NEUROSCIENCE WINTER CONFERENCE

17th International

Sölden Austria
April 7 -April 11 2015

Hotel Das Central

Preliminary Scientific Program

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

Program Committee:

Tobias Bonhoeffer
Nils Brose
Alois Saria
Stephan Schwarzacher

Organizer:

brainplatform.net e.U.

Conference Chair:

Alois Saria, Austria

Contributors:

- Austrian Neuroscience Association
- Das Central
- International Society for Neurochemistry
- TARGEAR, a project of the European Union FP7-People
- Elsevier GmbH
- Taylor & Francis Group Ltd

Exhibitors:

- Olympus Europa SE & Co. KG
- Sensapex Oy

Tuesday
April 7 Afternoon

15:30 - 16:30 Registration

16:30 - 17:00 Welcome Cocktail

17:00 - 19:00 Symposium 1

The energy barrier for vesicle fusion
Chair: **Matthijs Verhage (The Netherlands)**

Erwin Neher (Germany) Modulation of short-term plasticity at the Calyx of Held synapse
Christian Rosenmund (Germany) Modulators of vesicle fusogenicity
Jakob B. Sørensen (Denmark) Phosphatidylinositol 4,5-Bisphosphate uncaging potentiates exocytosis by activating synaptotagmin
L. Niels Cornelisse (The Netherlands) Supralinear modulation of synaptic strength through additive effects on the energy barrier for vesicle fusion

19:00 - 19:45 Keynote Lecture 1

Josep Rizo (USA) Elucidating the molecular mechanism of neurotransmitter release

Wednesday
April 8 Morning

08:15 – 09:00 Keynote Lecture 2

Hongjun Song (USA) Single-cell analysis of adult neural stem cells and neurogenesis

09:00 - 11:00 Symposium 2

Neuronal stem cells in development and disease
Chair: **Victor Tarabykin (Germany)**

Freda D. Miller (Canada) Translational mechanisms regulating mammalian neurogenesis
Verdon Taylor (Switzerland) Molecular determination of quiescent and binary cell fate choices in adult neural stem cells
Angela M. Kaindl (Germany) Stem cells in a novel human mid-hindbrain malformation, microcephaly and intellectual disability phenotype
David R. Kaplan (Canada) Genes and drugs regulating stem cell function and repair

11:00 - 11:20 Coffee Break

11:20 - 13:20 Special Interest Session 1

Epigenetics and brain imaging: How do environmental and developmental factors influence behaviour?
Chair: **Gunter Schumann (UK)**

Sylvane Desrivieres (UK) Contributions of neurogenesis in human cognition: Cellular and MRI studies
Tomas Ekström (Sweden) The functional genome at the genetic-epigenetic-environment intersection
Georgy Bakalkin (Sweden) Locus-specific repressive DNA methylation as mechanism of neuron-specific gene transcription in the human brain: in-depth analysis of opioid genes
Gunter Schumann (UK) The effect of the environment on brain activity and behaviour: Characterisation of epigenetic mediators

Wednesday
April 8 Evening

16:00 – 18:00 Symposium 3

Structure and function of the synapse
Chairs: **Stephan Sigrist (Germany)** and **Uri Ashery, (Israel)**

Vladan Lucic (Germany) Quantitative analysis of synaptic structure by cryo-electron tomography
Benjamin Cooper (Germany) Molecular and morphological correlates of vesicle priming in neurosecretory cells
Stephan Sigrist (Germany) Shedding light on the functional organization of active zones
Uri Ashery (Israel) A combined optogenetic-knockdown strategy reveals a major role of tomosyn in mossy fiber synaptic plasticity
Timothy A. Ryan (USA) New layers of synaptic transmission control

18:00 - 18:20 Coffee Break

18:20 - 20:20 Symposium 4

Structural plasticity of perisynaptic astroglia
Chair: **Dmitri Rusakov (UK)**

Valentin Nägerl (France) Structure-function analysis of tripartite synapses
Yann Bernardinelli (Switzerland) Dynamics of astrocytic processes influence excitatory synapses stability
Alfonso Araque (USA) Structural and functional plasticity of astrocyte process and dendritic spine interactions
Dmitri Rusakov (UK) Astroglia withdraw from synapses during LTP

Thursday
April 9 Morning

08:15 - 09:00 Keynote Lecture 3

Bernhard Bettler (Switzerland) Deconstructing $GABA_B$ receptors

09:00 - 11:00 Symposium 5

Trans-synaptic signaling
Chairs: **Nils Brose (Germany)** and **Markus Missler (Germany)**

Thomas Biederer (USA) Trans-synaptic SynCAM interactions organize synapse formation and neuronal connectivity
Joris DeWit (Belgium) The sorting receptor SorCS1 interacts with neurexin and regulates synaptic proteome composition
Garret Anderson (USA) Beta-neurexin proteins at the synapse: Functional link with the endocannabinoid signaling system
Jürgen Klingauf (Germany), Markus Missler (Germany) Neuroligin-induced hippocampal synapses on functionalized micropatterns

Thursday
April 9 Evening

16:00 - 18:00 Symposium 6

Coordinated migration of excitatory and inhibitory neurons
Chairs: **Annette Gaertner (Belgium)** and **Stephan Schwarzacher (Germany)**

Laurent Nguyen (Belgium) MicroRNA targeting of CoREST controls polarization of migrating cortical neurons
Simon Hippenmeyer (Austria) Genetic dissection of neuronal migration using mosaic analysis with double markers
Christine Metin (France) Control of cortical interneuron migration by N-cadherin
Orly Reiner (Israel) Regulation of radial neuronal migration by polarity molecules

18:00 - 18:20 Coffee Break

18:20 - 20:20 Symposium 7

microRNAs in the nervous system - supported by the International Society for Neurochemistry
Chair: **Michaela Kress (Austria)**

Hermona Soreq (Israel) From mice to men: Fine tuning of cholinergic signaling by non-coding RNAs
Gerhard Schratt (Germany) miRNA function in synapse development and plasticity
Claudia Verderio (Italy) Glia-to-neuron shuttling of miR-146a via extracellular microvesicles modulates synaptotagmin I translation in neurons
Michaela Kress (Austria) microRNAs in nerve injury and neuropathic pain

Friday
April 10 Morning

08:15 - 09:00 Keynote Lecture 4

Alejandro Schinder (Argentina) A novel view of neurogenesis and memory encoding in the dentate gyrus

09:00 - 11:00 Symposium 8

Structural dynamics of adult neurogenesis
Chair: **Stephan Schwarzacher (Germany)** and **Paul Lucassen (Netherlands)**

Robert Hevner (US) Dentate Gyrus Development: A Movable Niche
Stephan Schwarzacher (Germany) Structural maturation and integration of adult newly generated hippocampal neurons: Is there a critical phase?
Nora Abrous (France) Influence of spatial learning on development and structural maturation of newly formed neurons
Mirko Schmidt (Germany) The novel Notch ligand EGFL7 governs adult neurogenesis *in vivo*
Paul Lucassen (Netherlands) Adult neurogenesis: Structural plasticity in relation to stress and depression

11:00 - 11:20 Coffee Break

11:20 - 13:20 Special Interest Session 2

Hearing function and dysfunction in man and experimental animals - supported by TARGEAR, a project of the European Union FP7-People
Chair: Josef Syka (Czech Republic)

Wei Lui (Sweden) Ultrastructure and macromolecular organization of the human cochlea
Marlies Knipper (Germany) BDNF gathered cochlear driving force improves auditory fidelity, risking hyperactivity without gain after injury
Isabel Varela-Nieto (Spain) Age-related hearing loss: Apoptosis, autophagy and senescence: The role of IGF-1
Josef Syka (Czech Republic) Changes in the structure and function of the central auditory system with aging

Friday
April 10 Evening

16:00 - 16:45 Keynote Lecture 5

Moritz Helmsteadter (Germany) Connectomics: The dense reconstruction of neuronal circuits

16:45 - 17:05 Coffee Break

17:05 - 19:05 Symposium 9

Assembly and maintenance of the nodal complex in development, health and disease
Chair: **Sue Barnett (UK)**

Elior Peles (Israel) Assembly and maintenance of PNS nodes
Matthew Rasband (USA) Assembly and maintenance of CNS nodes
Jerome Devaux (France) Cell adhesion molecules at the nodal complex in health and disease
Hugh Willison (UK) Glycolipids at PNS and CNS nodes during maintenance and disease

19:05 - 20:00 Poster Session

20:00 Gala Dinner (free for Das Central hotel residents, others book at registration desk for 48,- € until Thursday evening)

Saturday
April 11 Morning

08:15 - 09:00 Keynote Lecture 6

Susanne Schoch (Germany) Transcriptional and epigenetic control of aberrant neuronal plasticity in epileptogenesis

09:00 - 09:30 Coffee Break

09:30 - 11:30 Symposium 10

Epigenetic mechanisms in epileptogenesis
Chair: **Günther Sperk (Austria)**

Katarzyna Lukasiuk (Poland) Methyl-CpG-binding domain protein 3 (MBD3) in the rat model of epilepsy
Katharina Kiese (Germany) Metabolic interference with epigenetic alterations in a rodent model of TLE
Christophe Bernard (France) Reprogramming of the hippocampal circadian clock in epilepsy
Günther Sperk (Austria) Changes in the expression of histone deacetylases in the hippocampus in models of temporal lobe epilepsy
Noora Puhakka (Finland) Chronic changes in microRNAs after after traumatic brain injury in the rat

11:30 End of meeting and departure

Friday
April 10
19:05 - 20:00
Poster Session

1. Roles for MicroRNAs in retinal pigmented epithelium development and function
Reut Ohana, Benjamin Weiman-Kelman and Ruth Ashery-Padan

2. The dynamic properties of receptive fields in the rat superior colliculus visual neurons
Gytis Baranauskas, Gytis Svirskis, Natasa Svirskiene, Tatiana Tkatch

3. A novel stimulus and analysis system for studying the neural mechanisms of natural language processing in the human brain
Seonmin Chung

4. Social interaction reward decreases p38 activation in the nucleus accumbens shell of rats
Salti A, Kummer KK, Dechant G, Saria A, El Rawas R

5. Uncoupling of mitosis and differentiation allows for fast and synchronous CNS development *in vivo*
Peter Engerer, Philip Williams, Sachihiro Suzuki, Takeshi Yoshimatsu, Prisca Chapouton, Nancy Obeng, Benjamin Odermatt, Leon Lagnado, Thomas Misgeld, Leanne Godinho

6. Active endocannabinoids are secreted on microglial microvesicles
Martina Gabrielli, Natalia Battista, Loredana Riganti, Ilaria Prada, Flavia Antonucci, Laura Cantone, Marta Lombardi, Michela Matteoli, Mauro Maccarrone, Claudia Verderio

7. Mild hypothermia therapy for patients with severe brain injury
Gal R., Smrcka M., Slezak M., Mrlian A.

8. Brian Hyland

9. *In vivo* imaging of microtubule dynamics in developing and diseased axons
Kleele T., Marinkovic P., Williams P., Herms J., Kerschensteiner M., Godinho L. and Misgeld T.

10. Cold stress induced RBM3-dependent neuroprotection is mediated by the reticulon protein RTN3
Giovanna Mallucci

11. Kainate receptors mobilize enocannabinoids in striatal spiny projection neurons
John Marshall, Jian Xu, Anis Contractor

12. Ethanol and caffeine on apoptosis in the cerebellum of UChB rats (voluntary ethanol consumers)
M. Martinez; F. S. N. Lizarte; D. P. C, Tirapelli; L. F. Tirapelli; L. G. Chuffa; P. F. F. Pinheiro; F. E. Martinez

13. Perceptual changes of "self" disrupt number sense
Q. Arshad, M. Pikovsky, U. Goga, Y. Nigmatullina, R.E. Roberts, P. Malhotra, R. Cohen-Kadosh, A.M Bronstein

14. Higher specific infectivity of exosomal prions
F. Properzi, M. Logozzi, H. Abdel Haq, E. Ferroni, C. Federici, L. Lugini, T. Azzarito

15. Short term energy deprivation is not sufficient to induce proteasome stress and stress response in neural cells
Pilchova I., Klacanova K., Dobrota D., Racay P.

16. Effect of febrile seizures on newborn hippocampal dentate granule cell morphology and function
Marjolein Raijmakers, Elke Clynen, Nick Smisdom, Sofie Nelissen, Bert Brône, Jean-Michel Rigo, Govert Hoogland and Ann Swijsen

17. EGFR mediates neuronal survival through regulation of glutamate transporters in cortical astrocytes
Jonathan Robson

18. Distinct neurexin-based complexes at GABAergic versus glutamatergic synapses
Astrid Rohlmann and Markus Missler

19. Search for causal variants in microRNA and biogenesis genes in epileptic encephalopathies
Jolien Roovers, Sophia Cammaerts, Mojca Strazisar, Johannes Lemke, Sarah Weckhuysen, Peter De Rijk, Peter De Jonghe, Jurgen Del-Favero, Arvid Suls, RES-EuroEPINOMICS

20. Why are the magnocellular and parvocellular systems sensitive to different spatial frequencies in visual signals?
Parameswari Shunmugam, K.A. Muthusamy, Vijayakumar Vengadasalam

21. Optogenetic investigation of chandelier synapses in developing and adult olfactory cortex
Qian-Quan Sun, Xinjun Wang, Weiguo Yang and Chunzhao Zhang

22. Febrile seizures persistently alter hippocampal $GABA_A$ receptor physiology
Swijsen A, Bernareggi A, Schipper S, Raijmakers M, Clynen E, Aalbers MW, Rijkers K, Dings J, Schijns O, Vles JSH, Rigo JM and Hoogland G

23. Lateralization of language function in epilepsy patients: An event-related potential (ERP) study of a visual word memory/recognition task
Karin Trimmel, Ekaterina Pataraia, Gerald Lindinger, Felicitas Huber, Jens Sachsenweger, Eduard Auff, and Michael Trimmel

24. Katarzyna Wilczynska

25. R-Phenibut exerts anti-nociceptive properties via the α_2-δ subunit of the voltage-dependent calcium channels
Liga Zvejniece, Edijs Vavers, Baiba Svalbe, Maija Dambrova

