NEUROSCIENCE WINTER CONFERENCE

14th International

Sölden Austria
April 10-April 14 2012

Central Spa Hotel

Final Scientific Program

Time schedule of plenary lectures, symposia and special interest sessions

List of Poster Sessions

List of Participants

Abstracts

Program Committee:

Tobias Bonhoeffer
Nils Brose
Alois Saria
Stephan Schwarzacher

Contributors:

- Austrian Neuroscience Association
- International Society for Neurochemistry
- Central Spa Hotel Sölden

Organizer:

brainplatform.net e.U.

Conference Chair:

Alois Saria, Austria

Tuesday
April 10

15:00 – 17:00 Registration

17:00 – 17:45 Keynote Lecture 1
Leslie Vosshall (USA)
The genetics of innate behavior: Courtship and feeding

17:45 – 18:00 Break

18:00 - 20:00 Symposium 1
Neural circuits and behavior in Drosophila
(Chair: Alexander Borst)

Ian Meinertzhagen (Canada) Progress towards the connectome of the Drosophila visual system
Alexander Borst (Germany) ON and OFF pathways in Drosophila motion vision
Michael Dickinson (USA) Straighten up and fly right: Visual navigation in Drosophila
Vivek Jayaraman (USA) Probing sensorimotor integration in Drosophila

Wednesday
April 11 Morning

08:15 – 09:00 Keynote Lecture 2
Martin Schwab (Switzerland)
Structural plasticity and hardware repair after CNS injury

09:00 – 11:00 Symposium 2
Cortical reorganisation following brain injury
(Chairs: Mathias Bähr & Melanie Wilke)

Melanie Wilke (Germany) Reorganisation of spatial networks following reversible lesions in thalamo-cortical circuits: fMRI and single cell studies in monkeys
Christian Gerloff (Hamburg) Reorganisation of the motor network following irreversible brain lesions in stroke patients
Giacomo Koch (Italy) Hyperexcitability of parietal-motor functional connections in the intact left-hemisphere of patients with neglect: Combined TMS and MRI studies in human patients
Andreas Luft (Switzerland) Neurophysiology-based approaches for neurorehabilitation

11:00 - 11:30 Coffee Break

11:30 - 13:30 Special Interest Session 1
ISN Symposium on Accumbens cholinergic interneurons as a therapeutic target in substance-use disorders: basic science (Chair: Gerald Zernig)
Ilka Diester (USA) Cholinergic interneurons control local circuit activity and cocaine conditioning
Louk Vanderschuren (The Netherlands) The neurobiology of social play behaviour in rats
Karine Guilleme (France) Simultaneous recording of accumbens network activity with microelectrode arrays and its neurochemical modulation with real-time neurochemistry
Gerald Zernig (Austria) Possible differential role of accumbens cholinergic interneurons in drug- vs social interaction reward

Wednesday
April 11 Afternoon

16:00 – 16:45 Keynote Lecture 3
Edvard Moser (Norway)
Brain maps for space

16:45 – 17:15 Coffee Break

17:15 – 19:15 Symposium 3
Axonal computation of neurotransmitter release (Chair: Dimitri Rusakov)

Beverley Clark (UK) Direct measurement of signalling in mammalian central axons
Dominique Debanne (France) Analog-digital signaling in hippocampal axons
Yuji Ikegaya (Japan) Functional and structural role of axon cables in synaptic output
Nigel Emptage (UK) The role of glutamate autoreceptors in transmitter release

Thursday
April 12 Morning

08:15 – 09:00 Keynote Lecture 4
Anne-Marie Craig (Canada)
Cell surface synaptic organizing complexes

09:00 – 11:00 Symposium 4
Ubiquitin-like proteins in nerve cell development and synaptic function and plasticity (Chairs: Damian Refojo & Nils Brose)

Nils Brose (Germany) Nedd4-family ubiquitin ligases in nerve cell development and differentiation
Damian Refojo (Germany) Needing Neddylation: A role for Nedd8 in neuronal development
Andreas W. Püschel (Germany) Regulation of neuronal polarity by Rap1 GTPases
Azad Bonni (USA) Regulation of neuronal connectivity by ubiquitin signaling

11:00 - 11:30 Coffee Break

11:30 - 13:30: Special Interest Session 2
Conditioned vulnerability elicited by metabolic insults occurring at birth: New paradigms for understanding neuropsychiatric disorders with delayed clinical onset
(Chair: Mario Herrera-Marschitz)

Peter J Gebicke-Haerter (Germany) Systems biology, epigenetics, and beyond: New paradigms and understandings on development of mental diseases
Andre Fischer (Germany) Epigenetics in schizophrenia
R. Andrew Tasker (Canada) Repetitive insults facilitate disease progression and disease diversity
Mario Herrera-Marschitz (Chile) Pharmacodynamics and pharmacokinetics studies showing that PARP-1 inhibition protects against the long-term consequences of perinatal asphyxia

Thursday
April 12 Afternoon

16:00 – 16:45 Keynote Lecture 5
Erin Schuman (Germany)
Local translation in neurons

16:45 – 17:15 Coffee Break

17:15 – 19:15 Symposium 5
The neurobiology of Sleep
(Chair: William Wisden)

Helmut Haas (Germany) Waking with the hypothalamus
Raphaelle Winsky-Sommerer (UK) Physiology and pharmacology of sleep: Novel insights into the role of GABAergic and adenosinergic transmission
Giorgio F. Gilestro (UK) Sleep in Drosophila Melanogaster
William Wisden (UK) Neuronal pathways of sleep and anaesthesia

19:15 – 20:30 Poster Session

Friday
April 13 Morning

08:15 – 09:00 Keynote Lecture 6
Gilles Laurent (Germany)
Theme and variations on STDP

09:00 – 11:00 Symposium 6
Stem cells and neurodegenerative diseases (Chairs: Govindan Dayanithi and Eva Sykova)

Zaal Kokaia (Sweden) Stem cells and stroke
Eva Sykova (Czech Republic) Treating spinal cord injury using an immortalized human neural stem cell line (SPC-01)
Lawrence Rajendran (Switzerland) Cellular complexity underlying Alzheimer's disease
Govindan Dayanithi and Eva Sykova (France and Czech Republic) Calcium homeostasis in stem cells

11:00 - 11:30 Coffee Break

Friday
April 13 Afternoon

16:00 – 16:45 Keynote Lecture 7
Idan Segev (Israel)
Inhibiting the brain - design principles

16:45 – 17:15 Coffee Break

17:15 – 19:15 Symposium 7
Imaging neuron subcellular organization and activity; from molecules to function (Chair: Daniel Choquet)

Daniel Choquet (France) A nanoscale view into the dynamic of AMPA receptor organization in synapses
Thomas Oertner (Germany) Synaptic plasticity: Adjusting weights or changing topology?
Valentin Nägerl (France) Dual-color superresolution imaging of synapses and glia cells in living brain slices using STED microscopy
Antoine Triller (France) The synapse as a statistical nanomachine

19:30 Gala Dinner (free for Central Spa hotel residents, others book at hotel front desk for 50,- €)

Saturday
April 14

08:15 – 10:15 Symposium 8
Neuroethology: Novel approaches to studying the brain in action (Chair: Georg Keller)

Florian Engert (USA) A neural circuit controlling motor learning in larval zebrafish
Richard Hahnloser (Switzerland) Auditory feedback and song learning
Michael Häusser (UK) Spatial navigation: The view from inside a single cell
Carl Petersen (Switzerland) Synaptic mechanisms of sensory perception

10:15 – 10:45 Coffee Break

10:45 – 12:45 Symposium 9
Neurogenesis and glial function and dysfunction in aging and neurodegeneration: The ultimate neural symbiosis (Chair: Jose Julio Rodriguez Arellano)

Djoher Nora Abrous (France) Neurogenic changes in aging
José Julio Rodriguez Arellano (Spain) Neurogenic impairment and recovery in Alzheimer's disease: A concomitant process with glial alterations
Alexei Verkhratsky (UK) Astroglial ionotropic receptors in neurodegeneration
Lydia Vargova (CZ) Changes in CNS diffusion parameters during aging and Alzheimer´s disease

12:45 End of meeting and departure

Thursday
April 12
19:15 - 20:30
Poster Session

1. Dynorphin mutations cause human neurodegenerative disorder spinocerebellar ataxia type 23
D.S. Verbeek, H. Watanabe, J. Jezierska, K.A. Artemenko, T. Yakovleva, Kurt F. Hauser, and Georgy Bakalkin

2. Lipophilicity as a determinant of procaine analogues binding to the rat $\alpha_3\beta_4$ nicotinic acetylcholine receptor
Cheffer A., Mustafa E.V., -do Amaral A.T., Ulrich H.

3. A nanoscale view into the dynamic of AMPA receptor organization in synapses
Mario Carta, Patrizio Opazo, Julien Veran, Daniel Choquet, Christophe Mulle and Françoise Coussen

4. Bilateral propagation of neuroinflammatory reaction in the dorsal root ganglia alongside neuroaxis after unilateral nerve injury and possible intrathecal signaling
Dubový P., Svíženská I., Klusáková I., Brázda V., Joukal M., Strejčková, L.

5. Two cases of rare form of Charcot-Marie-Tooth disease caused by mutation in Hexokinase 1 gene
Gabriková D.[1], Bernasovská J.[1], Mistrík M.[2], Tóthová I.[1]

6. Mild hypothermia therapy for patients with severe brain injury
Gal R., Smrcka M., Slezak M., Colonova M.

7. CCL2/MCP-1 as a possible mediator of noradrenaline neuroprotective actions
Madrigal JLM, Hinojosa AE, Leza JC

8. Induction of tolerance to cerebral ischemia/reperfusion injury with NMDA receptor antagonists two *in vivo* and *in vitro* models
D. Makarewicz, M. Kuszczyk., J.W. Lazarewicz

9. MR-spectroscopy of asparagine in hippocampus and human working memory functioning
Kozlovskiy S.A., Vartanov A.V., Pyasik M.M., Polikanova I.S.

10. The ALS disease protein TDP-43 is actively transported in motor neuron axons and regulates axon outgrowth
Fallini C., Bassell G.J., and Rossoll W.

11. Presynaptic cannabinoid-sensitive receptor GPR55 regulates neurotransmitter release in the brain
Sergiy Sylantyev, Thomas P. Jensen, Ruth A. Ross, Dmitri A. Rusakov

12. Knockout rat models for the study of neurodegenerative diseases
Schmidl S, Dan Fisher, Aaron McCoy, Edward Weinstein, and Xiaoxia Cui

13. Inhibitory control over spine dynamics by drug-unpaired environments
B. F. Singer, N. Bubula, V. Bindokas, P. Vezina

14. Inhibition of the Casein-kinase-1-epsilon/delta prevents relapse-like alcohol drinking
Stéphanie Perreau-Lenz, Valentina Vengeliene, Hamid R. Noori, Emilio V. Merlo-Pich, Mauro A. Corsi, Corrado Corti & Rainer Spanagel

15. Correlation between diffusion tensor imaging and relaxometry in Huntington's disease: A globus pallidus study
Michael Syka, Jiří Keller, Jiří Klempíř, Aaron M. Rulseh, Jan Roth, Robert Jech, Ivan Vořišek Josef Vymazal

16. EAG channels enable cost efficient neural coding in cockroach photoreceptors
Vähäsöyrinki M., Frolov R., Immonen E.-V., Salmela I, Weckström M.

17. Neddylation controls axonal and dendritic development in the mouse brain
Annette M Vogl, Marisa Brockmann, Boldizsar Czéh, Sebastian Giusti, Anna Moebus Florian Holsboer, Wolfgang Wurst, Chichung Lie, Jan M Deussing, Damian Refojo

18. Anticonvulsants failed to block whereas bumetanide suppressed the epileptiform activity in immature rat temporal cortex
Abdul Wahab, Klaus Albus, Uwe Heinemann

19. Evaluation of the anxiolytic effects of new phencyclidine derivatives in mouse elevate plus maze model
Kayvan Yaghoobi, Nima Naderi, Abbas Ahmadi, Zahra Shirazizand

20. Lateralized response in dynorphin A peptide levels after traumatic brain injury
Tatjana Yakovleva, Zubair Muhammad Hussain, Sylvia Fitting, Hiroyuki Watanabe, Ivan Usynin, Pamela E. Knapp, Stephen W. Scheff, Kurt F. Hauser and Georgy Bakalkin

21. Orexin-$GABA_A$ergic cross-talking events during lead-induced neurotoxicity of fish
Zizza M., Giusi G., Crudo M., Canonaco M. and Facciolo R.M.

