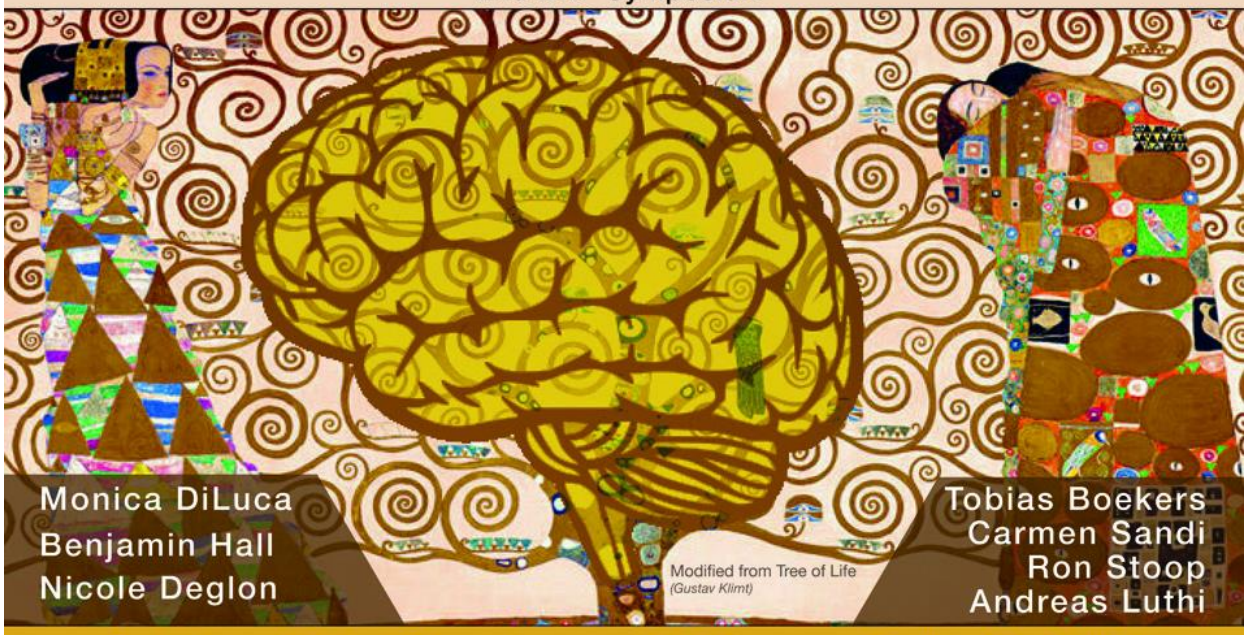
MOLECULAR **BASIS** of MENTAL **DISORDER**

2nd DNF Symposium



Registration and Abstract Submission: www.unil.ch/dnf Deadline: May 1st, 2015

Organizers: Camilla Bellone and Paola Bezzi

Friday 22nd May

Grand auditoire du DNF, Bugnon 9 - Lausanne

PROGRAM AND ABSTRACT BOOKLET

SECOND SYMPOSIUM OF THE DEPARTMENT OF FUNDAMENTAL NEUROSCIENCES, UNIVERSITY OF LAUSANNE

The DNF was founded in August 2012. It is part of the Faculty of Biology and Medicine (FBM: http://www.unil.ch/fbm/fr/home.html) of the University of Lausanne. It grew out of the former Department of Cell Biology and Morphology (DBCM).

At the DNF, there are about 80 scientists in 15 research groups, working mainly on basic and translational neuroscience. Together these include the majority of the neuroscientists in the University of Lausanne.

The DNF is playing a leading role in coordinating two major neuroscience collaborations:

- the **Lausanne Centre of Neuroscience (Pôle Lausannois de Neurosciences),** under establishment in collaboration with its partners in the Centre Hospitalier Universitaire Vaudois (CHUV) and the École Polytechnique Fédérale de Lausanne (EPFL)
- the **Lemanic Training Program in Neuroscience** (http://www.unil.ch/ln/home.html), in collaboration with the EPFL and the University of Geneva. This encompasses a series of thematic teaching programs at the Master and PhD levels.

The DNF encourages translational research by its policy of hosting research groups from clinical departments (Anesthesiology, Neurology, Neurorehabilitation, Neonatology) and by collaborating with clinical groups in the study of neurological and neuropsychiatric diseases, as well as diabetes. In addition, the DNF played a major role in creating the Centre de Neurosciences Psychiatriques at Cery, along with several other departments of the Faculty of Biology and Medicine (FBM).

Cellular imaging

The DNF's priority area of technological development is cellular imaging, including a considerable concentration of “state-of-the-art” techniques for both structural and dynamic imaging. As a consequence, the DNF is a leader in the development of imaging techniques within the FBM. Furthermore, the DNF hosts one of the two antennas of the FBM's Cellular Imaging Facility (CIF: http://cifweb.unil.ch/). This integration guarantees the transfer of research developments in cellular imaging to users of the CIF who come from other departments of the FBM.

PROGRAM

8h45 – 9h15 Opening Remarks (Camilla Bellone, Jean-Pierre Hornung)

9h15 – 9h50 **Monica Di Luca**, Dept. Pharmacological Science, University of Milano, Italy

Synaptic retention of GluN2A containing NMDA receptors: from mechanisms to pathology

9h50 – 10h15 Presentation of DNF (Paola Bezzi)

10h15 – 10h40 Coffee break

10h40 – 11h20 **Benjamin Hall,** Roche Pharma Research and Early Development Neuroscience, Ophthalmology and Rare Diseases, Roche Innovation Center Basel, Switzerland

Synaptic Mechanisms Underlying the Rapid Antidepressant Actions of Ketamine

11h20 – 12h00 **Tobias Boekers**, Institut für Anatomie und Zellbiologie Universität Ulm, Germany

Shanks and autism spectrum disorders (ASDs)

12h00 – 12h40 **Nicole Déglon**, Department of Clinical Neurosciences, Lausanne. Switzerland

Neurodegenerative diseases: genetic engineering to the rescue

12.40 - 15.00 Poster session / Lunch

15h00 – 15h40 **Carmen Sandi**, Brain Mind Institute Ecole Polytechnique Federale de Lausanne, Switzerland

Synaptic cell adhesion molecules link stress with alterations in social behavior and cognition

15h40 – 16h20 **Ron Stoop**, Center of psychiatric Neuroscience, University of Lausanne, Switzerland

Optogenetic dissection of oxytocinergic circuits underlying fear and social buffering

16h20 – 17h00 **Andreas Luthi**, Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

Deconstructing fear

17h00 -17h30 Concluding remarks, Poster prize (Jean-Pierre Hornung, Paola Bezzi, Camilla Bellone)

17h30 Cocktail Apero

SPEAKERS

Prof Monica Di Luca is Full Professor and Vice-Director of the Department Pharmacological Sciences University of Milano. Her primary research interest is related to brain and synaptic plasticity both in physiological and pathological conditions, with the primary aim to apply her basic findings to the cure of neurodegenerative diseases as Alzheimer and Parkinson Disease. In the last 15 years she focused on the understanding of the molecular mechanisms regulating the composition, the structural organization and the dynamic of the glutamatergic synapse. In this frame she was coordinator of one European Commission VI FP project (Synscaff) and of two VII FP projects (REPLACES, cPADS) and partner one VII FP project (SYMBAD).

For several years, Benjamin Hall has been an Adjunct professor at the Tulane University. Using a combination of techniques in mouse genetics, electrophysiology, molecular biology, and cellular imaging, he has studied the cellular and molecular mechanisms that regulate synapse development and function in the neocortex. Dr. Hall is now principal scientist at F. Hoffmann-La Roche Ltd at the Roche Innovation Center in Basel where he focus his research on the Synaptic Mechanisms Underlying the Rapid Antidepressant Actions of Ketamine.

Prof Tobias Bökers is Full Professor and the Director of the Institut für Anatomie und Zellbiologie at the University of Ulm. His primary research focuses on the molecular characterization of the postsynaptic density (PSD). Specifically, the laboratory have cloned and characterized several PSD proteins including ProSAP / Shank that are related to Autism spectrum disorders.

