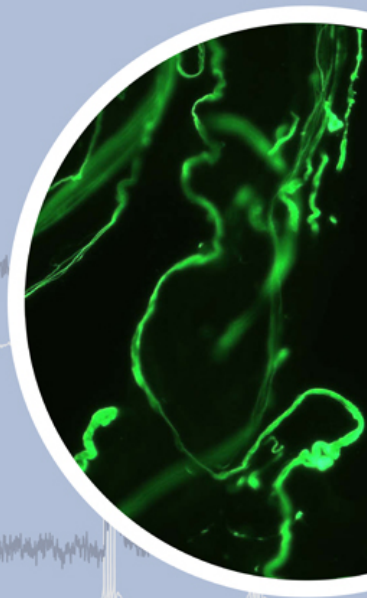


# 1<sup>st</sup> DNF symposium

May 9, 2014



## Keynote speakers:

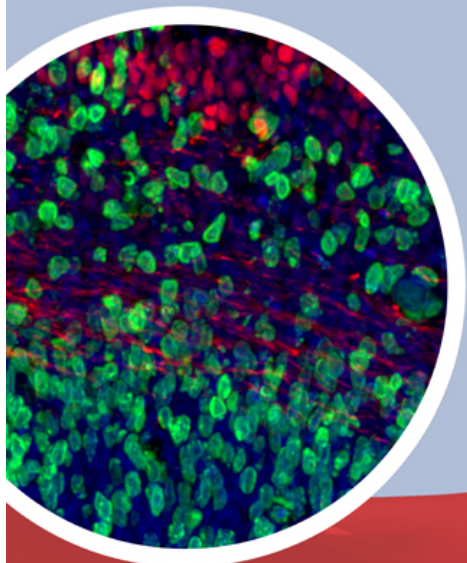
Maiken Nedergaard MD  
University of Rochester, USA

Tony Wyss-Coray PhD  
Stanford University, USA

## Local speakers:

Antoine Adamantidis PhD, University of Bern  
Anita Lüthi PhD, University of Lausanne  
Denis Jabaudon MD-PhD, University of Geneva  
Nicolas Toni PhD, University of Lausanne

Poster session/best poster prize



## Venue:

DNF, 9 rue du Bugnon, Lausanne

## Registration and Abstract Submission:

<http://www.unil.ch/dnf>

Deadline: April 10, 2014

Images: M. Krzisch, S. Astori Photos: M.Kielar, M. Pertin Conception: E. Bernardi

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Département  
des neurosciences  
fondamentales

**PROGRAM AND ABSTRACT BOOKLET**

# **WELCOME TO THE FIRST 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 of the University of Lausanne and grew out of the former Department of Cell Biology and Morphology. The name DNF reflects the shift in research focus towards cutting-edge neuroscience, including the entire modern array of molecular, cellular and behavioral methodologies.

This is **the very first international symposium organized by the DNF**. The combination of keynote lectures presented by international speakers with talks given by members of the DNF and the Lemanic Neuroscience Community has the aim of presenting the research directions of the DNF and indicating how they are embedded within the international scientific community. This first edition will be devoted to the subjects of **sleep physiology** and **neural stem cells-developmental plasticity**. In addition to this theme, poster sessions and short oral presentations will provide a broader overview over the research carried out at the DNF.

DNF Symposia will aim at fostering dialogues between our international invitees, our national colleagues, and the DNF members. They are intended to be inspiring one-day meetings that help discover parallels, synergies, convergences but also divergences and controversies between and within our areas of expertise. We hope that you will help to get this idea off the ground.

We are pleased to have you here today!  
Anita Lüthi and Nicolas Toni, Organizers

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**PROGRAM**

**Poster session : 9h-18h**

8h15-8h45: Registration and Poster Set-up

8h45-9h00: Opening Remarks

9h00-10h00: **Keynote lecture** Prof. Maiken Nedergaard

*The night life of astrocytes*

10h00-10h30: Coffee amid posters

10h30-11h00: **Lecture** Prof. Antoine Adamantidis

*Optogenetic deconstruction of sleep-wake states*

11h00-11h30: **Lecture** Prof. Anita Lüthi

*The sleep-deprived brain, a view from the NMDA receptor*

11h30-11h45: Coffee amid posters

11h45-12h45: **Blitz presentations** from selected posters

11h45-12h00: Poster 50, by David Bovard (see p.16)

12h00-12h15: Poster 40, by Michael van der Kooij (see p.18)

12h15-12h30: Poster 2, by Luc Stoppini (see p.34)

12h30-12h45: Poster 25, by Sebastiano Bariselli (see p.34)

12h45-14h15 Lunch buffet amid posters

14h15-15h15: **Keynote lecture** Prof. Tony Wyss-Coray

*Circulatory factors modulate brain aging and plasticity*

15h15-15h45: **Lecture** Prof. Denis Jabaudon

*Gene-circuit interactions during assembly of forebrain neurons*

15h45-16h15: Coffee amid posters

16h15-16h45: **Lecture** Prof. Nicolas Toni

*Astrocytes regulate adult hippocampal neurogenesis*

16h45-17h30: **Award** of Poster prizes

Concluding Remarks

17h30-19h00: Buffet dinner

## SPEAKERS

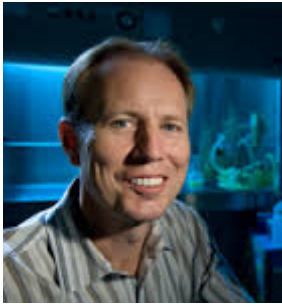


### ***Professor Maiken Nedergaard, MD, D.M.Sc.***

Dr. Nedergaard is a Professor at the University of Rochester at the Center for Translational Neuromedicine, where she is the Co-director. She holds the Frank P. Smith Chair in the Department of Neurosurgery, with secondary appointments in the Departments of Neurobiology and Anatomy, and Neurology. She obtained her pre- and postdoctoral education at the Universities of Copenhagen, Denmark, and at Cornell University Medical School. The Nedergaard lab's multiple interests range from basic research on neuron-glia interactions to their role in seizure disorders and cerebral blood flow. Forefront amongst her discoveries is the identification of the glymphatic system, a brain equivalent of the lymphatic system within which cerebrospinal fluid diffuses rapidly and mixes with interstitial fluids, thereby filtering metabolic byproducts that accumulate due to neuronal activity. Most recently, she published a landmark study in *Science* showing that the glymphatic system dramatically expands during sleep compared to waking – brain cleaning and detoxification is thus greatly facilitated during sleep, providing a novel and direct explanation for what we all generally consider sleep's restorative effect. Among her many honors was her election in 2008 to the Royal Danish Academy of Sciences in recognition of her role as a pioneer in brain research, who has demonstrated that brain cells known as astrocytes play a role in a host of human diseases. More recently, she was elected member of Academia Europaea and of the Royal Academy of Pharmacy of Spain. She has filed several patents and is a pioneer in research on the neural basis of acupuncture.