List of Participants

Name	Last name	Country	Name	Last name	Country	Name	Last name	Country
Nora	**Abrous**	France	Tatjana	**Kleele**	Germany	Jolien	**Roovers**	Belgium
Garret	**Anderson**	USA	Jürgen	**Klingauf**	Germany	Kobi	**Rosenblum**	Israel
Alfonso	**Araque**	USA	Marlies	**Knipper**	Germany	Christian	**Rosenmund**	Germany
Uri	**Ashery**	Israel	Michaela	**Kress**	Austria	Dmitri	**Rusakov**	UK
Ruth	**Ashery-Padan**	Israel	Anders	**Kristensen**	Denmark	Timothy	**Ryan**	USA
Mathias	**Bähr**	Germany	Kai	**Kummer**	Austria	Miklós	**Sántha**	Hungary
Georgy	**Bakalkin**	Sweden	Lothar	**Kussmaul**	Germany	Alois	**Saria**	Austria
Christine	**Bandtlow**	Austria	Simon	**Kyaga**	Sweden	Alejandro	**Schinder**	Argentinia
Gytis	**Baranauskas**	Lithuania	Peter	**Lederer**	Germany	Mirko	**Schmidt**	Germany
Sue	**Barnett**	UK	Wei	**Liu**	Sweden	Susanne	**Schoch**	Germany
Christophe	**Bernard**	France	Paul	**Lucassen**	The Netherlands	Gerhard	**Schratt**	Germany
Yann	**Bernardinelli**	Switzerland	Vladan	**Lucic**	Germany	Jörg	**Schulz**	Germany
Bernhard	**Bettler**	Switzerland	Katarzyna	**Lukasiuk**	Poland	Gunter	**Schumann**	
Thomas	**Biederer**	USA	Mathias	**Lundberg**	Sweden	Stephan	**Schwarzacher**	Germany
Ulrik	**Bølcho**	Denmark	Volker	**Mack**	Germany	Alison	**Semmonds**	Australia
Nils	**Brose**	Germany	Giovanna	**Mallucci**	UK	Parameswari	**Shunmugam**	Malaysia
Young	**Choi**	Republic of Korea	John	**Marshall**	USA	Stephan	**Sigrist**	Germany
Seonmin	**Chung**	USA	Marcelo	**Martinez**	Brazil	Hongjun	**Song**	USA
Benjamin	**Cooper**	Germany	Christine	**Métin**	France	Jakob	**Sørensen**	Denmark
Lennart	**Cornelisse**	The Netherlands	Freda	**Miller**	Canada	Hermona	**Soreq**	Israel
Joris	**De Wit**	Belgium	Markus	**Missler**	Germany	Tim	**Spencer**	USA
Sylvane	**Desrivieres**	UK	Hans-Peter	**Mofors**	Sweden	Günther	**Sperk**	Austria
Jérôme	**Devaux**	France	Patrik	**Mofors**	Sweden	Walter	**Stühmer**	Germany
Wouter	**Duyck**	Belgium	Heidi	**Müller**	Denmark	Qian-Quan	**Sun**	USA
Florian	**Eich**	Olympus Europe	Valentin	**Nägerl**	France	Ann	**Swijsen**	Belgium
Tomas	**Ekström**	Sweden	Erwin	**Neher**	Germany	Josef	**Syka**	Czech Republic
Rana	**El Rawas**	Austria	Laurent	**Nguyen**	Belgium	Eva	**Sykova**	Czech Republic
Betina	**Elfving**	Denmark	Peter	**Ovesen**	Denmark	Victor	**Tarabykin**	Germany
Peter	**Engerer**	Germany	Yun-Zu	**Pan**	USA	Verdon	**Taylor**	Switzerland
Florian	**Engert**	USA	Elior	**Peles**	Israel	Dersehilign	**Teshome**	Austria
Martina	**Gabrielli**	Italy	Jorge	**Petrera**	Denmark	Pernille	**Thomasen**	Denmark
Annette	**Gaertner**	Belgium	Margaret	**Pikovsky**	UK	Karin	**Trimmel**	Austria
Roman	**Gal**	Czech Republic	Francesca	**Properzi**	Italy	Mikko	**Vähäsöyrinki**	Finland
Frank	**Gillardon**	Germany	Noora	**Puhakka**	Finland	Isabel	**Varela-Nieto**	Spain
Ole	**Greiner-Tollersrud**	Norway	Peter	**Račay**	Slovakia	Edijs	**Vavers**	Latvia
Moritz	**Helmstaedter**	Germany	Marjolein	**Raijmakers**	Belgium	Claudia	**Verderio**	Italy
Robert	**Hevner**	USA	Matthew	**Rasband**	USA	Matthijs	**Verhage**	The Netherlands
Simon	**Hippenmeyer**	Austria	Orly	**Reiner**	Israel	Niels	**Wellner**	Denmark
Kenneth	**Hovis**	USA	Christiane	**Riedl**	Austria	Katarzyna	**Wilczynska**	UK
Brian	**Hyland**	New Zealand	Willem H.	**Rijks**	The Netherlands	Hugh	**Willison**	UK
Angela	**Kaindl**	Germany	Josep	**Rizo**	USA	Elisabeth	**Wintersteller**	Austria
David	**Kaplan**	Canada	Jonathan	**Robson**	Austria	Tongtong	**Zhao**	USA
Katharina	**Kiese**	Germany	Astrid	**Rohlmann**	Germany	Ágnes	**Zvara**	Hungary
						Liga	**Zvejniece**	Latvia

Abstracts Speakers

Abstracts are listed alphabetically according to presenting author

Missing abstracts have not been forwarded to the meeting secretariat.

Influence of spatial learning on development and structural maturation of newly formed neurons

Abrous DN

INSERM U862, Bordeaux, France

Adult hippocampal neurogenesis is a multistep process characterized by the birth of thousand new cells per day, the fate of which depends on life experiences. In particular, spatial learning has been shown to promote the survival and to accelerate the maturation of newborn neurons that were 2 week-old at the end of the learning. This “epigenetic” specification of neo-networks are specific of new neurons, are long lasting, depend upon the level of cognitive demand and NMDA receptors.

How NMDA receptors are involved in these learning-evoked changes remains to be addressed. NMDA receptor antagonists may act directly on immature newborn neurons or indirectly on GABAergic interneurons. Among these interneurons, parvalbumin-expressing ones (PV) regulate adult neurogenesis and express functional NMDA receptors suggesting that remodelling of these inhibitory inputs are involved in learning-evoked changes in adult neurogenesis. I will show that spatial learning accelerates the arrival of PV-GABAergic inputs onto the soma of these newborn neurons. Then to deepen our understanding, we develop in rats a method that combines rabies virus-mediated retrograde tracing with retroviral labelling of new granule cells. Our preliminary data confirmed the recruitment of PV neurons in learning-evoked changes in structural plasticity.

Beta-neurexin proteins at the synapse: Functional link with the endocannabinoid signaling system

Garret Anderson

α- and β-neurexins are presynaptic cell-adhesion molecules implicated in autism and schizophrenia. Although β-neurexins are expressed at 10- to 100-fold lower levels than α-neurexin, we find that conditional knockout of β-neurexins with continued expression of α-neurexins dramatically decreases the release probability of excitatory synapses. The β-neurexin knockout phenotype was rescued by neurexin-1β in a splice site-dependent manner, and attenuated by CB1-receptor antagonists that block endocannabinoid signaling. In synapses formed by CA1-pyramidal neurons onto burst-firing subiculum neurons, presynaptic deletion of β-neurexins enhanced endocannabinoid-mediated inhibition of synaptic transmission, and blocked LTP. These phenotypes were also attenuated by CB1-receptor antagonists, again implicating endocannabinoid signaling. Moreover, deletion of β-neurexins in CA1 neurons impaired contextual fear memory, suggesting that β-neurexin-dependent endocannabinoid signaling is behaviorally significant. Thus, our data show that β-neurexins control synaptic strength in a subset of excitatory synapses, revealing an unexpected role for β-neurexins in the endocannabinoid-dependent regulation of neural circuits.

Structural and functional plasticity of astrocyte process and dendritic spine interactions

Alfonso Araque

Department of Neuroscience, University of Minnesota, Minneapolis, USA

Experience-dependent plasticity of synaptic transmission, which represents the cellular basis of learning, is accompanied by morphological changes in dendritic spines. Astrocytic processes are intimately associated with synapses, structurally enwrapping and functionally interacting with dendritic spines and synaptic terminals by responding to neurotransmitters and by releasing gliotransmitters that regulate synaptic function. While studies on structural synaptic plasticity have focused on neuronal elements, the structural-functional plasticity of astrocyte-neuron relationships remains poorly known. We have investigated the structural changes of astrocytic processes elicited by stimulation protocols that induce synaptic plasticity and the subsequent consequences on the synaptic regulation by astrocytes. Evidence will be presented showing that stimuli that induces hippocampal synaptic long-term potentiation (LTP) enhance the motility of synapse-associated astrocytic processes. This motility increase is relatively rapid, starting < 5 min after the stimulus, and reaching a maximum in 20-30 min ($t_{1/2}$ = 10.7 min). It depends on presynaptic activity and requires G protein-mediated calcium elevations in astrocytes. The structural remodeling is accompanied by changes in the ability of astrocytes to regulate synaptic transmission. Sensory stimuli that increase astrocyte calcium also induce similar plasticity in mouse somatosensory cortex in vivo. Therefore, structural relationships between astrocytic processes and dendritic spines undergo activity-dependent changes with metaplasticity consequences on synaptic regulation. These results reveal novel forms of synaptic plasticity based on structural-functional changes of astrocyte-neuron interactions.

Supported by: Human Frontier Science Program (RGP0036/2014).

A combined optogenetic- knockdown strategy reveals a major role of tomosyn in mossy fiber synaptic plasticity

Yoav Ben Simon[1,2], Alma Rodenas-Ruano[3], Karina Alviña[3], Pablo E. Castillo[3] and **Uri Ashery**[1,2]

[1] Department of Neurobiology, Wise Faculty of Life Sciences, and
[2]Sagol School of Neuroscience, Tel Aviv University, Tel Aviv, Israel
[3] Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine of Yeshiva University, Bronx, New York NY, USA

Neurotransmitter release probability (P_r) largely determines the dynamic properties of synapses. While much is known on the role of presynaptic proteins in transmitter release, their specific contribution to synaptic plasticity is unclear. One such protein, tomosyn, is believed to reduce P_r by interfering with SNARE complex formation. Tomosyn is enriched at hippocampal mossy fiber-to-CA3 pyramidal cell synapses (MF-CA3), which characteristically exhibit low P_r, strong synaptic facilitation and pre-synaptic PKA-dependent LTP. To evaluate tomosyn's role in MF-CA3 function, we transduced dentate granule cells with anti-tomosyn shRNA, in tandem with an optogenetic channel. Using this technique in mouse hippocampal slices we selectively activated neurons with reduced tomosyn levels. We found that facilitation, LTP, and PKA-induced potentiation were impaired at tomosyn-deficient synapses. These findings indicate that tomosyn is a key regulator of plasticity at the MF-CA3 synapse and highlight the power of a tandem expression approach when studying the role of presynaptic proteins.

Reprogramming of the hippocampal circadian clock in epilepsy

Christophe Bernard

INS - Institut de Neurosciences des Systèmes, Inserm UMR_S 1106, Marseille, France

The circadian clock has been extensively studied in the suprachiasmatic nucleus. In the latter, gene and protein expression vary during the night and day cycle, thus changing the molecular architecture. Surprisingly, we found that hundreds of genes and proteins also oscillate in a circadian fashion in the hippocampus, many of which are transcription factors. Hence, according the time of the day, the molecular architecture of the hippocampus, and possibly its functioning mode, may be different. In an experimental model of epilepsy, we found a full reprogramming of the hippocampal circadian clock. Most of the oscillating genes are not oscillating anymore, new genes are oscillating, and common oscillating genes show a phase shift. The functional consequences of these alterations remain to be investigated. Our results also indicate that some results looking at gene and protein expression in health and disease may require reinterpretation.

Dynamics of astrocytic processes influence excitatory synapses stability

Yann Bernardinelli

Astrocytic structures are frequently observed in close association with excitatory synapses, providing a morphological entity for bidirectional interactions with neurons. Synaptic activity regulates astrocytic process dynamics in the synapse periphery, which in turn influence longterm synaptic fate.

Deconstructing $GABA_B$ receptors

Bernhard Bettler

Department of Biomedicine, University of Basel, Basel, Switzerland.
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We are interested in how $GABA_B$ receptors control neuronal functions. Research over the past decade showed that cloned $GABA_B$ receptors do not reproduce the diversity of native receptor signaling. We therefore initiated a search for proteins that regulate $GABA_B$ receptor responses in their native context. In collaboration with Bernd Fakler (University Freiburg iBr) we used an unbiased proteomic approach that combines antibody-based affinity-purification with high-resolution quantitative mass spectrometry to identify $GABA_B$ receptor-associated proteins. Surprisingly, many of the identified proteins have not been implicated in the signaling of G-protein coupled receptors before. Finding the physiological functions of these proteins is therefore a challenging task. We adopted a strategy in which we systematically analyze the effects of proteins on receptor function and trafficking in a panel of assays in heterologous cells. We additionally probe the *in vivo* functions of these proteins in overexpression, knock-down and knock-out experiments. To address the molecular mechanism underlying functional effects of receptor-associated proteins we also identify their binding partners (besides the receptor) using reverse proteomics. My presentation will cover $GABA_B$ receptor-associated proteins for which this strategy has enabled us to identify a functional role.

Trans-synaptic SynCAM interactions organize synapse formation and neuronal connectivity

Thomas Biederer, Ph.D.

Department of Neuroscience, Tufts University School of Medicine, Boston, Massachusetts 02111, USA

Trans-synaptic interactions have emerged as key elements in guiding synapse development and maturation. Proteins of the SynCAM (Synaptic Cell Adhesion Molecule) family are trans-synaptic immunoglobulin proteins that organize excitatory synapses. Mapping the topography of the synaptic cleft, we demonstrate that SynCAM 1 marks the perimeter of excitatory synapses. Functionally, SynCAM 1 initially drives and then maintains synapse numbers *in vivo*. Loss of SynCAM 1 results in fewer synapses with altered ultrastructure. SynCAM 1 adhesion contributes to synapse development and network function in diverse brain areas that include the hippocampus and the retina. To delineate the signaling pathways that underlie synapse development, we screened for regulators by proteomic analysis of synaptic membranes purified from wild-type and SynCAM 1 knock-out mice. This identified Farp1 as an intracellular postsynaptic binding partner of SynCAM 1 that activates the GTPase Rac1 and triggers a retrograde signal regulating active zone composition. In addition, Farp1 promotes dendritic arborization, providing insights into how postsynaptic and dendritic development may be coordinated. Together, these results highlight how trans-synaptic interactions control synapse development and neuronal wiring.