List of Participants

Last name	Name	Country
Abrous	Nora	France
Bähr	Mathias	Germany
Bakalkin	Georgy	Sweden
Betz	Heinrich	Germany
Bonhoeffer	Tobias	Germany
Bonni	Azad	USA
Borst	Alexander	Germany
Brose	Nils	Germany
Chang	Hao-Teng	Taiwan
Cheffer	Arquimedes	Brazil
Choquet	Daniel	France
Clark	Beverley	UK
Coussen	Françoise	France
Craig	Anne-Marie	Canada
Dayanithi	Govindan	France
Debanne	Dominique	France
Dickinson	Michael	USA
Diester	Ilka	USA
Dubovy	Petr	CZ
Emptage	Nigel	UK
Engert	Florian	USA
Fischer	André	Germany
Gabrikova	Dana	Slovakia
Gal	Roman	CZ
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Gerloff	Christian	Germany
Gilestro	Giorgio F.	UK
Guilleme	Karine	France
Haas	Helmut	Germany
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Häusser	Michael	UK
Herrera-Marschitz	Mario	Chile
Hovis	Kenneth	Qatar
Ikegaya	Yuji	Japan
Jayaraman	Vivek	USA
Keller	Georg	Germany
Koch	Giacomo	Italy
Kokaia	Zaal	Sweden
Lasbleiz	Christelle	France
Laurent	Gilles	Germany
Luft	Andreas	CH
Madrigal	Jose Luis Munoz	Spain
Makarewicz	Dorota	Poland
Meinertzhagen	Ian	Canada
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Nägerl	Valentin	France
Oertner	Thomas	Germany
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Petersen	Carl	CH
Püschel	Andreas	Germany
Pyasik	Maria	Russia
Rajendran	Lawrence	CH
Refojo	Damian	Germany
Rodriguez Arellano	J.J.	Spain
Rossoll	Wilfried	USA
Rusakov	Dmitri	UK
Russig	Holger	Germany
Salinas-La Rosa	Cesar	Australia
Sandberg	Mats	Sweden
Schmidl	Sabine	USA
Schuman	Erin	Germany
Schwab	Martin	CH
Segev	Idan	Israel
Singer	Bryan F.	USA
Spanagel	Rainer	Germany
Stühmer	Walter	Germany
Swinnen	Stephan	Belgium
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Sykova	Eva	CH
Tasker	Andrew	Canada
Thomsen	Christian	USA
Torro	Lauri	Finland
Triller	Antoine	France
Trimmel	Michael	Austria
Vähäsöyrinki	Mikko	Finland
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Vargova	Lydia	CZ
Verkhratsky	Alexei	UK
Vilotti	Sandra	Italy
Vogl	Annette	Germany
Vosshall	Leslie	USA
Wahab	Abdul	Pakistan
Wilke	Melanie	Germany
Winsky-Sommerer	Raphaelle	UK
Wisden	Bill	UK
Yaghoobi	Kayvan	Iran
Yakovleva	Tatiana	Sweden
Zernig	Gerald	Austria
Zizza	Merylin	Italy

Abstracts

Abstracts are listed alphabetically according to presenting author

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Sponsored by:

Neurogenic changes in aging

Abrous, DN

"Neurogenesis and Pathophysiology" laboratory, Institut F Magendie, , INSERM 862, Bordeaux France. University of Bordeaux 2, Bordeaux, France.

Aging is associated with cognitive dysfunction, which has been correlated to an alteration of hippocampal functioning Indeed, the hippocampal formation (HF) plays a crucial role in controlling cognitive functions, and is the brain region most vulnerable to ageing processes. The mammalian HF, in particular the dentate gyrus (DG), is an important site for the production of new neurons during adulthood. The aim of our work is to determine the role of adult neurogenesis in the appearance of age-related cognitive deficits. We have found that cognitively-impaired senescent rats display lower levels of neurogenesis than cognitively-unimpaired old rats. We have further shown that these inter-individual differences result from early deleterious life events. Indeed, prenatal stress orients neurogenesis in pathological ways for the entire life, and precipitates age-related cognitive impairments. More importantly, we have recently fond that the consequences of prenatal stress on hippocampal plasticity can be reversed by a form of postnatal environmental stimulation, neonatal handling. Finally, we have highlighted that inhibition of neurogenesis is one of the mechanisms by which glucocorticoids may fragilize cognitive functions during aging. Altogether these data strengthen that hippocampal neurogenesis plays a pivotal role in the development of pathological aging and reinforce the hypothesis of an early neurodevelopmental origin for psychopathological vulnerabilities in aging.

Dynorphin mutations cause human neurodegenerative disorder spinocerebellar ataxia type 23

D.S. Verbeek (1), H. Watanabe (2), J. Jezierska (1), K.A. Artemenko (2), T. Yakovleva (2), Kurt F. Hauser (3), and Georgy Bakalkin (2)

Dept. Genetics, Univ. Groningen, The Netherlands (1), Dept. Pharmac. Biosci., Uppsala Univ., Sweden (2), and Dept. Pharmacol. Toxicol., Virginia Commonwealth University, Richmond, USA (2)

Neuropeptides have not been previously identified as causative factors for neurodegenerative disorders. The spinocerebellar ataxias (SCAs) are a genetically heterogeneous group of neurodegenerative disorders characterized by progressive cerebellar ataxia, dysarthria and loss of the Purkinje cells. We have identified missense mutations in the prodynorphin (*PDYN*) gene to cause SCA23 in four Dutch families displaying progressive gait and limb ataxia. PDYN is the precursor protein for the opioid neuropeptides, alpha-neoendorphin, and dynorphins A and B (Dyn A and B). Dynorphins regulate pain processing and modulate the rewarding effects of addictive substances. Three mutations were located in Dyn A, a peptide with opioid activities, as well as non-opioid neurodegenerative actions. Two of these mutations resulted in excessive generation of Dyn A in a cellular model system. In addition, two of the mutant Dyn A peptides induced toxicity above that of wild type Dyn A in cultured striatal neurons. The fourth mutation was located in the non-opioid PDYN domain and was associated with altered expression of components of the opioid and glutamate system as evident from analysis of SCA23 autopsy tissue. Thus, alterations in Dyn A activities and/or impairment of secretory pathways by mutant PDYN may lead to glutamate neurotoxicity which underlies Purkinje cell degeneration and ataxia. This is the first demonstration of causative link between mutations in neuropeptides and neurodegenerative/neuropsychiatric disorders. Identification of such mutations will also provide further insight into neuropeptide functions.

Acknowledgments: R. Franklin Fellowship, University of Groningen, the Netherlands; the Swedish VR and FAS.

Regulation of neuronal connectivity by ubiquitin signaling

Azad Bonni

Department of Neurobiology, Harvard Medical School, Boston, MA

The assembly of neural circuits is essential for the proper development and function of the brain. Axon and dendrite differentiation culminating in synapse formation represent key developmental events that orchestrate the establishment of neural circuits. Our studies using the rat cerebellar cortex as a model system suggest that ubiquitin ligases play fundamental roles in the control of axon and dendrite development in neurons. We have identified a function for the ubiquitin ligase Cdh1-APC in the control of axon growth and patterning. In other studies, we have discovered that the major mitotic ubiquitin ligase Cdc20-APC drives the formation and elaboration of dendrites in postmitotic neurons in the mammalian brain. Cdc20 is concentrated at the centrosome in neurons, and the centrosomal localization is critical for Cdc20-dependent dendrite development. We have also found that the centrosome-associated protein HDAC6 promotes the ubiquitinated state of Cdc20 and thereby stimulates Cdc20-APC activity and dendrite growth. Our findings highlight the importance of ubiquitin ligases in the control of neuronal morphogenesis and connectivity, with important implications for brain development and plasticity.

ON and OFF Pathways in Drosophila Motion Vision

A.Borst

Max-Planck-Institute of Neurobiology, Martinsried, Germany

The fly nervous system computes local motion information from the local retinal brightness changes by an algorithm as specified in the Reichardt model. In this model, the luminance derived from one image pixel is low-pass filtered and subsequently multiplied with the instantaneous signal from the neighboring image pixel. This is done twice in two mirror-symmetrical subunits and the results of both subunits become finally subtracted. Much evidence has been collected in the past in favor of this computational model. However, due to the small size of the participating neurons, it is still unclear which neurons of the fly optic lobe constitute the Reichardt detector, and what the biophysical mechanisms are which correspond to formal operations like low-pass filtering and multiplication.

By combining genetic targeting of small, individual neurons with whole-cell patch recording from the large output cells in Drosophila, we have identified the two major input neurons feeding into the motion detector, namely lamina cells L1 and L2. We have shown that they split the visual signal into an ON and an OFF component, similar to the ON- and OFF-bipolar cells of the vertebrate retina (Joesch et al, 2010). Using cell-specific driver lines to silence L1 and L2, respectively, while simultaneously recording from tangential cells (Joesch et al, 2008), we found the visual responses to moving gratings to be reduced to about 50%, when either L1 or L2 output was blocked. A fundamental difference between the L1 pathway and the L2 pathway was uncovered when blocking L1 or L2 output while presenting moving edges of positive (ON) or negative (OFF) contrast polarity: blocking L1 eliminated the response to moving ON edges, whereas blocking L2 eliminated the response to moving OFF edges. The finding of two parallel input pathways in the fly visual system subsequently led to the discovery that there exist two separate, parallel motion detection systems, one for detecting the motion of ON-edges (dark-to-bright transition), the other for the detection of moving OFF-edges (bright-to-dark transitions) (Eichner et al, 2011). Knowing the cellular identity of the input neurons of the lamina, anatomy is presently guiding our way to identify the further constituents of these two motion detectors. Using specific expression lines, these cells will be tested by genetically blocking their output as well as by optogenetic activation, while whole-cell patch recording from the lobula plate tangential cells. With this strategy, we hope to unravel the cellular implementation of the Reichardt detector in full detail within the forthcoming years.

Joesch M, Plett J, Borst A, Reiff DF (2008): Response properties of motion-sensitive visual interneurons in the lobula plate of Drosophila melanogaster. Current Biology 18: 368-374.

Joesch M, Schnell B, Shamprasad VR, Reiff DF, Borst A (2010): ON and OFF pathways in *Drosophila* motion vision. Nature 468: 300-304.

Eichner H, Joesch M, Schnell B, Reiff DF, Borst A (2011): Internal structure of the fly elementary motion detector. Neuron 70: 1155-1164.

Lipophilicity as a determinant of procaine analogues binding to the rat $\alpha_3\beta_4$ nicotinic acetylcholine receptor

Cheffer A., Mustafa E.V., -do Amaral A.T., Ulrich H.

University of São Paulo, São Paulo, Brazil

Nicotinic acetylcholine receptors (nAChRs) were studied in detail in the past regarding their interaction with therapeutic and drug-addiction related compounds. Using a structure-activity approach, we have examined the relationship between the molecular features of a set of eight *para*-*R*-substituted *N,N*-[(dimethylamino)ethyl] benzoate hydrochlorides, structurally related to procaine and their affinity for the $\alpha_3\beta_4$ nAChR heterologously expressed in KXα3β4R2 cells. Affinity values (log $1/IC_{50}$) of these compounds for the $\alpha_3\beta_4$ nAChR were determined by their competition with [3H]TCP binding. Log $1/IC_{50}$ values were analyzed considering different hydrophobic and electronic parameters and those related to molar refractivity. These have been experimentally determined or were taken from published literature. In accordance with literature observations, the generated cross-validated quantitative structure-activity relationship (QSAR) equations indicated a significant contribution of hydrophobic term to binding affinity of procaine analogues to the receptor and predicted affinity values for several local anaesthetics (LAs) sets taken from literature. The predicted values by using the QSAR model correlated well with the published ones both for neuronal and electroplaque nAChRs. Our work also reveals the general structure features of LAs that are important for interaction with nAChRs as well as the structural modifications that could be done in order to enhance binding affinity.

A nanoscale view into the dynamic of AMPA receptor organization in synapses

Daniel Choquet

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Ionotropic AMPA glutamate receptors (AMPAR) mediate fast excitatory synaptic transmission in the central nervous system. Using a combination of high resolution single molecule imaging techniques and video-microscopy, we have previously established that AMPARs are not stable in the synapse as thought initially, but undergo continuous entry and exit to and from the post-synaptic density through lateral diffusion.
Single molecule approaches give access to the full distribution of molecule behaviors and overcome the averaging intrinsic to bulk measurement methods. They allow access to complex processes where a given molecule can have heterogeneous properties over time. We will present some recent developments in single molecule imaging technologies and their application to track single molecules in live neurons.
We have recently found a new function for this fast diffusion in controlling fast synaptic transmission. Upon consecutive synaptic stimulation at high frequency, synaptic transmission is depressed. This depression shapes the frequency dependent adaptation of individual synapses. AMPAR lateral diffusion allows fast exchange of desensitised receptors with naïve functional ones within or nearby the post-synaptic density. This participates to the recovery from depression in the tens of millisecond time range, in parallel with recovery from desensitization.
In addition, we now show that the Ca^{2+}/Calmodulin-dependent protein kinase II (CaMKII), which is critically required for the synaptic recruitment of AMPA-type glutamate receptors (AMPARs) during both development and plasticity, induces the synaptic trapping of AMPARs diffusing in the membrane. Furthermore, this CaMKII dependent AMPAR immobilization regulates short term plasticity. Thus, NMDA dependent Ca2+ influx in the post-synapse trigger a CaMKII and Stargazin dependent decrease in AMPAR diffusional exchange at synapses that controls synaptic function.

Refs:

1. Heine, M., Groc, L., Frischknecht, R., Beique, J.C., Lounis, B., Rumbaugh, G., Huganir, R.L., Cognet, L., and Choquet, D. (2008). Surface mobility of postsynaptic AMPARs tunes synaptic transmission. Science 320, 201-205.
2. Bats, C., Groc, L., and Choquet, D. (2007). The interaction between Stargazin and PSD-95 regulates AMPA receptor surface trafficking. Neuron 53, 719-734.
3. Ehlers, M.D., Heine, M., Groc, L., Lee, M.C., and Choquet, D. (2007). Diffusional Trapping of GluR1 AMPA Receptors by Input-Specific Synaptic Activity. Neuron 54, 447-460.
4. Groc, L., Lafourcade, M., Heine, M., Renner, M., Racine, V., Sibarita, J.B., Lounis, B., Choquet, D., and Cognet, L. (2007). Surface trafficking of neurotransmitter receptor: comparison between single-molecule/quantum dot strategies. J Neurosci 27, 12433-12437.
5. Triller, A., and Choquet, D. (2005). Surface trafficking of receptors between synaptic and extrasynaptic membranes: and yet they do move! Trends Neurosci 28, 133-139.
6. Groc, L., Heine, M., Cognet, L., Brickley, K., Stephenson, F.A., Lounis, B., and Choquet, D. (2004). Differential activity-dependent regulation of the lateral mobilities of AMPA and NMDA receptors. Nat Neurosci 7, 695-696.
7. Tardin, C., Cognet, L., Bats, C., Lounis, B., and Choquet, D. (2003). Direct imaging of lateral movements of AMPA receptors inside synapses. Embo J 22, 4656-4665.
8. Borgdorff, A.J., and Choquet, D. (2002). Regulation of AMPA receptor lateral movements. Nature 417, 649-653.