#### **Abstract: The night life of astrocytes**

We have recently described a macroscopic pathway in the central nervous system – the glymphatic system that facilitates the clearance of interstitial waste products from neuronal metabolism. Glymphatic clearance of macromolecules is driven by cerebrospinal fluid (CSF) that flows in along para-arterial spaces and through the brain parenchyma via support from astroglial aquaporin-4 water channels. The glymphatic circulation constitutes a complete anatomical pathway; para-arterial CSF exchanges with the interstitial fluid, solutes collect along para-venous spaces, then drain into the vessels of the lymphatic system for ultimate excretion from the kidney or degradation in the liver. The glymphatic system is only active during sleep. As such, this circulation represents a novel and unexplored pathway for understanding the biological necessity for sleep.



### ***Professor Tony Wyss-Coray, PhD***

Dr. Wyss-Coray is Associate Director of the Center for Tissue Regeneration, Repair and Restoration and Professor at the Department of Neurology and Neurological Sciences, Stanford University, USA. He was trained at the faculty of Sciences of the University of Bern from 1984-1992. After a postdoctoral training in the Department of Neuropharmacology at the Scripps Research Institute in San Diego from 1993-1995, he became staff scientist at the Gladstone Institute of Neurological Sciences in San Francisco (1996-2002) and then Professor at Stanford. Dr. Wyss-Coray studies the role of immune and injury responses in brain aging and neurodegeneration, pursuing the hypothesis that failing or dysfunctional immune responses underlie or contribute to the demise of the aging brain. He combines the study of mouse models with human clinical samples using cytomic, proteomic, and bioinformatic tools. His most recent studies show that systemic circulatory factors can modulate neurogenesis, neuroinflammation, and cognitive function in mice and that factors from young mice can rejuvenate the aging brain. He is the recipient of an NIH Director's Transformative Research Award, a Zenith award from the Alzheimer's Association, a distinguished scholar award from the John Douglas French Alzheimer Foundation and he is an inventor on multiple patents.

#### **Abstract: Circulatory factors modulate brain aging and plasticity**

Growing evidence links neurodegeneration with altered immune responses not only in the brain but in the periphery as well. In addition, age is the main risk factor for sporadic forms of neurodegenerative diseases and aging of peripheral organs may affect brain function. How the systemic environment affects brain health is largely unknown and while some of these interactions may involve cells entering the nervous tissue, it is likely that others are mediated by soluble factors. We use a combination of physiological methods to manipulate systemic aging and proteomic methods to try to identify factors that age or potentially rejuvenate the brain. Our findings point to systemic changes in immune responses and cellular signaling factors with aging and may be relevant for our understanding of age-related neurodegeneration.





### **Professor Antoine Adamantidis, PhD**

Dr. Adamantidis is an Assistant Professor in the Department of Neurology at the University of Bern and holds a joint appointment at the Department of Clinical Research. He is the Co-Director for the Zentrum für Experimentelle Neurologie (ZEN labs) at the Inselspital. He also has an Adjunct Professor position in the Department of Psychiatry at McGill University, Montreal, Canada. He obtained his pre- and postdoctoral education at the Universities of Liege, Belgium, and at Stanford Medical School, USA. His research objectives aim at investigating the wiring, firing dynamics and plasticity of the neural circuits regulating brain states in normal and pathological states using *in vitro* and *in vivo* optogenetics - a technology that he and his colleagues pioneered at Stanford University - combined to genetics and electrophysiological methods. His research program has been driven by questions such as: What defines a sleep/wake circuit? What is the relevance of neural discharge rate in controlling sleep-wake transitions and maintenance? How do pathophysiological symptoms of sleep disorders (narcolepsy, insomnia, etc.) relate to sleep-wake circuits dynamics? In his recent work, his laboratory identified a rapid-eye movement (REM) sleep circuit in the hypothalamus that controls switch and duration of REM sleep states in mammals. Dr. Adamantidis received several awards including the R. Broughton Young Investigator Award (Canadian Sleep Society), a Canadian Research Chair in Neural Circuits and Optogenetics, a NIH Pathway to Independence (PI) Award-K99/R00 (USA), NARSAD and Sleep Research Society Young Investigator Award (USA).

#### **Abstract: Optogenetic deconstruction of sleep-wake states**

Sleep is a primary and essential biological need for higher vertebrates and sleep-like states have been demonstrated in lower vertebrates. While the functions of sleep are still a matter of debate and may include memory consolidation, metabolism clearance and brain plasticity, the basic neurobiological mechanisms controlling sleep-wake state transitions and maintenance remain largely unknown. Over several decades, experimental evidence has identified distinct arousal- and sleep-promoting neural populations in the brain; however, how these nuclei act individually and collectively to promote and maintain sleep-wake states is unknown. We have recently applied optogenetic technology to the repertoire of techniques used in experimental sleep research to probe the function of hypocretins/orexins and melanin-concentrating hormone (MCH) neurons in arousal and rapid eye-movement sleep, respectively. This work further reveals their communication mode and defines possible networks for hypothalamic control of arousal and sleep.



***Professor Denis Jabaudon, MD, PhD***

Denis Jabaudon graduated from the University of Lausanne Medical School in 1995 and did his PhD in Beat Gäwhiler's lab at the Brain Research Institute of the University of Zürich, where he worked on mechanisms of synaptic plasticity using electrophysiological approaches. Following his neurology residency at the University of Geneva, he moved to Boston where he did his postdoc in Jeffrey Macklis's lab at Harvard Medical School, where he investigated the developmental molecular controls over the differentiation of distinct subtypes of cortical neurons. He has been an Assistant Professor in the Department of Basic Neurosciences at the University of Geneva since 2009. The main research interests of the lab are the genetic controls over the developmental connectivity of thalamic and cortical neurons, and gene-circuit interactions during development.

**Abstract: Gene-circuit interactions during assembly of forebrain neurons**

While the initial assembly of neurons into specific circuits largely relies on cell-intrinsic differentiation programs, reciprocally, circuit activity instructs cell-type specific gene expression programs later in development. We will discuss recent work from our laboratory illustrating how such developmental cross-talks between gene expression and circuit connectivity act to orchestrate the assembly of cognate neurons into functionally-specialized pathways.





