries.

Onecut (OC) transcription factors constitute a particular family of transcriptional activators present in the endoderm and in the CNS during embryonic development. They have been shown to control neuronal differentiation and migration in murine encephalon and ventral spinal cord but little is known about their role in spinal dorsal interneuron development.

Here, we report that OC factors are present in early-born dorsal interneurons. At embryonic day (e) 10.0, OC proteins are detected in 30 to 100% postmitotic interneurons of dorsal subpopulations dI2 to dI6. However, OC distribution is rapidly restricted to a few cells. At e12.5, OC factors are absent in dI2 while dI3 to dI6 subpopulations show 10 to 25% OC-positive neurons. This suggests that OC transcription factors may play an early role in the differentiation of dorsal interneurons. To address this hypothesis, loss-of-function and gain-of-function experiments have been undertaken, from which results will be presented.

Activation of cannabinoid receptor 1 induces ramification in primary microglia cells through activation of the signaling cascade PKCε-Src/Fyn-Raf-ERK1/2

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Microglia are the resident immune cells of the Central Nervous System, and are characterized by their ability to undergo morphological and functional transformation upon activation, triggered by many pathological conditions, such as infection, trauma, and neurodegenerative disorders. The mechanisms of how microglia transforms to its ameboid activated phenotype has been thoroughly studied, whereas how microglia assumes its ramified inactive phenotype, an important feature of development and of neuroinflammation, are less understood. Cannabinoids, plant-derived, synthetic, or endocannabinoids, are neuroprotective against excitotoxicity and acute brain damage, both in vivo and in culture. Exerting their action through activation of cannabinoid receptors 1 and 2 (CB1R, CB2R), may confer neuroprotection by blocking excitotoxicity, enhancing trophic factor support, or by suppressing neuroinflammation. Here, we report that activation of CB1R in primary murine microglia in culture triggers acute actin cytoskeleton alterations that induced in the long term ramification. The CB1R agonist R(+)-MA specifically stimulated tyrosine phosphorylation of several F-actin binding proteins, while activating ERK1/2. The time-course of R(+)-MA-induced ERK activation revealed a significant increase by 10min that lasted up to 30min and gradually decline thereafter. Similar time courses were established for the tyrosine kinase Fyn and of c-Raf, while pharmacological analysis with appropriate inhibitors showed that this Fyn/Raf/ERK pathway was $G_{q/11}$, and PKCε-dependent and that it emanated from the lipid rafts. Taken together our results may indicate a role of cannabinoid use in chronic inflammatory disorders of the CNS (AK is a National Foundation of Greece (IKY) scholarship recipient).

LH stimulation could potentiate the effect of inefective dose of morphine and induce morphine sensitization

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Orexin has a crutial role in drug- seeking behavior. Lateral hypothalamus (LH) is a main region for producing orexin and their projections to ventral tegmental area (VTA) play an important role in reward and addiction- related behavior.
Material and methods: In this study we investigate the role of LH stimulation and orexinergic system in morphine sensitization. Adult male Wistar rats were divided to two groups. All of animals implanted unilaterally into the LH and different doses of carbachol (62.5,125 and 250 nmol/0.5 µl saline) as a cholinergic agonist microinjected into this area for 3 days consecutively (sensitization period). In one of groups, the animals received carbachol during sensitization period alone but the other group received carbachol 5 min before administration of ineffective dose of morphine (0.5 mg/kg; s.c.) in a room distinct from which conditioning occurred. After 5 days, the conditioned place preference (CPP) paradigm was induced by ineffective dose of morphine (0.5 mg/kg) and CPP score represents by the differences in time spent in drug- and saline-paired compartment.
Results:The results revealed that using carbachol for stimulating LH (without morphine injection) could not affect morphine- induced CPP but, administration of carbachol and ineffective dose of morphine (0.5 mg/kg) simultaneously induced CPP significantly.
Conclusion: Our finding revealed that LH stimulation could potentiate the effect of inefective dose of morphine and induce morphine sensitization. It seems that there is an interaction between orexinegic system and opioid system in morphine sensitization.