Prof Nicole Déglon is Associate Professor and Director of Center for Research in Neuroscience (CRN) of the Department of Clinical Neurosciences of the CHUV. She is the had of the Cellular and Molecular Neurotherapy Lab (LNCM) which has the aim of (i) the development of relevant and predictive models of CNS diseases, in particular Huntington's disease (rare inherited disorder with middle-age onset characterized by impaired motor control and psychiatric problems) and Alzheimer's disease ; (ii) the analysis of disease mechanisms leading to neuronal degeneration; (iii) the identification and validation of therapeutic targets with a potential transfer to the clinic in collaboration with medical doctors in the Department of Clinical Neurosciences. The development of new treatments requires progress at many levels - from improved understanding of the disease and brain function to clinical testing of treatments.

Prof Carmen Sandi is Full Professor and Director of the Brain Mind Institute, School of Life Sciences, EPFL, Switzerland. She is had of the Laboratory of Behavioral Genetics which has the aim of investigating the impact and mechanisms whereby stress and personality affect brain function and behavior, with a focus on the social domain and, particularly, on aggression and social hierarchies. Specifically, the research in her laboratory is focused on: (i) the neurobiological mechanisms involved in the formation of social hierarchies, and their modulation by stress and anxiety; (ii) the mechanisms whereby early life stress enhances risk to develop psychopathology, with a main focus on the emergence of pathological aggression.

Prof Ron Stoop is Associate Professor at Center for Psychiatric Neuroscience (CNP), Department of Psychiatry, CHUV. His primary research is the basis of fear and anxiety. It is well known that one region in the brain is particularly involved in our anxiety and fear responses: the amygdala, a cluster of nuclei situated in the temporal lobe, anterior to the hippocampus with which is maintains strong anatomical and functional connections. The amygdala receives a large variety of different sensory inputs (auditif, visual, olfactory and gustatory) and projects to a great number of nuclei in the brain stem that control our visceral reactions. To study the regulation of these functions by the amygdala they use the rat as animal model. The approach they have chosen consists of a combination of in vitro and *in vivo* experiments.

Prof Andreas Lüthi is Full Professor at Friedrich Miescher Institute for Biomedical Research (FMI), which is affiliated with the Novartis Institutes for BioMedical Research (NIBR) and affiliated Institute of the University of Basel, Basel. He studies cellular mechanisms of learning and memory. Experience-dependent changes in behavior are mediated by long-term functional modifications in brain circuits. The laboratory is interested in understanding the underlying mechanisms at the molecular, cellular and circuit levels. As a model system, they use classical (Pavlovian) fear conditioning, a simple form of associative learning that is particularly suitable for study in rodents. The inability to control or inhibit inappropriate fear responses is a hallmark of human anxiety disorders. The research in his laboratory is focus on the cellular mechanisms underlying fear extinction, an associative learning process mediating inhibitory control of inappropriate fear behavior. Using a multidisciplinary and integrated experimental approach in mice, they combine *in vitro* and *in vivo* electrophysiology, imaging, molecular biology, genetics, and behavioral techniques to identify the synaptic and cellular constituents of neural circuits in the amygdala underlying the acquisition, encoding and extinction of fear memory - the microcircuitry of fear conditioning.

ORGANIZATION

Main organizers	Camilla Bellone, Paola Bezzi
Local organizing committee	Jean-Pierre Hornung (Director, DNF)
	Eric Bernardi (Logistics and Printing)
	Katerina Catalan-Seidl (Secretary)
	Kim Godat (Secretary)
	Alexandre Sandoval (Website)
Symposium poster design	27 Agency (http://www.27agency.com/)

ABSTRACTS

DNF

Camilla Bellone's group

Poster 1. Toward and Autistic VTA: electrophysiological and behavioral evidences for a Shank3 developmental role.

Sebastiano Bariselli, Stamatina Tzanoulinou, Eoin Cornelius O'Connor, Joanna Viguié, Francoise Georges, Christian Lüscher, Camilla Bellone

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ASDs (Autism Spectrum Disorders) constitute a class of neurodevelopmental diseases characterized by deficits in social interaction, increased stereotypies and cognitive impairments. Dopamine (DA) has been involved in reward-related behaviors as well as in the control of impulsive behaviors and cognition. Although few studies have suggested that alterations in DA signaling may contribute to the emergence of autism, direct evidences of the role of DA neurons in ASDs are still elusive. By Shank3 down-regulation in the Ventral Tegmental Area (VTA), we aimed to validate a region-specific genetic model of ASDs characterizing the postnatal maturation of excitatory transmission onto VTA DA neurons. In our model, we observed altered synaptic transmission, impaired AMPAR maturation and changes in DA neuron activity in vivo. In addition, we detected social behavior deficits, reminiscent of ASD-like symptoms. Importantly, chronic injections of a positive allosteric modulator of mGluR1 in vivo rescue the synaptic abnormalities and ameliorate the behavioral impairment, opening new possibilities for the ASD-treatment.

DNF

Paola Bezzi's group

Poster 2 - Astrocytic mitochondrial impairments in Prodh-deficient mice

Jan Lopatar, Tamara Zehnder, Francesco Petrelli, Joseph A. Gogos, Pierre Magistretti, Sylvain Lengacher and Paola Bezzi

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One of the main candidates risk gene for behavioral phenotype of 22q11 deletion syndrome (DS) is the gene encoding for proline dehydrogenase (PRODH). PRODH is a mitochondrial enzyme involved in the metabolism of L-proline. The expression of PRODH is typically ascribed to proliferating tissues (Polyak et al., Nature, 1997) where it regulates cell proliferation (Donald et al., Cancer Res, 2001) and metabolism (Liu et al., PNAS, 2012). The functional role of PRODH in the brain is not completely understood. We and others have found that PRODH expression in the brain is limited to the developing astrocytes in the frontal cortex (FC). The FC brain tissue during postnatal development showed enhanced glycolytic rates (Schulze and Harris, Nature, 2012), a situation that requires an extensive metabolic reprogramming that could render astrocytes more susceptible to mitochondrial perturbations (Fulda et al., Nature Rev Drug Disc, 2010). In the FC of PRODH-deficient mice (Paterlini et al., Nature Neurosci., 2005), the increase in the glycolytic rates is maintained but both the respiratory capacity and the ATP levels were significantly impaired. In physiological conditions, mitochondria exhibit a high transmembrane potential ($\Delta\Psi m$) which is generated by the respiratory chain and which is exploited for ATP generation. In PRODH deficient mice consistent with results obtained so far, we found a significant decrease of $\Delta\Psi m$ with respect to wild type mice of the same postnatal age. We concluded that PRODH expression during postnatal development is necessary to maintain a proper mitochondrial function.

Poster 3 - VMAT2 in astrocytes regulates morphology of pyramidal neurons in developing PFC by modulating extracellular levels of dopamine

Francesco Petrelli 1, Luca Pucci 1, Corrado Calì 1, Glenn Dallerac 2, Frank Kirchhoff 3, Nicole Déglon 4,5 Bruno Giros 6,7, Robert H. Edwards 8, Jean-Pierre Mothet 2, Paola Bezzi 1

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Neuromodulation of neuronal circuits of the prefrontal cortex (PFC) by monoamines influences synaptic plasticity and executive functions and may play critical roles in psychiatric disorders. The cellular mechanisms governing the homeostasis of monoamines in the developing PFC are not completely defined. Evidence that astrocytes express the whole enzymatic apparatus for the metabolism of monoamines (Youdim et al., 2006) suggests a possible involvement of astroglial cells in mechanisms governing monoaminergic homeostasis. Here we report that astrocytes located in PFC express VMAT2. Immunoperoxidase labeling followed by serial sections analysis and 3D reconstruction show that VMAT2- positive astrocytes in PFC contain dopamine (DA) and are strategically positioned between DAergic varicosities and glutamatergic synapses. The physiological relevance of VMAT2 in astrocytes is investigated by generating conditional transgenic mice in which VMAT2 is specifically deleted in GFAP expressing cells (here to referred to as aVMAT2cKO). Interestingly, VMAT2 deletion in astrocytes leads to a specific decrease in the extracellular levels of DA in the PFC. In a different set of experiments, by taking advantage of in vivo microdialysis and of transgenic mice overexpressing Gq protein-coupled receptor (GPCR) Mas-related gene A1 (MrgA1), we directly assess the competence of astrocytes for releasing DA. Finally, we analyse the effects of the decreased levels of DA in the developing PFC in the aVMAT2cKO on spine formation and dendritic growth. To this purpose aVMAT2cKO mice have been crossbreed with Thy1EGFP fluorescent mice. Taken together, these results highlight a critical role for VMAT2 in astrocytes in the regulation of DA levels and the normal development of pyramidal neurons in the PFC.