### ***Professor Anita Lüthi, PhD***

Dr. Lüthi obtained her PhD in 1995 from the Brain Research Institute of the University and the ETH of Zürich, where she used electrophysiological methods to dissect the roles of fast and slow forms of hippocampal glutamatergic synaptic transmission. She spent her postdoctoral period at Yale University, USA and started her interests in elucidating ionic mechanisms underlying oscillatory activity in neurons implicated in sleep rhythm generation. From 2000 on, as a Junior Group Leader at the University of Basel, she has pioneered the consequences of sleep deprivation on glutamatergic synaptic transmission and plasticity, showing that insufficient sleep materializes at the level of the primary excitatory communication sites in the brain. In 2008, she joined UNIL first as a Fellow of the Cloëtta Foundation, and from 2009 on, as a Tenure-Track Assistant Professor. Using electrophysiological recordings in combination with viral intervention techniques, she applies her expertise in ionic mechanisms of neural rhythmicity to manipulate brain waves generated during sleep, in an effort to eventually understand sleep's beneficial functions for the brain. She was promoted to Associate Professor in March 2014.

#### **Abstract: The sleep-deprived brain, a view from the NMDA receptor**

A major line of modern sleep research addresses the consequences of sleep loss on the expression of genes and proteins. The ultimate aim of this approach is to understand the mechanisms via which sleep affects neuronal function. However, sleep loss influences the expression of many genes and proteins. Hence, it is of obvious interest to identify specific proteins whose expression is altered by sleep loss and whose effect on defined, system-relevant, neuronal consequences can be understood in mechanistic detail. We have identified an obligatory role for NR2A-containing NMDA receptors in mediating the effects of sleep deprivation on hippocampal synaptic plasticity. Hippocampal synaptic plasticity in NR2A-knockout mice turns out to be insensitive to sleep loss, although the behavioral reaction to sleep deprivation is normal. As to the mechanistic basis of NR2A's role, we show that sleep deprivation augments the number of NR2A proteins on the spines of CA1 apical dendrites. Our work has now expanded beyond hippocampus to other brain circuits and to the situation of chronic sleep disruption.



### ***Professor Nicolas Toni, PhD***

Dr. Toni obtained his PhD from the University of Geneva in 2000, in the laboratory of Professor Dominique Muller, where he worked on the mechanisms of structural plasticity involved in the expression of long-term potentiation. He then pursued his postdoctoral training in the laboratory of Fred Gage, at the Salk Institute in San Diego, where he studied adult neurogenesis in the hippocampus. He then returned to Switzerland, where he worked for 2 years in pharmaceutical companies, in the development of new drug targets for Alzheimer's disease. He has been an SNF Assistant Professor in the Department of Fundamental Neurosciences since 2010. The main research interests of his laboratory are the mechanisms of synaptic integration of adult-born hippocampal neurons. Using viral-mediated gene-delivery tools, optical and electron microscopy, they are studying the mechanisms of synaptogenesis and their regulation by cell-intrinsic and extrinsic pathways. The structural identity of the neurogenic niche is also examined, using automated 3D electron microscopy, in an attempt to understand the key factors involved in the regulation of cell proliferation in the adult hippocampus.

#### **Abstract: Astrocytes regulate adult hippocampal neurogenesis**

Adult neurogenesis results in the constant addition of new neurons, which have increased plastic properties and participate to mechanisms of learning and memory. Unlike during development, several steps of adult neurogenesis from cell proliferation to neuronal differentiation and synaptic integration depend on cell-extrinsic mechanisms involving neuronal activity, pathological conditions or animal behavior. Here, we will show how the neurogenic niche is involved in the regulation of several steps of adult neurogenesis and discuss the relevance of these mechanisms for brain plasticity.

## **ORGANIZATION**

**Main organizers**

Anita Lüthi, Nicolas Toni

**Local organizing committee**

Professor Andreas Mayer (Director DNF)  
Eric Bernardi (Logistics and Printing)  
Katerina Catalan-Seidl (Secretary)  
Alexandre Sandoval (Website)

**Symposium Poster Design:**

Eric Bernardi

**Food catering service:**

CHUV

**We thank the DNF committee and the FBM for support  
throughout all stages of the organization!**



## ABSTRACTS

### TOPIC 1 NEUROSCIENCE OF COMPLEX SYSTEMS

#### Poster 1

**Presenter:** Fernandez Laura M.J.

**Affiliation:** DNF

#### **Diversity of sleep spindle rhythms in local areas of the mouse brain**

Laura M.J. Fernandez, Anita Lüthi.

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, and we recorded simultaneously local field potentials (LFP) from high-impedance (~10-12M $\Omega$ ) electrodes chronically implanted in the dorsal hippocampus (dCA1) and in somatosensory (S1 & 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 spindle 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 Ca<sup>2+</sup> 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, thereby providing a basis to assess their origin and function

#### Poster 17

**Presenter:** Lecci Sandro

**Affiliation:** DNF

#### **Acoustic stimulation as a tool for assessing sleep quality in mice**

Sandro Lecci, Ralf D. Wimmer, Jean-Yves Chatton, Anita Lüthi

Well-being depends on good sleep. Sleep quality (SQ) is typically quantified by the amplitude of low-frequency components in the power spectrum of the NREM sleep electroencephalogram, but, in humans, arousal thresholds also serve as measure of SQ. Here, we examined whether arousal through acoustic stimuli reports on SQ in freely-moving C57BL/6J mice chronically implanted for polysomnography (ECoG/EMG).

Acoustic arousal was tested in 4 different SQ conditions: early resting phase (control SQ), late resting phase (lowered SQ), after sleep deprivation (SD, increased SQ) and after injection of hypnotics (Zolpidem, 10 mg/kg, pharmacologically increased SQ). In all 4 conditions, varying SQ was ascertained via delta-power and sleep architecture measurements. Mice were then exposed to 3 different white-noise protocols (80 or 90dB for 20s; ramp from 60-90dB, 160s) and arousal success rates (ASRs) and latencies to arousal were quantified. Noise pulses at 90dB, but not at 80dB, faithfully correlated with changes in SQ, with ASRs lower for increased SQ (SD,  $33.37 \pm 4.01\%$ ; n=8, early resting phase,  $60.86 \pm 6.09\%$ ; n=8,  $p < 0.01$ ; values expressed as mean $\pm$ SEM), and tendentially higher for late in the resting phase. A first series of experiments indicates that ASR decreases with Zolpidem. Furthermore, the latency to arousals, as assessed with noise ramps, also reported on changes in SQ.

Together, we present here the ASR to a 90dB-20s acoustic stimulus as an index for both increases and decreases in SQ in natural and pharmacologically induced sleep, thus providing a useful tool for analyzing SQ in mouse models of sleep and neurological disorders.