Direct measurement of signalling in mammalian central axons

Beverley A Clark

Wolfson Institute for Biomedical Research, University College London, UK

Axons are thought to be reliable transmission lines, with every somatic action potential accurately representing the activation of axonal synaptic boutons. However, some axonal geometries, particularly highly branching ones, may be less secure, especially at high rates of activity, resulting in failures of transmission to their target neurons. Also, while all action potenitals are initiated in the axon, they may not always reliably invade the soma, and somatic recordings might not accurately detect all action potential output. As with all neuronal compartments, axonal excitability depends on the relative availability of voltage gated channels and it is now known that passive propagation of subthrehold activity can have considerable effects on axonal channel availablity and on spike initiation and propagation. To invesigate the impact of these features on a defined functional network we have used patch clamp and imaging approaches to directly measure the activity of myelinated axons in the olivocerebellar system, focussing on Purkinje cells, the output neurons of the cerebellar cortex and neurons of the inferior olive, which provide the important climbing fibre input to the Purkinje cells.

A nanoscale view into the dynamic of AMPA receptor organization in synapses

Mario Carta, Patrizio Opazo, Julien Veran, Daniel Choquet, Christophe Mulle and Françoise Coussen

CaMKII is key for long-term potentiation of synaptic AMPA receptors. Whether CaMKII is involved in activity-dependent plasticity of other ionotropic glutamate receptors is unknown. We identify a novel form of spike-timing dependent depression (STDP-LTD) of kainate receptor-mediated responses at hippocampal mossy fiber synapses which depends on Ca^{2+} influx, activation of CaMKII, and on the GluK5, kainate receptor subunit (KAR). CaMKII phosphorylation of three residues in the C-terminal domain of GluK5 subunit promotes surface expression of KARs, but concomitantly decreases its synaptic content. CaMKII activation also markedly increases lateral mobility of KARs, possibly by decreasing the binding of GluK5 to PSD-95. We demonstrate that the direct phosphorylation of GluK5 by CaMKII is necessary for STDP-LTD. We propose that CaMKII-dependent phosphorylation of GluK5 is responsible for synaptic depression by untrapping of KARs from the PSD and increased diffusion away from synaptic sites.

Cell Surface Synaptic Organizing Complexes

Ann Marie Craig

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Synapse development requires bidirectional coordinated signaling: 1) anterograde signals from the axon to cluster appropriate postsynaptic receptors and scaffolding molecules in the apposing dendrite, and 2) retrograde signals from the dendrite to cluster synaptic vesicles and fusion apparatus in the axon. A well-known synapse organizing complex at mammalian glutamate synapses are postsynaptic neuroligins and their partners presynaptic neurexins. Neuroligins alone presented on non-neuronal cells or on beads to axons induce local presynaptic differentiation of functional release sites. Conversely, neurexins alone presented to dendrites induce local postsynaptic differentiation, clustering postsynaptic scaffolds and neurotransmitter receptors. Thus, neuroligins and neurexins are 'synaptogenic'. Using the neuron-fibroblast coculture assay, we performed an unbiased screen for synaptogenic proteins. We screened a custom brain expression library in non-neuronal cells for proteins able to induce presynaptic differentiation in contacting axons. From this screen we re-isolated neuroligin-2 and netrin-G ligand-3 and isolated novel synaptogenic factors LRRTM1, Slitrk3, and the neurotrophin receptor TrkC. These postsynaptically localized organizers function through two families of presynaptically localized organizers, neurexins and receptor-type protein tyrosine phosphatases (PTPs). An emerging theme is that the presynaptically localized organizers have multiple postsynaptic partners. For example, neurexins bind in an isoform-specific code to LRRTM1,2 and cerebellin-GluRδ as well as to neuroligins. Furthermore, subsets of neurexin and PTP based complexes function selectively in excitatory or inhibitory synapse development. For example, TrkC-PTPσ functions specifically at glutamatergic synapses and Slitrk3-PTPδ at GABAergic synapses. The importance of these synapse organizing complexes for brain function is underscored by their linkage to neurodevelopmental psychiatric disorders, particularly autism.

Calcium homeostasis in stem cells

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Every cell or neuronal type utilizes its own specific organization of its Ca^{2+} homeostasis depending on its specific function and its physiological needs. Dysregulation of Ca^{2+} homeostasis leads to various complex scenarios that regulate a range of processes in all types of cells throughout the body, including cell function, growth, survival, aging, disease and cell death. Stem cell-based therapies are a potential avenue for cell replacement therapy in CNS injuries and neurodegenerative diseases. However, Ca^{2+} homeostasis and signalling mechanisms in stem cells are less well understood. One of our aims is to evaluate the role and physiology of Ca^{2+} and its signalling mechanisms to understand the functional properties of human embryonic stem cell-derived neural precursors (hESC NPs, CTL14 cell line) during long term propagation in culture (in vitro). We analyzed the physiopathological responses and pharmacological profiles of these cells in terms of their $[Ca^{2+}]_i$ responses to high K^+, ATP, glutamate and intracellular Ca^{2+}-releasing agents at different passages (P1 through P10) during the course of hESC differentiation. Our results show that these cells respond to different physiological stimuli by an increase in $[Ca^{2+}]_i$ that varies during the course of hESC differentiation. The number of cells responding to the above agents was higher in passage 7 (P7) NPs when compared to other passages. P7 hESC NPs express functional glutamate receptors, purinoreceptors and voltage-dependent Ca^{2+} channels and show spontaneous Ca^{2+} oscillations as typically observed in neuronal/endocrine cells.
In addition, mesenchymal stromal/stem cells (MSC) are multipotent cells that represent a promising tool for cell replacement therapies in many diseases and currently are being tested in a number of approved clinical trials for stroke, spinal cord injury, etc. We sought to study the mechanisms underlying their function and effect in regeneration and addressed whether mesenchymal cells of different origin differentiate in a similar manner under the same environmental conditions. We used two cell lines, rat adipose-derived stem cells and rat bone marrow stem cells. Our preliminary results show that pre-differentiation leads to an activation of Ca^{2+} signalling cascades and enhances the functional activity of the stem cells. While pre-differentiated MSC expressed functional voltage-gated Ca^{2+} channels, P2X and P2Y purinergic receptors, glutamate receptors and OT and AVP receptors, released Ca^{2+} from the endoplasmic reticulum via ryanodine receptors and also exhibited spontaneous $[Ca^{2+}]_i$ oscillations, the undifferentiated MSC expressed only purinergic receptors. These results indicate that understanding the functional properties of stem cells may allow us to better control their regenerative potential and help to improve strategies for their use in transplantation and treatment.

Analog-digital signaling in hippocampal axons

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In most neurons, information is transmitted to the postsynaptic neuron as discrete amounts of neurotransmitter released by the presynaptic neuron in an all-or-none or *digital* mode. However, subthreshold activity in the presynaptic element also determines the flow of neuronal information in an *analog* mode. We will discuss recent results showing analog-digital enhancement of synaptic transmission at excitatory connections established between CA3 pyramidal neurons.

Straighten up and fly right: Visual navigation in Drosophila

Michael Dickinson

One of the greatest challenges in neurobiology is in understanding how nervous systems can generate long seamless sequences of behavior, for example, the yearly migrations of birds, whales, or insects from the poles to the tropics. The research in my laboratory focuses on the flight behavior of fruit flies which - although not global migrants - do use flight to disperse over long distances and explore their local environment for food and mates. A successful flight sequence from take-off to landing involves many sensory-motor programs operating in series and parallel on different time scales. By applying various quantitative behavioral methods we are attempting to identify and isolate these different sensory-motor modules at the algorithmic level, with the ultimate goal of identifying the underlying circuits. My talk will focus on several critical visually-mediated components of flight behavior including take-off, navigation, predator avoidance, and landing.

Cholinergic Interneurons Control Local Circuit Activity and Cocaine Conditioning

Ilka Diester

Cholinergic neurons are widespread, and pharmacological modulation of acetylcholine receptors affects numerous brain processes, but such modulation entails side effects due to limitations in specificity for receptor type and target cell. As a result, causal roles of cholinergic neurons in circuits have been unclear. We integrated optogenetics, freely moving mammalian behavior, in vivo electrophysiology, and slice physiology to probe the cholinergic interneurons of the nucleus accumbens by direct excitation or inhibition. Despite representing less than 1% of local neurons, these cholinergic cells have dominant control roles, exerting powerful modulation of circuit activity. Furthermore, these neurons could be activated by cocaine, and silencing this drug-induced activity during cocaine exposure (despite the fact that the manipulation of the cholinergic interneurons was not aversive by itself) blocked cocaine conditioning in freely moving mammals.

Bilateral propagation of neuroinflammatory reaction in the dorsal root ganglia alongside neuroaxis after unilateral nerve injury and possible intrathecal signaling

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Wallerian degeneration following mechanical injury of the peripheral nerve is essential stimulus for cellular and molecular changes inducing neuropathic pain. Unilateral chronic constriction injury (CCI) of the sciatic nerve was performed aseptically in sixty fore rats. Neuropathic pain induction was tested by withdrawal threshold of mechanoallodynia and thermal hyperalgesia. Expression of TNFa and IL-6 protein as well as mRNA was investigated bilaterally by immunohistochemistry, Western blot, ELISA and in situ hybridization in both lumbar (L4-5) and cervical (C7-8) DRG following CCI for 1, 3, 7 and 14 days. In addition, FluoroRuby (dextran-TRITC) was injected intrathecally in the level of L4-L5 or C7-C8 spinal segments. Although mechanoallodynia and thermal hyperalgesia were detected predominantly in the ipsilateral hind paws from 1 to 14 days, levels of cytokine proteins and mRNA were enhanced bilaterally in both cervical and lumbar DRG. FluoroRuby penetrated from intrathecal space not only into DRG at the level of application but diffused also into DRG of remote spinal segments. Our results indicate that cytokine proteins and their synthesis in DRG may spread bilaterally from the spinal segments associated with injured nerve and per se are not completely involved in neuropathic pain induction and maintenance. A possible pathway for diffusion of signal molecules inducing changes in the remote DRG is cerebrospinal fluid of spinal intrathecal space.
This work was supported by the project CEITEC (CZ.1.05/1.1.00/02.0068) from European Regional Development Fund.

A neural circuit controlling motor learning in larval zebrafish

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A fundamental question in neuroscience is how neural circuits generate behavior and adapt it to changes in sensory feedback. Here we use two-photon calcium imaging throughout the brain of larval zebrafish while the paralyzed animals interact fictively with a virtual environment and rapidly adapt their motor output to changes in visual feedback. We describe the neural dynamics that occur during adaptive locomotion throughout the brain of the fish, and provide an anatomical map of their locations. A subset of these signals occurred during behavioral adjustments and are attractive candidates for the functional elements that drive motor learning. This work enables us to propose a specific model of a neural circuit that detects mismatches between expected and actual feed-back and adjusts the sensory motor transformation accordingly.

Epigenetics in schizophrenia

Andre Fischer

In addition to genetic variations, there is accumulating evidence that proper genome-environment interactions are central for cognitive function. Epigenetic mechanisms such as histone-modifications are key regulatory processes that mediate genome-environment interactions and eventually lead to changes in chromatin plasticity and gene-expression. Recent research suggested that dys-regulated histone-acetylation plays an important role in the pathogeneses of brain diseases and provided evidence that targeting histone-acetylation via the use of histone-deacetylase (HDAC) inhibitors could serve as a novel and promising therapeutic avenue to treat brain diseases. However, little is know on the role of individual 11 HDAC proteins in the adult brain.

Several studies reported that HDAC1 was found to be up-regulated in patients suffering from schizophrenia. Thus, we developed a viral-mediated approach to over-express neuronal HDAC1 in the adult mouse hippocampus and anterior cingulate cortex We show that acute over-expression of neuronal hippocampal HDAC1 specifically affects the extinction of contextual fear memories, while other cognitive abilities were unaffected. In subsequent experiments we use HDAC1 gain and loss of function approaches to show that under physiological conditions hippocampal HDAC1 is required for extinction learning via a mechanisms that involves H3K9 deacetylation and subsequent tri-methylation of target genes. In contrast modulation of HDAC1 in the ACC lead to schizophrenia like symptoms.

Two cases of rare form of Charcot-Marie-Tooth disease caused by mutation in Hexokinase 1 gene

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Charcot-Marie-Tooth disease (CMT) is the most frequent form of hereditary neuropathy. The CMT subtypes are phenotypically similar but genetically it is a very complex group of disorders. Mutations in more than 31 genes have been reported to cause various forms of CMT. Proteins coded by these genes are mostly myelin components, or they affect protein synthesis, transport or degradation and mutation can result in demyelinating form of CMT. Mutations in other proteins can be involved in axonal damage causing axonal type of CMT. In addition, some proteins can cause both demyelinating and axonal forms. Here we reposrt two cases of CMT type 4G (HSMN-Russe). Mutation in Hexokinase 1 gene (HK1), confirmed in these patients, was discovered recently and is known to be a founder mutation in Roma/Gypsy ethnic group. HK1 gene encodes a ubiquitous form of hexokinase which localizes to the outer membrane of mitochondria and serves as a major point of regulation of the energy-producing glycolytic pathway. Clinical and molecular characterization of two patients will be presented.