Key Word: LH, Sensitization, Carbachol, Morphine, Rat

Contrast normalization in cat primary visual cortex

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Sensory neurons code stimulus intensity in their spike rate and adjust their sensitivity by the mechanism of adaptation. In response to changes in the magnitude of the stimulus, however, it is not known whether all neurons in a local cortical network act in unison and adapt to the same set point, or whether responses in the local cluster are heterogeneous and effectively cover the entire stimulus range. Here we exploited two-photon calcium imaging *in vivo* to detect how a local network of neurons in the cat's visual cortex respond to changes in stimulus contrast. Cells were classified into two groups based on cortical depth, cells in layer 2 and cells at the border of layer 1 and 2, and according to the post mortem immunostaining of GABA and parvalbumin. We found that with instantaneous changes in contrast, adjacent neurons exhibit different thresholds of activation, so that the recruitment of active cell is spatially heterogeneous, and the thresholds of more superficial neurons were strikingly lower. With sustained contrast, most cells decrease their firing over seconds, but the parvalbumin positive neurons and more superficial neurons, show a slow increase in firing. These new observations indicate that the standard normalization model of contrast adaptation is oversimplified. We propose that the set point of normalization and its time course strongly depend on the role of the cell in the circuit.

Supported by EU SECO grant ETH-2-73246-08, and UZH grant 74603/72102-00 to KACM, and ETH grant 2010-010 to KACM.

Cav1.4 IT mouse as model for vision impairment in human congenital stationary night blindness type 2

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Mutations in the CACNA1F gene encoding the Cav1.4 Ca2+ channel are associated with X-linked congenital stationary night blindness type2 (CSNB2). Despite the increasing knowledge about the functional behaviour of mutated channels in heterologous systems, the pathophysiological mechanisms that results in vision impairment remain to be elucidated. Our work provides a thorough characterization of the novel IT mouse line that harbours the gain-of-function mutation I745T reported in a New Zealand CSNB2 family. Our data supported the hypothesis that a hyperpolarizing shift in the voltage-dependence of channel activation - as seen in the IT gain-of-function mutant - may reduce the dynamic range of photoreceptor activity. Morphologically, the retinal outer nuclear layer in adult IT mutants was reduced in size. Using cone-specific markers we observed aberrations in the cone morphology visible e.g. as shortening of cone outer segments Moreover, in some cones sprouting was detectable. Integrity of photoreceptor synapses was disrupted in IT mice. Synaptic morphology resembled immature synapses, a finding that supports the role of Cav1.4 for synapse development and maturation. In qRT-PCR experiments a reduction in expression of Cav1.4, beta2, and alpha2delta-4 subunits was evident in IT mice, likely to be explained by a photoreceptor loss. Intriguingly Cav1.3-mRNA was upregulated. The functional importance of retinal Cav1.3 channels, however, remains to be clarified. The IT mouse line serves as a specific model for the functional phenotype of human CSNB2 patients with gain-of-function mutations and may help to further understand the dysfunction in CSNB.

FWF-Support: P22526, SFBF44020 and the Medical University Vienna.

Network dynamics in resting state EEG of youths with Autism Spectrum Disorder

Bates, E., Seitzman, B., Coppers, K., and **Malaia, E**.

The recent rise in the prevalence of Autism Spectrum Disorder (ASD) has highlighted the need for neurobiological models of ASD. Developmental hypotheses suggest that atypical engagement of networks for local vs. globalized processing might underlie the diverse phenotypical profiles of ASD populations. In this experiment, we compared 32-channel EEG data of typically developing (TD) youths and youths with Autism Spectrum Disorder (ASD) during wakeful rest using network stability (microstate) and network diameter metrics (Sporns, 2013).