**Poster: 18**

**Presenter:** Perin Martina

**Affiliation:** DNF

**Orexin-A inhibits NMDA receptor function in the hippocampus: time-of-day dependence**

Martina Perin, Fabio Longordo, Christine Massonnet, Egbert Welker and Anita Lüthi

Orexin fibers are fundamental for the wake state projecting to the major wake-promoting centers, but, interestingly, they also extend to the hippocampus. Hippocampal NMDA receptors (NMDARs) undergo molecular modifications during prolonged waking. Are orexins implicated in this susceptibility? We studied orexin actions on NMDAR-mediated excitatory synaptic transmission in rat acute hippocampal slices. Orexin-A (ox-A, 100 nM) exerted a postsynaptic inhibitory effect on the NMDAR-mediated excitatory postsynaptic currents (NMDAR-EPSCs) at mossy fiber (MF) synapses ( $55.6 \pm 6.3\%$  of baseline). Moreover, it had minor inhibitory actions on NMDAR-EPSCs at CA3-CA1 synapses ( $70.8 \pm 6.8\%$ ), whereas it remained ineffective at NMDAR-EPSCs of other non-MF excitatory synapses in the CA3 area. Ox-A is released in the cerebrospinal fluid (CSF) following a circadian rhythm. We examined whether orexin effects in the hippocampus were time-of-day dependent. Bath application of ox-A decreased NMDAR-currents in slices prepared during the resting time of the rats, when CSF ox-A levels are low. Conversely, in slices prepared during the active period of the rats, when CSF ox-A levels are high, exogenous ox-A did not affect NMDAR-currents. This lack of effect was abolished when we interfered with the orexin system through intraperitoneal injections of almoxant (100 mg/kg), a dual orexin receptor antagonist, during the active period.

In conclusion, ox-A affects NMDAR function of at least two hippocampal synapses, with a major inhibition at the postsynaptic site of MF-CA3 connections. These actions are also exerted by endogenous orexin levels, which occlude the actions of exogenous orexins depending on the time-of-day.



## **Poster 9**

**Presenter:** Curto-Reyes Verdad

**Affiliation:** Pain Center, Department of Anesthesiology, CHUV and DNF

### **Nav1.7 regulation by beta-secretase BACE1 and neuropathic pain phenotype**

Verdad Curto-Reyes, Xavier Rezai, Guylène Kirschmann, Isabelle Décosterd

**Introduction:** Nav1.7 channel is expressed in the PNS and is crucial for the transmission of nociceptive information. Nav B subunits can be cleaved by BACE1, a secretase that regulates channel levels at the plasma membrane and also directly modulates Nav channel expression and properties. Here we investigate the modulation of Nav1.7 by BACE1 and its implication in pain responses.

**Methods:** Western blot and electrophysiological recordings were performed on HEK cells transfected with BACE1, Nav1.7 and B subunits in cultured DRG neurons and also in DRG and nerves of WT and BACE1 KO mice. Mice underwent SNI surgery and behavioural tests to evaluate pain responses.

**Results:** Transfected HEK cells showed a downregulation on total expression of Nav1.7 upon coexpression with BACE1, independently of the presence of B2 or B4 subunits. Expression of BACE1 did not modify Nav1.7 current density or activation/inactivation. Electrophysiology of WT and KO DRG neurons revealed no changes on nociceptor excitability. Conversely, Nav1.7 is increased on sciatic nerve but not in DRG of KO mice. Behavioral tests showed no changes of basal sensitivity in KO mice and neither after SNI surgery.

**Conclusions:** Cotransfection of Nav1.7 with BACE1 reveals a significant decrease of the expression of the channel, suggesting a direct effect of the secretase. Presence of B subunits does not influence this regulation, showing no effect of the cleavage of B subunits in Nav1.7 levels. Electrophysiology results in HEK cells and DRG neurons show no effect of BACE1 in cell excitability. In KO mice the increase of Nav1.7 expression in nerve shows no correlation with behavioral responses. BACE1 regulates Nav1.7 levels but have no phenotype on neuron excitability and is neither involved in nociceptive responses, or neuropathic pain.

## **Poster 30**

**Presenter:** Laedermann Cédric

**Affiliation:** DNF and Pain Center, Anesthesiology Department, CHUV

### **Metformin downregulates Nav1.7 via Nedd4-2: a new treatment for neuropathic pain?**

Cédric Laedermann and Isabelle Décosterd

**Background:** We recently demonstrated that Nedd4-2 ubiquitin ligase is a potent negative regulator of Navs in vivo. In the spared nerve injury (SNI) model of neuropathic pain, we observed that the substantial decrease of Nedd4-2 in nociceptive neurons led to concomitant increase of Nav1.7/Nav1.8 and hyperexcitability. Metformin is the most widely used drug for treatment of diabetes type 2. Its exact mechanism of action remains debated, but a role in activating AMP-activated kinase (AMPK) has been recurrently highlighted. AMPK has been reported to enhance Nedd4-2 activity in *Xenopus* oocytes. Interestingly, Metformin was demonstrated to reverse mechanical allodynia after SNI in rats. We hypothesize that Metformin, via AMPK activation, enhances Nedd4-2 activity, decreases Nav1.7 and restores a normal neuronal excitability.

Results: Metformin has no effect on Nav1.7 current in vitro (HEK293 cells). However, Metformin, in condition where Nedd4-2 is transfected in amount not sufficient to significantly decrease Nav1.7 current, is able to decrease Nav1.7 current. This is due to Nedd4-2 catalytic activity because Metformin has no effect on Nav1.7 current when an inactive Nedd4-2 mutant is coexpressed. In ex vivo experiments using primary culture of nociceptive neurons, Metformin also downregulates total Navs and Nav1.7 current (isolated with ProTxII). This effect was not observed in nociceptive neurons of Nedd4-2 knockout mice, confirming the Nedd4-2 dependent effect of metformin.

Perspective: We will test the effect of Metformin on SNI-induced neuropathic pain in Nedd4-2 knockout mice. In addition, we will analyze the CoLaus database and will determine whether diabetic patients treated with Metformin report less neuropathic pain than those receiving an alternative treatment.

### **Poster 23**

**Presenter:** Khadimallah Ines

**Affiliation:** DNF (1) and Department of Anesthesiology, CHUV (2)

### **Altered microRNAs expression in synaptic plasticity in the adult mouse barrel cortex**

Ines Khadimallah(1), Nathalie Wenger (1), Rudolf Kraftsik(1), Romano Regazzi(1), Guylène Kirschmann(2) & Egbert Welker(1)

In rodents, sensory experience alters the representation in layer IV of the barrel cortex. Excitatory and inhibitory interneurons, with the astrocytic network, modify the functional representation of the whisker follicles. Our group showed that whisker stimulation in adult mice induces depression of neuronal responses and insertion of new inhibitory synapses on spines. This form of plasticity is controlled by several gene regulatory mechanisms including the activation of genetic programs controlling the expression of microRNAs. To investigate the involvement of miRNA in cortical plasticity, we selected four miRNAs known to be implicated in other forms of synaptic plasticity: miR-125b miR-132 miR-137 and miR-138. After unilateral stimulation of three whiskers in the adult mouse, we compared the expression level of these miRNAs in stimulated and adjacent non-stimulated barrels using in situ hybridization with DIG-labeled probes. Whisker stimulation increases the expression of miR-132 after 3hrs of stimulation ( $p=0.02$ ) and miR-137 ( $p=0.03$ ; 24h of stim.), whereas it reduces the level of miR-125b ( $p=0.002$ ; 9h of stim.). No significant difference was detected for miR-138. In addition to these quantitative comparisons, we combined miRNA in situ hybridization and immunolabeling using various markers for neurons and astrocytes. Stimulation alters the degree of colocalization in the stimulated barrel. For example, double labeling of miR-138 and PSD95 is 35% increased in stimulated barrel as compared to the level of colocalization in adjacent non-stimulated barrel. These results indicate that microRNAs have a potential role in sensory activity-dependent cortical plasticity in the adult mouse by acting specifically within the different cellular components of the neocortical circuit.