Molecular and morphological correlates of vesicle priming in neurosecretory cells

Benjamin Cooper

Germany

To visualise different morphological synaptic vesicle states with high spatial resolution, we use an experimental approach combining rapid cryo-fixation by high-pressure freezing, freeze-substitutution, and 3D electron tomographic analysis of conventional (glutamatergic spine synapses) and ribbon synapses (photoreceptor ribbon synapses) in hippocampal and retinal model systems, respectively. Focusing on the molecular mechanisms underlying synaptic vesicle docking and priming, we systematically analysed the synaptic ultrastructural architecture and spatial arrangement of vesicles at active zones in a range of genetic mouse mutants lacking key presynaptic proteins, thereby enabling changes in nanostructural organization to be correlated with the specific synaptic dysfunction caused by respective mutations. Our data from hippocampal spine synapses reveals severe reductions in the pool of membrane-attached/docked vesicles in mutants lacking synaptic vesicle priming proteins (Munc13s, CAPSs) or SNARE (soluble N-ethylmalemide-sensitive factor attachment protein receptor) proteins (Syntaxin-1, SNAP25, Synaptobrevin-2), indicating that synaptic vesicle docking and priming steps may not reflect independent mechanisms, but rather describe the same molecular process, namely full or partial SNARE complex formation initiated by members from the UNC13/Munc13 protein family. Interestingly, however, we found that in contrast to conventional synapses, the absence of Munc13 priming proteins at retinal ribbon synapses has only a negligible impact on the number of docked/primed synaptic vesicles. These data indicate that photoreceptor-bipolar synapses utilize Munc13-independent mechanisms to prime synaptic vesicles for release and thus provide further experimental support for the hypothesis that ribbon synapses rely on a unique molecular ensemble to execute and maintain their specific mode of release.

Supralinear modulation of synaptic strength through additive effects on the energy barrier for vesicle fusion

L. Niels Cornelisse

The energy required to fuse synaptic vesicles with the plasma membrane is a major determinant in synaptic efficacy. Transition-state theory predicts that modulation of the energy barrier for vesicle fusion efficiently regulates synaptic efficacy because additive effects on this barrier are predicted to cause multiplicative effects on the fusion rate. To test this hypothesis experimentally, we developed a method to assess the fusion barrier using synaptic responses to hypertonic shock. Fitting a vesicle state model to these responses provides accurate estimates of the readily releasable vesicle pool, the rate constants for vesicle priming, unpriming, and fusion, and the fusion barrier height. We used ComplexinI/II deficient synapses and phorbol ester stimulation to demonstrate that additive effects on the fusion barrier indeed produce supralinear effects on synaptic transmission. The additive/multiplicative relationship between energy barrier and fusion rate demonstrated here provides an explanation for unexplained non-linear effects of genetic/pharmacological perturbations on synaptic transmission.

Contributions of neurogenesis in human cognition: Cellular and MRI studies

Sylvane Desrivières

Institute of Psychiatry, Psychology & Neurosciences, King's College London, UK

Genetic factors exert lasting influences on brain structures and functions associated with behaviour and predisposition to disease. Experimentations in animals have revealed functional relevance of neurogenesis for normal cognitive processes –such as learning and memory– and neurodegeneration. Yet, little is known about the genes that contribute to cognition in humans and how they affect brain and behaviour across the lifetime.

We have started to address this, in the largest and most comprehensive genetic neuroimaging studies to date, analysing brain scans acquired by magnetic resonance imaging in the context of two international neuroimaging Consortia, IMAGEN and the Enhancing NeuroImaging Genetics through Meta-Analysis (ENIGMA) Consortium.

These studies have enabled the identification of the first functional gene variant that associates with region-specific decrease in cortical thickness in 14y-old teenagers. They also led to the first genome-wide association studies (GWAS) of the volumes of seven subcortical regions and intracranial volume (ICV), in which eight loci influencing the volumes of the putamen, caudate nucleus, hippocampus –brain regions associated with movement, motivation, learning and memory– and ICV were identified.

Nonetheless, more refined methodologies than GWAS, taking into consideration developmental aspects and the polygenic nature of brain measures will be necessary to broaden this understanding. I will present data illustrating how combining appropriate stem cell-based genomics and GWAS approaches have contributed to enhancing our understanding of human brain function in health and disease.

Cell adhesion molecules at the nodal complex in health and disease

Jérôme Devaux

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The high density of voltage-gated sodium (Nav) channels at the nodes of Ranvier allows the rapid saltatory propagation of the action potentials along the axons. Cell adhesion molecules play an important function in the formation of the node and of the paranodal regions which flank the nodes. The complex gliomedin/NrCAM/neurofascin-186 is crucial for the initial aggregation of Nav channels at hemi-nodes. In addition, the complex Caspr1/contactin-1/neurofascin-155 dictates the formation of the paranodal axo-glial complex and participates to the formation of the nodes. In a recent study, we demonstrated that the node of Ranvier is the primary site of the immune attack in patients with Guillain-Barré syndrome (GBS) or chronic inflammatory demyelinating polyneuropathy (CIDP). GBS and CIDP are groups of neuropathies that affect peripheral nerves. We found that a subset of patients show antibodies against gliomedin, contactin-1, or neurofascin-155. In particular, antibodies to contactin-1 are associated with CIDP patients showing sensory ataxia. Using animal models, we demonstrated that the passive transfer of IgG against these cell adhesion molecules induced a severe pathology associated with conduction loss. Notably, antibodies to gliomedin induced a progressive neuropathy in Lewis rats characterized by demyelination and disruption of the nodal complex. These latter results indicate that cell adhesion molecules play an important role in the stabilization of Nav channels at nodes, and that the alteration of the axo-glial complex together with paranodal demyelination induces the dismantling of Nav channel clusters. Cell adhesion molecules thus play important function in myelin physiology and are potential targets of autoantibodies in human inflammatory neuropathies.

The sorting receptor SorCS1 interacts with neurexin and regulates synaptic proteome composition

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The formation, maintenance and plasticity of synapses require the assembly and turn-over of synaptic protein complexes. However, the mechanisms that regulate synaptic proteome composition remain elusive. Here, we identify the sorting receptor SorCS1 (Sortilin-related CNS expressed 1) as a key regulator of the synaptic proteome. Analysis of the SorCS1 complex reveals close association with a number of synaptic proteins, including adhesion molecules and glutamate receptors. The synaptic adhesion molecule neurexin is a major binding partner of SorCS1, and SorCS1 regulates neurexin surface expression. Quantitative proteomic analysis of cultured SorCS1-deficient neurons reveals a marked decrease in surface abundance of a broad range of receptors, including neurexin. Reduction of *SorCS1* levels in vivo broadly affects synaptic proteome composition, particularly receptors regulating synaptic adhesion and excitatory synaptic transmission. Our observations indicate that SorCS1 is required to maintain surface expression and abundance of neuronal receptors in vitro and in vivo. Mutations in *SORCS1* have been associated with autism, ADHD, and Alzheimer's disease, suggesting that misregulation of the synaptic proteome may contribute to the etiology of synaptopathies.

Keywords
Synapse formation, adhesion, trafficking, endosome, glutamate receptor

2-3 Lines on content
Neurexins are synaptic adhesion molecules with key roles in synapse development and function. We identify the sorting receptor SorCS1 as a novel binding partner of neurexin, and show that SorCS1 regulates neurexin expression on the neuronal cell surface. Our results identify SorCS1 as a novel neurexin interactor and show that SorCS1 is a key regulator of surface proteome composition in cultured neurons and of synaptic proteome composition in vivo.

The functional genome at the genetic-epigenetic-environment intersection

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Mechanisms involving environmental-induced changes of the functional genome, act through epigenetic modifications.
Our strategies for understanding the role of epigenetics in disease include early life exposures, genetic makeup and environmental assaults, e.g. smoking. All these may set the epigenome in “poised” states which do not nessecarily lead to immediate phenotypic changes, but may do so later on if the “proper” environmental impact arrives. We have e.g. shown that the mode of birth delivery, as well as duration of labor, can influence the specific DNA methylation in hematopoietic stem cells, that may have later consequences for disease, as has been suggested by epidemiological data.
Studies of epigenetics are also needed in order to understand the impact of the classic genetic variations of complex genetic disease. Our recent results suggest that integrated genetic and epigenetic analyses enhance the resolution of information, to identify genetic risk alleles for disease. By using a causal inference testing algorithm with genetic, epigenetic, and phenotypic data, we further show a dependence on DNA methylation for the penetrance of several risk alleles in rheumatoid arthritis, suggesting DNA methylation as a mediator of genetic risk for disease.
Since epigenetic profiles may in part be dependent on the genotype, the genetic make-up is in a way a confounder when studying epigenetic traits caused by environmental factors. We therefore performed pairwise comparisons of genome wide DNA methylation in blood from monozygotic twins discordant for rheumatoid arthritis. Together with mathematically performed cell type adjustment algorithms, this method reveals both non-genetically influenced phenotypically associated DNA methylation, as well as skewed cell type distribution between disease and health.

Connectomics: The dense reconstruction of neuronal circuits

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The mapping of neuronal connectivity is one of the main challenges in neuroscience. Only with the knowledge of wiring diagrams is it possible to understand the computational capacities of neuronal networks, both in the sensory periphery, and especially in the mammalian cerebral cortex. Our methods for dense circuit mapping are based on 3-dimensional electron microscopy (EM) imaging of brain tissue, which allows imaging at nanometer-scale resolution across substantial volumes (typically hundreds of micrometers per spatial dimension) using Serial Block-Face Scanning Electron Microscopy (SBEM). The most time-consuming aspect of circuit mapping, however, is image analysis; analysis time far exceeds the time needed to acquire the data. Therefore, we developed methods to make circuit reconstruction feasible by increasing analysis speed and accuracy, using a combination of crowd sourcing and machine learning. We have applied these methods to circuits in the mouse retina, mapping the complete connectivity graph between almost a thousand neurons, and we are currently improving these methods for the application to much larger neuronal circuits in the cerebral cortex. Using these methods, we want to measure the similarity between neuronal networks in the cortex of different individuals and different species in search for the algorithms of sensory perception, search for engrams of sensory experience in the cerebral cortex, and ultimately understand the alterations in neuronal network structure in psychiatric disease.

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Genetic dissection of neuronal migration using mosaic analysis with double markers

Simon Hippenmeyer

Coordinated migration of newly-born neurons to their prospective target area is an essential prerequisite for correct neuronal circuit assembly in the developing brain. However, the precise cellular and molecular mechanisms regulating neuronal migration, in particular at the single cell level, are not well understood. It is therefore essential to develop tools that allow the visualization and manipulation of migrating neurons at high resolution. MADM (for Mosaic Analysis with Double Markers) technology offers a unique genetic approach to visualize and concomitantly manipulate individual clones of genetically defined neurons at the single cell level in mice. MADM employs Cre recombinase/loxP-dependent interchromosomal recombination to reconstitute two split marker genes (green GFP and red tdTomato), and can thereby label sparse clones of homozygous mutant cells in one color (e.g. green), wild-type cells in the other color (e.g red) and heterozygous cells in yellow (both markers together) in an otherwise unlabeled background. Major MADM applications include, but are not limited to: single neuron labeling and 4D tracking; lineage analysis; circuit tracing, and conditional knockouts in small populations of neurons. We have recently extended the MADM technology; and assess the cell-autonomous and non-autonomous mechanisms regulating the sequential steps of cortical projection neuron migration with unprecedented single cell resolution.

Stem cells in a novel human mid-hindbrain malformation, microcephaly and intellectual disability phenotype

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During development, the mid-hindbrain, comprised of brainstem (midbrain, pons, medulla oblongata) and cerebellum, forms at the anterior part of the neural tube. The latter is divided into transient segments, or rhombomeres, through localized expression of genes. This segmentation is highly important for the proper development of various brain regions, and several proteins are known to contribute to localized gene expression. Therefore, developmentally related structures are often co-affected in diseases that are associated with early midbrain-hindbrain developmental disorders.

We identified a novel mid-hindbrain malformation phenotype in two affected children of a consanguineous family of Kurdish-Turkish descent. Clinically, the patients display facial dysmorphism, microcephaly, muscular hypotonia, and intellectual deficit with a severe speech delay. Cranial magnetic resonance imaging (MRI) revealed microencephaly, hypoplasia of the pons and grooves, and caudal vermis or cerebellar hypoplasia in patients. Whole exome sequencing revealed a homozygous nonsense mutation in a gene associated with localized gene expression, but not linked to any disease so far. A disruption of this gene causes neural precursors to remain in a cycling mode, leading to an expansion of the precursor pool and reduced neurogenesis. These processes likely contribute to the microcephaly phenotype of our patients.

Genes and drugs regulating stem cell function and repair

David Kaplan

Our group studies how genes and signaling proteins regulate stem cell function in the developing and adult brain. Stem cells must be maintained, undergo proliferation and differentiation when they receive the appropriate cues to build the brain during development, and regulate aspects of learning, memory and repair in the adult. Here, I will discuss new mechanisms we recently identified on how stem cells are maintained and mobilized, and how environmental influences such as maternal infection and diabetes perturb these processes.

There is a desperate need for new and effective therapies for neurological and wound healing indications. We have developed and used various drug screening platforms as well as translation of our basic research findings to discover drug candidates for cognitive dysfunction as a result of acute head trauma, wound healing, alopecia, peripheral neuropathy, and Parkinson's disease. Our findings suggest that academic drug discovery efforts can rapidly and inexpensively identify drug candidates for intractable neurological and wound-related conditions, and new signaling pathways and proteins regulating stem cell and axonal function.

Metabolic interference with epigenetic alterations in a rodent model of TLE

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Epigenetic mechanisms are self-perpetuating, posttranslational modifications of nuclear proteins (acetylation, methylation, phosphorylation, etc.), and DNA (methylation) that can produce lasting alterations in chromatin structure and gene expression patterns. They are increasingly recognized as fundamental regulatory processes in central nervous system development, synaptic plasticity, and memory. Epigenetic alterations are implicated in many neurological disorders including autism, bipolar disorders, schizophrenia, brain tumors, neurodegeneration, and more recently epilepsy.

We used a massive parallel sequencing approach to map genome-wide alterations in DNA methylation in a chronic rat TLE model. Sequencing of mRNA was used in same specimens for complementary gene expression profiling and integration with methylome data. Unsupervised clustering of an epigenetic mark, i.e. DNA methylation, successfully separated chronic epileptic from non-epileptic animals (Kobow et al., 2013). Further, aberrant methylation patterns could be inversely correlated with gene expression changes. Our data support the idea that epigenetic gene regulation may be critical in epileptogenesis and propagation of the chronic disease state. To further address this issue, we use an in vitro model of primary neuronal cell culture transiently stimulated with Glutamate to analyse the time-dependent epigenetic changes following a seizure-like event. We hope to distinguish the temporal and spatial epigenetic changes that lead to the molecular, anatomical, and functional reorganization of cells and networks respectively in support of a proepileptogenic phenotype.