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Mild Hypothermia Therapy for Patients with Severe Brain Injury

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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. 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. 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 affects and maximum cerebral protection as well as an exact selection of patients with TBI suitable for deliberate hypothermia is a task for the future.

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Systems biology, epigenetics, and beyond:
New paradigms and understandings on development of mental diseases.

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Our results from human post-mortem tissue support the notion that fundamental functions of the synapse are disturbed in schizophrenia. The highly significant upregulation of MLL3, an histone methyl-transferase, in superior temporal cortex of the patients underscores the importance of epigenetic modifications and the crucial impact of environmental influences on the development of the disease. The generally disappointing results from pure genetic investigations (candidate genes, polymorphisms, GWAS, CNVs) have put epigenetic studies in the center of recent research interests. In contrast to the rather stable DNA sequence, DNA methylations and histone modifications appear to be subject to lifelong changes. For this reason, they are excellent targets to study the development of health and disease. Unfortunately, many investigations – in humans in particular – suffer from groupwise comparisons between patients and controls by pooling individuals of different ages. Here, we present an approach to use the available data to introduce the dynamic aspect of time. The example of MLL3 shows an age-dependent increase of expression in the controls, which means that differences of its expression between patients and controls become indistinguishable in old individuals. Some more examples of genes showing time-dependent changes of expression are shown. These genes are good candidates to study dynamically modified epigenetic patterns leading to the transcriptional changes. The ensueing results would provide a deeper understanding of epigenetic mechanisms of gene regulation.

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Reorganisation of the motor network following irreversible brain lesions in stroke patients

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Stroke is the 2nd most common single cause of death and the most frequent cause of permanent disability in industrialised countries. The WHO estimates that 15 million people suffer from stroke each year and 5 million are left permanently disabled. From a systems neuroscience perspective, stroke is an acute irreversible damage to a neural network. Depending on which function the affected network had been subserving, deficits can be devastating, i.e., loss of speech and mobility, or very subtle. The acute stroke phase (phase 1) is characterized by an extended breakdown of neural function and metabolism. Subsequently (phase 2), a generalized increase of neuronal excitability and metabolism can be detected and is thought to be the basis for reorganisation of the affected neural network. Finally (phase 3), in the course of weeks and months, new network properties are shaped and allow for at least partial recovery in many patients. The focus of the present talk will be the motor network in humans. Network properties of the human motor system can be readily addressed with non-invasive techniques such as EEG, MEG, transcranial magnetic stimulation (TMS) and functional magnetic resonance imaging (fMRI). In the first part, basic principles of motor network analysis in the human brain will be explained. In the second part, specific findings in stroke patients will be demonstrated. Finally, possibilities of active network modulation by transcranial brain stimulation will be outlined.

Sleep in *Drosophila Melanogaster*

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Sleep is a mysterious activity. All animals that have been tested so far, from the simplest invertebrates to mammals, have shown to possess and to require the fundamental characteristics of sleep, independently of the size or the complexity of their nervous systems. This remarkable conservation across evolution suggests that the core function of sleep has to be sought at the basic cell biological level of neuronal function, namely that sleep is an intrinsic requirement of any neuronal network and, possibly, every neuron (or cell?).

So far, efforts in investigating the function of sleep have mostly focused on electrophysiological analysis of the sleeping brain in a very descriptive fashion. Over the past 60 years we have acquired a great amount of information about the electroencephalogram correlates of sleep and wakefulness in mammals but, albeit helpful on a number of aspects, this knowledge could not yet shed a light on the function of sleep. In short, the question of ``why do we sleep?'' still remains unanswered.

My goal is to tackle the problem with an approach based on genetics and cell biology, using the fruit fly Drosophila melanogaster as animal model. In particular, I am interested in unveiling the connections between sleep and memory consolidation, with focus on the synaptic dynamics of this phenomenon. The talk will outline some of my previous and recent work on the topic and focus on three specific questions: 1) what happens to molecular correlates of synaptic strength during sleep and wakefulness; 2) can we interfere with sleep by genetically or environmentally modifying synaptic strength; 3) why is *Drosophila* the best model to answer these questions.

Simultaneous recording of accumbens network activity with microelectrode arrays and its neurochemical modulation with real-time neurochemistry

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The nucleus accumbens (NAc) is an important neural component of the brain reward system. It receives and integrates multiple sources of information from different brain regions to regulate goal-directed behaviors and action selection. This integrative function is modulated by ascending neurochemical modulator systems, most prominently by midbrain dopamine neurons. To understand NAc integrative function and its neurochemical modulation during goal-directed behaviors, it is crucial to simultaneously record large numbers of neurons and monitor neurotransmitter release with the same time resolution. Today this can be achieved by coupling chronic extracellular recordings and fast-scan cyclic voltammetry – a real-time neurotransmitter monitoring technique with subsecond temporal resolution. In this talk, these basic methods and their advantages will be presented and illustrated with the example of drug-seeking behaviors.

Waking with the hypothalamus

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The regulation of sleep and waking, feeding and drinking, body temperature and activity, i.e. the administration of energy is essential for the whole body homoeostasis. The waking brain is activated through neuronal pathways ascending from the brain stem reticular formation (ARAS) and reaching the cerebral cortex by a dorsal route through the thalamus and a ventral route, including the hypothalamus and the basal forebrain. In spite of the early description of sleep disorders in patients with hypothalamic lesions, the posterior hypothalamus has only recently been recognized as a major waking center, with the histaminergic neurons in the tuberomamillary nucleus and the orexinergic/hypocretinergic neurons in the neighbouring perifornical area playing leading distinct but complementary roles: the peptides being responsible for behavioural motor aspects, histamine for EEG activation and cognitive aspects. These and other wake-active cholinergic and aminergic nuclei are inhibited by GABAergic neurons from the sleep-active preoptic area. The timing of sleep and waking is determined by the circadian factor (clock-genes) and the accumulation of sleep pressure (adenosine). Recent advances in the understanding of sleep-waking regulation have opened new therapeutic avenues.

Auditory feedback and song learning

Richard Hahnloser

What is the role of the auditory forebrain and auditory feedback in vocal learners such as the zebra finch? To support motor learning based on an auditory template provided by a tutor, motor neurons must receive acoustic information about the song template as well as auditory feedback. It is currently unknown how motor, memory, and feedback signals interact in the brain, though several models have been proposed, including forward and inverse models, and reinforcement learning. In my talk I will present data recorded in singing zebra finches that provides new insights into the algorithms for song learning.

Spatial navigation: the view from inside a single cell

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Neurons in the medial entorhinal cortex fire action potentials at regular intervals in space, giving rise to a remarkable grid-like arrangement of firing fields (Hafting et al., Nature 2005). How grid cell firing arises from the combination of intrinsic conductances, network connectivity and synaptic activity remains unclear. Solving this question critically depends on knowledge of subthreshold membrane potential dynamics in grid cells in awake, behaving animals. We have performed patch-clamp recordings from neurons of the medial entorhinal cortex in awake, head-restrained mice navigating on a spherical treadmill. Visual and somatosensory feedback was provided by a virtual reality environment. Stellate cells in layer II, which constitute the majority of grid cells, were identified by their characteristic electrophysiological characteristics, and by their morphology following biocytin labelling. A subset of stellate cells showed multi-peaked firing fields, reminiscent of grid cell firing on a linear track. While depolarizing steady-state current injections failed to induce significant subthreshold oscillations in stellate cells, they showed pronounced membrane potential fluctuations in the theta frequency range during movement, indicative of theta-modulated synaptic input. This increase in theta power amplitude upon movement onset was more pronounced in stellate cells than in non-stellate neurons of the MEC. These findings should provide critical constraints for models of grid cell genesis.

Pharmacodynamics and pharmacokinetics studies showing that PARP-1 inhibition protects against the long-term consequences of perinatal asphyxia.

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The effect of two putative PARP-1 inhibitors, nicotinamide and theophylline, was compared on the long-term consequences associated to perinatal asphyxia. As previously reported, perinatal asphyxia produced a decrease in dopamine release (by ~50%) evaluated *in vivo* with microdialysis 3 months after birth. That effect was observed on dopamine and metabolites, but not on serotonin and its metabolite levels. Also, perinatal asphyxia produced a decrease of exploratory behaviour (by ~50%) evaluated in the same period by several behavioural models, including an open field paradigm assessing thigmotactic cues. Nicotinamide (3x daily doses, 0.8 mmol/kg, i.p., started 1h after delivery), but not theophylline (0.14 mmol/kg, i.p., the same schedule as nicotinamide) prevented the effect of perinatal asphyxia on dopamine release and on exploratory behaviour, expanding previous results.

The issue of PARP-1 inhibition by the drugs was investigated with an *in vitro* assay detecting the formation of biotinylated polyADP-riboses (pADPr). It was found that a single dose of nicotinamide (0.8 mmol/kg, i.p.) produced a significant, long-term inhibition of PARP-1 activity, both in brain and peripheral (heart) tissue of neonates, reaching a >80% inhibition when assayed 24h after drug administration. In contrast, a single dose of theophylline (0.14 mmol/kg. i.p.) produced a significant inhibition of PARP-1 activity in heart only, detectable 1 and 8h after drug administration, suggesting a lower potency and lower brain availability at the tested dose of theophylline as compared to that of nicotinamide.

The pharmacokinetics of the drugs was explored in neonates with *in vivo* microdialysis, with a probe inserted into the brain and other subcutaneously. Nicotinamide showed a long-term peripheral (>30 μM) and brain (>10 μM) distribution, while theophylline showed a long-term peripheral distribution (>15 μM), but reaching significant levels (>5 μM) in the brain only 20 and 40 min after the systemic (i.p.) administration, decreasing thereafter to under detection limits (Allende et al. 2012).

Taken together these pharmacodynamics and pharmacokinetics studies, it can be concluded that nicotinamide, but not theophylline, is able to induce a therapeutically relevant inhibition of PARP-1 activity, protecting the animals from the long-term consequences of perinatal asphyxia, supporting the hypothesis that PARP-1 inhibition constitutes a strategy for lessening the long-term effects produced by perinatal asphyxia (Herrera-Marschitz et al 2011; Neurotox Res 19: 603-627).

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Functional and structural role of axon cables in synaptic output

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Chemical neurotransmission is conventionally considered a local event that occurs at synaptic junctions. However, several pioneering studies have recently demonstrated that the membrane potential of the neuronal cell body prior to action potential (AP) generation affects neurotransmission at synapses that are distant from the cell body. Although the underlying mechanisms remain controversial, the long-distance effect of the axon is mediated, at least in part, by an analogue-like distortion of the AP waveform; APs originating from more depolarized membrane potentials are broader, and the broadened APs facilitate downstream synaptic transmission. Here, we report that the range of this somatic influence is spatially restricted by not only axonal path length but also a branching-dependent decrease in axon diameter. Cell-attached recordings of APs from axon branches of a hippocampal CA3 pyramidal cell revealed that an AP was broadened following a 20-mV depolarization of the soma and reverted to a normal width during propagation down the axon. The narrowing of the AP depended on the distance travelled by the AP and on the number of axon branch points through which the AP passed. These findings were confirmed by optical imaging of AP-induced calcium elevations in presynaptic boutons, suggesting that the somatic membrane potential modifies synaptic outputs near the soma but not long-projection outputs. Consistent with this prediction, whole-cell recordings from synaptically connected neurons revealed that depolarization of presynaptic CA3 pyramidal cells facilitated synaptic transmission to nearby CA3 pyramidal cells, but not to distant pyramidal cells in CA3 or CA1. Moreover, we found that APs are subject to waveform modulation while they travel down axons. The waveforms of axonal APs increased in width in response to local application of glutamate and an adenosine A1 receptor antagonist to the axon shafts, but not to other unrelated axon branches. Uncaging of calcium in peri-axonal astrocytes caused AP broadening through ionotropic glutamate receptor activation. Therefore, axonal geometry enables the differential modulation of synaptic output depending on target location.

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Probing sensorimotor integration in Drosophila

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Drosophila melanogaster is a genetic model organism with many experimental advantages, including the ability to genetically manipulate and record from specific sub-populations of neurons. We aim to uncover general principles of sensorimotor integration by using *in vivo* physiology in tethered walking and flying flies during active behavior. I will summarize our findings from such recordings in the optic lobe, where we have found that lobula plate tangential cells change the magnitude and tuning of their responses to wide-field motion stimuli during behavior. I will also discuss our preliminary efforts to characterize neural responses from the fly central complex, a deeper region of the insect brain that has been implicated in a variety of visuomotor behaviors including visual learning, orientation memory, and place learning.

Hyperexcitability of parietal-motor functional connections in the intact left-hemisphere of patients with neglect: combined TMS and MRI studies in human patients

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The peculiar role of the right PPC in controlling visual-spatial attention is thought to be exerted trough its remote control over other areas such as the visual cortex or the frontal cortex within the same hemisphere, but also trough controlling the activity of the contralateral hemisphere. Several theories based on behavioral data from neglect patients have been proposed to explain how the intact human brain controls spatial attention. Heilman's hemispatial theory (Heilman and Van Den Abell 1980) has proposed that the RH directs attention to both visual hemifields, whereas the LH directs attention to the RVF only (Mesulam 1981). Alternatively, Kinsbourne's theory has proposed a mechanism of hemispheric rivalry or competition, in which each hemispheres is responsible for orienting attention toward the contralateral visual field and balance between parietofrontal circuits in the two hemispheres is achieved through reciprocal inhibition (Kinsbourne 1977).
We recently used a novel trifocal TMS method, to directly test the hypothesis that the asymmetry of visuo-spatial function in healthy subjects could be due to an unbalance of interaction between the hemispheres, with a right parietal advantage. The results demonstrated that the right PPC inhibits the activation state of the contralateral parieto-frontal connection more strongly than the left PPC does. Indeed, to obtain detailed anatomical information about the white matter pathways that mediate these neurophysiological interactions, we used diffusion tensor imaging (DTI). TMS/DTI analysis revealed that the effect we found is mediated by a transcallosal pathway located in the posterior portion of the CC. We proposed that this peculiar interhemispheric inhibition may represent an important neurophysiological mechanism at the basis of the well known asymmetry of visuo-spatial functions.