### **Poster 50 - Poster selected for a Blitz oral presentation**

**Presenter:** Bovard David

**Affiliation:** Department of Pharmacology and Toxicology, UNIL, Rue du Bugnon 27, 1005 Lausanne

## **CNGA4 ion channels in the mouse vomeronasal neurons: new roles in association with TRPC2**

David Bovard, Julien Brechbühl, Monique Nenniger Tosato, Marie-Christine Broillet

Mammalian pheromones are key chemical signals in the regulation of social behaviors such as aggressivity or sexual mating. The detection of pheromones, which takes place in sensory neurons of the vomeronasal organ (VNO), implies the activation of the transient receptor potential canonical channel 2 (TRPC2) as the final effector. While the role of this protein is now well understood, some other channel proteins expressed in the VNO remain without a function. This is the case for the cyclic nucleotide-gated channel 4 (CNGA4). CNGA4, forms a heteromeric cationic channel in the main olfactory epithelium with two others CNG subunits. It increases the sensitivity of this channel to cyclic nucleotides and is also important for the calmodulin-dependent odor adaptation. As no other CNG subunits are present in the VNO, the role of CNGA4 in this organ might be completely different.

We therefore decided to study the role of the CNGA4 protein in the VNO. We observed the protein to be expressed in axons, dendrites and on the microvilli of the vomeronasal sensory neurons. We found that it indeed plays a role in the pheromone signaling pathway as mice lacking the CNGA4 protein display a modified social behavior. These modified behaviors were similar to those observed with TRPC2<sup>-/-</sup> mice. We thus hypothesized that CNGA4 and TRPC2 might interact and form a heteromeric channel. We further observed with in vitro experiments using HEK cells as an expression system that CNGA4 could directly interact with TRPC2 acting either as a chaperon or as a subunit of a heteromeric channel. These results give us some new insights on the combined roles of these vomeronasal transduction ion channels.

### **Poster 77**

**Presenter:** Moine Fabian

**Affiliation:** Department of Pharmacology and Toxicology, UNIL, Rue du Bugnon 27, 1005 Lausanne

## **Deciphering the danger signaling pathway in the mouse Grueneberg ganglion neurons**

Fabian Moine, Julien Brechbühl, Marie-Christine Broillet

Mammals use different strategies to detect the chemicals present in their environment. The mouse olfactory system detect a large variety of odorants and pheromones and is subdivided in four subsystems, which are the main olfactory epithelium (MOE), the vomeronasal organ (VNO), the septal organ (SO) and the Grueneberg ganglion (GG). The GG, on which we focus our studies, has been proposed to mediate the detection of alarm pheromones (APs) in mice. Recently, we have been able to identify the precise chemical structure of one mouse AP; the SBT (2-sec-butyl-thiazoline). Since then, we have also identified new ligands activating the mouse GG neurons that are chemically related to the identified mouse AP. They share similar features with the sulfur-containing volatiles released by the predating carnivores. Calcium imaging experiments helped us to determine the molecular receptive range of GG neurons, allowing further comparisons with the chemical properties of neurons expressed in other olfactory subsystems. We are now trying to identify the source of APs production and using molecular and immunohistochemical

techniques, we are now also trying to decipher which signaling elements are implicated in the APs transduction pathway of mouse GG neurons.

**Poster 54**

**Presenter:** De Araujo Salgado Isabel

**Affiliation:** University of Fribourg

**Cortical circuits matching body metabolic signals and behavior**

Isabel De Araujo Salgado and Christophe Lamy

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 glucoprivation by an i.p. administration of 2-deoxyglucose (2DG) decreased anxiety-like traits and compulsion behaviors in mice. This metabolic challenge also induced c-fos expression in a subpopulation of cells in IC, suggesting a putative link between IC metabolic-responsive neurons and behavior. To investigate the underlying cellular mechanisms we performed experiments on acute cortical slices. Whole-cell electrophysiological recordings further evidenced a set of neurons located in deep IC layers 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.

**Poster: 40- Poster selected for a Blitz oral presentation**

**Presenter:** van der Kooij Michael

**Affiliation:** EPFL, School of Life Sciences - Brain Mind Institute, Laboratory of Behavioral Genetics, Station 19, 1015 Lausanne

**Social ranking depends on anxiety-profile and is controlled by the mesolimbic midbrain**

Michael A. van der Kooij, Fiona Hollis, Laura Lozano, Carmen Sandi

In a social hierarchy individuals are ranked based on competitive performance. Limited knowledge is available on the determinants for social ranking which is surprising seeing that the outcome of competitive encounters has important consequences for future behavioral interactions and health. Using adult male rats, we investigated the role of trait anxiety in social dominance. The anxiety-profiles were identified based on behavior in the elevated plus maze. Animals were pair-wise matched for body-weight but differed in anxiety-profile (high- vs. low-anxiety). Pairs were introduced to a novel/neutral cage after which animals display offensive behavior; social dominance was determined by summarizing the durations of offensive behavior. High-anxious individuals predominantly ended up subordinate during social encounters. Peripheral pretreatment with the anxiolytic benzodiazepine diazepam reduced anxiety and enhanced competitive behavior for high-anxious rats. The peripheral effects of diazepam on anxiety and social dominance were recapitulated by intra-VTA (ventral tegmental area) micro-infusion. Moreover, the effects of diazepam on social

dominance in high-anxious rats were subject to the participation of the nucleus accumbens (NAc). Intra-NAc micro-infusion of a D1-agonist enhanced social dominance (whereas a D2-agonist lacked behavioral effect). The effects of intra-VTA Diazepam on social dominance were blocked by intra-NAc-shell pretreatment with a D1-antagonist. Our results highlight the personality trait anxiety in social hierarchies and emphasize mesolimbic dopaminergic mechanisms for the mediation of these effects. We suggest that anxiolytics acting on the mesolimbic dopamine system could reduce the predisposition for a low rank in highly anxious individuals.