Poster 4 - Astrocytes may be behind the pathogenesis of autism.

Luca Pucci1, Francesco Petrelli1, Corrado Calì1, Glenn Dallerac2, Frank Kirchhoff3, Nicole Déglon4,5 Bruno Giros6,7, Robert H. Edwards8, Jean-Pierre Mothet2, Paola Bezzi1

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Monoaminergic systems appear early during brain development suggesting that, in addition to their later function in synaptic transmission, monoamines could have important roles in neuronal proliferation, migration, differentiation and maturation. Dopaminergic dysfunctions during the critical period, in particular, may regulate fundamental aspects of late neuronal differentiation. Although the largest number of brain cells expressing proteins of dopamine metabolism are astrocytes, whether dopaminergic neuromodulation is controlled solely by neurons, or also by other brain cells such as astrocytes, has never been investigated. Using conditional gene inactivation and viral-mediated gene replacement in vivo we found that a subset of cortical astrocytes expressing vesicular monoamine transporter 2 (VMAT2) governs extracellular dopaminergic tone allowing for adequate synaptic transmission and plasticity as well as efficient maturation of cognitive performance. The robust cognitive and behavioural deficits in transgenic animals where VMAT2 is conditionally deleted in astrocytes demonstrate, moreover, a causal role for the deletion of this gene in the genesis of cognitive and social dysfunctions typically associated to autistic-like behaviours. Our study shows that astrocytes, like neurons, are integral component of the dopaminergic neuromodulation in the developing brain. Support contributed: NCCR Synapsy and NCCR TransCure to P. Bezzi

Poster 5 - Homer1 scaffold proteins govern Ca^{2+} dynamics in normal and reactive astrocytes
Lara Buscemi, Vanessa Ginet, Jan Lopatar, Vedrana Montana, Luca Pucci, Paola Spagnuolo, Vladimir Grubišić, Anita Truttman, Carlo Sala, Lorenz Hirt, Vladimir Parpura, Julien Puyal and Paola Bezzi
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In astrocytes, the intracellular calcium (Ca2+) signalling mediated by activation of metabotropic glutamate receptor 5 (mGluR5) is crucially involved in the modulation of many aspects of brain physiology including gliotransmission. Here we show that the interaction of mGluR5 with Homer1 scaffolding proteins governs mGluR5-mediated Ca2+ signalling leading to release of glutamate from developing astrocytes. We show that in the critical early postnatal weeks astrocytes express the long splice variants Homer1b/c in their processes, where they cluster with mGluR5 at sites displaying intense local Ca2+ activity. We show that the structural and functional significance of the Homer1b/c-mGluR5 interaction is to relocate endoplasmic reticulum (ER) to the proximity of the plasma membrane and to optimize both global and local Ca2+ signalling. Conversely, the overexpression of the short splice variant Homer1a precludes the mGluR5-ER interaction and disrupts glutamatergic Ca2+ signalling and exocytosis of glutamate. In an in vivo model of neonatal hypoxia-ischemia, Homer1a is upregulated in reactive astrocytes of the lesion border that have impaired global and local glutamatergic Ca2+ signalling. The upregulation of Homer1a in reactive astrocytes might thus have a protective role by limiting the intensity and duration of excessive mGluR5 activation in pathological neuroinflammatory conditions. In summary, the interaction of mGluR5 with Homer1b/c appears as the molecular mechanism linking ER, strategically located in the astrocytic processes, to the plasma membrane, with pathophysiological significance on both local Ca2+ homeostasis and overall neuroglial signalling.

Poster 6- Contribution of HCAR1 pathway to neuronal network activity in physiological and epileptic conditions

A.-B. Rocher1, C. Schmuziger 1, S. Offermanns 2, J. Puyal 1, J.-Y. Chatton 1

1. Department of Fundamental Neurosciences, University of Lausanne, Switzerland 2. Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany

We have recently found evidence for a potential role of hydroxycarboxylic acid receptor 1 (HCAR1) in the brain (Bozzo et al., 2013). Activation of HCAR1 by lactate or selective agonists leads to a concentration-dependent decrease in the activity of mouse cortical neurons in primary culture. These findings open up perspectives for considering HCAR1 pathway as a novel efficient negative feedback to modulate neuronal network activity in vivo, with potential beneficial effects in epileptic conditions. Our project aims at characterizing the expression of HCAR1 in different brain regions, species and conditions (epileptic vs. control). In parallel, the effects of HCAR1 activation on neuronal network activity in acute brain slices and on mouse behavior are being investigated in wild-type and HCAR1 knock-out (KO) mice in both control and epileptic conditions. We first demonstrated that none of the tested anti-HCAR1 antibodies previously used in the literature is specific. We confirm however using qRT-PCR that HCAR1 mRNA transcripts are present in mouse primary neuronal cultures and mouse brain. We also detected for the first time mRNA transcripts in human cortex. Preliminary data suggest that HCAR1 agonist application decreases UP-state frequency of slow wave oscillations in acute mouse entorhinal slices. We also observed that HCAR1-KO mice have a higher rate of status epilepticus episodes 2-h post-pilocarpine injection. These studies may reveal novel functional effects of lactate and related monocarboxylates in the brain and provide a mechanistic basis of the beneficial effects of ketogenic diets—in which HCAR agonists are found elevated—for the treatment of intractable epilepsy. Supported by Swiss League Against Epilepsy, Novartis Foundation, SNF# 31003A_159513.

Poster 7 - Insights into the evolutionary history of NSF

Nickias Kienle Tobias H. Klöpper Dirk Fasshauer

Department of Fundamental Neurosciences Faculty of Biology and Medicine University of Lausanne (UNIL) Rue du Bugnon 9 CH-1005 Lausanne

The ATPase NSF (N-ethylmaleimide-sensitive factor) is indispensable for vesicular trafficking between the compartments of the eukaryotic cell. Fusion of a transport vesicle with its target membrane is fueled by SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins that spontaneously zipper into tight four-helix bundle complexes between membranes. This process is best characterized for Ca2+-dependent release of neurotransmitters at synapses. After fusion, SNARE complexes are disassembled by NSF and its co-factor SNAP (soluble N-ethylmaleimide-sensitive factor attachment protein). ATP-driven disassembly by NSF recharges SNARE proteins for consecutive rounds of fusion. While specific sets of SNARE proteins catalyze vesicle fusion in different trafficking steps in the cell, usually only one copy of NSF is required for their disassembly. So far, the evolutionary origins of NSF are ill-defined. In this study, we have shed more light on this by using hidden Markov models and phylogenetic reconstruction. We included the closest NSF homologs into our analysis, the Cdc48 family (i.e. Cdc48, VCPl, PEX1, PEX6, SPAF, SPAFl, and YTA7). All of these factors play important roles in key biological processes of the eukaryotic cell. Generally, we found the eight factors to be present in all major eukaryotic lineages, suggesting that they represent the repertoire of the last eukaryotic common ancestor (LECA). Since prokaryotes possess only one family member, Cdc48, if any, it is likely that the different Cdc48 family members, including NSF, functionally diverged during the rise of eukaryotes.

Poster 8 - Understanding Molecular Evolution of Vesicular Trafficking Proteins by using Multiple Sequence Information

Nicee Srivastava, Nickias Kienle, Tobias Kloepper, Dirk Fasshauer.

DNF-UNIL, Rue du Bugnon 9, 1005 Lausanne.