Neuroligin-induced hippocampal synapses on functionalized micropatterns

Jürgen Klingauf and Markus Missler
(joint presentation)

To analyze the dynamics of presynaptic exo- and endocytosis with high-resolution nanoscopy and live-cell imaging (spinning disc, TIRFM) in unprecedented detail, we developed a novel neuronal culture preparation, henceforth referred to as`xenapses´. Using microstructured coverslips functionalized by click chemistry with extracellular domains of the essential synaptic cell adhesion molecule neuroligin as an artificial postsynapse, we are able to induce hippocampal neurons to form large flat varicosities as purely presynaptic terminals `en face´ directly on the host substrate. 4Pi microscopy revealed the presence of several synaptic marker proteins like synaptophysin, VGlut or bassoon, whereas postsynaptic PSD-95 labeling was mostly absent. Electron microscopy (CTEM, FIB-SEM) confirmed that these xenapses harbor several hundred synaptic vesicles in several clusters near the plasma membrane facing the substrate, and show typical hallmarks of active zones. The isolated presynaptic varicosities are maintained and stable for at least two weeks following transfection of fluorescent probes like pHluorin-tagged constructs, enabling us to observe individual fusion events and to monitor the behavior of other synaptic molecules such as voltage-dependent calcium channels. Since neuroligins have previously been shown to induce the formation and differentiation of hybrid synapses of heterologous cells through interaction with presynaptic neurexins, our novel, purely presynaptic preparation also allows us to dissect existing hypotheses on the differentiation of excitatory versus inhibitory terminals and to correlate particular trans-synaptic complexes with distinct release properties.

BDNF gathered cochlear driving force improves auditory fidelity, risking hyperactivity without gain after injury

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The accomplishment of spatial and temporal cortical resolution in the central auditory system is a process that is assumed to be initiated by the first auditory experience. Experience driven activity has been suggested to trigger intracortical inhibition towards shaping the central sound resolution in dependency of brain-derived neurotrophic factor (BDNF). The experience dependent driving force for this process has so far not been specified further. Using mice with conditional deletion of BDNF under the Pax2 promotor and the TrkC promotor, we here demonstrate that the deletion of BDNF in the cochlea but not in the CNS reduced sound sensitivity and noise vulnerability. Extracellular recording of inferior colliculus neurons of $BDNF^{Pax2}$-mutant mice revals that peripheral BDNF improves auditory fidelity (response, thresholds, latency, and dynamic range) by increasing inhibitory strength along the auditory pathway. After tauma, this BDNF auditory driving force can be lost as occurs in WT but not KO mice. As a consequence higher spontaneous firing rates without correspondent increase in stimulus-related responses occur. Data are discussed in the context of BDNF gathered cochlear driving force that improves sensory outcomes with sensory experience on risk to generate central hyperactivity without gain when this driving force is lost in the adult system after injury.

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MicroRNAs in nerve injury and neuropathic pain

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Nerve injury to a peripheral nerve initiates regenerative processes of neuronal axons but frequently is complicated by a pathological neuro- immune response leading to persisting neuropathic pain. The communication pathways linking between signals regulating inflammation, regeneration and pain are still incompletely understood. Here, we report that the interleukin-6 signal transducer gp130 involved in inflammation and neuron regeneration (1-3) may convey bidirectional body-brain pain messages through microRNA (miRNA) regulators of neuroinflammation and neuroregeneration. Next generation non-biased sequencing of mouse miRNAs from dorsal root ganglia (DRG), spinal cord, hippocampus and pre-frontal cortex (PFC) highlighted tissue-specific differences in miRNA changes induced by spared nerve injury (sni) compared to sham operation, with largest differences in the PFC of injured over control mice (295 PFC miRNAs 30% over- or under-regulated compared to 124 hippocampal miRNAs). Furthermore, mice with ablated gp130 in sensory neurons (SNS-gp130$^{-/-}$), which show a delay in peripheral nerve regeneration and a protection from maintained nerve injury-induced pain, presented generally limited sni-induced miRNA differences in the pain pathway. These differences were smaller than the effect of sham operation, suggesting causal involvement of miRNA changes not only in neuroregeneration but also in neuropathic pain reactions. Specifically, we localized ngf targeted miR-21 (4) in neuronal cell bodies within the DRG and found miR-21 significantly up-regulated on day 7 and day 28 after nerve lesion in wt mice. Reintroduction of gp130 with viral vectors into gp130 deficient neurons in vitro recovered expression of nociceptor specific transducer ion channel TRPA1 and the deficit in neurite outgrowth but not the reduced miR-21 levels associated with gp130 depletion. Our findings demonstrate that miRNAs participate in communicating body-brain messages associated with nerve injury and call for testing the potential of micro-RNAs as therapeutic targets for treating chronic pain and nerve injury.

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Quantitative analysis of synaptic structure by cryo-electron tomography

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Neuronal synapses contain a complex network of filaments whose precise organization and functions are not yet understood. We have investigated this network by cryo-electron tomography, a method that allows visualization of vitrified, fully hydrated biological samples at molecular resolution. The wealth of information present in cryo-tomograms of synapses from animals that have altered expression levels of target proteins is a challenge for the interpretation of the visualized structures that we address by both image processing and biochemical methods.

We have previously developed a basic structural model of synaptic vesicle mobilization and release whereby the number and nature of vesicle tethers indicate vesicle progression towards release. More recently, we found out that RIM1Î± KO synapses showed significant alterations in vesicle distribution and synaptic vesicle tethering to the AZ, while a pharmacological activation of Munc13 lead to changes in short tethers. Synapses from mice having genetically altered synuclein isoform levels showed changes in tethering, as well as synaptic vesicle connectivity, defined as the number of vesicles linked via short filaments to other synaptic vesicles. This argues that genetic manipulations of synuclein isoform levels profoundly affect the organization of the presynaptic terminal and indicate that synucleins are likely to have multiple functions at the synapse. More generally, our quantitative characterization of synapses allowed us to build a correspondence between various structural properties and physiological states and to extract information that is not accessible by other methods.

Recent instrumentation development are promising to further improve the quality of cryo-tomograms. Better transfer properties and lower noise brought by direct electron detector devices and electron microscope phase plates are already increasing the achievable resolution. Furthermore, focused ion beam thinning of vitrified samples allows recording cryo-tomograms of thicker samples, such as synapses of neurons grown in culture.

Ultrastructure and macromolecular organization of the human cochlea

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The human ear is deftly sensitive to sound. At behavioral thresholds, the basilar membrane (BM) produces minute vibrations in the range of 0.1 nm, a distance similar to the diameter of a single hydrogen atom. This sensitivity depends on delicate structure of human cochlea that is elaborate to examine since it is located deep in the skull base, surrounded by hard bone, and rapidly undergoes autolytic change. Regardless of these obstacles outstanding morphological results (LM, TEM and SEM) have been obtained during years using both post-mortal perilymphatic perfusion and peri-operative biopsy techniques. Here, we display TEM, SEM and immunohistochemical results based on inner ear specimens obtained at surgery as well as after post-mortal perilymphatic fixation. Cellular and molecular preservation in collected samples at surgery allows protein identification, localization and quantification using confocal immunohistochemistry. Cell culture can also be used. Here, we present some recent results obtained at our laboratories in Innsbruck and Uppsala with particular focus on clinical relevancy.

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Methyl-CpG-binding domain protein 3 (MBD3) in the rat model of epilepsy

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MBD3 protein (*methyl-CpG-binding domain protein 3*) belongs to evolutionarily conserved family of proteins which are characterized by the presence of a methyl-CpG-binding domain (MBD), which is recognizing methylated CpG dinucleotides in the DNA. There is little data on the role and expression of MBD3 in brain pathology.
In the present study we examined the localization of MBD3 mRNA and protein in the brain and MBD3 expression level in the temporal lobe structures in control animals and following amygdala stimulation induced status epilepticus. We demonstrated that MBD3 mRNA is widely expressed in the brain. MBD3 transcript was present in neurons, oligodendrocytes and astrocytes both in control and epileptic animals. We detected also widespread MBD3 protein expression in the nuclei of neurons, mature oligodendrocytes, resting and activated astrocytes, but not in microglia in control brains and 14 days after stimulation. In normal brain, 98,0-99,7% neurons contain MBD3 protein in the temporal lobe structures. Following status epilepticus the percentage of MBD3 positive neurons increased in the piriform cortex and in the lateral nucleus of the amygdala. Moreover, we observed a significant increase in MBD3 immunofluorescence level in neurons in epileptic rats compared to control animals. We characterized the protein complex containing MBD3 and concluded that it assembles with MTA, HDAC and RBBP, components of NuRD. Finally, using chromatin immunoprecipitation combined with deep sequencing, we demonstrated differences in occupancy of DNA regions by MBD3 protein between control and stimulated animals.
Obtained results indicate that complex NuRD, containing MBD3 protein, can play a role in brain pathology.

Control of cortical interneuron migration by N-cadherin

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In the developing brain, cortical GABAergic interneurons migrate long distances from the medial ganglionic eminence (MGE), where they originate, to the cortex where they settle among principal cells and differentiate. Migration is the result of the interplay between intrinsic cell motility properties and extrinsic signals that influence and guide cell movements. Among those extrinsic signals, both chemical and physical cues can influence cell adhesion, cell polarity and neuritic outgrowth.
We have recently shown in mouse embryos that MGE GABA interneurons can sense extrinsic cues not only at their leading edge but also in the centrosomal region. The centrosome of these migrating MGE cells can organize a primary cilium at the cell surface. Signals transduced in the primary cilium control the escape of future GABAergic interneurons from their tangential routes toward the cortical plate. We have also analyzed the role of an adhesive signal, the cell adhesion molecule N-cadherin, which is expressed by both MGE cells and by the cells in their migratory pathway and in cortical plate. Here we show that N-cadherin controls MGE cells motility and directionality in both tangential paths and during cortical plate colonization. Using biomimetic substrates of N-cadherin, we also show that N-cadherin activates leading process outgrowth and favors fast and synchronous centrosomal and nuclear movements without preventing docking of the centrosome to the plasma membrane. Analyses of MGE cells from mouse embryos expressing a GFP-myosinIIB fusion protein show abnormal myosin IIB dynamics in these cells. Our results identify N-cadherin-mediated cell-cell interactions as signals that stimulate MGE cell motility and contribute towards maintaining cell polarity.

Translational mechanisms regulating mammalian neurogenesis

Freda D. Miller

One of the fundamental questions in human biology is how a pool of proliferating neural precursor cells (NPCs) in the embryo can ultimately generate the complex functional neural circuitry that comprises the adult mammalian brain. In that regard, this lecture will focus upon the precursors that generate the mammalian cerebral cortex, describing emerging evidence that translational mechanisms play a key role in regulating the genesis of appropriate numbers of cortical neurons, and describing how this might go awry in genetic disorders that cause cognitive dysfunction.

Neuroligin-induced hippocampal synapses on functionalized micropatterns

Jürgen Klingauf and **Markus Missler**
(joint presentation)

To analyze the dynamics of presynaptic exo- and endocytosis with high-resolution nanoscopy and live-cell imaging (spinning disc, TIRFM) in unprecedented detail, we developed a novel neuronal culture preparation, henceforth referred to as`xenapses´. Using microstructured coverslips functionalized by click chemistry with extracellular domains of the essential synaptic cell adhesion molecule neuroligin as an artificial postsynapse, we are able to induce hippocampal neurons to form large flat varicosities as purely presynaptic terminals `en face´ directly on the host substrate. 4Pi microscopy revealed the presence of several synaptic marker proteins like synaptophysin, VGlut or bassoon, whereas postsynaptic PSD-95 labeling was mostly absent. Electron microscopy (CTEM, FIB-SEM) confirmed that these xenapses harbor several hundred synaptic vesicles in several clusters near the plasma membrane facing the substrate, and show typical hallmarks of active zones. The isolated presynaptic varicosities are maintained and stable for at least two weeks following transfection of fluorescent probes like pHluorin-tagged constructs, enabling us to observe individual fusion events and to monitor the behavior of other synaptic molecules such as voltage-dependent calcium channels. Since neuroligins have previously been shown to induce the formation and differentiation of hybrid synapses of heterologous cells through interaction with presynaptic neurexins, our novel, purely presynaptic preparation also allows us to dissect existing hypotheses on the differentiation of excitatory versus inhibitory terminals and to correlate particular trans-synaptic complexes with distinct release properties.

Modulation of short-term plasticity at the Calyx of Held synapse

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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 is observed only. 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. Also, evidence will be presented that the DAG-dependent process (mimicked by application of PdBu) is strongly Ca^{++} dependent. The joint modulation by both agents can best be modeled by assuming a multiplicate effect on the rate-constant of superpriming.

MicroRNA targeting of CoREST controls polarization of migrating cortical neurons

Laurent Nguyen

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The migration of cortical projection neurons is a multistep process characterized by dynamic cell shape remodeling. The molecular basis of these changes remains elusive and the present work describes how microRNAs (miRNAs) control neuronal polarization during radial migration. We show that miR-22 and miR-124 are expressed in the cortical wall where they target components of the CoREST/REST transcriptional repressor complex, thereby regulating *doublecortin* transcription in migrating neurons. This molecular pathway underlies radial migration by promoting dynamic multipolar-bipolar cell conversion at early phases of migration, and later stabilization of cell polarity to support locomotion on radial glia fibers. Thus, our work emphasizes key roles of some miRNAs as epigenetic safeguard that controls radial migration during cerebral corticogenesis.

Chronic changes in microRNAs after traumatic brain injury in the rat

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Objective. Traumatic brain injury (TBI) is estimated to cause 10-20% of all acquired epilepsies. After the initial damage caused by direct mechanical force to the head, secondary damage develops over time consisting of molecular changes that underlie the subsequent reorganization of neuronal networks. The little evidence available suggests that changes in the expression levels of microRNAs controls some part of the alterations found in the expression of hundreds of genes after TBI.

Hypothesis. Changed microRNA level alters gene expression of its targets. These changes in molecular networks regulates post-injury reorganization of neuronal circuits.

Materials and methods. TBI was induced with lateral fluid-percussion injury to adult rats (n=6). Five sham-operated rats served as controls. At 3 months after TBI sampling of the dentate gyrus was done for total RNA extraction. Gene expression arrays were used to examine separately levels of microRNAs and mRNAs. Further, we used bioinformatics approach to assess putative targets for altered microRNAs.

Results. We found 12 microRNAs to be down-regulated ($p<0.05$) at 3 months after TBI. At the same time, 654 mRNAs were up-regulated and 212 down-regulated ($p<0.05$). According the Ingenuity Pathway Analysis three top molecular and cellular functions among altered genes were cellular movement, cellular growth and proliferation, and cell death and survival. Decreased microRNA levels highly correlated with increased levels of their known and putative mRNA targets.
Conclusion. Our results demonstrate long-lasting change after TBI in microRNAs which can explain altered gene expression levels of protein coding genes.