**Poster: 68**

**Presenter:** Mutel Sophie

**Affiliation:** DNF

**Influence of background strain on gender-dependent anxiety-like behavior of 5HT1a-receptor knockout mice**

Sophie Mutel, Christine Fülling, Alessandro Cumbo, Jean-Pierre Hornung

Constitutive serotonin 1a receptor (5HT1aR) knockout (KO) mice of different background strains all exhibit higher levels of anxiety-related behavior in common conflict tests such as the elevated plus maze (EPM) and open field (OF). Published data on gender differences of 5HT1aR KO mice, however, has provided less uniform results. While 5HT1aR KO mice bred on C57BL6 and 129/SV background show a gender difference on anxiety-related measures in the OF, no such difference is seen in the EPM (129/SV) or forced swim test (C57BL6). Hence, it is our aim to further analyze the differences between male and female 5HT1aR KO mice not only on behavioral, but also on morphological and molecular levels.

We found that male 5HT1aR KO mice on a 129/SV/C57BL6 mixed background show an increased arborization of oblique dendrites in the hippocampus when compared to WT mice, whereas female KO mice lack such an increase. We are now running behavioral experiments such as memory and learning tasks as well as open field and EPM to reveal some behavioral consequences of different amounts of arborization within the *stratum radiatum* of the CA1 region of the hippocampus.

In addition, we are investigating the impact of the background strain of 5HT1aR KO mice on gender differences by further back-crossing our animals onto the C57BL6 background. Up to date we are analyzing the behavior of the third generation of back-crossing.

**Poster 6**

**Presenter:** Fan Jui-Lin

**Affiliation:** Institute of Sport Sciences and Department of Physiology, UNIL, Rue du Bugnon 7, 1005 Lausanne

**AltitudeOmics: The effect of high altitude ascent and acclimatisation on cerebral blood flow regulation**

Jui-Lin Fan, Andrew W. Subudhi, Oghenero Evero, Nicolas Bourdillon, Bengt Kayser, Colleen G. Julian, Ronney B. Panerai, Andrew T. Lovering, Robert C. Roach

Adequate oxygen supply to the brain is critical to maintain brain function. Hypoxia presents a unique challenge in maintaining sufficient cerebral oxygen delivery (DO<sub>2</sub>). We assessed by

ultrasound cerebral blood flow (CBF: internal carotid, vertebral arteries and middle cerebral artery velocity [MCAv]) and arterial blood pressure (index of cerebral autoregulation; CA) during rest and hypercapnic breathing (MCAv-CO<sub>2</sub> slope; index of cerebrovascular function) in 21 healthy subjects at sea-level (SL) and upon arrival at 5260m (ALT1) and after 16 days of acclimatisation (ALT16). Cerebral DO<sub>2</sub> was calculated as the product of arterial oxygen content (CaO<sub>2</sub>) and flow in each respective artery and summed to estimate global CBF. Global CBF increased ~70% upon arrival at ALT1 (P<0.05) and returned to SL values at ALT16 as a result of changes in cerebral vascular resistance. A reciprocal pattern in CaO<sub>2</sub> maintained global cerebral DO<sub>2</sub> across acclimatisation. MCAv-CO<sub>2</sub> slope was elevated by ~79% upon arrival at ALT1 and further increased by ~89% at ALT16 (P<0.05). Indexes of CA were reduced upon arrival at ALT1 (P<0.05), but did not change with acclimatisation at ALT16 (P>0.10). Cerebral DO<sub>2</sub> was well maintained upon acute exposure and acclimatisation to hypoxia. Cerebrovascular function was enhanced with acclimatisation to high altitude, but these changes did not mitigate the reduction in CA associated with hypoxic exposure.

**Poster: 46**

**Presenter:** Samara Chrysanthi

**Affiliation:** Department of Medical Genetics, UNIL, Rue du Bugnon 27, 1005 Lausanne

**Neuropeptide Y in demyelinating neuropathies of the Peripheral Nervous System (PNS)**

Chrysanthi Samara\*, Poirot Olivier\*, Prunotto Andrea, Verdier Valerie, Bergmann Sven, Chrast Roman

\*These authors contributed equally to the work

Neuropeptide Y (NPY) is a peptidergic neurotransmitter that is implicated in pain modulation in the nervous system. After peripheral nerve injury NPY levels are increased in dorsal root ganglion neurons. It has been suggested that NPY upregulation is directly driven by axonal injury. Our results in genetic mouse models of peripheral demyelinating neuropathy further suggest that impairment of myelinating Schwann cells may be sufficient to trigger NPY expression in associated sensory neurons. Moreover, Schwann cell physiology may be also reciprocally affected by neuronal NPY production. Our observations indicate that NPY plays a critical role in sensory neuron-Schwann cell communication.

**Poster: 64**

**Presenter:** Castillo Ximena

**Affiliation:** DNC, CHUV, Bugnon 46, 1011 Lausanne (1)

Physiology Department, UNIL. Bugnon 7, 1005 Lausanne (2)

**Monocarboxylates neuroprotection in cerebral ischemia**

Ximena Castillo(1), Katia Rosafio(2), Luc Pellerin(2) and Lorenz Hirt(1).

Brain energy metabolism is a complex compartmentalized process. Although glucose is the brain energy substrate by excellence, other metabolic intermediates like lactate, pyruvate, glutamate, glutamine or acetate have been shown to be oxidized for energy production (Zielke et al., 2009). We have previously shown that L-lactate administered during reperfusion exerts long lasting protection in mice against the ischemic damage after transient middle cerebral artery occlusion (tMCAO) (Berthet et al., 2009; 2012). New



evidence has risen concerning the presence and possible involvement of the Hydroxy-Carboxylic Acid Receptor-1 (HCA1) in nervous system effects of lactate (Bergersen et al., 2013; Bozzo et al., 2013). Considering this, the objective of the present work is to elucidate whether the neuroprotective effects of lactate are exerted following its metabolism or acting through the HCA1 receptor. We used in vitro, in vivo and protein expression analysis approach to analyze the effect exerted by different monocarboxylates after oxygen-glucose deprivation or tMCAO. The primary endpoints were cell survival 48 hours after the insult, lesion size and neurological performance. In vitro, the administration of lactate (L and D isoforms), pyruvate and the HCA-1 receptor agonist 3-5 DHBA improved cell survival, while the administration of acetate and glucose did not. In vivo, the administration of D- Lactate also reduced lesion size and improved the neurological performance. Preliminary results indicate HCA1 expression is increased 24h after tMCAO, in the structures surrounding the lesion. Further experiments using HCA1  $-/-$  mice are needed to confirm the physiological relevance of these findings.

## TOPIC 2

### CELLULAR AND MOLECULAR NEUROSCIENCE

**Poster: 19**

**Presenter:** Pellegrini Chiara

**Affiliation:** DNF

#### **Modulation of molecular substrates of thalamic sleep rhythms through synaptic NMDA receptors**

Chiara Pellegrini, Anita Lüthi and Simone Astori

Thalamic circuits are reliable and stereotypic pacemakers for the generation of certain sleep rhythms. Yet, it is well known that sleep oscillations vary in intensity, not only in time, but also locally and globally in the brain due to circadian, homeostatic and use-dependent regulatory influences. Here, we asked: are thalamic pacemaker cells rigid elements in sleep rhythmogenesis or do they undergo activity-dependent modifications. Burst discharge in thalamic neurons through T-type calcium channels (CaV3) is a well-described molecular mechanism underlying sleep rhythm generation, therefore, we set out to explore regulation of CaV3 signaling through glutamatergic synaptic inputs.