Stem cells and stroke

Zaal Kokaia

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Ischemic stroke, which is caused by occlusion of a cerebral artery, is an acute neurodegenerative disorder and represent one of the leading causes of death and disability in adult humans. It leads to focal tissue loss and the death of multiple cell types. Neuronal plasticity and reorganization of neural circuitries contribute to varying degrees of spontaneous recovery, but most patients exhibit persistent motor, sensory, or cognitive impairments. There are virtually no treatments to support efficient functional recovery in stroke patients. Stem cell–based approaches hold much promise as potential novel treatments to restore function after stroke. Indeed, transplantation of stem cells or their derivatives in animal models can improve function by replacing the neurons and glia cells lost in neurodegenerative diseases and through trophic actions, and modulation of inflammation. Endogenous neural stem cells are also potential therapeutic targets because they produce neurons and glial cells in response to injury and could be affected by the degenerative process. Clinical trials are ongoing but there is currently no proven stem cell-based therapy for stroke. Significant hurdles remain before these findings of basic research can be responsibly translated to novel therapies. In particular, we need to better understand mechanisms of action of stem cells after transplantation in stroke-lesioned brain and learn how to control proliferation, survival, migration, and differentiation in the pathological environment of grafted and endogenously derived stem cells.

Theme and variations on STDP

Gilles Laurent

Recent results concerning the format of representations for odors in the olfactory system of insects, and the complex interplay between circuit architecture (fan out, fan in, lateral inhibition, connection density), network dynamics (oscillations, synchrony and importance of short time delays) and synaptic plasticity (STDP rules, reinforcer signal, reward assignment problem, and solution) will be presented.

CCL2/MCP-1 as a possible mediator of noradrenaline neuroprotective actions

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Noradrenaline (NA) is known to prevent the neuronal damage associated to several pathologies, particularly Alzheimer's disease, where the reduction of NA levels in brain appears to be one of the main causes of its progression. In vitro studies showed that NA can protect neurons against different types of injuries. The analysis of the mechanisms through which NA exerts this protection indicates that the chemokine monocyte chemoatractant protein 1 (MCP-1 or CCL2) could be one of the main mediators involved. Treatment of primary cultures of rat astrocytes with NA caused the induction of MCP-1. This MCP-1 provided protection to neuronal cultures against excitotoxicity caused by direct treatment with glutamate or by exposure to ischemic conditions. Accordingly, the elevation of brain NA levels by the treatment of mice with the NA precursor L-DOPS increased the production of MCP-1 by astrocytes.

Being a chemokine, MCP-1 participates in immune responses by attracting different cell types to injury sites. In order to analyze if MCP-1 effect on microglia could be toxic for neurons, markers of microglia activation were analyzed in primary cultures of rat microglia treated with different concentrations of MCP-1. While we could not observe MCP-1 induction of microglial activation or production of neurotoxic agents, we were able to detect that it induces microglial proliferation as well as the induction of the trophic factor IGF (insulin-like growth factor).

Induction of tolerance to cerebral ischemia/reperfusion injury with NMDA receptor antagonists two *in vivo* and *in vitro* models

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Previous results of our and other studies have shown that preconditioning of the primary cultures of cortical and cerebellar neurons by brief exposing them to NMDA receptor antagonists for up to 96 hours before the insult induces tolerance to necrosis and apoptosis-inducing conditions including excitotoxicity, that damage neurons. The mechanisms of this phenomenon remain unclear, also neuroprotective potential of preconditioning with NMDA receptor antagonists in brain ischemia has not previously been studied. Thus, the aim of the present study was to check if NMDA receptor antagonism induces tolerance in the experimental models of cerebral ischemia/reperfusion both in vivo and in vitro. Tolerance to the *in vitro* ischemia / reperfusion - resembling challenge by preconditioning with different NMDA receptor antagonists including MK-801 and memantine was studied in the primary cultures of rat cerebellar granule cells exposed at 7 DIV for 90 min to oxygen and glucose deprivation (OGD). NMDA receptor antagonists were applied for 30 min 24 or 48 h before OGD. *In vivo* hypoxia-ischemia (H-I) was induced in 7-day-old rats. In the neonatal rats exposure to 7% O_2 in N_2 was used for hypoxic preconditioning (H-P) as a positive control, while for pharmacological preconditioning two NMDA receptor antagonists MK-801 (3 mg/kg) and memantine (5 mg/kg) were injected i.p. The animals were preconditioned 24, 48, 72 and 96 hours before the insults, and the brain damage was evaluated two weeks later. Our results demonstrated that both MK-801 and memantine induced significant tolerance to neuronal injury in both tested models *in vivo* and *in vitro*. In vitro studies demonstrated, that significant viability was observed when the cultures were preconditioned with memantine 24 h and with MK-801 48 h before OGD. Our *in vivo* studies showed that MK-801 administered in all studied time points almost completely reduced brain damage compared to the untreated H-I group, while H-P and preconditioning with memantine were less effective. These data demonstrate for the first time ischemic tolerance induced by MK-801 and memantine preconditioning in two different experimental models.

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Progress towards the connectome of the *Drosophila* visual system

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Anatomical analysis of visual circuits in the fly's optic lobe has always been frustrated by the >60 morphological cell types in the medulla, the optic lobe's second, largest and most complex neuropile, but now yields to approaches using serial-section EM (ssEM). ssEM reconstructions are facilitated because the medulla is arrayed as ~750 columns each with the same 10 repeating input elements, and layered in 10 strata respected by medulla cell arbors and those input elements, which are: R7, R8; five L-cells, centrifugal cells, C2, C3; and T1. So far, 39 columnar neurons are reconstructed from a single column: 17 transmedulla (Tm/TmY), 7 intrinsic (Mi), and 6 amacrine (5 distal and 1 proximal) cells, and a further 9 uncharacterised types; together with many T4 and tangential cells. Receiving matched photoreceptor input in the first neuropile -- or lamina, from R1-R6, L1 and L2 provide input to independent motion-sensing pathways: L1 to Mi1, L5, C2, C3 and overlapping Tm3 cells; and L2 to Tm1 and Tm2, partners at postsynaptic tetrads, and also L5. L4 is also postsynaptic to Tm2 but lies outside its column. Single-cell transcript profiling suggests that L1 is glutamatergic while L2/L4 and Tm2 express nicotinic acetylcholine receptors that mediate fast transmission, Their circuits transmit to retinotopically posterior columns in both lamina and medulla. Tm2 thus appears to integrate cholinergic sign-conserving inputs from neighbouring columns to mediate detection of front-to-back motion generated during forward motion.

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Brain maps for space

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This talk will focus on the neural substrate of our 'sense' of space. I will show how the entorhinal cortex and hippocampus of the mammalian brain forms a continusously updated map of external space that includes both present and past information. We have shown that cells in the entorhinal cortex are part of a universally applicable map for space, consisting of multiple functionally specialized cell types entangled in a complex neural network. Particular attention will be given to the grid cell – a cell type that we discovered in entorhinal cortex in 2005. Grid cells fire if and only if the animal is at certain positions in space. These positions form a regularly spaced hexagonal pattern that covers the entire space available to the animal, much like the grid lines of a geometric map, or the cross-points of graphics paper. The spatially periodic activity pattern defines distances as well as directions. I will provide evidence showing that the grid map is divided into functionally independent modules of grid cells with different grid spacing and grid orientation. I will further show that grid cells co-localize with head-direction cells and border cells – cell types that signal directions and geometric boundaries, respectively – and that output from grid cells and border cells form the basis for place signals downstream in the hippocampus. Collectively this network of specialized space cells generates a dynamically updated map of current location that may be used when we try to find our way from one place to the next.

Dual-color superresolution imaging of synapses and glia cells in living brain slices using STED microscopy

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Neuronal synapses are complex structures composed of pre- and postsynaptic membrane specializations ensheated by glia processes, forming elementary functional compartments for rapid and flexible signaling in the central nervous system. Understanding how synapses are built during development and modified by experience is a central theme for neuroscience.
However, as they are typically very small (< 1 mm) and dynamic and reside inside three-dimensional, light-scattering tissue, it is difficult to study them by conventional, diffraction-limited light microscopy.
However, major advances in superresolution imaging and fluorescence labeling are greatly improving our ability to investigate the inner life and dynamics of synapses using life-cell imaging approaches. We have previously shown that superresolution STED microscopy is a powerful technique for live-cell imaging of synapse morphology using YFP as a genetically encoded volume-label.
We will review our recent progress in adapting STED microscopy for live-cell nanoscale imaging deep inside biological tissue and in two colors simultaneously. Specifically, we will demonstrate the powerful potential of these methodological advances for several applications concerning superresolution imaging of synapses: 1) spine plasticity and actin dynamics using lifeact 2) nanoscale imaging up to 100 mm deep below tissue surface and 3) dual-color live-cell imaging of pre- and postsynaptic structures as well as glia cells with nanoscale spatial resolution.

Synaptic plasticity: adjusting weights or changing topology?

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Activity-dependent changes in synaptic strength are thought to represent a cellular mechanism for learning and memory formation. Long-term imaging experiments *in vivo* and *in vitro* demonstrate that spines can persist over long time periods and at the same time are subject to significant structural remodeling upon induction of large-scale synaptic plasticity. Yet, how the current state of a single synapse determines its fate during a plasticity event is not well understood. The relationships between long-term plasticity, synaptic strength and persistence of individual synapses over extended periods of time are not fully understood.

We used an optogenetic approach to control and read out synaptic activity with light. With our all-optical method we monitored pre- and postsynaptic morphology as well as calcium signals at identified synapses in mature organotypic cultures of the hippocampus over one week. We found that spines continuously adjust their shape without a net volume change at the population level, indicating that individual synaptic inputs fluctuate whereas the sum of synaptic weights remains constant. Optogenetic induction of long-term depression (LTD) at identified synapses had no immediate effect on spine volume, but days later led to pruning of spines. Analyzing postsynaptic calcium signals, we found that synapse elimination could be predicted from the initial release probability (p_r). Robust depression and subsequent spine elimination occurred in low-p_r synapses whereas reliable transmission protected synapses from depression-induced elimination. Our results suggest that LTD not only changes synaptic weights but triggers complete removal of specific neuronal connections. Interestingly, depending on the state of their presynaptic release machinery, individual synapses interpret the same low-frequency protocol quite differently, and, after a period of strong depression, may fully restore their initial strength. Thus, what appears to be ‘stable LTD’ in field recordings is actually a binary decision between recovery or death at the level of individual synapses.

Synaptic mechanisms of sensory perception

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A key goal of modern neuroscience is to understand the neural circuits and synaptic mechanisms underlying sensory perception. Here, I will discuss our efforts to characterise sensory processing in the mouse barrel cortex, a brain region known to process tactile information relating to the whiskers on the snout. Each whisker is individually represented in the primary somatosensory neocortex by an anatomical unit termed a ‘barrel’. The barrels are arranged in a stereotypical map, which allows recordings and manipulations to be targeted with remarkable precision. In this cortical region it may therefore be feasible to gain a quantitative understanding of neocortical function. We have begun experiments towards this goal using whole-cell recordings, voltage-sensitive dye imaging, viral manipulations, optogenetics and two-photon microscopy. Through combining these techniques with behavioral training, our experiments provide new insight into sensory perception at the level of individual neurons and their synaptic connections.

Regulation of neuronal polarity by Rap1 GTPases

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The establishment of neuronal polarity in hippocampal neurons is directed by a pathway that depends on the sequential activity of several GTPases. Rap1 GTPases perform a central function in this pathway and are necessary and sufficient to specify axonal identity. The analysis of conditional knockout mice for Rap1a and Rap1b shows that Rap1 GTPases are required for axon formation in the hippocampus not only in cultured neurons but also in vivo.
Rap1B accumulates in the growth cone of a single neurite in stage 2 neurons before they are polarized morphologically. This restriction of Rap1B localization is an essential step in neuronal polarization and is mediated by its destruction through the proteasome. We identified the E3 ubiquitin ligase Smurf2 as the enzyme that modifies inactive, GDP-bound Rap1B to initiate its degradation. The selective degradation of inactive Rap1B mediates its removal from minor neurites that contain a low level of active GTPase while the active Rap1B in the future axon is protected from proteolysis.
In addition to Smurf2, Rap1B is also regulated by Rheb and the mTOR pathway. Knockdown of Rheb by RNAi or inhibition of mTOR blocks the formation of axons while activation of mTOR induces supernumerary axons. One of the targets for mTOR is Rap1B. Activation of the mTOR pathway in neurons increases the amount of Rap1B and expression of Rheb induces supernumerary axons in an mTOR- and Rap1B-dependent manner. The mTOR-dependent translation of Rap1B may balance its degradation by the proteasome. While loss of Smurf2 alone induces multiple axons and suppression of Rheb results in the loss of axons, neurons establish normal neuronal polarity with a single axon when both pathways are blocked by knockdown of Smurf2 and Rheb.