Elucidating the molecular mechanism of neurotransmitter release

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Neurotransmitter release is controlled by a highly sophisticated protein machinery. Results from multiple groups including ours have led to a model of release whereby: i) the SNAREs syntaxin-1, synaptobrevin and SNAP-25 form SNARE complexes that bridge the vesicle and plasma membranes to catalyze membrane fusion; ii) NSF/SNAPs disassemble SNARE complexes; iii) Munc18-1 binds to a self-inhibited 'closed' conformation of syntaxin-1 and, together with Munc13, orchestrates SNARE-complex assembly; iv) synaptotagmin-1 acts as the Ca^{2+} sensor that triggers fast release; and v) complexins play dual, active and inhibitory roles in a tight interplay with synaptotagmin-1. Recently, we resolved the paradox that emerged from the findings that Munc18-1 and Munc13 are essential for neurotransmitter release in vivo, yet syntaxin-1/SNAP-25-liposomes can fuse efficiently with synaptobrevin-synaptotagmin-1-liposomes in vitro. Thus, we showed that such fusion is abrogated by NSF/α-SNAP because they disassemble the syntaxin-1/SNAP-25 complex and then fusion requires Munc18-1 and Munc13, which mediate SNARE complex assembly in an NSF/SNAP resistant manner. Moreover, we have uncovered a tight interplay between the different domains of Munc13 in these reconstitution assays that correlates with the roles of these domains in neurotransmitter release and presynaptic plasticity. In structural studies using nuclear magnetic resonance experiments that measure lanthanideinduced pseudocontact shifts, we have elucidated the major synaptotagmin-1-SNARE complex binding mode in solution. The dynamic structure revealed by our data supports a model whereby, upon Ca^{2+} influx, synaptotagmin-1 release the inhibition caused by complexin and cooperates with the SNAREs to bring the synaptic vesicle and plasma membranes together to induce fast membrane fusion.

New layers of synaptic transmission control

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Release of neurotransmitter is triggered by action-potential gated influx of Ca^{2+} into nerve terminals in the vicinity of docked synaptic vesicles at the plasma membrane. Small changes in Ca^{2+} influx leads to large changes in exocytosis efficiency reflecting the non-linear nature of the calcium-exocytosis coupling. The magnitude of Ca^{2+} influx is dictated by properties of voltage-gated Ca^{2+} channels in the active zone as well as the amplitude and kinetics of the action potential as this controls the opening of the channels and dictates the driving force for Ca^{2+} ion entry. My lab has been developing and using optical tools to measure both the local action potential waveform and Ca^{2+} influx at nerve terminals to determine how these are modulated by active zone proteins and by classical G-protein coupled pathways such as $GABA_B$ receptors.

A novel view of neurogenesis and memory encoding in the dentate gyrus

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The dentate gyrus is the first relay station in information flow from the entorhinal cortex towards the hippocampus, and it plays a crucial role in memory processing. A remarkable feature of the dentate circuitry is the high degree of plasticity conveyed by its ability to generate and integrate new principal neurons (granule cells, GCs) through life. Extensive evidence indicates that adult-born GCs are important for specific forms of memory, and my laboratory is focused on understanding the precise modifications of local dentate networks produced by the incorporation of newly generated GCs. Adult-born GCs develop and connect over several weeks before they achieve complete morpho-functional maturation. Our recent findings indicate that developing GCs at different stages of maturation may play distinct roles in memory encoding. In my talk I will focus on recent experimental data applying optogenetics and chemogenetics to dissect changes in local microcircuits by adult neurogenesis, highlight the critical role of GABAergic interneurons, and propose a novel view on how newborn GCs may contribute to memory encoding.

The novel Notch ligand EGFL7 governs adult neurogenesis *in vivo*

Mirko Schmidt

In neurobiology the dogma on the unchangeability of the adult mammalian brain and its inability to give rise to new neurons has been challenged since the early nineties. Generally, it is now accepted that neurogenesis occurs in the adult brain and originates from neural stem cells (NSCs) that reside in stem cell niches such as the subventricular zones (SVZ) in the lateral walls of the lateral ventricles. A central molecular pathway regulating NSCs and adult neurogenesis is the Notch signaling cascade.
Previously, we identified the secreted epidermal growth factor-like protein 7 (EGFL7) as a novel non-canonical Notch ligand promoting neuronal differentiation of NSCs *in vitro* that acts by competing with canonical Notch ligands of the Jagged type. In order to determine whether or not EGFL7 regulates NSCs *in vivo* we explored an EGFL7 knock-out mouse model and performed cerebro-ventricular injection studies of adenoviruses encoding for EGFL7 into the lateral ventricle of adult mice. Further, we performed expression analyses and quantitative mass spectrometry (SILAC) in order to understand the molecular mechanism behind EGFL7's impact on NSCs. Last, we analyzed the population of newborn neurons in the olfactoric bulb and correlated the data with mouse behavior. The combination of *in vivo* models and comprehensive signaling studies revealed that EGFL7 exerts a profound and Notch-dependent effect on the architecture of the NSC niche in the SVZ. In conclusion, EGFL7 governs adult neurogenesis *in vivo* and is an important player in the regeneration of the adult brain.

Transcriptional and epigenetic control of aberrant neuronal plasticity in epileptogenesis

Susanne Schoch

Temporal lobe epilepsy (TLE) is one of the most common seizure disorders in adults. In acquired epilepsies, like TLE, spontaneous seizures begin after an initial injury to a healthy brain as a consequence of trauma, status epilepticus, infection or stroke. The underlying mechanisms during its etiopathogenesis, collectively referred to as epileptogenesis, are still poorly understood. However, it is now well established that the changes occurring during this period that ultimately cause alterations in neuronal network function involve significant changes in gene-expression patterns, both transiently and long-term. Accumulating evidence suggests that transcriptional mechanisms play an important role in the transient regulation of gene activity and that modulation of the chromatin structure mediates long-term changes in gene expression. We have recently found the transient transcriptional upregulation during the latent phase of epileptogenesis of the pore-forming T-type Ca2+ channel subunit CaV3.2 to be critical for the development of recurrent spontaneous seizures and of neuropathological alterations. In the next step we have analyzed the signaling cascades involved in CaV3.2 transcriptional regulation and could identify two transcription factors, early growth response 1 (Egr1) and the zinc sensor metal-regulatory transcription factor-1 (MTF1), can activate CaV3.2 gene expression in vitro and in vivo. Intriguingly, the activating effect of Egr1 in vitro in counterbalanced by repressor element 1 (RE-1) protein-silencing transcription factor (REST). rAAV-mediated overexpression in mice hippocampi in vivo of a dominant-negative variant of Egr1 or MTF1 significantly reduced the pilocarpine-induced CaV3.2 upregulation. Thus, Egr1 and MTF1 can regulate CaV3.2 promoter activity and mRNA expression and hence, the size of ICaT. These findings may provide new perspectives for pharmacological intervention aimed at epigenetic and/or promoter regulation for preventing the process of epileptogenesis.

miRNA function in synapse development and plasticity

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Our research group is interested in the role of microRNAs (miRNAs), a large class of small non-coding RNAs, in synapse development and plasticity in mammalian neurons, as well as the potential impact of miRNA regulation on higher cognitive functions and neurological disease. During the last years, we have identified key neuronal miRNAs and their targets that are involved in dendrite and spine morphogenesis in rat hippocampal neurons. One of these miRNAs is part of a large imprinted, mammalian-specific miRNA cluster. Induced expression of the miRNA cluster by neuronal activity is required for dendritic arborization and the downscaling of excitatory synapses, a form of homeostatic plasticity that is frequently disturbed in neurodevelopmental and psychiatric disorders. Accordingly, validated target genes of this miRNA cluster are frequently deregulated in neurological disease. Mechanistically, cluster miRNAs are regulated at the level of transcription, dendritic transport and by a novel competing endogenous RNA encoded by a gene frequently mutated in autism-spectrum disorders (ASD). Our results point to a function of microRNAs in the control of synapse homeostasis and raise the possibility that impaired miRNA function could contribute to synaptic dysfunction in neurodevelopmental disorders, including ASD.

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The effect of the environment on brain activity and behaviour: Characterisation of epigenetic mediators

Gunter Schumann

In this presentation we are reporting the effect of environmental influences, including psychosocial stress and substance use on brain activity during reinforcement-related behaviour i.e. reward processing, behavioural inhibition and emotional processing, and their epigenetic mediation. (1) We show that life events influence brain activity of a network emanating from the parahippocampal gyrus during emotional processing, and that these changes are associated with anxiety measures in adolescent girls. Epigenetic analyses are ongoing. (2) Brain activity in the subthalamic nucleus during behavioural control is associated with escalation of alcohol drinking. A gemone-wie methylation study revealed that both, alcohol drinking as well as brain activity during behavioural control; are associated wuth hypermethylation in the 3**:**-protein-phosphatase-1G (PPM1G) gene locus. (3) Ventral striatal activity during reward processing is associated with OPRL1 methylation, psychosocial stress and alcohol abuse. Together our data provide evidence for a behaviourally relevant epigenetic mediation of environmental effects on brain activity.

Single-cell analysis of adult neural stem cells and neurogenesis

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Santigao Ramon Cajal famously deemed the mature central nervous system as a place where “everything may die, nothing may be regenerated”. Studies in the last decades instead have revealed tremendous plasticity of the mature brain. Probably, the most striking form of structural plasticity in the adult mammalian brain is continuous generation of new neurons in discrete regions through a process named adult neurogenesis. Adult neurogenesis arises from neural stem cells within specialized niches and is regulated by various experience. Using adult mouse as an experimental model, my laboratory has been addressing basic questions related to adult neurogenesis in the hippocampus. In particularly, we have been developing ‘singe-cell” technology to investigate adult neural stem cells and neurogenesis in vivo. I will present results from our latest studies on revealing transcriptome landscape of adult neurogenesis from single-cell RNA-sequencing and dynamic properties of adult neural stem cells from clonal analysis.

Phosphatidylinositol 4,5-Bisphosphate uncaging potentiates exocytosis by activating synaptotagmin

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Regulated secretion relies on exocytosis, the Ca^{2+}-dependent fusion of intracellular vesicles or organelles with the plasma membrane. Whether and how the membrane lipid phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$] controls the last exocytosis step - fusion triggering - is unknown. To investigate this, we created a membrane-permeant, photoactivatable $PI(4,5)P_2$ derivative, allowing us to increase $PI(4,5)P_2$ levels in secretory cells and neurons rapidly and non-invasively by optical uncaging. We demonstrate that $PI(4,5)P_2$ potentiates neurosecretion and increases synaptic release probability in the presence, but not in the absence, of the Ca^{2+} sensor synaptotagmin-1. To understand this effect we built a mathematical model of synaptotagmin-driven membrane apposition by cooperative binding to Ca^{2+} and $PI(4,5)P_2$ using parameter values determined *in vitro* and showed that this model is sufficient to account for the complex kinetic properties of neurosecretion and $PI(4,5)P_2$ uncaging. Thus, we conclude that $PI(4,5)P_2$ might be a key determinant of exocytosis triggering, which together with Ca^{2+} supports synaptotagmin-driven membrane apposition and membrane fusion.

From mice to men: Fine tuning of cholinergic signaling by non-coding RNAs

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Continuous communication between the nervous and the immune system is essential both for maintaining homeostasis and for ensuring rapid and efficient response to stressful and infection insults. Non-coding and microRNA (miRNA) regulators provide exciting and challenging models for studying this communication in anxiety and inflammation. Global genomic analyses show that miRNAs co-evolved with their target transcripts (Barbash et al. Mol Biol Evol 2014) to efficiently control neuronal signaling pathways and enable contribution to the development of higher brain functions while avoiding damaging evolutionary impact. Specifically, miRNA controllers of acetylcholine signaling (CholinomiRs (Nadorp & Soreq Front Mol Neurosci 2014)) modulate both anxiety and inflammation reactions to external insults through physiologically relevant bidirectional competition on interaction with their targets. We found rapid increases of the evolutionarily conserved neuro-modulator acetylcholinesterase (AChE)-targeted CholinomiR-132 in acute stress (Shaltiel et al. Brain Struct Funct 2013), intestinal inflammation (Maharshak et al. Inflamm Bowel Dis 2013) and post-ischemic stroke, inversely to its drastic reduction in the Alzheimer's disease brain (Lau et al. EMBO Mol Med 2013). Furthermore, single nucleotide polymorphisms interfering with the AChE-silencing capacities of the primate-specific CholinomiR-608 associate with elevated trait anxiety, inflammation and diverse aging-related diseases in human volunteers (Hanin et al. Hum Mol Genet 2014), whereas long non-coding RNAs complementary to such miRNAs are modulated in Parkinson's disease and by deep brain stimulation (Soreq et al. PLoS Comput Biol 2014). Deepened understanding of the evolution and complexity of neuronal non-coding RNAs may highlight their role in the emergence of human brain functions while enhancing the ability to intervene with diseases involving cholinergic signaling impairments.

Changes in the expression of histone deacetylases in the hippocampus in models of temporal lobe epilepsy

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Histone acetylation and deacetylation are mechanisms contributing to epigenetic regulation of gene expression. Deacetylation of histone proteins contributes to transcriptional silencing of gene expression, whereas histone deacetylation augments gene expression. Histone deacetylation is carried out by histone deacetylases (HDAC) comprising four classes of enzyme families comprising together 11 HDAC isoforms. To investigate the possible contribution of individual HDACs in the development of epilepsy, we explored possible changes in the expression of individual HDACs in two mouse models of temporal lobe epilepsy, 1) a model using unilateral intra-hippocampal injection of kainic acid (KA) and 2) i.p. injection of piocarpine. In the KA model, EEGs were continuously recorded by telemetry for 4 weeks. After an initial status epilepticus, the mice exhibited about two spontaneous seizures per day. They were killed at different intervals (4, 12, 24, 48 hrs, and 14 and 28 days) n. Coronar sections were obtained and subjected to *in situ* hybridization for HDAC 1-11 mRNAs.