Mimicking elevated thalamocortical synaptic activity by enhancing ambient glutamate levels with TBOA resulted in increased CaV3-currents in cells of the nucleus Reticularis thalami (nRt) ( $60 \pm 13\%$  above baseline,  $n=5$ ,  $p < 0.05$ ), as measured in patch-clamp recordings in acute slices. This facilitation was mimicked by brief bath-application of NMDA ( $102 \pm 22\%$ ,  $n=8$ ,  $p < 0.01$ ) and largely suppressed by blockade of GluN2C-NMDARs with PPDA ( $11 \pm 4\%$ ,  $n=7$ ,  $p > 0.05$ ). Surprisingly, CaV3-current increase was absent in CaV3.2<sup>-/-</sup> mice ( $6 \pm 5\%$ ,  $n=10$ ,  $p > 0.05$ ), but not in CaV3.3<sup>-/-</sup> mice ( $103 \pm 16\%$ ,  $n=5$ ,  $p < 0.01$ ). This suggests a glutamatergic mechanism recruiting CaV3.2 channels through activation of GluN2C-NMDARs that are expressed at both cortico-nRt and thalamo-nRt synapses. Preliminary results indicate a facilitation of CaV3-currents after repetitive photoactivation of cortical afferents (10Hz trains) ( $45 \pm 14\%$ ,  $n=4$ ,  $p < 0.05$ ), which was sensitive to PPDA ( $15 \pm 9\%$ ,  $n=4$ ,  $p > 0.05$ ). These data suggest that cortical inputs might trigger activity-dependent changes in the molecular cores of thalamic sleep rhythmogenesis.

**Poster 35**

**Presenter:** Astori Simone

**Affiliation:** DNF

#### **Time-of-day dependent changes in NMDAR subunits at intrathalamic synapses**

Simone Astori and Anita Lüthi

The sleep-wake cycle is regulated by circadian and homeostatic mechanisms setting the levels of network excitability and manifesting at synaptic sites through changes in receptor expression. NMDA receptors (NMDARs) represent a substrate of sleep-wake regulation with a recognized susceptibility to sleep loss and to wake-related neuromodulators, e.g. adenosine. Whether modifications in NMDAR transmission occur in thalamic areas and affect sleep rhythmogenesis is relatively unexplored. We previously showed that thalamic

inputs to the nucleus Reticularis thalami (nRt) express NMDAR-dependent plasticity and possess GluN2C- in addition to GluN2A- and GluN2B-NMDARs. Here, we examined GluN2 variations across the sleep-wake cycle in acute slices prepared at different times of day from 3-week-old mice (12h:12h cycle, lights on at 7AM). At the end of the light period, GluN2A and GluN2C contributions to NMDAR currents displayed a minimum and a maximum, respectively, as estimated by sensitivity to antagonists (6PM VS. 6AM: 50nM NVP-AAM077  $21.1 \pm 1.8\%$  vs.  $38.7 \pm 4.3\%$   $n=6,7$ ,  $p < 0.01$ ; 500nM PPDA  $47.8 \pm 6.4\%$  vs.  $27.4 \pm 3.8\%$ ,  $n=8,7$ ,  $p < 0.05$ ). This trend was reversed by activation of adenosine receptors (100nM CCPA, preincubated), which shifted GluN2A-C contributions to levels found in the dark phase (6PM in CCPA: NVP-AAM077  $33.5 \pm 3.0\%$ ,  $n=6$ ; PPDA  $29.0 \pm 1.7\%$ ,  $n=5$ ;  $p < 0.05$  vs. control). CCPA did not modify GluN2A-C components during the dark phase ( $p > 0.05$  vs. control), suggesting occlusion through endogenous adenosine accumulations. We also observed that GluN2C activation potentiated nRt T-currents (~2-fold increase), likely sustaining thalamic rhythmogenesis. These data suggest that GluN2 subunit switch might encode the homeostatic adjustment of thalamic excitability across the sleep-wake cycle. Supported by SwissNSF.

**Poster: 13**

**Presenter:** Deftu Alexandru

**Affiliation:** DNF

**The chemokine CXCL1 can modulate the activity of TRPV1 and K<sup>+</sup> channels**

Alexandru Deftu, Violeta Ristoiu, Isabelle Décosterd, Marc Suter

**Introduction.** CXCL1 enhances neuronal excitability by increasing Na<sup>+</sup> and K<sup>+</sup> currents. The aim of this study was to investigate the effect of CXCL1 on DRG neurons and spinal cord microglia reactivity, which could have significance in pain sensibility.

**Methods.** Adult male Wistar rats (100-150g) were sacrificed and L4, L5 and L6 DRG were dissociated and kept in culture at 37°C and 5% CO<sub>2</sub>. HEK293 cells transfected with TRPV1 and CXCR2 were maintained in similar conditions. Adult male C57BL/6 mice (20-24g) were sacrificed and lumbar spinal cord was incubated in the presence of papain for 30min at 30°C and microglia were kept in culture until experiment.

**Results.** The short term incubation (4h) with 1.5nM CXCL1 induced a significant dose-dependent reduction of TRPV1 desensitization, as shown by calcium microfluorimetry measurements in IB4(+) neurons ( $56.2 \pm 5.0 \Delta F/F_0$ ,  $n=45$  and control  $41.7 \pm 3.7 \Delta F/F_0$ ,  $n=60$ ,  $P < 0.05$   $EC_{50}=4.1nM$ ) and by patch-clamp recordings in HEK293 cells transfected with CXCR2 and TRPV1 ( $91.0 \pm 14.4pA$ ,  $n=25$  and control  $57.9 \pm 6.9pA$ ,  $n=26$ ,  $P < 0.05$ ). The acute application of 4nM CXCL1 induced a transient current in HEK293 cells stably transfected with TRPV1 ( $216.5 \pm 40.4pA$ ,  $n=13$ ) and a calcium influx in 23% of IB4(+) neurons ( $0.37 \pm 0.06 \Delta F/F_0$ ,  $n=27$ ) mediated by TRPV1. A time course of electrophysiological recordings of spinal cord microglia revealed in the first day after culture a delayed rectifier outward potassium (K<sub>dr</sub>) current that decreased after 2-4 days, during which we measured an inwardly rectifying potassium (K<sub>ir</sub>) current.

**Conclusions.** Short term incubation and acute application of CXCL1 modulated TRPV1 activity. In culture, spinal microglia presents different electrophysiological properties which changes in a time-dependent manner.