Vesicular trafficking is an essential molecular machine controlling the transport system of the cell. Currently, it is becoming clear that the important proteins participating in vesicular trafficking are highly conserved, not only between different species but also between different vesicle trafficking steps. Previous phylogenetic analyses showed that the interacting proteins of the vesicle fusion apparatus arose by duplication and diversification of prototypic protein machinery. It is conceivable that these proteins might show common patterns of episodes of duplication and diversification. A better insight about the evolution, function and interaction surfaces of the key factors involved in vesicle fusion can be obtained by exploring their sequence information. To perform a comprehensive sequence analysis, a tool with different analysis options and visualizations was developed. As a first step towards exploring sequence information, the tool was used to identify intra-protein coevolving residues by combining coevolutionary analysis methods with network theory methods. This approach was applied on the SM protein family, which has been studied for two decades, but their molecular role is still debated. The coevolving residues obtained were found to be lying in distant regions in the tertiary structure of the protein. The result appears to hint at conformational changes and allosteric coupling as discussed in the recent literature. In order to understand the functional relevance of the coevolving sites obtained, we are further analyzing the results. This involves visualizing all coevolving sites detected, validating them by running the methods on simulated alignments and comparing the results obtained on the other proteins of vesicle fusion apparatus.

Poster 9 - Role of Munc18c in vesicle exocytosis

Czuee Morey, Dirk Fasshauer

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The Sec1/Munc18 (SM) protein family plays an essential role in vesicle fusion processes of eukaryotic cells. The SM proteins are suggested to function in the regulation of SNARE-mediated vesicle fusion, primarily by binding to the SNARE protein syntaxin (Syx). Munc18a, the SM protein involved in synaptic exocytosis, binds to the closed conformation as well as the N-peptide of Syx1a, while some other SM proteins are supposed to bind only the N-peptide. Munc18a inhibits SNARE complex assembly in vitro, however, its precise role is not clear since it is shown to be important for vesicle fusion in vivo. In order to better understand SM proteins we are studying a close homolog of Munc18a called Munc18c. It is a ubiquitously expressed vertebrate protein that has a preference for Syx4. It is mainly studied in regulated exocytosis of GLUT4 vesicles in response to insulin. The crystal structure of Munc18c is solved in association with the N-peptide of Syx4, but its interaction with rest of the Syx4 protein is not clear. Also, the influence of Munc18c on SNARE complex formation is debated. We now investigate the Munc18c-Syx4 binding mode and its influence on SNARE assembly kinetics using biochemical and biophysical methods. Our analyses indicate that Munc18c also interacts with the “closed conformation” of Syx4. Moreover, the presence of Munc18c slows down the SNARE assembly reaction. Vertebrates have three Munc18 isoforms involved in exocytosis in different tissues that interact with four secretory syntaxin isoforms, but their interaction patterns are not mapped clearly. Since SM proteins are suggested to give specificity to the SNARE interaction, the preference of the secretory SM proteins for the syntaxin isoforms was also studied.

Poster 10 - Looking for the first nervous system : Neurosecretory Cells and Body Plan of the Early-Diverging Metazoan, Trichoplax adhaerens

Frédérique Varoqueaux

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Chemical synapses evolved early in animal evolution, and relatively simple nervous systems can be found in early branching animals, such as jellyfish. By contrast, sponges or placozoans appear not to be equipped with bona fide synapses. In a previous study, we had discovered that choanoflagellates, a group of mostly single-celled eukaryotes that is thought to be the closest known sister group to animals, already possess a primordial secretion machinery that may have served as a starting point for the evolution of the more complex machinery found in animals, in particular in vertebrates. We now took a closer look at the very early stage of the development of the neuronal secretory apparatus by studying the placozoan Trichoplax adhaerens, an organism positioned near the root of the animal tree. Sequencing of its genome has revealed that Trichoplax has a remarkable set of genes that control cell differentiation and cell-cell communication in more complex animals, including vertebrates. While much is known about Trichoplax genome, much less was known about its structure. Using confocal and electron microscopy, we show that Trichoplax has a well-organized body plan with specialized cells deployed at specific locations. Interestingly, we found that neuronal proteins are expressed in gland cells, suggesting that these cells have a neurosecretory function that might control locomotor and feeding behaviors of Trichoplax. As gland cells do not appear to form synapses, they seem to constitute an ancestral rather than a derived and simplified version of a neuronal cell. FV is financed by a Marie-Heim-Vögtlin grant from the Swiss National Science Foundation (SNSF).

Poster 11 - Contribution of estrogen and NR2B subunit to the Htr1aKO mouse phenotype

Alexandre Pinault , Alessandro Cumb, Julien Ackermann, Peggy Mittaud, Silvia Pedrani, Sophie Mutel, Jean-Pierre Hornung

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Our laboratory study the phenotypic alteration in 5HT1a-deficient mice (KO). Adult KO male mice display exuberant dendritic growth of oblique dendrites in CA1 pyramidal neurons (CA1-PN). This is associated with GluN2B synaptic enrichment at puberty. Starting at puberty, CA1-PN oblique dendrite arborization in KO female mice returns progressively to WT values. We hypothesize that estrogen receptors (ERs) activation contributes to the morphological phenotype observed in the KO female mice. In organotipic cultures, GPER-1 activation, a specific ER coupled to a G-protein, regulates dendritic morphology of CA1-PN. The blockage of DAPK1 phosphorylation, a downstream target of GPER-1, regulates the dendritic morphology by decreasing GluN2B availability at the synapse. Moreover, neonatal GPER-1 agonist injection in KO male reduced the dendritic morphology in adult CA1-PN. In acute hippocampal slices, field recordings confirmed GluN2B synaptic enrichment in P25 KO male. Five Hz stimulation protocols resulted in a stronger synaptic strength in CA1-PN of KO male than KO female mice. At P40, GluN2B is equally distributed in KO male and female mice, while 5Hz stimulation increases synaptic strength in Schaffer collateral synapses only in male KO mice. This correlates with the increased dendritic arborization of oblique dendrite limited to male KO mice. Our current data suggest that loss of 5-HT1a impacting on GluN2B levels leads to long term dendritic and synaptic alterations. Estrogen rise occurring at puberty in females compensate this defect by activating GPER-1 which interact with GluN2B to rescue the phenotype still present in adult male KO mice characterized by an uncompensated GluN2B enrichment.

Poster 12 - The impact of 5HT1a receptor on dendritic arborization of hippocampal pyramidal neurons and associated behavior

Christine Fülling, Sophie Mutel, Alexandre Pinault, Peggy Mittaud, Jean-Pierre Hornung

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Serotonin 1a receptor knockout (5HT1aR-KO) mice exhibit increased anxiety-like behavior, a feature also found in humans exhibiting an htr1a promoter polymorphism. Focusing on the hippocampus - the region that harbors the highest 5HT1aR expression in the forebrain - we identified a role for this receptor in regulating dendritic arborization. Constitutive male 5HT1aR-KO mice showed increased secondary branches of pyramidal neurons in the dorsal hippocampal CA1 and altered synaptic plasticity. In contrast, female 5HT1aR-KO mice do not show these alterations, suggesting a gender-dependent rescue mechanism. Next we aimed at understanding whether these gender-dependent differences would translate into behavior. We observed that female 5HT1aR-KO performed similar as compared to wild-type conspecifics. Male 5HT1aR-KO mice, however, displayed increased anxiety-like behavior and impaired spatial learning in comparison to wild-type males. Furthermore, maternally deprived 5HT1aR-KO males exhibit abnormal social approach towards aggressive conspecifics, hinting towards abnormal olfactory processing, which was confirmed in the buried food test. As olfaction is influenced by adult neurogenesis we are currently investigating to which extend the impaired survival of adult born neurons of 5HT1aR KO animals is involved in the olfactory deficits seen in these animals. Furthermore, we are investigating the possibility of an overall effect of the 5HT1aR KO on cell survival during the early postnatal period. The gender difference described in this study as well as differences in olfactory processing and motivation add to the phenotype of the 5HT1aR-KO animals and give further hints for the understanding of the contribution of this receptor to anxiety-like behavior and depression.