MR-spectroscopy of asparagine in hippocampus and human working memory functioning

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Introduction. Recent studies show, that asparagine level in hippocampus might correspond with memory functioning. For example, decreased asparagine level in hippocampus results in spatial memory deficiency in mice (Kwon et al., 2000). Furthermore, it is known, that asparagine affects normal long-term potentiation (LTP) process in rat hippocampus (Kapai et al., 2004). In present study we compared asparagine level in both hippocampi with working memory tests performance in healthy humans.
Methods. Participants in the study were 8 right-handed females (mean age – 59±16) without neurological and mental disorders. All subjects performed working memory tests – verbal and spatial N-back task (1-back & 2-back variations). Asparagine levels in hippocampi of both hemispheres were measured with proton magnetic resonance spectroscopy performed with 3T MRI scanner. As a control measurement we used asparagine level in inferior parietal cortex. The received data was analyzed by calculating non-parametric correlations (Spearman, $p < 0.05$) between individual behavioral and biochemical measurements.
Results and conclusions. According to the obtained data, significant correlations between asparagine level in right hippocampus and verbal N-back task were revealed. Moreover, these correlations were revealed in both 1-back variation of the task (0.91) and 2-back variation (0.79). Asparagine level in left hippocampus correlates negatively (-0.68) with spatial 1-back task performance. No relevant correlations between asparagine level in inferior parietal cortex and memory tests performance were revealed. Thus, relatively high asparagine level in right hippocampus associates with verbal memory tests performance, whereas decreased asparagine level in left hippocampus correlates with spatial memory tests performance.

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Cellular Complexity Underlying Alzheimer's Disease

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Synaptic dysfunction leads to neurodegeneration observed in age-related disorders such as Alzheimer's disease (AD). AD presents itself with two distinguishing features in terms of its pathogenesis, one, the formation of neurofibrillary tangles, and the other, amyloid plaques. These plaques contain the β-amyloid peptide (Aβ), which either in the plaque-associated form or in its soluble oligomeric form is thought to set in a cascade of events that eventually leads to neurodegeneration. The neurotoxic Aβ peptide is derived from a large type I transmembrane protein, the amyloid precursor protein (APP). APP is cleaved sequentially by two enzymes termed β- and γ-secretases leading to the formation of the Aβ peptide. The key players in the processing of APP, i.e. β-, γ-secretase and the substrate APP itself, are all membrane associated and hence are subjected to regulation by the lipid environment and membrane trafficking. Our work showed that these amyloidogenic cleavages occur in early endosomes followed by the routing of the cleaved product to late endosomes. Subsequently, Aβ peptides can be released from the cells via the novel exosomal pathway. Moreover, targeting a transient state analog, β-secretase inhibitor to endosomes inhibited the secretase more efficiently than its soluble counterpart suggesting a novel therapeutic strategy.
Our findings suggested that membrane trafficking regulates APP metabolism and that cell biological principles can be exploited to devise novel therapeutic strategies. In order to understand the role of other proteins that regulate this process, we performed functional genomic screen and have identified several signaling proteins and pathways involved in the processing of APP. The ultimate goal is to understand how such networks contribute to the onset of Alzheimer's disease and to develop strategies for early diagnosis and therapy.

Needing Neddylation: A role for Nedd8 in neuronal development

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The developmental processes orchestrating neuronal development and synaptogenesis not only explain the complexity of the wiring in the mature brain but also help understanding the biological substrate of neurodevelopmental disorders such as autism or mental retardation. Most of the synaptic contacts are made between presynaptic axonal boutons and dendritic spines, postsynaptic microcompartments where membrane trafficking, calcium metabolism and protein synthesis and degradation are tightly controlled. Only in the post-synapse, more than 400 different proteins act in concert to mediate and control neural transmission. Therefore, a precise temporal and spatial control of protein synthesis and stability is required within spines.

Ubiquitylation and other Ubiquitin-like proteins (UBLs) pathways like sumoylation, change the function, localization, partner interaction or stability of target proteins by triggering a covalent binding of the ubiquitin-like tags to the targets proteins. Both Ubiquitin and Sumo are post-translational modifications involved in a myriad of neuronal functions: neuronal survival, dendritic arborization, axonal growth, spine formation, synaptic pruning and trafficking of Glu receptors, among others. However, the putative role of other ubiquitin-like proteins on the CNS remains unknown. This is particularly surprising for the case of Nedd8 (“neural precursor cell expressed, developmentally down-regulated gene 8”) an UBL sharing 56% with Ubiquitin that we have recently found to be highly expressed in the brain. Similarly to other UBLs, the neddylation pathway proceeds in the three classical biochemical steps of activation (E1), conjugation (E2) and ligation (E3). The different proteins involved in the Nedd8 conjugation pathway are highly expressed in neurons and more importantly the activity of the pathway change during the developmental time windows where dendritogenesis and synaptogenesis take place. Thus we have intensely explored the role that neddylation exert on different aspects of dendritic development, spine formation and synaptic stability. These results as well as some of the mechanisms behind including the critical functional actions that neddylation exert on PSD95, the main scaffold protein of the postsynaptic density, will be discussed.

Neurogenic impairment and recovery in Alzheimer's disease: a concomitant process with glial alterations.

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Neurodegenerative diseases including Alzheimer's disease (AD) have been mainly associated with neuronal dysfunction and alterations ignoring, somehow, the involvement of neuroglia in their apparition, evolution and treatment. Neuroglial cells are fundamental for brain homeostasis and they represent the intrinsic brain defence system. In fact, the human brain is formed by neuronal networks embedded into astroglial syncytia. The astrocytes perform numerous functions providing for overall brain homeostasis, assisting in neurogenesis, determining the micro-architecture of the grey matter and defending the brain through evolutionary conserved astrogliosis programmes. Astroglial cells are engaged in neurological diseases determining the progression and outcome of neuropathological processes including AD. The recently acquired knowledge also allows us to regard neurodegenerative diseases as gliodegenerative processes, in which glial cells determine the progression and outcome of neuropathological processes such as AD. We have recently probed this active pathological role, by showing: (i) an astroglial generalised atrophy with a concomitant astrogliosis just restricted to Ab plaques presence ii) alterations in glutamate glial metabolism and (iii) an early resting microglial recruitment in the affected areas, even before the presence of activated/macrophagic microglial cells. These neuroglial alterations appear in parallel with a marked reduction of cell proliferation and neurogenesis in both hippocampus and subventricular zone, appearing even earlier that the AD associated pathological hallmarks, plaques and tangles. Thus, the concomitant glial and neurogenic alterations are fundamental for the disruption of neural networks connectivity together with the neurotransmitters imbalance, underliying the mnesic deficits associated with AD; and new therapeutic approaches targeting simultaneously these changes might be of major relevance in the treatment of the disease.

The ALS disease protein TDP-43 is actively transported in motor neuron axons and regulates axon outgrowth

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease specifically affecting cortical and spinal motor neurons. Cytoplasmic inclusions containing hyperphosphorylated and ubiquitinated TDP-43 are a disease hallmark, and mutations in the gene encoding TDP-43 have been directly linked to the development of ALS. TDP-43 is a ubiquitous DNA/RNA-binding protein whose nuclear role in pre-mRNA splicing has been characterized. However, the selective vulnerability and axonal degeneration of motor neurons in ALS poses the question of whether TDP-43 may have an additional role in the regulation of the cytoplasmic and axonal fate of mRNAs, processes important for neuron function. To investigate this possibility, we have characterized TDP-43 localization and dynamics in primary cultured motor neurons. By using a combination of cell imaging and biochemical techniques, we demonstrate that TDP-43 is localized and actively transported in live motor neuron axons, and that it colocalizes with well-established axonal mRNA-binding proteins. Expression of the TDP-43 C-terminal fragment led to the formation of hyperphosphorylated and ubiquitinated inclusions in motor neuron cell bodies and neurites, and these inclusions specifically sequestered the RNA-binding protein HuD. Additionally, we showed that overexpression of full length or mutant TDP-43 in motor neurons caused a severe impairment in axon outgrowth, which was dependent on the C-terminal protein-interacting domain of TDP-43. Taken together, our results suggest a role of TDP-43 in the regulation of axonal growth, and suggest that impairment in the post-transcriptional regulation of mRNAs in the cytoplasm of motor neurons may be a major factor in the development of ALS.

Presynaptic cannabinoid-sensitive receptor GPR55 regulates neurotransmitter release in the brain

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The G protein-coupled receptor 55 (GPR55) is present in the brain, and in cell culture expression systems it induces elevations of intracellular Ca^{2+}. However, its adaptive neurobiological significance is not known. To probe the role of these receptors in the brain, we combine patch-clamp electrophysiology, live immuno-fluorescence labelling, and two-photon excitation imaging of presynaptic and postsynaptic Ca^{2+} at individual CA3-CA1 synapses in acute hippocampal slices. We find that GPR55 activation transiently increases release probability at these synapses while triggering Ca^{2+} store-dependent presynaptic Ca^{2+} elevations at axonal boutons projected in area CA1 by CA3 pyramidal cells. These effects are abolished by genetic deletion of GPR55 or by the GPR55 antagonist cannabidiol, a constituent of *Cannabis sativa.* Cannabidiol also inhibits short-term synaptic potentiation and the associated slow presynaptic Ca^{2+} rises at CA3-CA1 connections in wild type but not GPR55 knockout animals. Pharmacological dissection of metabolic cascades suggests that the underlying mechanism involves synthesis of phospholipids in the presynaptic cell, but not endocannabinoids 2-AG or anandamide. Our results unveil a role for GPR55 signalling in the brain, also suggesting a target for the documented link between the cannabidiol content of cannabis and effects on cognitive function.

Knockout rat models for the study of Neurodegenerative Diseases

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Compared to the mouse, the rat is an optimal species for studying human neurological disorders. Several characteristics contribute to their advantage including: their less anxious behavior, similar neuronal circuitry to humans, flexible learning ability and a larger size that makes them amenable for surgical manipulations. Zinc finger nucleases (ZFNs) have successfully been used to create genetic modifications in rats. Here we report creation and characterization of several knockout models of neurodegenerative diseases. Brain-derived neurotrophic factor (Bdnf) has been implicated to play critical roles in the integrity of the nervous system. Polymorphisms in the Bdnf gene have been associated with increased susceptibility to Alzheimer's disease, Parkinson's disease, bipolar disorder, depression, epilepsy and eating disorders. Bdnf -/- rats demonstrate a dramatic locomotor impairment and significant deficiencies in size and are not viable past 2 weeks of age. Bdnf +/- rats were analyzed by locomotor activity assay and contextual fear conditioning assay and demonstrated deficiencies in locomotion and freezing response relative to their WT littermates.
Parkinson's disease (PD) is the second most common neurodegenerative disease that affects millions of individuals worldwide. The majority of PD cases are sporadic, however there are less common familial forms that are linked to specific genes. We have created the first genetic knockout rat models for the study of PD. Specifically, Lrrk2, Lrrk1, Parkin (Park2), Pink1 and DJ-1 (Park7) genes were disrupted. Phenotypic testing is underway. Available data will be discussed.

Local Translation in Neurons

Erin Schumann

An individual neuron in the brain possesses approximately 10,000 synapses, many of which are hundreds of microns away from the cell body, which can process independent streams of information. During synaptic transmission and plasticity, remodeling of the local proteome occurs via the regulated synthesis and degradation of new proteins. I will discuss previous and current studies aimed at understanding how local protein synthesis contributes to synaptic function and plasticity. Using deep-sequencing techniques and bioinformatic approaches, we now describe the local transcriptome- a large population of previously undetected mRNAs that code for synaptically relevant proteins.

Structural Plasticity and Hardware Repair after CNS Injury

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Large spinal cord or brain injuries lead to life-long major functional impairments. In contrast, small lesions of the CNS often have a good prognosis with extensive functional recovery; the underlying mechanisms are not well understood, however. Major changes in the neuronal wiring including formation of new circuits and maps were found after spinal cord lesions in adult rats. A spinal cord injury transecting the hindlimb corticospinal tract (CST) induced spontaneous sprouting of the lesioned fibers in the upper spinal cord leading to new connections of former hindlimb CST fibers to the forelimb. Forelimb sensory connections also expanded into the former hindlimb motor cortex. A similar re-wiring and map shift was observed after focal cortical strokes: destruction of the forelimb cortex led to sprouting of hindlimb fibers into the cervical spinal cord. These anatomical changes may represent part of the basis for the functional recovery e.g. of forelimb movements observed behaviourally. However, in all these cases extent and length of fiber growth was limited to about 0.2 - 2 mm.

20 years ago, the presence of specific neurite growth inhibitory factors in myelin of the CNS was discovered. The membrane protein Nogo-A is currently the most potent known neurite growth inhibitor. Function blocking antibodies against Nogo-A have been generated and applied to rats and macaque monkeys with spinal cord injuries as well as animals with strokes in the sensory-motor cortex. Biochemical readouts showed an up-regulation of growth specific proteins. On the anatomical level, injured fibers showed enhanced regenerative sprouting as well as long-distance regeneration with formation of large terminal arbors. Simultaneously, spared fiber tracts showed enhanced compensatory sprouting, often covering relatively long distances. In animals with cortical strokes, fibers from the intact corticobulbar or corticospinal systems crossed the midline, supplying innervation to the denervated brain stem or spinal cord under the influence of anti- Nogo-A antibodies. Behavioral tests for locomotion, grid and beam walk, swimming, as well as skilled forelimb reaching showed marked improvements of functional recovery in the Nogo-A antibody treated injured animals. In collaboration with Novartis, a human anti-human Nogo-A antibody was generated. The antibody enhances regeneration of corticospinal fibers and recovery of precision movements of the paralysed hand after high cervical hemisections in adult monkeys. A clinical trial in acutely spinal cord injured patients is currently ongoing.