A cross-correlation time series analysis was used to produce weighted, undirected graphs corresponding to functional brain networks. The stability of these networks was assessed by use of both the L1- and L2-norms for matrix entries. For stable networks identified with the L1-norm, there was a significantly larger number of stable networks observed in the resting condition across both populations. Furthermore, the mean duration of the stable networks during the resting condition was significantly longer in youths with ASD. The L2-norm, however, yielded no significant results.

Children with ASD also had a larger mean network diameter in the resting condition, indicating high level of correlation between the connected nodes. The overall results suggest that in its resting state, the brain of ASD participants is engaged differently that that of TD participants: the periods of quasi-stability are longer, and there is higher correlation in node activity within each microstate. The overall method appears promising, and further analysis of task-related EEG data is needed to develop interpretive metrics correlating behavioral performance and activity patterns of brain networks.

Cortical plasticity following perceptual learning

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Perceptual learning is a cognitive phenomenon whereby perceptual capabilities improve with training. The neural substrate of perceptual learning is not well understood but probably involves multiple brain regions, one of which is the neocortex. Our work focuses on how learned information is encoded at the functional level by individual cortical neurons. Our model is the primary auditory cortex (A1) of the mouse. We study how different subpopulations of neurons in A1, e.g. inhibitory neurons and excitatory pyramidal cells, encode the learned information.
First, to study perceptual learning in mice, we developed an automated assay in a learning chamber that we named “the Educage”. The Educage is designed to train groups of mice (up to 6 mice simultaneously) on a two-tone ’go no-go’ discrimination task. Once the procedure is learned, task difficulty is gradually increased by decreasing the difference between the two tones. Using this procedure, mice became experts in this task and reached their perceptual limits within thousands of trials. Second, to study the physiological correlates of learning in A1, we used *in vivo* two photon targeted patch clamp to asses basic response properties of inhibitory and excitatory neurons of layer 2/3. As inhibitory neurons, we targeted Parvalbumin positive (PV^+) interneurons, the largest inhibitory subpopulation of the cortex. We used transgenic mice expressing TdTomato in PV neurons and used unlabeled neurons as controls (PV^-). We compared the frequency receptive fields and other response properties (e.g. response latency, spontaneous and evoked firing rate) of PV^+ and PV^- neurons in A1 of expert and naïve mice. To date, the data we collected already reveals that PV^+ and PV^- neurons changed in unique ways following learning. We suggest that specific modifications in inhibitory circuits within layer 2/3 of A1 contribute to auditory perceptual learning.

Effects of loud noise exposure on sound processing in the mouse primary auditory cortex

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Exposure to loud sounds damages the function of the inner ear and induces profound changes in the central parts of the auditory system. Acoustic trauma results in changes in the receptive fields of cortical neurons, contributing to the development of tinnitus. However, the role of different classes of cortical neurons in the development of various trauma-induced receptive field changes has not yet been resolved. We evaluated the effects of acute acoustic trauma on the functional properties of neurons in the mouse primary auditory cortex (A1) using single-unit extracellular recordings and two-photon calcium imaging in vivo.

Mice (C57Bl/6, 8-18 weeks) were anaesthetized with ketamine and xylazine and acoustic trauma was induced by 125 dB SPL white noise. Three 5-minute exposures to noise were followed by an additional 15-minute noise exposure. Responses of neurons to two sets of acoustical stimuli (broad-band noise and pure tones) were recorded before and after each successive noise exposure. Extracellular unit activity was recorded with 16-channel electrodes and two-photon imaging experiments were performed using the calcium indicator OGB-1. In addition, auditory brainstem responses were measured in separate experiments using the same noise exposure protocol to reveal the extent and character of peripheral damage.