Poster 13 - Diversity of sleep spindle rhythms in local areas of the mouse brain

Laura M.J. FERNANDEZ, Anita LUTHI

Université de Lausanne Département des Neurosciences Fondamentales Bugnon 9 | CH-1005 Lausanne |Switzerland

Non-rapid eye movement sleep (NREMS) is a natural behavioral state during which various rhythmic electrical activities develop in the brain. Among those, sleep spindles, occurring as 0.5-1s oscillatory events in the 9-15Hz frequency range, are supposedly important in brain plasticity and memory consolidation. However, spindles represent a comparatively minor component of the rodent NREMS EEG (~8% of total spectral power) and appear prominently only at periods of NREMS-REMS transitions. Additionally, restricted knowledge of the spatiotemporal occurrence of spindles in different cortices hinders insights into their functional role. To overcome this limit, here we explored sleep in head-restrained mice, assessing behavioral states through conventional polysomnography (EcoG/EMG), and we recorded simultaneously local field potentials (LFP) from high-impedance (10-12 MOhm) electrodes chronically implanted in the dorsal hippocampus (dCA1) and in somatosensory (S1 and S2), auditory (A1), piriform (Pir), and medial prefrontal (mPFC) cortices. While EcoG displayed typical NREMS activities, LFP recordings indicated local variation of spindle power from one site to another. Discrete spindles events could also be reliably detected and showed differences in power and frequency depending on brain area. Exploration of the functional organization of spindles indicated a higher correlation for parietal than frontal areas. Finally, as spindles originate from the reticular thalamic nucleus (nRT) through CaV3.3-type Ca2+ channel-dependent bursts (Astori et al., 2011), we are currently exploring nRT-bursting activity during spindle generation in CaV3.3KO animals. Our study provides a functional topology of sleep spindle activity in mouse brain and a basis to assess their origin and function.

Poster 14 - Optogenetic activation of glutamatergic afferents into the reticular thalamic nucleus of mouse

Gil Vantomme, Chiara Pellegrini, Zita Rovó, Anita Lüthi

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The reticular thalamic nucleus of the mouse (nRt) is a GABAergic nucleus surrounding the dorsal thalamus that is strongly innervated by thalamic and cortical glutamatergic projections relevant for its involvement in large-scale thalamocortical oscillations, such as spindle rhythms in sleep. In spite of this heavy glutamatergic innervation, still little is known about its synaptic characteristics and innervation patterns across the different functional sectors of this nucleus. We took an optogenetic approach to selectively activate the cortical projections to nRt in acute slice preparations of young adult NTSR1-Cre;Ai32 mice (Madisen et al., 2012) that express the light-activated ChR2 in thalamically projecting layer VI cortical neurons. Brief flashes of LED light (455 nm, 0.05-0.1 s) produced large excitatory postsynaptic currents (EPSCs) in nRt neurons recorded in the whole-cell patch-clamp configuration around -60 mV at room temperature that were entirely blocked by DNQX (0.04 mM), an AMPA receptor blocker. Repetitive light pulses (10x, 20 Hz) evoked a train of EPSCs showing a progressive increase in amplitude, consistent with the presynaptic facilitatory characteristics of the cortical synapses. A small NMDA-component of the synaptic response could be discerned at positive holding potentials (+40 mV). Virally induced expression of ChR2 in only the primary somatosensory cortex elicited similar EPSCs specifically in the posterior part of the nRt. These findings indicate that optogenetics will be useful to specify the functional characteristics and the topology of the cortical drive into nRt.

Poster 15 - Strategic position of thalamic reticular nucleus between subiculum and anterior thalamus: relevance for head direction system?

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The anterior thalamic nuclei (ATN) are abundantly and reciprocally connected with the hippocampal formation with parallel recurrent loops, and have the capacity to profoundly shape hippocampal spatial and mnemonic information processing. Cells in the subiculum and anterior thalamus discharge as a function of the animalâ€™s head direction (HD) in the horizontal plane, but independent of its behavior and location in the environment. The reticular thalamic nucleus (TRN) controls thalamic throughput of sensory information through feedback inhibition. Neurons in the dorsal part of TRN also receive subicular projections, yet this innervation was never functionally addressed in the context of HD system. TRN could provide a potential source of synchronization among the HD neurons in the ATN, but we donâ€™t know yet how the TRN is integrated in the hippocampo-thalamic loops. We used both anatomical and functional techniques to determine the nature of subicular afferents of the TRN. Retrograde and anterograde tracers were injected into the rostral TRN and posterior subiculum, respectively to validate the previously described projections. Channelrhodopsin-expressing viruses were then injected into the subicular complex, and two-three weeks of postinjection in vitro and in vivo electrophysiological recordings were performed. Using in vitro acute brain slices we found that the subicular neurons discharge reliably upon optical stimulation and that the thalamic neurons are synaptically connected to these neurons and generate depressant glutamatergic EPSCs with a pronounced AMPA/NMDA ratio. Preliminary in vivo data suggest that trains of laser stimulation trigger repetitive firing in the dorsal TRN neurons under urethane anesthesia.

Poster 16 - Modulation of molecular substrates of thalamic rhythmogenesis through synaptic NMDA receptors

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Thalamic circuits are reliable and stereotypic pacemakers for the generation of sleep rhythms. Yet, it is well known that sleep oscillations vary in intensity, locally and globally in the brain, due to circadian, homeostatic and use-dependent regulatory influences. In the nucleus Reticularis thalami (nRt), sleep rhythmogenesis is governed by low-threshold spiking through CaV3-type calcium channels. In addition, glutamatergic synapses in nRt express GluN2C-containing NMDA receptors that contribute to thalamic excitability and that have been linked to the emergence of pathophysiological oscillations. We explored synaptically mediated modulation of nRt discharge using selective optogenetic activation of cortical layer VI afferents in slices from NtsR1-CreXChR2floxed mice. We found that repetitive activation of cortical inputs (10Hz trains) induced a long-lasting increase of postsynaptic potentials in nRt cells (29±9%, n=7, p0.05). Whereas AMPAR-mediated currents were not altered by the train stimulation (6±6%, n=6, p>0.05), CaV3-currents were potentiated (39±8%, n=8, p>0.05). Increase of CaV3-currents could be mimicked by agonistic activation of NMDARs with brief superfusion of NMDA (30 microM) (97±25%, n=7, p0.05), and, surprisingly, absent in CaV3.2-/- mice (6±5%, n=10, p>0.05). Altogether, our data indicate that repetitive activation of GluN2C-NMDARs facilitate recruitment of CaV3.2 channels, thus promoting nRt excitability. This suggests that cortical drive triggers activity-dependent changes in specific molecular cores of thalamic sleep rhythmogenesis.
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Poster 17 - Fine tuning c-jun N-terminal kinase (JNK)-mediated neuroprotection by subcellular targeting of JNK inhibition in excitotoxic conditions

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The c-Jun NH2-terminal kinases (JNK) have been implicated in the pathophysiology of several brain disorders including those due to excitotoxicity. Paradoxically JNK have also important physiological roles in brain development, neuroregeneration, cytoarchitecture and neuronal plasticity. This JNK duality is partly due to the diversity of JNK targets localized in various cellular compartments. Clarification of the compartmental segregation of JNK actions is therefore required to target JNK pathological functions specifically, and thus to develop a clinically safe and effective neuroprotective strategy against brain disorders involving excitotoxicity. In this study, we evaluated the effects on cell viability of cell-permeable JNK-inhibitory peptides containing a nuclear localization sequence (TAT-NLS-JBD) or a nuclear exclusion sequence (TAT-NES-JBD) in both normal and excitotoxic conditions in cortical neuronal cultures. Our results showed that TAT-NLS-JBD (2 µM) protects (by 80%) against an excitotoxic dose of NMDA (100µM, 6h) while TAT-NES-JBD (2 µM) is strongly neurotoxic even in normal conditions. This compartment-specific effect was confirmed both by transiently transfecting neurons with compartment-targeted JNK-inhibitory plasmids or in vivo in a rat neonatal model of stroke. These results indicate that cytosolic JNK is mainly involved in physiological roles while nuclear JNK mediates excitotoxicity. Altogether these results provide better understanding of JNK multifunctionality and identify crucial JNK nuclear substrates involved in excitotoxicity.