Inhibitory control over spine dynamics by drug-unpaired environments

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Brief elevations in c-Fos expression in Nucleus Accumbens neurons following re-exposure to an amphetamine-paired but not drug-unpaired environment suggest that expression of this immediate early gene serves as a marker for neurons which process drug-context information. In contrast, ΔFosB displays a long-lasting context-independent increase following repeated drug exposure, a neuronal expression pattern that overlaps with c-Fos activation following exposure to drug-paired stimuli. Therefore, when conditioning accrues, a proportion of ΔFosB(+) cells may process this information. Previous research has revealed brain-region specific changes in dendritic architecture following repeated systemic amphetamine administration. Given the well documented relationship between conditioning and dendritic spine growth, we hypothesized that dendritic changes may be restricted to c-Fos(+) and ΔFosB(+) neurons which may encode this behavioral information. Labeled cells were injected with the carbocyanine neuronal tracer DiI in order to characterize their dendritic spine morphology. One week after withdrawal from repeated amphetamine, NAcc FosB(+) cells showed an increase in the basal frequency (no challenge) of spines with medium head diameters. This increase was absent in activated c-Fos(+) neurons 30-minutes after re-exposure to a drug-unpaired environment, but preserved after exposure to a drug-paired environment. Consistent with these results, significant decreases in levels of the actin binding proteins Arp2 and p-cofilin were observed following exposure to the drug-unpaired environment. These results support the existence of long-lasting conditioning-related changes in dendritic spine properties following repeated amphetamine and the inhibitory regulation of these changes by drug-unpaired environments.

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Inhibition of the Casein-kinase-1-epsilon/delta prevents relapse-like alcohol drinking

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During the last decade it has been shown that circadian clock genes have more than a simple circadian time-keeping role being implicated in the development of psychiatric disorders, such as drug addiction. Human genetic association studies indicate that one of the components of the circadian molecular clock-work - the casein-kinase 1ε/δ (CK1ε/δ) - might be involved in the etiology of addictive behavior. The present study was initiated to study the specific role of CK1ε/δ in alcohol relapse-like drinking using the "Alcohol Deprivation Effect" model. The effect of CK1ε/δ inhibition was therefore tested on alcohol consumption in long-term alcohol drinking rats upon re-exposure to alcohol after deprivation using a four-bottle free choice paradigm with water, 5%, 10%, and 20% ethanol solutions. The inhibition of CK1ε/δ with systemic PF-670462 (0, 10, 30 mg/kg) injections dose-dependently decreased, and at a higher dosage even prevented the alcohol deprivation effect as compared to vehicle-treated rats. The impact of the alcohol deprivation effect during the treatment was further characterized using non-linear regression analyses on the daily profiles of drinking and locomotor activities. These analyses further revealed a strong inhibition of the daytime alcohol-intake typically observed in control rats upon alcohol re-exposure. Our data suggest that inhibition of CK1ε/δ might be considered as a new treatment possibility for alcohol-dependent patients.

Correlation between diffusion tensor imaging and relaxometry in Huntington's disease: A globus pallidus study

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Huntington's disease (HD) is an inherited autosomal dominant neurodegenerative disorder with progressive impairment of motor and cognitive functions and behavioral abnormalities. The clinical features of HD are closely related to the degeneration of the basal ganglia, predominantly the striatum. The main striatal output structure, the globus pallidus, strongly accumulates ferritin-bound iron, which is believed to change the diffusion tensor in a systematic way. To test the hypothesis that this same effect exists in the iron-rich basal ganglia of HD patients, we examined changes in the globus pallidus using a DTI WIP sequence and a T2 relaxometry CPMG sequence. Quantitative MR, clinical data and genetic data (the number of CAG repeats) were obtained from 14 HD patients. Coregistered MR parameters such as the T2 relaxation rate (RR), fractional anisotrophy (FA) and mean diffusivity (MD) were analysed by 2 independent readers in FSL and further processed in GraphPad. Bonferroni's correction was used for multiple statistical comparisons. Significant correlations between RR and FA (R2=0.84), between RR and MD (R2=0.66), between FA and MD (R2=0.85) and between CAG and RR (R2=0.59) were found. A trend towards a significant correlation between CAG and FA (R2=0.44) was noted. No significant correlation between CAG and MD or between MR and clinical parameters was found. We also found that there were no significant hemispheric differences in the MR parameters and no significant difference between HD patients and age-matched healthy controls. These results indicate that FA in the globus pallidus may have the potential to be used as a biomarker of iron accumulation in future studies of HD patients.

Keywords: Huntington's disease, Iron, Ferritin, MRI, DWI, DTI, T2 relaxation time, Fractional anisotropy, Mean diffusivity, BG, Globus pallidus

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Treating spinal cord injury using an immortalized human neural stem cell line (SPC-01)

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In recent years, both embryonic and adult stem cells have been the subject of widespread investigation to evaluate their therapeutic potential in brain and spinal cord injury (SCI). Great efforts have been made to ameliorate the deleterious effects of SCI and to improve locomotor function during recovery by the transplantation of various types of adult as well as embryonic or fetal stem cells. We used an immortalized stem cell line (SPC-01) derived from human fetal spinal cord tissue for the treatment of a balloon-induced spinal cord compression lesion in rats. Prior to in vivo experiments, we tested the ability of the cells to differentiate in vitro, specifically, their ability to differentiate into motoneurons. To check the functional characteristics of these SPC-01 cells, we measured the intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) in individual cells after 14 days of differentiation in culture. We found that 50% of the tested cells (n=24) responded to depolarization induced by high K^+ (50 mM KCl) by a transient increase of ($[Ca^{2+}]_i$) and that these responses were decreased by 50% by the Ca^{2+} channel blocker nicardipine (n=5), suggesting the presence of high voltage activated Ca^{2+} channels in this cell population. In addition, about 14% of these cells exhibited spontaneous $[Ca^{2+}]_i$ oscillations, typically observed in neuronal cells. The $[Ca^{2+}]_i$ signals in these cells suggest that SPC-01 cells differentiated into functional motoneurons in vitro. As a model of SCI, a balloon-induced compression lesion at the Th8-9 level was used in adult male Wistar rats. Suspensions of SPC-01 cells ($5x10^5$ cells in 5ml of culture medium) were implanted into the lesion one week after SCI (n=22), while the control group (n=16) was injected with saline. SPC-01 cells were labeled with poly-L-lysine-coated superparamagnetic nanoparticles prior to transplantation in order to track their subsequent migration and fate in vivo using MRI. Locomotor (BBB) and sensitivity (plantar) tests were performed weekly for two months. Animals transplanted with SPC-01 cells displayed significantly better motor and sensory improvement compared to the controls. Two months post-implantation (PI), the SPC-01 cells robustly survived in the lesion as confirmed by MRI, Prussian Blue staining and staining for the human mitochondrial marker MTC02. Many of the SPC-01 cells expressed the astrocytic marker GFAP. At two months PI we found 25% of the implanted cells to be positive for Nkx 6.1, while at four months PI the cells were positive for ChAT and Islet2, specific motoneuron markers . Morphometric evaluation revealed that the spinal cord white matter was spared to a significantly greater extent in the transplanted rats, while positive staining for GAP43 suggested that the SPC-01 cells supported endogenous neurite sprouting and regeneration. Our results demonstrate that the transplantation of SPC-01 cells into the acutely injured rat spinal cord improves functional outcome by partially bridging the spinal cord cavity and by providing trophic support to the spared axons. In chronic lesions, HPMA-RGD hydrogel bridges seeded in vitro with cells were implanted into SCI. The hydrogels filled the post-traumatic cavity, and the SPC-01 cells in the hydrogel differentiated into astrocytes and neurons. In addition, host neurofilaments, blood vessels and Schwann cells infiltrated the implant. The combination of SPC-01 cells with biomaterials thus appears to be a suitable approach to the treatment of chronic spinal cord injury.

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Repetitive insults facilitate disease progression and disease diversity

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It is now well accepted that many neurological diseases and disorders originate around the time of birth but are characterized by delayed clinical onset. Further, while the initiating perinatal event(s) may be similar (eg. hypoxia) the resulting clinical condition may be quite different. These observations imply that (1) perinatal trauma confers a functional change in brain development that results in enhanced susceptibility to neurological disease, and (2) that environmental interactions throughout life contribute to disease diversity and clinical signs. In an attempt to understand both the initiation and progression of neurological dysfunction we have developed models in which a common neurodevelopmental insult (administration of low-dose domoic acid [a selective AMPA/kainate ligand] in the rat) produces neurochemical and neuropathological changes in brain development that progress in a reproducible temporal sequence throughout life. Using both in vitro (organotypic hippocampal slice cultures) and in vivo (changes in behaviour, pathology and response to pharmacological challenge) approaches we have described both the mechanisms involved in early neurochemical changes in the brain and the altered phenotypic responses to different environmental challenges later in life. These combinations result in alterations in stress-response, seizure threshold, attentional processing and cognitive function consistent with established adult animal models of both temporal lobe epilepsy and schizophrenia.

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The synapse as a statistical nanomachine

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A complex molecular assembly accounts for receptor accumulation at synapses and "synaptic plasticity" derives partly from modifications of postsynaptic receptor number resulting from receptor trafficking. Single-molecule approaches give access to the full distribution of molecule behaviors and overcome the averaging intrinsic to bulk measurement methods. They allow access to complex processes where a given molecule can have heterogeneous properties over time. Recent developments in single-molecule imaging technologies have been followed by their wide application in cellular biology and are leading to the unraveling of new mechanisms related to molecular movements. They are shaping new concepts in the dynamic equilibria of complex biological macromolecular assemblies such as synapses related to lipid and receptor lateral diffusion. Altogether, these experimental and conceptual approaches, provides a new framework for the understanding of the dynamic regulation of synaptic molecular composition during plasticity i.e. learning and memorizing. I will present the case of synaptic inhibition.

EAG channels enable cost efficient neural coding in cockroach photoreceptors

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Although the ether-á-go-go (EAG) K^+ channels are ubiquitously expressed in neurons their physiological role is largely unknown. Here we show that the EAG constitutes the delayed rectifier type K^+ conductance in cockroach (*P. americana*) photoreceptors. Pharmacological evidence for the identity of the conductance is supported by characterization of the biophysical properties that are known to be unique to the EAG: (1) strong Cole-Moore shift and (2) deceleration of the activation in the presence of external divalent cations. The EAG channels participate on establishing the resting potential and they contribute to the information processing by setting the membrane gain and speed. Interestingly, two forms of plasticity were also discovered. High functional variability in the light adaptation properties of the cockroach photoreceptors is matched by the correlated variability in the voltage-gated properties of the EAG channels. In addition, the EAG channels were found to be inhibited by the increase in the cytocolic calcium concentration attributable to the light stimulation. This light dependent inhibition provides large K^+ conductance to accurately process information about light intensity transients, while recovering the large membrane gain for the small amplitude light induced currents under prolonged light adaptation. The variable and strong light adaption in the cockroach photoreceptors combined with the matched and adaptively adjusted membrane filtering by the EAG channels provides an intriguing example of neural mechanisms behind energy efficient neural processing.

The neurobiology of social play behaviour in rats

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Inbetween weaning and puberty, the young of all mammalian species, including humans, display a characteristic form of social interaction known as social play behaviour or rough-and-tumble play. This form of social behaviour is highly rewarding and essential for the development of social and cognitive skills. Our research focuses on elucidating the neural underpinnings of social play behaviour in adolescent rats. Pharmacological analysis of the neurotransmitter systems involved has revealed an important role for interacting opioid, cannabinoid and dopaminergic neurotransmission in the modulation of social play behaviour. This is in keeping with the rewarding properties of social play, as these neurotransmitter systems have been widely implicated in the positive subjective properties of food, sex and drugs. Investigation of the brain regions involved has identified the nucleus accumbens and amygdala, respectively, as important sites of action for opioids and endocannabinoids on social play. Furthermore, analysis of immediate early gene expression patterns after social play has shown activation of medial and orbital prefrontal cortical regions, the dorsal and ventral striatum and amygdala, as well as reduced activity in habenula. Pharmacological inactivation studies have so far confirmed important roles for the nucleus accumbens and medial prefrontal cortex in the regulation of social play behaviour. Together, our studies show that interacting opioid, cannabinoid and dopaminergic systems within the corticolimbic circuits underlying incentive motivation and reward modulate the expression of social play behaviour.

Changes in CNS diffusion parameters during aging and Alzheimer´s disease

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Extrasynaptic transmission, mediated by the diffusion of neuroactive substances in the extracellular space (ECS), plays an important role in short- and long-distance communication between nerve cells. The ability of a substance to reach extrasynaptic high-affinity receptors via diffusion depends on the ECS diffusion parameters, i.e. the size of the ECS volume and the presence of diffusion barriers represented by, for example, fine astrocytic processes or extracellular matrix (ECM) molecules. These barriers channel the migration of molecules in the ECS, so that extracellular diffusion in certain brain regions (e.g., the hippocampus or myelinated white matter) is inhomogeneous and may be facilitated in a certain direction, that is, the diffusion is anisotropic. Anisotropy allows for specificity of extrasynaptic transmission and might be important for "cross-talk" between synapses and synaptic plasticity. Changes in the ECS diffusion parameters during aging and in two experimental models of Alzheimer´s disease (APP23 and triple-transgenice mice) were determined by the real-time tetramethylammonium (TMA) method using ion-selective microelectrodes and by diffusion-weighted MRI, which measures the apparent diffusion coefficient of water (ADC_W). Decreases in the ECS volume, ADC_{TMA} and ADC_W and the disappearance of anisotropy found in the hippocampus of aged animals were accompanied by learning deficits, morphological changes in astrocytes and ECM loss. Compromised extracellular diffusion reflected by decreased ADC_{TMA} values was also found in both experimental models of Alzheimer's disease despite a larger ECS volume, probably associated with amyloid plaque deposition. Our data show that structural changes in the nervous tissue developing during aging or Alzheimer´s disease alter the diffusion of neuroactive substances in the ECS, which in turn affects both synaptic as well as extrasynaptic transmission in the hippocampus and thus the formation of memory traces and cognitive functions. Supported by GACR 309/09/1597 and GACR P304/11/0184.