We observed different dynamics of spiking responses to broadband noise before and after the first 5-minute acoustic trauma (n=86 neurons). A subset of neurons decreased their sound-evoked responses (n=13, 15 %), while another subset (n=20, 23%) increased the responses. Almost half of the neurons (n=38, 44%) did not change their sound-evoked activity and 15 neurons (18 %) remained unresponsive. Interestingly, neurons with decreased responsiveness had significantly narrower spikes than other sound-responsive neurons. Neurons with unchanged or increased responses displayed an increased jitter and higher spontaneous activity after the acoustic trauma. Frequency response areas of individual neurons showed distinct changes after the first noise trauma, with an overall increase of activity at the flanks of responsive areas accompanied by a decrease of activity at the initial receptive field. We obtained analogous results from two-photon imaging. After the first 5-minute exposure most neurons displayed shifts in their best frequencies towards lower frequencies. The observed effects suggest that an acute acoustic trauma selectively disrupts activity of inhibitory interneurons in the auditory cortex leading to specific changes in tuning and response dynamics of other neurons. To explore directly the changes in activity of cortical interneurons after acoustic trauma, we use calcium imaging in the auditory cortex of PV-Cre/TdTomato mice (expressing TdTomato in parvalbumin-positive interneurons) before and after noise-induced damage.

Study of proteins associated with epileptic seizures in primary hippocampal cultures under basal and stimulated conditions

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Primary cultures of hippocampal neurons and associated glia are a useful method for investigating changes in proteins implicated in the underlying pathology of epileptic seizures. Specifically, changes in the expression of connexins and proteins within astrocytes that modulate extracellular glutamate levels are associated with increased neuronal excitability and neurocytotoxicity. Initially, we characterized the basal expression of connexin 43 (Cx43) and glutamate transporters (GLT-1 and GLAST) using qPCR in tissues isolated from d5 neonatal Sprague-Dawley rats, adult rats, and d5 hippocampal and cortical cultures. We found the basal mRNA levels of Cx43 and the glutamate transporters to be expressed at similar levels in our cultured cells when compared to known in vivo levels. To investigate the stimulatory effect of low-Mg^{2+}/high-K^{+} (seen in epileptic seizures) on protein expression, we established primary hippocampal cultures from d5 neonates and studied changes using immunocytochemistry. Under stimulatory seizure conditions for 2-hr and 24-hr time points, the level of Cx43 was increased while the levels of GLAST and GLT-1 were decreased when compared to basal levels. Furthermore, glutamine synthetase (a protein responsible for converting extracellular glutamate into glutamine) was decreased at both time points. We also found that the MAP kinase ERK, a marker of neuronal activation, was increased 30 minutes after stimulation. Taken together, our primary hippocampal culture conditions mimic cellular conditions associated with epileptic seizures in vivo and would therefore be a useful model to screen for potential new anti-epileptic drugs.

Role of K_V channels in activity-dependent biphasic changes of Schaffer collateral fiber volleys

Benjamin Owen

The unmyelinated hippocampal Schaffer collateral axons originate from CA3 pyramidal cells and synapse onto CA1 pyramidal cells. Previously, we observed that distal Schaffer collateral responses (extracellular fiber volleys) undergo biphasic changes during high-frequency stimulation (HFS) and burst stimulation, with a period of hyper-excitability, characterized by enhanced fiber volley amplitude, followed by amplitude depression. These changes in fiber volleys were frequency-dependent, with larger enhancements seen at higher frequencies or shorter inter-burst intervals. To examine the possible roles of voltage-gated potassium (K_V) channels in excitability changes, we recorded fiber volleys from *in vitro* hippocampal slices before and after application of the non-specific blockers tetraethylammonium (TEA) and 4-aminopyridine (4-AP) during HFS and burst stimulation. Application of TEA caused larger, briefer enhancements to fiber volley amplitudes, and caused more amplitude depression, while application of 4-AP caused briefer, but not larger, enhancements to fiber vollley amplitudes, and increased the amplitude depression like TEA. To test involvement of specific K_V subtype(s), we recorded fiber volleys before and after applying the following specific blockers: UK78282 (K_V1.4), tityustoxin (K_V1.2), kaliotoxin (K_V1.1/1.2/1.3), or XE-991 (K_V7). None of the specific blockers altered the fiber volley response to HFS or burst stimulation, however, suggesting redundant function with other K_V channels able to compensate for the selective block of a single K_V channel subtype, or involvement of a different K_V channel subtype.