Our data show distinctly different and specific expression patterns for individual HDAC mRNAs indicating rather specific changes in the expression of numerous genes after KA-induced seizures. The changes follow different time courses and different anatomical patterns with graded responses in individual hippocampal subfields. These distinctly different changes may be related to different phases of epileptogenesis including 1) acute seizures during the status epilepticus, the phases of 2) manifestation of spontaneous seizures or 3) manifest epilepsy. For example expression of class I HDACs 1 and 2 mRNAs was significantly decreased in the ipsi- and contralateral granule cell layer between 4 and 48 hrs after KA injection. These bilateral *decreases* (also affecting the CA1 and CA3 sectors of the hippocampus) may be caused by the initial status epilepticus and may be related to the rapid expression of a variety of proteins such as immediate early genes or BDNF. This initial phase was followed by *increased* expression of the class I HDACs 2 and 3 after 24 hrs to 7 days. These changes may induce a compensatory down-regulation of gene expression of related proteins and coincide with the manifestation of spontaneous behavioral seizures. At the long time intervals (14 and 28 days after KA injection) mRNA levels of HDACs 5 and 9 mRNAs were markedly increased in the KA injected granule cell layer. This prominent change paralleled development of a pronounced unilateral dispersion of the granule cell layer. Class II HDACs 5 and 9 may be exported from the nucleus to the cytoplasm and may there exert their enzymatic activity e.g. on cytoskeletal proteins altering lamination of granule cells. In contrast, in the pilocarpine model, where no granule cell dispersion develops, we observed at the same interval (14 days after pilocarpine) pronounced down-regulation of the same HDACs (HDAC 5 and 9) in all hippocampal subfields. This change may be related to spontaneous seizures becoming manifest at this interval.

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Changes in the structure and function of the central auditory system with aging

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With the prolongation of human life, the number of people suffering from age-related hearing loss continuously increases, yet there is no efficient method of treatment with the exception of hearing aids or cochlear implants. The age-related apoptotic decrease in the number of inner ear receptors, outer and inner hair cells, is well known, however, the changes in the central auditory system have been described to a lesser extent. The results of animal experiments show that one of the significant age-related changes is the deterioration of inhibitory transmission in the central auditory pathway. Age-related changes in the auditory system are also present in the levels of calcium-binding proteins.. Both in human subjects and experimental animals, aging is accompanied by a worsening of temporal resolution as demonstrated by increased gap detection thresholds or by poor detection of speech in noisy environments. Our results from examining the human auditory cortex with magnetic resonance spectroscopy show that aging is connected with a significant decrease in the levels of glutamate and N-acetylaspartate. With MR morphometry we were able to reveal age-related decrease in the volume of gray matter in the primary auditory cortex, planum temporale and gyrus frontalis superior. Also functional MR imaging highlights differences between activation of the auditory cortex in old subjects and young controls, with larger activation in old subjects mostly in the non-dominant hemisphere. All these results allow us to envisage the development of more biologically oriented treatments of presbycusis in the future.

Age-related hearing loss: Apoptosis, autophagy and senescence: The role of IGF-1

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Presbyacusis is a neurodegenerative disorder that affects approximately half of the population over 60 years old. Hearing loss (HL) occurs when the sensory cells and neurons of the cochlea degenerate and die. Genetic and environmental factors contribute to the progression of HL, being noise the main environmental noxious agent for human hearing. There is no restorative treatment for deafness but functional replacement by means of prosthesis. Therefore, prevention and treatment of HL is an unmet medical need. The described pathophysiological mechanisms involved in HL include oxidative stress, excitotoxicity and inflammation, resulting in synaptic loss, axonal degeneration, and apoptosis of spiral ganglion neurons. These mechanisms are shared with other neurodegenerative disorders. Neuroinflammation is an essential element in the progression of injury and cell loss, and a target for cell protection strategies. Autophagy and senescence are cellular processes involved in development and ageing, whose contribution to inner ear structures and function will be discussed in the context of their regulation by IGF-1.

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Glia-to-neuron shuttling of miR-146a via extracellular microvesicles modulates synaptotagmin I translation in neurons

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Astrocytes and microglia release extracellular vesicles (EVs) upon activation, which participate to glia-to-neuron signalling. Using miRNA real-time PCR panels we identified a set of miRNAs differentially expressed in EVs produced by pro-inflammatory compared to pro-regenerative microglia. Among them there was miR-146a, a glial-enriched microRNA, which is altered in brain disorders and targets neuron specific genes. We showed that glia-derived EVs transfer their miR-146a cargo to cultured neurons, as proved by a *Renilla* luciferase-based specific sensor, and decrease immunoreactivity of a validated miR-146a target, i.e. the synaptic vesicle protein synaptotagmin I. Additionally, by visualizing single EV-neuron contacts driven by optical manipulation we revealed highly dynamic interaction between EVs and neurites, with EVs moving along neuronal processes. More stable contacts occurred between EVs and the cell bodies, where EVs stayed attached to the neuronal surface up to 2h after adhesion, ruling out the possibility that EVs undergo rapid internalization or full fusion with cell membrane. Further investigation is ongoing to identify surface proteins mediating EVs-neuron interaction and to clarify whether EVs can open a transient pore to transfer their cargo to neurons.
Our study sheds light on an unexpectedly regulated trafficking outside neurons of miRNA-storing EVs, and on capability of glia-derived EVs to modulate neuronal gene expression.

Glycolipids at PNS and CNS nodes during maintenance and disease

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Gangliosides are widely expressed sialylated glycosphingolipids with multifunctional properties in different cell types and organs. In the nervous system, they are highly enriched in both glial and neuronal membranes where they act as targets for autoantibodies in paralytic disorders. Mice lacking complex gangliosides due to targeted ablation of the B4galnt1 gene that encodes beta-1,4 N-acetylegalactosaminyltransferase 1 (GalNAc-transferase; GalNAcT$^{-/-}$) develop normally before exhibiting an age-dependent neurodegenerative phenotype characterised by marked behavioural abnormalities, central and peripheral axonal degeneration, reduced myelin volume and loss of axo-glial junction integrity. The cell biological substrates underlying this neurodegeneration, and the relative contribution of either glial or neuronal gangliosides to the process are unknown. To address this, we generated neuron-specific and glial-specific GalNAcT rescue mice crossed on the global GalNAcT$^{-/-}$ background (GalNAcT$^{-/-}$-*Tg(neuronal)* and GalNAcT$^{-/-}$-*Tg(glial)*), and analysed their behavioural, morphological and electrophysiological phenotype. Complex gangliosides, as assessed by thin layer chromatography, mass spectrometry, GalNAcT enzyme activity, and anti-ganglioside antibody immunohistology were restored in both neuronal and glial GalNAcT rescue mice. Behaviourally, GalNAcT$^{-/-}$-*Tg(neuronal)* retained a normal 'wild-type' phenotype throughout life, whereas GalNAcT$^{-/-}$-*Tg(glial)* resembled GalNAcT$^{-/-}$ mice, exhibiting progressive tremor, weakness and ataxia with ageing. Quantitative electron microscopy demonstrated GalNAcT$^{-/-}$ and GalNAcT$^{-/-}$-*Tg(glial)* nerves had significantly increased rates of axon degeneration and reduced myelin volume whereas GalNAcT$^{-/-}$-*Tg(neuronal)* and wild-type appeared normal. The increased invasion of the paranode with juxtaparanodal Kv1.1, characteristically seen in GalNAcT$^{-/-}$ and attributed to a breakdown of the axo-glial junction, was normalised in GalNAcT$^{-/-}$-*Tg(neuronal)* but remained present in GalNAcT$^{-/-}$-*Tg(glial)* mice. These results indicate that neuronal rather than glial gangliosides are critical to the age-related maintenance of nervous system integrity.

Abstracts Posters

Abstracts are listed alphabetically according to presenting author

Roles for MicroRNAs in retinal pigmented epithelium development and function

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Normal vision depends on the retinal pigmented epithelium (RPE), a monolayer of tightly connected polarized pigmented epithelia that support and maintain photoreceptor (PR) activity and survival. Herein, we uncover the roles of microRNA (miRNA), small regulatory non-coding RNAs, in the developing RPE. To this end we inactivated *Dicer1*, a key mediator of miRNA biosynthesis, using the *DctCre* transgene, in which Cre is expressed in the developing RPE after the specification. MiRNAs were found to be dispensable in the maintenance of RPE precursor fate during embryonic development; however, they were essential for tissue maturation and for acquisition of key properties of the RPE, including expression of visual cycle genes, pigmentation and cell adhesion. In addition to being required for the RPE itself, miRNA function in the RPE affected maturation of the adjacent PR, specifically the morphogenesis of the PR's outer segments. The unbiased transcriptomic data of the mutant RPE exposed the families of miRNAs and the predicted complex gene regulatory network that participate in the differentiation of the RPE and the generation of functional PRs.

The dynamic properties of receptive fields in the rat superior colliculus visual neurons

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The concept ‘a receptive field’ is widely used in vision research. A receptive field is defined as the area of vision in which visual stimuli can evoke a response in a neuron. Outside this area no visual stimuli can evoke a response. Typically receptive fields are determined by probing with a small, less than 5 degrees in diameter stimuli although more complex stimuli such as slits and gratings are also used for receptor field determination. It has been shown that in the superficial layer of rodent superior colliculus neurons typical size of receptive fields range from 5 to 40-60 degrees in diameter, in the majority of cases it does not exceed 25 degrees. Here we show that in the neurons of the rat superior colliculus a small stimulus of 1.5-3.5 degrees in width evoked responses from a vision area of 15-35 degrees in diameter in good agreement with previous reports. However, larger stimuli of 7.5-15 degrees could evoke responses from almost all tested areas, corresponding to the receptive fields of >90-100 degrees in diameter. Although this result was obtained mainly with multi-unit recordings, a number of identified single units showed this property too. This result cannot be explained by reflections or as a response to diffuse light because a decrease in stimulus contrast did not reduce the area from which responses could be obtained. Preliminary data show that receptive fields in response to the moving stimuli increased with stimulus size also. These results show that in the rat superior colliculus neurons can receive direct and/or indirect inputs from most of the retinal ganglion cells and the size of a receptive field is a dynamic, stimulus dependent property.

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A novel stimulus and analysis system for studying the neural mechanisms of natural language processing in the human brain

Seonmin Chung

Human speech perception during interpersonal communication involves the processing of auditory stimuli with visual stimuli from the lip movements of each speaker. Traditional experiments on language perception in the human brain used tightly controlled sets of stimuli in the experimental design, including short phrases and repetitions of words to analyze the effects on neurological recordings. However, critics, like proponents of corpus-based linguistics, argue that simple language stimuli outside the context of a conversation fail to capture the full spectrum of linguistic complexity during natural speech. We present a novel experimental strategy and data design to study natural language use in epileptic patients undergoing electrocorticography while watching a Hollywood movie that contains many instances of natural interpersonal speech. Detailed analyses were created based on the language components available, involving multisensory speech and linguistic parsing of human language based on phonology, lexical access, semantics, and syntax: the study of the cognitive impacts of sounds, neurological effects of the meanings of words, denotation of the language used, and sentence composition. This experimental system promotes a more comprehensive understanding of the neural implementation of speech by allowing a shift from studying language in confined experimental conditions and introducing innovative approaches to studying the brain's capacity for understanding multimodal and naturalistic language.

Social interaction reward decreases p38 activation in the nucleus accumbens shell of rats

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We have previously shown that animals acquired robust conditioned place preference to either social interaction alone or cocaine alone. Recently it has been shown that p38, a member of mitogen-activated protein kinase family that regulates both physiological and pathological processes, is abnormally activated in the nucleus accumbens after morphine conditioned place preference (CPP) as assessed by western blot. It has been also reported that injections of the p38 kinase inhibitor SB203580 in the nucleus accumbens dose dependently impaired amphetamine CPP. In this study, we aimed to investigate the expression of the activated form of p38 (pp38) in the nucleus accumbens of (1) control rats that received saline in both compartment of the CPP, (2) rats that expressed cocaine CPP and (3) rats that expressed social interaction CPP 1 hour, 2 hours and 24 hours after the CPP test. We intended to check separately in the nucleus accumbens core and the nucleus accumbens shell using immunohistochemistry if there is any increase of P38 activity after cocaine CPP (drug) versus social interaction CPP (non-drug).

We did not find any change in p38 activation in the nucleus accumbens core or shell one hour or two hours after the CPP test. Unexpectedly, 24 hours after the CPP test, our results show no increase in p38 activation after cocaine CPP in the nucleus accumbens but instead a decrease in p38 activation after social interaction CPP in the nucleus accumbens shell. Furthermore, social interaction CPP-associated decrease in pp38 in the nucleus accumbens shell reached the levels of naïve untreated animals. These effects of social interaction reward on p38 activation were paralleled by a decrease in pCREB levels in one of the stress related region namely the basolateral amygdala.

Altogether, these results show that p38 is implicated in social interaction CPP and suggest an effect anti-stress of social interaction reward.

Uncoupling of mitosis and differentiation allows for fast and synchronous CNS development *in vivo*

Peter Engerer, Philip Williams, Sachihiro Suzuki, Takeshi Yoshimatsu, Prisca Chapouton, Nancy Obeng, Benjamin Odermatt, Leon Lagnado, Thomas Misgeld, Leanne Godinho

The prevailing view of neuronal development is that specific ontogenetic events occur in a defined sequence. Thus, following cell-cycle exit, newly generated neurons are believed to migrate to specific locations and differentiate, acquiring molecular and morphological features that permit their integration into synaptic circuits. How these events are coordinated to accommodate the generation of a rapidly developing nervous system is not well understood. Using the zebrafish retina as a model for *in vivo* CNS development, we show that mitosis and neuronal differentiation are largely independent of each other. Rather than dividing at a stereotypic point in their developmental trajectory, we find that vsx1+ progenitors of retinal bipolar interneurons can undergo mitosis at different stages of differentiation. For example, late-dividing vsx1+ progenitors already target their neuronal processes to synaptic neuropil, reposition their soma to their final stratum of residence prior to mitosis and show gene expression dynamics similar to the post-mitotic bipolar cells that surround them. Intriguingly, the differentiation of post-mitotic and progenitor cells towards mature bipolar cells appears to be locally regulated rather than being time-locked to mitosis. We propose that uncoupling of mitosis and differentiation allows for accelerated neuronal development and synchronizing neuronal differentiation within a local population. Our findings are compatible with a reinterpretation of previous observations from neuronal development in mammals, and hence reveal a new neuro-developmental strategy that might be operating in a wide range of species and brain structures.

Active endocannabinoids are secreted on microglial microvesicles

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Endocannabinoids (eCBs) are bioactive lipids which primarily influence synaptic communication within the nervous system. They are synthesized by neurons but also by microglia, especially under neuroinflammatory conditions. To exert their function, eCBs travel across the intercellular space. However, how eCBs move extracellularly remains obscure. Our recent evidence indicates that reactive microglia release extracellular vesicles (EVs), which may represent an ideal vehicle for the transport of hydrophobic eCBs. Hence, in this study we investigated whether microglial EVs carry eCBs and may influence neurotransmission.