Poster 18 - STRUCTURAL ANALYSIS OF THE NEUROGENIC NICHE IN THE ADULT HIPPOCAMPUS

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Adult mammalian hippocampal neurogenesis consists in the generation of new neurons from neural precursors located in the subgranular zone of the dentate gyrus. Learning experiences and neuronal activity increase adult neurogenesis and increasing or reducing neurogenesis leads to corresponding changes in memory performances, suggesting a role of these cells in learning and memory. Hippocampal neurogenesis is tightly regulated by the stem cell's highly specialized microenvironment, called the neurogenic niche, which can potentially include every brain cell type: neurons, microglia, endothelial cells, astrocytes and oligodendrocytes. Understanding the interaction between these cells and the adult neural stem cell, Radial Glia Like cell (RGL) is crucial for a full understanding of the function of the neurogenic niche. Here, we are using morphological approaches to identify RGL, niche cells and the nature of their interactions.

Poster 19 - Synaptic Integration of Adult-Born Hippocampal Neurons is locally controlled by astrocytes

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By taking advantage of the mosaic expression of genes interfering with exocytosis in astrocytes, we examined the synaptic integration of new neurons in individual astrocytic territories. We found that dendritic spine density and maturation depend on the functional integrity of astrocytes they intersect, indicating that the synaptic integration of new neurons requires spatially-restricted, astrocytic cues. Synaptic deficits were accompanied by reduced extracellular levels of D-serine and were restored by D-serine treatment. These results reveal that, in the adult brain, mature astrocytes release D-serine, which is required for the synaptic integration of new hippocampal neurons.

Poster 20 - Astrocytes-released molecules regulate the proliferation of adult neural progenitor cells in the dentate gyrus

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The adult hippocampal neurogenic niche regulates adult neural stem/progenitor cell (aNSCs) proliferation and a major contributor to the neurogenic niche are astrocytes. Here we tested the effect of astrocyte-conditioned medium (ACM) on the mouse hippocampal adult neurogenesis. We found that ACM increased aNSC proliferation in vivo and in vitro and resulted in increased neurogenesis. In contrast, the experimental block of astrocytic vesicular release reduced aNSC proliferation in the dentate gyrus and ACM from blocked astrocytes did not increase proliferation in vivo or in vitro. These results indicate that astrocytes release molecules that regulate the proliferation of aNSCs in the dentate gyrus.

Poster 21- High-resolution two-photon analysis of astrocytic Ca^{2+} dynamics using synthetic and genetically-encoded Ca^{2+} indicators

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Astrocytes display a wide spectrum of calcium transients in response to neuronal activity that may in turn provoke the reciprocal modulation of the synaptic network. Classically, these transients were reported to last several seconds to tens of seconds, and recruit large areas of the astrocytic structure. In the last years, however, there has been also reported the existence of remarkably fast (sub-second) "focal" Ca2+ transients mainly located in astrocytic processes (reviewed in Volterra et al., Nature Rev Neurosci 2014). The appearance of such focal events requires activation of astrocytic GPCRs by synaptic activity (sensitive to Bafilomycin A1 incubation) and is blocked by interfering with GPRC or IP3 signaling in the astrocytes (sensitive to GDP-beta-S, heparin, IP3R2 knockout (Di Castro et al. Nat Neurosci 2011)). To date, these observations required the use of small, exogenously loaded, organic calcium indicators, which have the advantages of high temporal and signal fidelity, but also have drawbacks. The salt versions of these dyes require patch-clamping a cell that may perturb somatic responses and possibly change the diffusion speed of free Ca2+ in the cytoplasm. Bulk-loading cells with an AM-version of these dyes decreases loading specificity and could reduce the dynamic range and signal-to-noise ratio (SNR).

Genetically-encoded Ca2+ indicators (GECIs) have the potential to address some of these shortcomings. Because the latest generations of GECIs now approach the sensitivity and speed of the small organic dyes, they may in principle be able to report the entire continuum of Ca2+ responses in astrocytes. Notably, however, the groups that have recently used GECIs in astrocytes have so far reported only the longer-lasting calcium transients (duration ca. 5-10s, see e.g. Bonder and McCarthy J Neurosci 2014, Haustein et al. Neuron 2014), suggesting that these GECI may not equal the sensitivity of organic indicators in reporting the population of faster and smaller Ca2+ transients.

Therefore, we have decided to undertake a systematic comparison between acutely loaded small molecule dyes (Fluo-4-AM and Rhod2-AM), and a genetically encoded cytosolic GCaMP3 in unperturbed hippocampal astrocytes of adult (P30-40) mice, under the optimal loading and imaging conditions for these dyes.

In order to minimize the potential for significant side effects typically associated with viral infection, we took advantage of a novel transgenic mouse line conditionally expressing GCaMP3 upon the activation of a Cre-Lox system (Otsu et al. Nat Neurosci 2015). We have tested several astrocytic promoters, and noted that GCaMP3 expression was highly specific to astrocytes when activated by a tamoxifen-triggered GFAP-CreERT2 line (Hirrlinger et al. Glia 2006) in the hippocampal dentate gyrus (DG).

We were able to adequately load Fluo-4 and express GCaMP3 in astrocytes, allowing the direct comparison of the two dyes in the same type of process segments at least up to 20 um from the somatic region using multiphoton imaging setup in the fast speed line-scan mode. Additionally, because of good spectral separation of Rhod2 and GCAMP3, we performed simultaneous measurement of the signals reported by the two dyes co-loaded into the same process. We observed that in general Fluo-4AM, Rhod2-AM and GCaMP3 can faithfully report the existence of longer (duration of several seconds) Ca2+ transients at a frequency matching that previously reported for "expanded" events recorded with Fluo-4 loaded through the patch pipette (Di Castro et al. Nat Neurosci 2011). For this sub-population of events, GCAMP3 has exhibited a superb

SNR matching the quality of pipette-loaded organic dyes. Additionally, under these conditions, GCAMP3 exhibited a greater dynamic range as compared to Rhod-2 AM. However, GCaMP3 tended to under-report the occurrence of the faster (sub-second duration), smaller Ca2+ transients even compared to the two AM-loaded organic dyes.

In conclusion, the GCaMP3 generation of GECI is already able to report good part of the fast Ca2+ events in astrocytes with high fidelity. As for the faster events, in the future, it is likely that the newer generations will parallel the quality and utility of Fluo-4, which remains a valid standard.

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Poster 22 - Role of astrocyte TNFα signalling in synaptic and behavioral alterations

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TNFα is a cytokine that, at basal levels, exerts a physiological control on hippocampal synaptic functions1,2, including via astrocyte-dependent glutamate release3,4,5. Here we investigated the synaptic effect of TNFa at 10-fold the constitutive concentration to mimic the enhanced cytokine levels in pathological conditions. Of particular interest in this context, are the evidences that TNFa is enhanced in anxious-depressive states, notably in patients with major depression6, and that icv TNFα injection is pro-depressant in rodents7 and, conversely, TNFR1 ablation is antidepressant8. Our synaptic data in the hippocampus show that high TNFa induces long-lasting increase of excitatory transmission at perforant path-granule cell (PP-GC) synapses via activation of pre-synaptic NR2b-containing NMDAR. The effect is TNFR1-dependent (as shown by using TNFR1-/- mice) and TNFR1 signaling occurs in astrocytes (as shown by re-expressing conditionally TNFR1 only in these cells). Given the relevance of the studied synaptic circuit to cognitive function, notably to contextual memory, we next evaluated if enhanced TNFa is responsible, via the identified astrocyte-dependent mechanism, of induction of an impaired cognitive endophenotype. To this end, we utilized Experimental Auto-immune Encephalomyelitis (EAE), a murine model of Multiple Sclerosis, as a pathological condition notoriously associated to enhanced brain TNFa9. Indeed, we found in the hippocampus of EAE mice parenchymal inflammation with increased TNFα, specifically in the dorsal region, which is critical for contextual learning. Moreover, excitatory transmission in the underlying circuit (PP-GC synapses) was enhanced, very similar to the effect induced by application of exogenous TNFα. Not only, the synaptic alteration in EAE mice depended on the same astrocyte TNFR1 signaling cascade and required NR2b-NMDAR activation. Finally, and importantly, EAE mice showed a clear impairment of contextual memory in the fear conditioning test. In conclusion, parenchymal TNFa elevation in dorsal hippocampus is responsible, via astrocyte signaling, of altered excitatory synaptic function and impaired cognitive function, an endophenotype that may contribute to the global syndromic state of several psychiatric conditions, including anxious-

depressive states. The relevance to these states will be directly investigated in future studies using ad hoc models of depression, such as chronic social defeat, in which we already reported increased plasma TNFa levels.