Modulating synaptic function through neurexophilin/α-neurexin complex formation

Astrid Rohlmann

Neurotransmission at different synapses is remarkably variable and synaptic cell-adhesion molecules such as neurexins and their ligands are candidates to organize this process. While presynaptic neurexins affect transmission and contact formation by acting together with several postsynaptic molecules, the role of neurexophilins (Nxph), tightly-bound presynaptic ligands of α-neurexin, is unclear. In contrast to α-neurexin, Nxph are only expressed in subpopulations of neurons: endogenous Nxph1 is restricted to inhibitory interneurons of many brain regions, and Nxph3 is even more limited to glutamatergic layer 6 neurons in the neocortex and to the vestibulocerebellum. To test the hypothesis that neurexophilins modulate neurotransmission when co-expressed with α-neurexin, we generated two transgenic mouse models under control of Thy1.2 promoters which target GFP-tagged Nxph1 and Nxph3 to excitatory synapses which normally do not contain these molecules. Extensive morphological analysis confirms that both Nxph-GFP variants are ubiquitously expressed in most cortical pyramidal neurons, and localized to the synaptic cleft. We demonstrate that this ectopic overexpression of the Nxph1/α-neurexin complex leads to recruitment of $GABA_B$ and $GABA_A$ receptors to excitatory synapses. In addition, electrophysiology shows that short-term plasticity as assessed by paired-pulse facilitation is impaired at these transgenic terminals. Similarly, we observe for the ectopic Nxph3/α-neurexin complex that recruited $GABA_B$ receptors are involved in altered spontaneous release: the frequencies of miniature excitatory postsynaptic currents were decreased in these transgenic mice compared to littermate controls. Together, our findings suggest that neurexophilins can serve as a local modulator of neurotransmission, in concert with α-neurexin.

Activation of GABA A receptors of medial prefrontal cortex produces anxiolytic-like response

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In the present study, we have investigated the effects of GABAergic systems in the medial prefrontal cortex (MPFC) of rats, using the elevated plus maze test of anxiety. Rats were anaesthetized with ketamine and xylazine and special cannulas were inserted stereotaxically into the MPFC. After 1 week recovery, the effects of intra-MPFC administration of GABAergic agents were studied. Result shows that bilateral injection of the GABAA receptor agonist muscimol (0.25, 0.5and 0.75 µg/rat) produce an anxiolytic-like effect, shown by specific increases in the percentage of open-arm time (%OAT) and percentage of open arm entries (%OAE). Intra-MPFC administration of the GABAA receptor antagonist bicuculline (0.25, 0.5 and 1 µg/rat) produces significant anxiogenic-like behaviour. However, Intra-MPFC injection of GABAB receptor agonist baclofen (0.05, 0.1and 0.2 µg/rat), and GABAB receptor antagonist CGP35348 (5, 10 and 20 µg/rat) have not altered %OAT and %OAE significantely. In conclusion, results of the present study demonstrate that the GABAergic system of the MPFC modulate anxiety-related behaviors of rats via GABAA receptors.

Prion protein facilitates synaptic vesicle release by enhancing release probability