Astroglial ionotropic receptors in neurodegeneration

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Astrocytes possess a diverse assortment of ionotropic transmitter receptors, which enable these glial cells to respond to many of the same signals that act on neurones. Ionotropic receptors mediate neurone-driven signals to astroglial cells in various brain areas including neocortex, hippocampus and cerebellum. Glutamate and ATP are the major neurotransmitters responsible for signalling in neuronal-glial networks. Ionotropic glutamate receptors in astrocytes are represented by AMPA and NMDA types. Glial and neuronal NMDA receptors are functionally and structurally different; the glial receptors are weakly sensitive to the extracellular magnesium block, which may indicate a predominant expression of the NR3 receptor subunit. Astroglial ionotorpic P2X receptors are territorially restricted; P2X-medaited responses were hitherto found only in cortical astrocytes. Cortical astrocytes express $P2X_{1/5}$ purinoceptors that are characterised by very high sensitivity to ATP (EC_{50} ~ 50 nM) and weak desensitization. Stimulation of neuronal afferents in mice neocortex triggers complex glial synaptic currents (GSCs) mediated by NMDA, P2X and AMPA receptors and glutamate transporters. In addition, astrocytes demonstrate spontaneous "miniature" GSCs resulting from quantal release of neurotransmitters. Maturation and ageing of the brain of mice (from 1 to 21 months) affects the density of ionotropic receptors in astrocytes and their role in GSCs generation. The AMPA receptor-mediated component is the largest in young animals and progressively declines with age. The P2X and NMDA components of GSC are smallest in young, maximal in adult (3 and 6 months old) and once more decrease in old mice, probably reflecting the remodelling of neuronal-glial circuitry. Our results demonstrate that fast synaptic transmission between neurones and astrocytes in neocortex that may be involved in information processing in neuronal-glial networks undergoes remodelling during brain maturation and ageing.

Neddylation controls axonal and dendritic development in the mouse brain

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Among the ubiquitin family members, ubiquitin and SUMO were implicated in the regulation of various neurobiological processes such as dendritic growth, synaptic plasticity and neurotransmission among others. Dysregulation of the ubiquitin-proteasome and SUMO-system is associated with several neurological disorders in humans, including Alzheimer and Parkinsons disease. Nevertheless the function of other ubiquitin-like proteins in the brain, like Nedd8, remains elusive. Analyzing the expression pattern of the molecules involves in the neddylation cascade we found that Nedd8 and Ubc12 mRNA are highly and ubiquitously expressed throughout the embryonic and adult mouse brain. Western blot analysis of mouse brain extracts from different developmental stages showed revealed a changing pattern of neddylated target proteins. Besides, GFP-Ubc12 and GFP-Nedd8 fusion constructs expressed in primary neurons show somatodendritic and axonal localization of both proteins which were also present in dendritic spines.

These expression mapping studies strongly encouraged us to continue exploring the putative role of the Nedd8 conjugation pathway in early neuronal development.

To address the role of neddylation during neuronal development we employed dissociated primary neuronal cells and in utero electroporation apporoaches two well characterized methods and models to stud neuronal growth and differentiation. Inhibiting neddylation by expressing a dominant-negative Ubc12-C111S or shRNA constructs against Nedd8 and Ubc12 in hippocampal and cortical neurons resulted in a strongly reduced growth and branching of the axon as well as the dendritic tree.

Using viral approaches and *in utero* electroporation of mouse embryos to study cortical and hippocampal development, we found that neddylation also controls axonal and dendritic growth *in vivo*.

The details of these complex approaches as well as the putative mechanisms behind will be discussed.

The Genetics of Innate Behavior: Courtship and Feeding

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The biological drive to eat and have sex is important for all sexually reproducing species. These drives, however, must be carefully controlled for animals—including humans—to function normally. The sensations of hunger and satiety ensure that animals eat at appropriate times and avoid either undereating or overeating. The same concept of hunger and satiety can apply to sex drive. Work in my laboratory is aimed at understanding the drive mechanisms that control feeding and sex. We use *Aedes aegypti* mosquitoes to study feeding behavior. Because this insect is also a vector of dangerous infectious diseases such as yellow fever and Dengue virus, the work has important public health implications. Female mosquitoes require a blood meal to complete egg development and are attracted to human hosts via multiple sensory cues including emitted body odor, heat, and carbon dioxide in the breath. We are using genetics to understand how sensory cues are integrated to lead to host-seeking behavior. We use the fly *Drosophila melanogaster* to study sex drive and have identified a small number of neurons that both controls male fly sex drive and the precise duration of copulation. Recent advances from my group in both research areas will be discussed.

Anticonvulsants failed to block whereas bumetanide suppressed the epileptiform activity in immature rat temporal cortex

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Objectives: The incidence of seizures is high in childhood and pharmacoresistance is more intense in this age group. In this study we investigated the age specific effects of antiepileptic drugs using hippocampal-entorhinal cortex slices prepared from different age groups of rats. We also evaluated the role of Na^+ -K^+ -2Cl^- (NKCC1) co-transporter in pharmacoresistance.
Methods: Frequently recurring seizure like events (SLEs) presumably representing status epilepticus were induced by 4-aminopyridine in acute rat hippocampal-entorhinal cortex slices obtained from postnatal day 3-19 (P3-P19), and the effects of carbamazepine, phenytoin, valproic acid and phenobarbital were examined. In addition, bumetanide was tested, which blocks the NKCC1 co-transporter, and also acetazolamide, which blocks the carbonic anhydrase and thereby the accumulation of bicarbonate inside neurons.
Results: The efficacy of all antiepileptic drugs in blocking SLEs was dependent on postnatal age, with low efficacy in P3-P5 slices and high efficacy in older age groups. In P3-P5 slices, valproic acid and phenobarbital increased both tonic and clonic seizure-like activities in the CA3, whereas phenytoin and carbamazepine blocked tonic-like but prolonged clonic-like activity. Similar to the effects of other antiepileptic drugs, the seizure-suppressing effects of acetazolamide increased with postnatal age. In contrast to standard antiepileptic drugs, bumetanide showed high efficacy in younger age group.
Conclusion: We conclude that pharmacoresistance may be inherent to very immature tissue and suggest that expression of the NKCC1 co-transporter might contribute to pharmacoresistance.

Physiology and pharmacology of sleep: novel insights into the role of GABAergic and adenosinergic transmission"

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The 24-h sleep-wake cycle is a fundamental phenomenon across the animal kingdom. Poor quality of sleep or its inappropriate timing has many adverse effects on our daily life and is typically associated with disturbances of waking performance (e.g., sleepiness, difficulty concentrating). Sleep-wake disorders are highly prevalent both in adults and children. They are also commonly associated with a number of conditions including cardiovascular diseases, anxiety, depression, psychiatric disorders and neurodegenerative diseases. Several neurotransmitter systems are involved in the regulation of the sleep-wake cycle, among which the GABAergic and adenosinergic systems, which are the respective targets of the most prescribed sedative-hypnotics and the psychostimulant, caffeine.
GABA and $GABA_A$ receptors play a major role in sleep and insomnia. Recent advances in the characterisation of $GABA_A$ receptors heterogeneity and the development of compounds selective for particular $GABA_A$ subtypes allow to more precisely dissecting the mechanisms underlying physiological and pharmacological sleep. We will present recent data that highlight the importance of $GABA_A$ receptors' heterogeneity in relation to the mechanism of action of existing and novel hypnotics, and the contribution of extrasynaptic $GABA_A$ receptors, mediating tonic transmission, to sleep. We will also discuss recent studies further elucidating the role of adenosine in sleep.
Taken together, the information presented here aims to provide new perspectives to the mechanisms underpinning the control of sleep and wakefulness, as well as the pharmacological treatment of sleep-wake disorders.

Neuronal pathways of sleep & anesthesia

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The induction of safe and reversible loss of consciousness (LOC) is a pillar of modern medicine. It also poses, however, one of the most long-standing puzzles in neuroscience. General anaesthesia is a routine procedure, but we do not really understand how it works. Although it is clear that general anaesthetics act at a relatively small number of targets, finding the links between these receptors and anaesthetic-induced loss of consciousness presents an important challenge. In this talk I will elaborate our overall hypothesis that anaesthetics produce their sedative/hypnotic effects by recruiting natural sleep pathways, and discuss how α_2-adrenergic receptor agonists such as the sedative/hypnotic agent dexmedetomidine (DEX) can induce a sleep-like state.

Evaluation of the anxiolytic effects of new phencyclidine derivatives in mouse elevate plus maze model

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The aim of this study was to investigate the anxiolytic effects of Phencyclidine (1-(1-phenylcyclohexyl) piperidine, PCP, I) and some of its derivatives (M, F, L, B, S, P) using elevated plus maze test. Phencyclidine and its derivatives (M, F, L, B, S, P) were administrated intraperitoneally (i.p.) at the dose of 10 mg/kg to male mice. Anxiety-like behaviors were assessed using the elevated plus maze (EPM) test. EPM results revealed an increase in time spent in open arms in mice treated with PCP as well as compounds M, L, P, and B at the administered dose. Moreover, an increase in the number of open arm entry was observed with compounds M, P, and B. However, compounds P, B and S increased the locomotion of animals which would be considered as a side effect of these compounds. Based on the elevated plus maze results, it is concluded that compounds M and L can be considered as potential anxiolytic compounds with less side effects which is likely related to high electron donating of methoxy group and hydrophilic property of hydroxyl groups on these compounds.
Key words: Phencyclidine derivatives, Anxiety-like behavior, Elevated plus maze test, Mice.

Lateralized response in dynorphin A peptide levels after traumatic brain injury

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Traumatic brain injury (TBI) induces a cascade of primary and secondary events resulting in impairment of neuronal networks that eventually determine clinical outcome. The dynorphins, endogenous opioid peptides have been implicated in secondary injury and neurodegeneration in rodent and human brain. To gain insight into the role of dynorphins in brain response to trauma, we analyzed short term (1 day) and long term (7 day) changes in dynorphin A (Dyn A) levels in the frontal cortex, hippocampus and striatum induced by unilateral left-side or right-side cortical TBI in mice. TBI effects were significantly different from those of sham operation (Sham), while the Sham also produced noticeable effects. Both Sham and TBI induced short-lasting changes, and long-term changes in all three regions. Two types of responses were generally observed. In the hippocampus, Dyn A levels were predominantly altered ipsilateral to the injury. In the striatum and frontal cortex, injury to the right (R) hemisphere affected Dyn A levels to a greater extent than that to the left (L) hemisphere. The R-TBI but not L-TBI produced Dyn A changes in the striatum and frontal cortex at 7 days after the injury. Effects of the R-side injury were similar in the two hemispheres. In naive animals, Dyn A was symmetrically distributed between two hemispheres. Thus, trauma may uncover an existing lateralization in the mechanism mediating the response of Dyn A-expressing neuronal networks in the brain. These networks may differentially mediate effects of left and right brain injury on the lateralized brain functions.

Possible differential role of accumbens cholinergic interneurons in drug- vs social interaction reward

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Converging evidence from several independent laboratories[1-6] including our own[4-6] suggests that drug-associated stimuli acquire control over an individual's behaviour through the activation of a very localized group of neurons, i.e., cholinergic interneurons, in a portion of the nucleus accumbens (Acb) that is located medial of the anterior commissure, comprising both core (AcbC) and shell (AcbSh) subregions of the Acb. Of note, lesioning the AcbC or the AcbSh before the concurrent acquisition and expression of conditioned place preference (CPP) for cocaine vs social interaction[7] shifted the balance from a stronger CPP for cocaine (AcbSh lesion) toward social interaction CPP (AcbC lesion), suggesting that drug reward vs social interaction reward is mediated preferentially in one of these Acb subregions. Finally, in vivo microdialysis before, during, and after the CPP sessions[8] revealed a differential neurotransmitter release pattern (transmissome) for social interaction vs cocaine. These findings suggest that accumbens core ACh interneuron activity is differentially affected by contextual stimuli associated with social interaction vs contextual stimuli associated with cocaine. **Financial support:** FWF M1169-B18, FWFP18787-B05 and VEPPP.

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Orexin-$GABA_A$ergic cross-talking events during lead-induced neurotoxicity of fish

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Recent studies have focused their attention on cross-talking mechanisms between orexin (ORXR) and $GABA_A$ ($GABA_AR$) receptors that are known to play a regulatory role on different physiological activities, although no information is available about their role during environmental toxicity especially in fish. In view of this, we exposed the ornate wrasses *Thalassoma pavo* for 96 hours to 1.6 mg/L of $PbNO_3$ in combination with daily intraperitoneal administrations of $GABA_AR$ agonist (muscimol 0.1 µg/g body weight) or its antagonist (bicuculline 1 µg/g body weight) and data were compared to both Pb-exposed fish and controls, i.e. fish that was never exposed to any treatments. From behavioral analyses, it appeared that muscimol completely neutralized Pb-dependent enhancement of time spent swimming, as displayed by its great ($p<0.01$) capacity of reducing this behavior while bicuculline moderately ($p<0.05$) strengthened metal-induced feeding reductions. At molecular level, *in situ* hybridization revealed that Pb+muscimol treatment accounted for moderate reductions of ORXR mRNA levels in some encephalic regions such as the torus longitudinalis (-37%) whereas very great ($p<0.001$) up-regulations were detected in the corpus of the cerebellum (+91%). As expected, Pb+bicuculline induced a general down-regulation in most brain areas. Interestingly, amino cupric silver stain technique showed a protective property of muscimol against Pb-mediated neurodegenerative mechanisms, that were instead strongly enhanced following bicuculline administration. Overall, these data supply important indications about $GABA_AR$-ORXR cross-talking events during metal neurotoxicity of fish.