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CHUV

Poster 23 - Genome editing with viral-delivered CRISPR/Cas9 system for Huntington's disease

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Huntington's disease is a neurodegenerative disorder caused by a pathological CAG expansion at the 3' end of the first exon of the huntingtin gene (HTT). Currently, there is no efficient treatment for HD. A promising approach consists to directly repair the mutant HTT gene using targeted site-specific nucleases. The recently described Clustered Regularly Interspaced Short Palindromic Repeats system (CRISPR) offers the possibility to direct a bacterial nuclease, Cas9, using a single-guide RNA sequences (sgRNA) to a specific DNA target site. Recognition of the target sequence allows Cas9 to perform DNA double-strand breaks (DSB), which can be repaired by non-homologous end joining (NHEJ) by the cell or by homologous recombination (HR) in the presence of a DNA template. In this study, we propose to characterize and optimize the CRISPR system for in vitro and in vivo DNA repair, with an application for Huntington's disease. As a proof-of-principle of gene editing with this technology, we targeted an artificial sequence containing fluorescent reporter genes to facilitate the readout in HEK 293T cells. Using the surveyor assay and restriction fragment length polymorphism (RFLP), we were able to reach up to 50% of DSB formation and up to 25% of HR both in transient transfection and using lentiviral vectors expressing Cas9 and the sgRNA. Targeting of a genomic integrated fluorescent reporter sequence by the CRISPR system in primary cultures of cortical neurons and astrocytes result in an efficient gene disruption, leading to the loss of the fluorescence of the reporter gene. Furthermore, a comparable efficiency was observed when LV expressing the CRISPR system are injected into the mouse brain and we are currently evaluating this powerful technology to disrupt the mutant HTT.

Poster 24 - Tollip, a modulator of intracellular inflammatory signaling, reduces the response to a bacterial toxin in the brain.

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Neuroinflammation is thought to have an important role in the physiopathology of neurodegenerative diseases. Inflammatory signaling cascades in the brain constitute a double-edged sword, eliminating pathogens and debris of apoptotic cells but producing inflammatory mediators that are neurotoxic if released at a high concentration for a prolonged period. Intracellular modulators of signal transduction act to reduce the intensity and facilitate the termination of these inflammatory reactions. Tollip was first described in Lausanne in 2000 as a negative modulator of the inflammatory response in immune cells. It has been later on demonstrated to reduce inflammation in experimental colitis, but its role in the brain remains unknown. Therefore, we have explored the potential protective role of Tollip in neuroinflammation provoked by an acute lipopolysaccharide (LPS) injection. Knock-out Tollip-/- and wild-type Tollip+/+ mice were subjected to an intracerebral low dose (0.1 μg) LPS injection and analyzed 6 hours later. In the absence of inflammatory stimulus, the intracerebral level of inflammatory mediators was not modified by the suppression of the Tollip gene. In contrast, inflammatory response to the LPS challenge was amplified in the absence of Tollip. Indeed, no significant increases of IL-6, IL-1β, iNOS, IFNγ and IL-10 were observed in WT mice, whereas these inflammatory mediators were respectively increased 15 -, 39 -, 10 -, 6 - and 7 -fold in Tollip knock-out mice. Our data suggest that Tollip reduces the early-phase of the inflammatory response to a low dose of LPS, and support the idea of a protective effect of Tollip in neuroinflammation.

Poster 25 - Toward a pharmacological control of gene therapy for Parkinson's disease

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The safety and tolerability of AAV vectors as tools for gene therapy in the brain has been established by several pioneer clinical trials. However, for some therapeutic genes, prolonged uncontrolled expression can lead to adverse effects. Therefore, given the irreversibility of the administration method, gene expression should be adjusted to the patients needs and if necessary, arrested. A clinically-acceptable genetic system allowing to control the concentration of the therapeutic gene product does not exist. The main challenge is to obtain a genetic switch responding to a clinically-approved drug inducer at a dose which does not elicit adverse effects. The BrainVectors group (http://www.brainvectors.org) has developed a highly sensitive inducible AAV vector whose activity depends on the antibiotic doxycycline (AAV-DoxON). We are evaluating the potential of this new vector for pharmacologically-controlled gene therapies in a neuroprotective therapeutical approach consisting in the delivery of a transgene coding for a neurotrophic factor called Glial cell line-derived Neurotrophic Factor (GDNF) in the striatum, a target brain region for the treatment Parkinson's disease. Combining a single AAV-DoxON-GDNF intracerebral injection by stereotaxic neurosurgery and oral treatment with doxcycline resulted in a drug dose-dependent GDNF concentrations in the striatum. Strikingly, our data suggest that biological effects of GDNF relevant to its therapeutic efficacy can be obtained with clinically-approved sub-antimicrobial doses of doxycycline commonly prescribed for long-term treatment of inflammatory diseases of the skin (Rosacea) and of the teeth surrounding tissue (Periodontitis).

Poster 26 - Evaluation of Redox Dysregulation in the Pathology of Schizophrenia Using Induced Pluripotent Stem Cell Technology

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Schizophrenia (SZ) is a disorder that involves genetic and environmental factors. A decrease of glutathione (GSH), a major cellular antioxidant, was shown in patient's brain and CSF. Furthermore, polymorphisms in the key synthetizing enzyme for GSH were found associated with the disease. These observations lead to the hypothesis that redox dysregulation is a main hub in this disorder. In this study, we set up a method based on fluorescence imaging to identify the redox state of thiol residues in a GSH deficient mouse model (Gclm-/-). Our long-term objective is to use induced pluripotent stem cells (iPSC) to examine the impact of oxidative stress on neurons derived from a well-characterized cohort of SZ patients. We established the conditions for thiol labelling by fluorescence in WT mice brain slices and evaluated its sensitivity. Then, we investigated redox state of cells in WT and GBR-treated Gclm-/- mice, GBR being a dopamine reuptake inhibitor that induces additional oxidative stress. In parallel, we have started to generate iPSC from patient's fibroblasts and to derive them into neurons. The ratio between oxidized and reduced thiols was increased in GBR-treated Gclm-/- compared to WT mice, suggesting a more oxidized cellular environment. This ratio will be measured in iPSC-derived neurons from patient's fibroblasts that we are currently producing. This method together with other approaches will allow to assess whether the redox state is also altered in iPSC-derived neurons from patients. Ultimately, application of this method to iPSC may pave the way to individualized therapies.

Poster 27- Involvement of the agmatinergic system in the depressive-like phenotype of CRTC1-deficient mice

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Recent studies have highlighted the involvement of the arginine-decarboxylation product agmatine in depression. Most notably, it has been shown that this compound has antidepressant properties in rodents and that the agmatinergic system is impaired in mood disorders patients. Our group has generated a CRTC1-deficient mouse line which presents an important behavioural and molecular depressive-like phenotype, as well as blunted responses to classical antidepressants. Microarray analysis showed an increased expression of agmatinase (the agmatine-degrading enzyme) in the cortex of Crtc1-/- mice, suggesting that the agmatinergic system of these mice might be impaired. We were therefore interested in investigating the link between this system and the CREB-CRTC1 pathway. We confirmed the increased agmatinase expression displayed by Crtc1-/-mice at the mRNA and protein levels and found that it was overexpressed in the hippocampus and prefrontal cortex (PFC) of these animals. Immunohistochemical data showed that Crtc1-/-mice display more agmatinase-expressing cells than wild-type mice in several brain regions, including the PFC and the CA1 region of the hippocampus. We also observed that agmatinase was mainly expressed in parvalbumin and somatostatin interneurons. At the behavioural level, we found that acute agmatine treatment was able to reduce the increased immobility time displayed by Crtc1-/- mice in the Forced-Swim Test. We also observed that agmatine could rapidly induce BDNF translation, a mechanism that could underlie its antidepressant effects. Altogether, these data support the involvement of the agmatinergic system in the depressive-like phenotype of Crtc1-/-mice, and also allow a better understanding of the agmatinergic system and its putative role in depression.