First we analyzed the eCB content of EVs and found a clear enrichment of N-arachidonoylethanolamine (AEA) in EVs relative to parental microglia. This analysis revealed higher AEA levels in EVs shed from the plasma membrane (microvesicles), compared to those which originate from the endocytic compartment (exosomes).

To bioassay the activity of vesicular AEA, we used patch clamp analysis of miniature inhibitory post-synaptic currents (mIPSC) on rat hippocampal primary culture. Exposure of neurons to microvesicles (MVs) induced a significant decrease in mIPSC frequency, mimicking the well-known inhibitory action of CB1 receptor agonists. The involvement of vesicular AEA in this phenomenon was inferred from the ability of the CB1R antagonist SR141716A to block the reduction of mIPSC frequency evoked by MVs. Western blot analysis showed an increase in ERK phosphorylation in neurons exposed to MVs, which was completely inhibited by SR141716A. This indicate that CB1R activation by AEA-storing MVs translates into downstream signaling.

Finally, the use of biotin-AEA revealed an affinity of AEA for MV membrane, indicating that AEA travels in association with MVs surface. Consistent with a surface localization of AEA, broken MVs depleted of their luminal cargo maintain their capability to decrease mIPSC frequency.

Overall, this study shows that microglial MVs carry AEA on their surface to stimulate CB1R on target GABAergic neurons and demonstrates that extracellular vesicular transport of eCB play a crucial role in the modulation of inhibitory transmission.

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Mild hypothermia therapy for patients with severe brain injury

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Background and Goal of Study: The authors present a group of patients with severe head injuries in which deliberate mild hypothermia was carried out together with standard protocol according to EBIC.

Materials and Methods: We have prospectively analysed 100 patients with severe head injury (GCS 4-8, admitted to our hospital from 2002 to 2006) randomised into a group with (n = 47) and without (n = 53) hypothermia. The influence of hypothermia on ICP, CPP and neurological outcome was analysed in the context of the extent of the primary brain damage.

Results: Patients with normothermia and primary lesions (n = 25) - mean values: GCS on admission 4,54, ICP 19,56, CPP 72,83, GOS 3,52. Patients with normothermia and extracerebral hematomas (n = 28): GCS 4,15, ICP 16,9, CPP 71,45, GOS 2,9. Patients with hypothermia and primary lesions (n = 27): GCS 4,65, ICP 12,77, CPP 76,3, GOS 3,78. Patients with hypothermia and extracerebral hematomas (n = 20): GCS 4,55, ICP 14,92, CPP 77,62, GOS 4,59. The difference in GOS between the hypothermic and normothermic group of patients after 6 month was not statistically significant.

Conclusions: Hypothermia decreased ICP and increased CPP regardless to the type of brain injury. Hypothermia was not able to improve outcome in patients with primary brain lesions, however it significantly improved outcome in patients with extracerebral hematomas who were threatened by the secondary ischemic brain damage. Determining optimal temperature with minimal side effects and maximum cerebral protection as well as an exact selection of patients with TBI suitable for deliberate hypothermia is a task for the future.

Brian Hyland

Midline thalamus is implicated in linking visceral and exteroceptive sensory information with behavior, including attribution of incentive salience to cues. However, whether neuronal activity is modulated with temporal precision by cues and actions in real time is unknown. Using single neuron recording and a Pavlovian visual-cue/liquid-reward association paradigm, we discovered phasic responses to sensory cues, appropriately timed to modify information processing in output targets, and differentially reward-modulated tonic modulations within and between trials, which may have distinct arousal functions. Many neurons also responded to repetitive licks, consistent with sensorimotor integration. Further, some neurons with "hypercomplex" lick responses were activated only by the 1st rewarded lick, and only if that lick were also part of a conditioned-response sequence initiated earlier, consistent with binding action decisions to their ensuing outcome. This rich repertoire of complex responses provides electrophysiological evidence for midline thalamus as site of complex information integration for reward-mediated behavior.

In vivo imaging of microtubule dynamics in developing and diseased axons

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Microtubules are major cytoskeletal components of all eukaryotic cells. In neurons, microtubules play key roles in polarization, transport and remodeling. Disturbances of microtubule organization occur early in neurodegenerative diseases, underscoring the importance of intact microtubules in maintaining neuronal structure and function. This makes microtubules interesting structures to study in nervous system health and disease. Growing and shrinking microtubules dynamically associate with a number of proteins, including plus-end-tracking proteins (+TIPs), which accumulate at plus-ends. Microtubule dynamics can thus be studied by fluorescently tagging +TIPs, a technique that has thus far been applied in vitro and in non-mammalian model systems. To assay such cytoskeletal remodeling by *in vivo* imaging in the mammalian nervous system, we generated transgenic mice that express a +TIP, EB3, fused to yellow fluorescent protein controlled by a neuron-specific promotor. Using two-photon and wide-field microscopy, we studied microtubule dynamics *in vivo* in the peripheral and central nervous system. In time-lapse recordings, the status of the microtubular cytoskeleton could be monitored in acute (axotomy) and chronic (amyotrophic lateral sclerosis) models of axonal injury, as well as under the acute influence of microtubule-modifying drugs and chronically via cranial windows. We found that an increase in microtubule dynamics is an indicator of imminent axon degeneration after injury or possibly in neurological disease. Such injury-induced acute axonal degeneration could be reduced by titrating microtubule-stabilizing drugs to partially block microtubule destabilization. In addition to being an early indicator of axon degeneration, we also found an increase in EB3-comet density during axon regeneration and developmental reorganization. This suggests that increased microtubule dynamics might be a general “plasticity tag” for axons that can be read out by our novel approach.

Cold stress induced RBM3-dependent neuroprotection is mediated by the reticulon protein RTN3

Giovanna Mallucci

The cold shock protein, the mRNA chaperone RNA binding motif 3 (RBM3), plays a central role in mediating the neuroprotective effects of cooling. Cold-shock also results in reduction in global protein synthesis rates, by inhibiting both initiation and elongation. To further understand these processes and how they are related, we undertook a genome-wide analysis of the changes that accompany cold stress, using transcriptional, translational and miRNA profiling of cooled cells and mouse brain. We found that while cooling causes little change in either the trancriptome or in miRNA expression, there is extensive reprogramming of the translatome. This leads to changes in the initiation and elongation rates of mRNAs of specific chaperone proteins, including RBM3, known to be upregulated in cooling. Importantly, we found that RBM3 specifically binds a number of mRNAs, including the reticulon protein 3 (RTN3) involved in synaptic plasticity. Cooling-mediated RBM3 induction in vivo increased levels of RTN3 expression, and knockout of RTN3 abolishes the neuroprotective effects of cooling in prion diseased mice. Lentivirally-mediated over-expression RTN3 in the presence of RBM3 knockdown rescues synaptic number and function, restores behavioural deficits and increases survival in these animals. The data support that RTN3 is the principal effector of the neuroprotective effects of cooling that are initiated by RBM3 induction.

Kainate receptors mobilize endocannabinoids in striatal spiny projection neurons

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The striatum is the main input structure of the basal ganglia, integrating information from the thalamus and cortex to control planning and modulation of movement. The principal neurons of the striatum, spiny projection neurons (SPNs), express high levels of kainate receptors, members of the ionotropic glutamate receptor family, yet their functional roles within SPNs have not been fully characterized. Kainate receptors play diverse modulatory roles at synapses throughout the brain in regions where they are expressed. In addition to contributing to excitatory post-synaptic currents (EPSCs) they have been shown to presynaptically regulate neurotransmitter release and in some cases are metabotropically coupled to intracellular signaling pathways—activating downstream signaling independent of ionotropic currents. Here we show that kainate receptors are present at postsynaptic sites in SPNs where they are activated by endogenously released glutamate. We also show that activating kainate receptors with a low concentration of agonist, that produces little or no inward current, reduces glutamate release at excitatory synapses onto SNPs. In a subset of cells, this effect is blocked with a cannabinoid type 1 (CB1R) antagonist, indicating that activating kainate receptors leads to endocannabinoid (eCB) mobilization. Cannabinoid signaling itself has a well-established role in the striatum playing a crucial role in different forms of striatal plasticity. Furthermore, this effect of low-agonist activation of kainate receptors is sensitive to a number of downstream pharmacological manipulations that suggest kainate receptors mobilize eCBs through a metabotropic function—signaling that is independent of ion flux through the channel pore. These experiments suggest a novel and significant role for kainate receptors in tuning striatal synapses and regulating striatal activity.

Ethanol and caffeine on apoptosis in the cerebellum of UChB rats (voluntary ethanol consumers)

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Ethanol alters motricity, learning, cognition and cellular metabolism in the cerebellum. The combination of ethanol with caffeine by consuming "energy drinks" is becoming increasingly popular among young people. We showed the effects of simultaneous use of ethanol and caffeine on apoptosis in the cerebellum of UChB rats. The adult rats were divided into three groups (n=14/group): 1. UChB: rats fed with 1:10 (v/v) ethanol ad libitum (free choice for water or ethanol) drinking from >1.9g of ethanol/Kg body weight/day, 2. Control: free choice for water and 3. UChB/caffeine: free choice for water or ethanol+caffeine 300mg/L. The treatments occurred from day 100 till day 150, totalizing 50 days of ethanol/caffeine ingestion. Cerebellar sections were subjected to immunohistochemistry and gene expression for Real Time-PCR (RT-PCR) for Caspase-3, XIAP and insulin-like growth factor 1-receptor (IGFR-1). The results showed a significant increase in Caspase-3 and XIAP in the UChB rats and a reduction of these expressions in UChB+caffeine rats. Regarding the IGFR-1, there was an increase in the UChB and UChB+caffeine rats. The simultaneous ingestion of ethanol and caffeine reversed the ethanol damages acting caffeine with a possible neuroprotective effect.

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Perceptual changes of “self” disrupt number sense

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Numerical and spatial representations are proposed to be intertwined in the human brain. Previous research has shown that shifts in spatial attention secondary to eye movements, lateral head turns or whole body rotations can all modulate numerical cognition as predicted by the theoretical framework for numerical-spatial interactions. However, to date the perceptual component has not been dissociated from the oculomotor component with respect to its impact upon numerical cognition. In this study we separately employ two different experimental protocols to induce perceptual changes in self-motion in either the visual or vestibular systems, whilst controlling for eye movements. Implementing a visual motion stimulus (optokinetic stimulation) we observed a differential modulation of numerical cognition based upon the perceptual state of the subject. When the perceptual state of *world-motion* was induced with the visual stimulus, rightward motion biased subjects towards smaller numbers, whereas leftward motion biased subjects towards larger numbers. However, when the perceptual state of *self-motion* was induced using exactly the same visual stimulus, subjects were biased towards larger numbers irrespective of the direction of the visual motion. Implementation of vestibular rotations, either perceived or subliminal, did not differentially modulate numerical cognition. Thus our results provide the first evidence that visually, but not vestibular mediated perceptual changes can modulate numerical cognition, independently of either spatial attention or eye movement mediated biases. Finally, these findings have potential clinical implications as they demonstrate that perceptual state can alter an individual’s number-sense, suggesting that perceptual deficits could impact numerical cognition in disorders such as dyscalculia.

Higher specific infectivity of exosomal prions

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Prion diseases or transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative disorders of humans and animals. During the disease, there is an accumulation in brain of an abnormal partially protease-resistant form of the prion protein (PrPSc). This is a misfolded form of the host-encoded protease-sensitive cellular prion protein (PrPc). It is generally believed that, after binding with PrPSc the PrPc converts to the misfolded pathological form, triggering neurotoxicity.
Nevertheless recently many solid data have questioned the equivalence of PrPSc to prions, primarily by showing that in some cases prion infection occurs in absence of PrPSc. In addition, to date, it has not been possible to reproduce infectivity by in-vitro approaches, which challenges the protein only content of prions. The nature of the TSE infectious agent is certainly not as simple as it was originally thought and remains at present elusive, delaying the finding of successful diagnostic and therapeutic strategies.
To better understand the nature of prions we used a novel experimental model: large scale preparations of exosomes released by chronically infected cells.
Exosomes are nanovesicles of 50-90nm released by the majority of cells and they have been shown to contain prions when released by infected cells in-vitro. As they are a much less complex material compared to brain tissue the finding of cofactors involved in prion infectivity could be greatly facilitated.
Our results confirmed that both prion infectivity and PrP^{Sc} released by chronically infected cells are mainly associated to nano-vesicles. In addition, an estimation of specific infectivity relative to the PrP^{Sc} content showed for the first time that the PrP^{Sc} released by cells on exosomes is at least thirty times more infectious than PrPSc retained in cell lysates. This is a crucial finding that clearly confirms that not all PrP^{Sc} molecules associate with the same level of prions. Interestingly infectivity levels of Triton-treated exosomes are significantly increased. This results suggest that lipid rafts retain the highest infectious PrP^{Sc} species and might have a role in prion propagation.

Short term energy deprivation is not sufficient to induce proteasome stress and stress response in neural cells

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Accumulation of polyubiquitinylated protein aggregates due to translation arrest is considered as the main cause of delayed neuronal death observed after transient global brain ischemia. However, the relationship between ischemia-induced inhibition of protein synthesis and accumulation of protein aggregates is still not clear.
The aim of this study was to compare effect of proteasome stress and chemically induced ischemia on cellular stress response and viability of neuroblastoma SH-SY5Y and glioblastoma T98G cells. Proteasome stress was induced by treatment of cells with bortezomib, inhibitor of proteasome 26S complex. To mimic ischemic energy deprivation, cells were temporally treated with sodium azid, inhibitor of cytochrome c oxidase, in combination with 2-deoxyglucose, inhibitor of glycolysis. Neuroblastoma cells were more sensitive to bortezomib than glioblastoma cells and death of neuroblastoma cells occurred significantly faster than death of glioblastoma cells. Treatment of SH-SY5Y cells with sodium azid/2-deoxyglucose for 15 minutes was associated with cell death observed 24 hours after treatment while glioblastoma cells were resistant to the same treatment. Treatment of both SH-SY5Y and T98G cells with bortezomib was associated with accumulation of polyubiquitinylated protein aggregates and increased expression of HSP70. These typical cellular responses to proteasome stress, observed also after transient global brain ischemia, were not observed after treatment of SH-SY5Y cells with sodium azid/2-deoxyglucose.
Our results indicate that temporal energy deprivation might be not sufficient to induce significant proteasome stress and point to possibility of direct effect of ischemia on proteasome 26S complex.