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Cellular prion (PrP^C) has been implicated in several neurodegenerative disease conditions as a result of protein misfolding. In humans, prion disease such as Creutzfeldt-Jacob Disease occurs typically with a sporadic origin where uncharacterised mechanisms induce a spontaneous misfolding of PrP^C. The consequences of misfolded prion signalling in disease progression is well characterised but little is known about the physiological roles of PrP^C. Here we investigated wild-type mouse prion signalling on synaptic function as well as the effects of a disease-relevant mutation within PrP^C (PrP^{P101L}) which resembles human disease-associated phenotypes when expressed in mice. Our data show that expression of PrP^C at the *Drosophila* NMJ leads to enhanced synaptic responses as seen in larger miniature synaptic currents which are caused by enlarged presynaptic vesicles. PrP^{P101L} expression leads to a reduction of both parameters relative to PrP^C. PrP^C enhances synaptic release probability and quantal content but reduces the size of the ready-releasable vesicle pool as shown by independent methodological approaches. These changes are less pronounced following expression of the mutant, PrP^{P101L}. A behavioural test revealed that prion expression caused an increase in foraging activities consistent with enhanced synaptic release and stronger muscle contractions. Neither protein displayed misfolded prion properties. This data uncovers new functions of PrP^C at the synapse with a disease-relevant mutation in PrP^C leading to altered and diminished functional phenotypes. Our findings provide novel evidence that prion enhances synaptic strength via increasing release probability with simultaneous reduction in the number of release-ready vesicles confirming an endogenous role of PrP^C signalling at the synapse.

Disruption of the circadian system in patients with neuropsychiatric disorders

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Most if not all neuropsychiatric disorders are accompanied with disruption of sleep/wake cycle. The underlying mechanisms are unknown, however, recent results suggested that these symptoms might be related with malfunction of the circadian system. The aim of our study was to ascertain whether the circadian system is affected in two disorders, Smith-Magenis syndrome (SMS) and bipolar disorder (BD). To assess the functional state of the system, we used daily profiles of melatonin levels in saliva as a marker of the central circadian clock in the brain, and clock gene expression profiles in buccal mucosa cells as a marker of the peripheral clocks. Our results demonstrated that SMS and BD patients exhibited abnormalities in the daily profiles of melatonin levels in saliva compared with healthy controls. The profiles in SMS and BD were affected in a different way, but in both diseases the melatonin levels were elevated during the daytime. In SMS, the individual clock gene expression profiles in the buccal cells were mutually desynchronized, whereas in controls the genes were expressed in synchrony. In BD, the clock gene expression profiles remained mutually synchronized, but they were phase-advanced during manic episode compared with depressive episode. Our results demonstrate that in various neuropsychiatric disorders, a disease-specific alteration of function of the circadian system is present.

Sponsored by the Czech Science Foundation grant P30412G069.

Lateralization of language function in epilepsy patients: An event-related potential (ERP) study of a word/pseudoword task

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Introduction: Assessment of language lateralization is important in epilepsy surgery to minimize postoperative language deficits. Although the Wada test is considered the gold standard for language lateralization assessment, non-invasive substitutes such as fMRI represent the current clinical standard, however showing discordance rates of ~15%. Event-related potentials (ERPs), especially the language-related negative component around 200-400ms, are associated with language processing and are therefore expected to reflect language lateralization. **Method:** The study was based on a 2 (Lateralization; left vs. right hemisphere) × 2 (Region; anterior vs. posterior) × 2 (Stimulus; words vs. pseudowords) × 2 (Group; patients vs. controls) repeated ANOVA design with Group as a between factor. Scalp EEG was recorded from 64 standard locations in 14 drug-resistant focal epilepsy patients and 20 healthy controls (all right-handed). ERP areas of 45 words and 45 pseudowords (randomly presented; 1000ms presentation time; ISI 2700-3200ms) were analyzed in the 200-400ms epoch. Language fMRI was routinely obtained in epilepsy patients. **Results:** ANOVA showed a significant interaction of Lateralization × Region ($F(1,27)=6.86$; $p=0.01$), indicating negative potentials over the left hemisphere (independent of region) and positive potentials over the right hemisphere (more positive at anterior compared to posterior regions). Effects were independent of Group and of Stimulus type. **Discussion:** Receptive language functions were lateralized to the left hemisphere in healthy controls and in epilepsy patients, for whom also fMRI indicated a left-lateralized language function. Results indicate that scalp-derived ERPs are a promising tool to investigate lateralization of language functions epilepsy patients.