Poster 28 - Cell-type specific expression of mutant huntingtin in the mouse striatum with AAV2/5 viral vector.

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Huntington's disease (HD) is a rare neurodegenerative disease caused by an autosomal dominant mutation on the huntingtin gene (HTT). Despite ubiquitous expression of the mutant HTT, a selective vulnerability of medium spiny neurons (MSNs) of the striatum is observed at the early stages of the disease. This is why most research has focused on mechanisms of MSNs dysfunction. Recent data have suggested the implication of astrocytes in the disease, and an increasing number of studies have demonstrated the essential role of these cells in neuronal functions. These underline the need to better characterize neuron-astrocyte interactions in HD. Our strategy consists to model the disease using cell-type specific viral vectors expressing mutant HTT in the striatum. Adeno-Associated Viruses (AAV) offer the possibility to shift their tropism from neurons to astrocytes using specific capsids or cell-type specific promoters. In this study, we propose to characterize a viral HD model using an AAV2/5 expressing mutant HTT under the control of either a neuronal or an astrocytic promoter. We shown that the combination of AAV2/5 and the chicken β actin promoter offered the possibility to express in vivo a reporter gene specifically in neurons whereas using a GFAP-derived promoter results in a strong astrocytic tropism. Replacement of this reporter gene by mutant HTT led to a cell-type specific formation of aggregates. We furthermore characterized several molecular and behavioral hallmarks relevant to HD and their evolution in these models. This will allow a better understanding of neurons-astrocytes interactions, and their respective contribution to HD.

Poster 29 - Genetics of intellectual disability

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Although intellectual disability affects 1-3% of the population, it is one of the least understood health problems. It is estimated that genetic lesions account for half of the currently undiagnosed cases. Despite recent successes in identifying some of the mutations responsible, it has been suggested that up to 1,000 more genes remain to be uncovered. The large number of intellectual disability syndromes is due to many causal pathophysiological mechanisms. The diversity of mechanisms results in an array of quantifiable neuroanatomical abnormalities. To identify genes related to intellectual disability, we are collaborating with the Sanger Mouse Genetics Project (MGP), allied to the International Mouse Phenotyping Consortium (IMPC), to systematically study the neuroanatomy of the MGP/IMPC knockout mouse strains using a standardized set of 78 brain parameters. So far, we have assessed brain defects in 825 knockout mouse mutants. These preliminary data yielded success with the identification of 40 known intellectual disability genes including Ap4e1, Cenpj, Chd7, Mcph1, Sc4mol and Ube3b demonstrating the pertinence of our approach. We also discovered 41 other genes including Mta1, Ccdc104, Caprin2 and Dusp3, which when disrupted caused modification of brain structures. Our study is the largest screen of brain morphology from the MGP/IMPC. It shows that we can detect abnormalities in about 10% of knockout mouse mutants, and that these translate into human pathology. This offers a complementary resource to human genetic studies.

Poster 30 - MRI and MRS characterization of Crtc1 knock-out mice limbic structures: investigating neurobiology of mood disorders

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In vivo MRI and MRS are two non-invasive techniques of choice for investigating and monitoring metabolic and structural changes in mood disorders, which are poorly understood. Discrepancies between human studies reflect however the lack of comprehension of their pathophysiology and reinforces the need for an endophenotypic characterization, which can be provided by animal models. We have investigated the metabolic and volumetric status of a previously reported mouse model of mood disorders lacking an important brain plasticity gene, Crtc1 (CREB-regulated transcriptional coactivator 1). Crtc1 knock-out animals are considered as relevant for studying mood disorders since they show neurobehavioral depressive-like endophenotypes as well as late-onset obesity together with monoaminergic system dysfunctions. Metabolic and volumetric profile alterations were determined with T2-weighted MRI together with 1H-MRS of prefrontal cortex (PFC), dorsal/ventral hippocampus and amygdala. KO mice showed reduced glutamate (-12%, p=0.0056) and GABA (-26%, p=0.03) in PFC, whereas a marked reduction of the energy metabolite phosphocreatine (-20%, p=0.03) was visible in the dorsal hippocampus. KO mice showed also a strong ventricle shrinkage correlating (p=0.01) with swelling of surrounding gray matter. Such alterations are similar to some human findings in mood disorders, which suggested that the GABA/glutamatergic system or the energy metabolism are impaired in some specific regions of the brain. This mouse model will thus allow a better understanding of the molecular mechanisms underlying the changes of MRI/MRS markers observed in human mood disorders.

Poster 31 - Imaging lithium pharmacokinetics in the brain in real time

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Magnetic Resonance Imaging (MRI) is a powerful noninvasive tool to study anatomy, physiology and function in vivo in both healthy and disease organs. Classical MRI is based on the detection of proton (1H) signal from water molecules; however it is possible to measure the magnetic resonance (MR) signal of other atoms such as the two stable lithium isotopes, namely 7Li and 6Li. Lithium MR studies are usually based on the detection of 7Li because of its higher natural isotopic abundance (92.58%), higher magnetic moment and more favorable relaxation properties than 6Li. 7Li MR is nevertheless a non-sensitive technique requiring long acquisition times that limits its imaging applications in vivo. The recent development of hyperpolarized MR enables to enhance the signal of small molecules that can be used as contrast agents for angiography and perfusion MRI. The long in vivo relaxation time of 6Li makes it an attractive hyperpolarized contrast agent. The present study explores new MRI opportunities relying on hyperpolarized 6Li as contrast agent. Given the toxicity of lithium in vivo we restrict our implementation to its pharmaceutical concentrations solely. We demonstrate that hemoglobin oxygenation can be readily detected by its effect on hyperpolarized 6Li relaxation time. Additionally we show that it is possible to monitor sub-millimolar concentrations of hyperpolarized 6Li in the brain in real time. We observed that Li ions penetrate deeper into the brain tissue within the time span of the measurement (18 s). Those finding opens new opportunities to assess the effect of Li+ on cerebral function.

Poster 32 - Characterization of dendritic structure and dynamics of superficial VIP cells in the mouse somatosensory cortex

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A fraction of the cortical vasoactive intestinal polypeptide (VIP)-expressing interneurons are thought to specialize in disinhibitory control of pyramidal cells. Some VIP neurons located in superficial cortical lamina bear spines. Here we sought to provide insights into the spine dynamics on these cells in order to further our understanding of how long-range excitatory inputs may modulate local disinhibition. We used VIP-Cre transgenic mice in combination with Cre-dependent AAV vectors encoding GFP to image spiny superficial VIP neurons in the mouse somatosensory cortex in vivo. Anatomical reconstructions suggest that the morphology of these spiny neurons display similarities to a previously described class of multi-polar VIP neurons in layer 2. Longitudinal imaging data suggest that the spine dynamics are different from those on pyramidal cells. A relatively large fraction of spines has intermediate life times (varying from several days to weeks), but the total population of spines is less stable than on pyramidal cells. These data suggest that the levels of disinhibition are adjustable though the dynamic regulation of the strength and source of excitatory inputs on VIP cells.

Poster 33 - Metabolic responsive neurons of the insular cortex

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Signals from peripheral organs are known to influence mental processes. Neuroimaging studies have confirmed that cortical areas respond to changes in body physiological conditions and that these fluctuations affect psychology and behavior. Despite their important clinical implications, the pathways underlying these effects have been little explored. We investigated the role of insular cortex (IC) as an interface between interoceptive sensing and cognitive and emotional responses. In vivo fasting and glucoprivation, by an i.p. administration of 2-deoxyglucose (2DG), decreased anxiety-like behaviors in mice. These metabolic challenges also induced c-fos expression in a subpopulation of cells in IC, suggesting that this cortical area contains neurons responding to metabolic changes which could account for the modulation of anxiety-like behavior during fasting and glucoprivation. To investigate the underlying cellular mechanisms we performed experiments on acute cortical slices. Whole-cell electrophysiological recordings further evidenced a set of neurons that respond to glucose in a cell-autonomous fashion, with either a glucose-inhibited or a glucose-excited phenotype. We are now looking at the identity of these neurons and characterizing the biophysical and molecular components of their responses to glucose changes.