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Effect of febrile seizures on newborn hippocampal dentate granule cell morphology and function

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Febrile seizures (FS) are a risk factor for the development of temporal lobe epilepsy. Previously, we have shown that experimental FS alter inhibitory synaptic input and postsynaptic GABA(A) receptor function of dentate granule cells (DGC). Moreover, we showed that the survival of DGC, born after seizures, increases. Newborn DGC are therefore hypothesized to contribute to the development of a hyperexcitable hippocampal network and to play a role in the development of temporal lobe epilepsy in later life.

In this study, we analyzed the structural and functional integration of newborn DGC into the existing hippocampal network. Therefore, we used an established model where FS are induced in 10-day old rat pups by subjecting them to heated air. Newborn DGC were labelled one day after treatment by a stereotactic injection of eGFP-expressing retroviral particles in the dentate gyrus. One, four and eight weeks after induction of FS, confocal Z-stacks were acquired allowing morphological analyses of GFP positive DGC. Spontaneous inhibitory postsynaptic currents were studied using the whole-cell patch-clamp technique applied on GFP positive DG cells in hippocampal slices.

One week after FS, dendritic length is significantly increased compared to controls. Moreover, four and eight weeks after seizures, Sholl analyses revealed that dendritic complexity is increased in FS animals. From these data we conclude that experimental FS alter dendritogenesis in post-FS born DGC. These changes are expected to result in an increased connectivity of the hippocampal network.

Key words:
Febrile seizures
Neurogenesis
Dentate granule cells
Morphological analyses
Whole-cell patch-clamp

EGFR mediates neuronal survival through regulation of glutamate transporters in cortical astrocytes

Jonathan Robson, PhD

The Epidermal Growth Factor Receptor (EGFR) plays an important role in the development of various organs including skin, heart, lungs, intestine and brain. Brain development is severely compromised in EGFR knock-out mice with a progressive degeneration of the frontal cortex and the olfactory bulbs occurring in the early postnatal period. In contrast, mice with a conditional ablation of the EGFR in either neural precursors ($EGFR^{\Delta Nes}$) or astrocytes ($EGFR^{\Delta Gfap}$) do not develop the cortical degeneration observed in EGFR knock-out mice. We show that $EGFR^{\Delta Nes}$ and $EGFR^{\Delta Gfap}$ mice establish a proper Blood Brain Barrier and perform reactive astrogliosis in response to brain injury and pathogenic insults but are more sensitive to kainic acid (KA) induced epileptic seizures compared with control mice. These KA induced epileptic seizures could not be significantly ameliorated with NMDAR or AMPAR antagonists despite EGFR ablated mice showing reduced expression of NMDAR in the neocortex. Utilizing the EGFR knock out murine model we show that loss of EGFR in the cortical astrocytes, but not midbrain astrocytes, results in a loss in the number and function of glutamate transporters, the critical transport system that regulates the activity of the excitatory neurotransmitter glutamate. Our results illustrate an excitotoxic mechanism to explain the hypersensitivity and neurodegeneration observed in EGFR ablated brains and provide evidence of a novel means to treat patients with neurodegenerative diseases.

Distinct neurexin-based complexes at GABAergic versus glutamatergic synapses

Astrid Rohlmann and Markus Missler

Neurexins (Nrxn) are mostly presynaptically and neuroligins (Nlgn) postsynaptically located binding partners with essential roles in neurotransmission. Mutations in their genes are genetically linked to neurodevelopmental disorders such as autism and schizophrenia. Since an imbalance between excitatory and inhibitory neuronal activity has been hypothesized as a pathomechanism for these disorders, it is of great importance that particular isoforms of Nrxn/Nlgn and their additional interaction partners may be preferentially associated with glutamatergic or GABAergic synapses. For example, Nlgn-2 has been exclusively localized at GABAergic terminals, similar to neurexophilin-1 (Nxph1), an exclusive ligand of α-Nrxn isoforms, which is selectively expressed in inhibitory interneurons. Their closely related isoforms, in contrast, behave differently: Nlgn-1 is localized at excitatory terminals and Nxph3 is expressed in a subpopulation of glutamatergic neurons. However, α-Nrxn variants themselves occur in both types of neurons, and play essential roles in excitatory and inhibitory synapses because deletion of α-Nrxn in mice led to ubiquitously reduced spontaneous and evoked transmission. Our poster will summarize available information on the specific role of Nrxn/Nlgn variants and their additional binding partners at excitatory versus inhibitory synapses. We present our recent data on the immunoEM localization and biochemical binding properties in distinct Nrxn-based complexes.

Search for causal variants in microRNA and biogenesis genes in epileptic encephalopathies

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The role of miRNAs in the pathomechanism of epilepsy is increasingly investigated. Variants in miRNA genes can have a drastic effect on their processing and functioning. However, a mutation analysis of these genes has never been performed for the epileptic encephalopathies (EE). We anticipate that variants in brain-expressed miRNAs can alter the expression of their target genes, leading to the severe phenotype of EE.

We screened miRNA genes in EE patients and their healthy parents using 'Multiplex Amplification of Specific Targets for Resequencing' (MASTR) assays (Multiplicom NV). The first assay amplifies 289 human brain-expressed miRNAs and was used to screen 119 trios, 13 duos, 2 quartets and 54 probands with EE. Furthermore, we created and optimized a second MASTR assay containing 183 additional miRNA genes and the coding region of six genes involved in miRNA biogenesis (*AGO2*, *DICER1*, *DGCR8*, *DROSHA*, *TARBP2, XPO5*). miRNA genes were selected based on (1) an in literature described link between the microRNA gene and epilepsy, (2) described expression during brain development, (3) validated microRNA-target interactions between microRNAs and epilepsy genes and (4) in-house CNV data. The second assay was used to screen 45 trios, 4 duos, 2 quartets and 38 probands with EE. Further screening in currently ongoing. Prioritization of variants was done using different inheritance models, the position and predicted impact on the structure of the miRNA and their frequency in controls.

So far, we identified one *de novo* variant, which is present in the mature miRNA and predicted to severely disrupt the secondary structure of the miRNA. Functional follow up is now started to evaluate the impact of the variant on the miRNA biogenesis and function.

Identification of miRNAs linked to EE will lead to novel insight into the underlying pathomechanisms of epilepsy.

Why are the magnocellular and parvocellular systems sensitive to different spatial frequencies in visual signals?

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During reading, the separation of neural signals in the temporal and spatial domains in the Magnocellular and Parvocellular pathways is achieved through different visual mechanisms leading to unique frequency bandwidths for each pathway. This creates clean non interfering signal streams for the transmission of signals carrying unique content to the brain. Visual signals in different temporal and spatial regimes are collected via different spatial and time domain windows. In this study we show how the width of the fovea determines a lower bound for the allowed spatial frequencies of visual signals that are conveyed through the midget ganglions to the parvocellular pathway. The magnocellular pathway is served by parosal ganglions situated away from the fovea and cover a much broader area. We will show how this distribution of ganglions will allow visual signals with much lower spatial frequencies to be conveyed through the parosal ganglions to the magnocellular pathway. Some observation on the allowed temporal frequencies of visual signals in the two pathways are also provided.

Optogenetic investigation of chandelier synapses in developing and adult olfactory cortex

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The abstract summarize our recent published (Wang et al., 2014 J Physiol; Sun et al., 2014, PLoS One) as well as unpublished new results. GABAergic terminals of chandelier cells exclusively innervate the axon initial segment (AIS) of excitatory neurons. Although the anatomy of these synapses is well-studied in several brain areas, relatively little is known about their physiological properties and developmental plasticity. Using vesicular γ-aminobutyric acid transporter VGAT-ChR2 expressing mice and a novel 'laserspritzer' approach, we investigated the functional properties of axo-axonic synapses (AASs) in the cortex of mice. AASs are in close proximity to NaV channels located at the AIS. AASs are selectively activated by a 5 μm laserspritzer placed in close proximity to the AIS. For the first time, we revealed that the laserspritzer induced AAS- IPSCs persist in the presence of TTX and TEA but not 4-AP. Next, using gramicidin-based perforated patch recordings, we found that the AAS activation alone can be sufficient to inhibit action potential generation and epileptiform activities *in vitro.* Our anatomical and physiological results lead to the novel conclusions that: 1) AASs innervate the entire length of the AIS, as opposed to forming a highly concentrated cartridge, 2) AAS exhibited profound plasticity during the postnatal critical periods that is dependent on neuronal activity and intrinsic neural modulators and 3) an important role of AAS in olfactory habituation and discrimination tasks is demonstrated.

Febrile seizures persistently alter hippocampal $GABA_A$ receptor physiology

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Febrile seizures (FS) are the most common type of childhood seizures, affecting 2-3% of the children between 3 months and 5 years. Correlative clinical studies have linked early-life FS to temporal lobe epilepsy (TLE) later in life. Insight into the cellular mechanisms underlying FS-induced epileptogenesis is crucial for a rational drug design to treat TLE. Our present study aims at elucidating whether altered hippocampal GABAergic signalling can be a link between childhood FS and TLE in adulthood.

We make use of an animal model for FS-induced epileptogenesis in which FS are elicited in 10-day old rat pups by hyperthermia. Normothermia littermates serve as control. One week later, $GABA_A$-receptor ($GABA_AR$)-mediated neurotransmission is determined by whole-cell patch-clamp of granule cells in acute hippocampal slices. For a translational approach, we collected freshly frozen hippocampal biopsies from TLE patients with and without a FS history, as well as non-neurological human post-mortem controls. Hippocampal membranes are isolated from the frozen specimen and transplanted into *Xenopus laevis* oocytes allowing the incorporation of human $GABA_ARs$ in the oocyte plasma membrane. GABA-evoked currents are recorded by two micro-electrode voltage-clamp on oocytes.

Using the FS model, we previously found a decrease in spontaneous inhibitory activity and an increased sensitivity of the $GABA_AR$ to GABA. Using human hippocampal samples, we observed that epilepsy patients with a history of FS are characterized by a reduced sensitivity of the $GABA_AR$ to GABA, compared to patients without a history of FS or controls. This study demonstrates that FS cause a long-lasting alteration in hippocampal $GABA_AR$ functioning.

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

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Introduction: Assessment of language lateralization is important in epilepsy surgery to avoid postoperative language deficits. Although the Wada test is considered the gold standard for language lateralization, non-invasive substitutes such as fMRI represent the current clinical standard, but show discordance rates of ~15%. Event-related potentials (ERPs), especially the language-related negative component around 400ms, are related to language processing and are therefore expected to reflect language lateralization. **Method:** The study was based on a 2 (Lateralization; left vs. right hemisphere) × 2 (Stimulus; memorized words vs. new words) × 2 (Group; patients vs. controls) repeated ANOVA design with Group as a between factor. Scalp EEG was recorded from 64 standard locations in 27 drug-resistant focal epilepsy patients and 28 healthy controls (all right-handed) during a visually presented word recognition task, where abstract nouns had to be memorized and later recognized from a larger word list. ERP areas of memorized and new words (45 trials each; randomly presented; stimulus presentation time 1000ms; ISI 2700-3200ms) were analyzed in the 400-600ms epoch. Language fMRI was routinely obtained in epilepsy patients. **Results:** ANOVA showed a significant interaction of Lateralization × Stimulus × Group ($F(1,53)=4.13$; $p=0.047$), indicating a more negative potential for memorized words over the left compared to the right hemisphere, and this was more pronounced in patients compared to controls, whereas no effects appeared for new words. The individual comparison of the mean ERP area showed a left-sided lateralization in 80% of epilepsy patients. **Discussion:** ERP of recognition of memorized words were lateralized to the left hemisphere in healthy controls and epilepsy patients. In patients, single-subject laterality indices showed 80% concordance with fMRI results. Results indicate that scalp-derived ERPs of memorized words are a promising tool to investigate lateralization of receptive language functions and verbal working memory in epilepsy patients.

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Nerve Growth Factor (NGF) signaling pathway is critical in peripheral nervous system development and is an important mediator of neuropathic and inflammatory pain in adults. Mutations in either NGF or TrkA receptor genes in humans cause severe disorders manifested by insensitivity to pain. Moreover, administration of NGF causes pain in rodents and human studies, which can be prevented by NGF-scavenging monoclonal antibodies. Since very little is known about NGF pathway in human nociceptive systems, here we use human induced pluripotent stem cells-derived sensory neurons in compartmentalized microfluidics devices to study kinetics, dynamics and consequences of specific inhibition of the pathway. Here, to investigate the mechanics of retrograde transport of ligand/receptor complexes, we use Quantum Dot-labelled NGF for real-time imaging at a single molecule level. Moreover, we study the perturbation of the NGF pathway with specific and potent small molecule inhibitors of TrkA receptor tyrosine kinase and find that they not only prevent progression of downstream signaling but negatively affect the clathrin-mediated endocytosis of the receptor. As a consequence the retrograde transport of the TrkA receptor-containing endosomes is less efficient without changing the kinetics of the endosomal transport.

Altogether, this study demonstrates the importance of TrkA receptor phosphorylation in NGF-mediated endocytosis and subsequent retrograde transport of NGF/TrkA complexes in human sensory neurons, which will contribute to a better understanding of nociceptor function in pain.

R-Phenibut exerts anti-nociceptive properties via the α_2-δ subunit of the voltage-dependent calcium channels

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R-Phenibut is the optically pure and pharmacologically active form of racemic phenibut. For more than 20 years phenibut is clinically used as an anxiolytic, mood elevator and nootropic drug. It has been suggested, that its pharmacological activities are mediated through $GABA_B$ receptors. Structurally, R-phenibut is related to baclofen and gabapentin (GBP), drugs that both mimic the chemical structure of the neurotransmitter GABA. Baclofen is a $GABA_B$ receptor-active compound, but GBP does not bind to these receptors and exerts its anti-nociceptive activity through binding to the α_2-δ subunit of the voltage-dependent calcium channel (VDCC).

The [^{3}H]GBP binding experiments revealed that the affinity constants for R-phenibut, baclofen and GBP in a rat brain membrane preparation were 26, 156 and 0.05 μM, respectively. The anti-nociceptive effects of R-phenibut were tested using the formalin-induced paw-licking test in mice, and it was found that pre-treatment with R-phenibut decreased the nociceptive response in both phases of the test in a dose-related manner. The anti-nociceptive activity of R-phenibut was blocked by a $GABA_B$ receptor-selective antagonist CGP35348, but this only occurred during the first phase of the test.

Our data suggest that R-phenibut binds to the α_2-δ subunit of the VDCC with 4-times higher affinity than to the $GABA_B$ receptor. The anti-nociceptive activity of R-phenibut in the second phase of the formalin-induced paw-licking test is associated with its effect on the α_2-δ subunit of VDCCs rather than through activity at $GABA_B$ receptors. In conclusion, our results provide experimental evidence for GBP-like anti-nociceptive properties of R-phenibut, which might be used clinically in treatment of neuropathic pain disorders.

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