Neuronal expression of complex gangliosides is necessary for the maintenance of axon and axo-glial junction integritiy

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Mice and humans lacking complex gangliosides initially develop normally before exhibiting an age-dependent neurodegenerative phenotype. More specifically, complex gangliosides are necessary for the maintenance of axo-glial junction structure. It is unclear whether aberrant axonal or glial expression of complex gangliosides has the greater impact on maintaining axon and axo-glial junction integrity.

To study the role and necessity of ganglioside expression in each neural cell type, GalNAc transferase knockout (GalNAcT KO, that cannot synthesis complex gangliosides beyong GM3/GD3) mice were developed that selectively express gangliosides either neuronally (GalNAcT driven by the neurofilament promoter; NFL-Tg), or in myelin (GalNAcT driven by the proteolipid protein promoter; PLP-Tg). Here we compare and characterise wild type (WT), GalNAcT KO, neuronal and glial rescue aged mice (>4 months).

The neuronal ganglioside rescue mouse reverted to a normal gross phenotype, whereas the glial rescue mouse did not. Ganglioside expression in nerves, as determined by anti-ganglioside monoclonal antibodies, was absent in GalNAcT KO, and was comparable between WT and neuronal rescue nerve. GalNAcT KO and glial rescue mice showed impaired performance on the rotarod and impaired grip strength compared to WT, while neuronal rescue mice were the same as WT. Quantitative EM demonstrated a significant increase in axon degeneration in GalNAcT KO mice and glial rescue mice. This was not seen with neuronal rescue mice, which had equivalent ultrastructure to WT. While GalNAcT KO and glial rescue mice demonstrate an invasion of the paranode with juxtaparanodal Kv1.1 staining which signifies a breakdown of the axo-glial junction, neuronal rescue mice demonstrated normalization of nodal protein immunostaining in the distinct domains also present in WT.

These results indicate that neuronal rather than glial expression of complex gangliosides in the nervous system is sufficient to rescue the phenotype of the GalNAcT KO mouse and that neuronal expression is therefore critical to the maintenance of the integrity of the axon and axo-glial junction.

Ca^{2+}-binding Calmyrin 2 functions in endocytosis in hippocampal neurons

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Recent studies indicate importance of Ca^{2+} in the regulation of endosome fusion and vesicular transport, but the identification and elucidation of the roles of particular Ca^{2+} sensor proteins in endocytosis is not complete. Calmyrin 2 (CaMy2, Cib2) belongs to the Ca^{2+}-binding EF-hand calmyrin protein family. Calmyrins are structurally most similar to Neuronal Ca^{2+} Sensors (NCSs). NCS proteins regulate many neuronal functions including neuron development, membrane trafficking and synaptic plasticity. Importance of such NCSs as calcineurin and hippocalcin in the regulation of endocytosis was also indicated.

Recently we have found that CaMy2 is preferentially expressed in hippocampus and cortex neurons (Blazejczyk et al., 2009). Endogenous CaMy2 was present in neurites and the Golgi apparatus, and was found in the membranous fraction. Here we report identification of first neuronal CaMy2 protein targets. In accordance with the subcellular localization in neurons, CaMy2 interacts with Rab proteins involved in early endosome trafficking and fusion. The interaction was identified in extracts of rat brain by pull-down using CaMy2 as bait, followed by mass spectrometry, and confirmed by coimmunoprecipitation. Moreover, we demonstrated colocalization of CaMy2 with Rab proteins on endocytic vesicles of rat hippocampal neurons in primary culture. Knock-down of CaMy2 with shRNA in hippocampal neurons greatly affected the distribution of Rab-positive vesicles. Our data suggest that CaMy2 is a novel Ca^{2+}-sensor that could be involved in endocytosis.

This work was supported by the Polish Research grants N301 109 32/3854, NN301 335239, and the EU FP7/2007-2013 Programme, grant no 229676 „Proteins in Health and Disease", HEALTH-PROT.

17th International Neuroscience Winter Conference

April 7 - 11, 2015

Das Central, Sölden, Austria

www.winterneuroscience.org/2015