**Poster: 55**

**Presenter:** Suter Marc

**Affiliation:** Pain Center, Department of Anesthesiology, CHUV (1) and DNF (2)

**L1612P, a new paroxysmal extreme pain disorder-causing Nav1.7 mutation shows unique combination of electrophysiological and clinical properties**

Marc R Suter(1), Muriel Schaller(1), Isabelle Décosterd(1), Christian Wider(2)

Background: Paroxysmal extreme pain disorder (PEPD) is a chronic disease characterized by episodes of excruciating pain which is produced by mutations in the SCN9A gene encoding for the alpha-subunit of the Nav1.7 sodium channel. Disease-causing mutations impair the inactivation of the channel. We here describe the electrophysiological properties of a previously unknown PEPD causing mutation (L1612P) and its response to amitriptyline, a first line drug for neuropathic pain and strong sodium channel blocker as well as to cold.

Methods: Whole-cell Patch clamp recordings from HEK293 cells transfected with WT or mutant Nav1.7 plasmid was performed.

Results: The mutation induced a huge 30.9mV depolarizing shift of the steady-state fast inactivation (SSI) curve ( $V_{1/2}$  from  $-61.8 \pm 4.5$  (WT) to  $-30.9 \pm 2.2$ mV (L1612P)) with a depolarizing shift of the activation curve ( $V_{1/2}$  from  $-9.0 \pm 7.2$  (WT) to  $0.0 \pm 1.9$ mV (L1612P)). The theoretical increase in the window current was corroborated by a significant difference in ramp current ( $1.8 \pm 1.4\%$  (WT) and  $3.4 \pm 0.7\%$  (L1612P)). The L1612P channel also demonstrated a slower current decay, the absence of persistent current and a shorter repriming. Amitriptyline shifted the SSI curve to more hyperpolarized values similarly for the WT ( $-7.96$ mV) and the mutant channel ( $-8.51$ mV). Cold exposure improved recovery from inactivation in the mutated channel.

Conclusions: This newly described PEPD-causing mutation of SCN9A shows a unique combination of electrophysiological features. The hyperpolarizing shift induced by amitriptyline on SSI cannot compensate for the mutation-induced depolarizing shift. Amitriptyline showed mitigated effect in the treatment of the affected patient. AMI could nevertheless be helpful in PEPD associated with mutation causing a lesser shift in SSI.

The clinical improvement with exposure to cold could be explained by temperature effect on sodium channel properties.

**Poster: 58**

**Presenter:** Gwiazda Karolina

**Affiliation:** Department of Pharmacology and Toxicology, UNIL, Rue du Bugnon 27, 1005 Lausanne

**Role of subunits interactions in different conformational states of Acid-Sensing Ion Channel 1a**

Karolina Gwiazda and Stephan Kellenberger

Acid-sensing ion channels (ASICs) are proton-activated  $\text{Na}^+$  channels implicated in the expression of fear, pain perception and diseases associated with a tissue acidification. ASIC subunits are composed of two transmembrane domains, intracellular N- and C-termini and a large ectodomain with the sub-domains palm, thumb, finger, knuckle and  $\beta$ -ball. Three subunits form a functional channel. Here, we focused on inter-subunit interactions and their

importance for channel activity. Identified residue pairs in the crystal structure were mutated into Cys in human ASIC1a. Oxidation/reduction treatments were applied and functional consequences of knuckle- $\beta$ -ball and palm-thumb interactions of adjacent subunits were investigated. Oxidation-induced, inter-subunit disulfide bond formation between the palm and the thumb domains led to a significant decrease in current amplitude, in part due to an acidic shift of ASIC1a activation. This permanent effect was only reversed by the treatment with a reducing agent. This indicates that disulfide bond formation between palm domain of one subunit and the thumb of another favors the closed state of ASIC1a. Another relevant inter-subunit interaction was identified between residues of the knuckle and the  $\beta$ -ball of adjacent subunits. The corresponding ASIC1a double Cys mutant presented a leak current unlike WT ASIC1a-expressing cells, suggesting that a disulfide bond was formed during the expression phase and locked a proportion of the channels in an open conformation. This leak current was prevented by incubation in a reducing medium and was not observed in the single Cys mutants. In summary, this study identifies functionally relevant inter-subunit interactions between the knuckle and  $\beta$ -ball as well as the palm and the thumb domains of ASIC1a.

**Poster: 22**

**Presenter:** Bonifacio Gaetano

**Affiliation:** Department of Pharmacology and Toxicology, UNIL, Rue du Bugnon 27, 1005 Lausanne

**Coordinated movements occur during ASIC1a activity**

Gaetano Bonifacio, Claudia Igutti Suenaga Lelli, Stephan Kellenberger

Acid-sensing ion channels (ASICs) are H<sup>+</sup>-gated and Na<sup>+</sup>-conducting channels predominantly expressed in the nervous system. They are associated with many physiological and pathological states such as neuronal degeneration after ischemia and nociception. The crystal structure of the chicken ASIC1 isoform has been revealed in the desensitized and the open state. Functional studies indicate that protonation of key residues in the extracellular loop triggers conformational changes leading to channel opening. However the molecular mechanisms linking protonation to the opening and closing of the gate have not been clarified yet. In this study we used voltage-clamp fluorometry (VCF) to reveal activity-associated movements occurring on the different ASIC1a domains. 20 different fluorophore positions located in the thumb, palm, finger and knuckle domains and in the extracellular pore entry showed VCF signals. The timing of fluorescence changes suggests a complex sequence of movements upon pH change. When the pH of the extracellular solution was lowered to activate ASICs, rapid conformational changes were observed in the thumb, finger, knuckle and extracellular pore entry, followed by slower movements in the palm. The kinetics of fluorescence and current signals were compared to each other in order to assess whether the timing of the fluorescence signal corresponded to an effective channel transition. Some of the residues tested were found to be closely related to channel desensitization or recovery from desensitization. Moreover we demonstrated an outward movement of the finger domain relatively to the  $\beta$ -ball. This is the first extensive analysis of activity-dependent conformational changes in ASICs which sheds new light on the gating mechanisms of this channel.

**Poster: 34****Presenter:** Alijevic Omar**Affiliation:** Department of Pharmacology and Toxicology, UNIL, Rue du Bugnon 27, 1005 Lausanne**Subtype-specific modulation of acid-sensing ion channel (ASIC) function by 2-guanidine-4-methylquinazoline.**

Omar Alijevic and Stephan Kellenberger

Acid-sensing ion channels (ASICs) are neuronal Na<sup>(+)</sup>-selective channels that are transiently activated by extracellular acidification. ASICs are involved in fear and anxiety, learning, neurodegeneration after ischemic stroke, and pain sensation. The small molecule 2-guanidine-4-methylquinazoline (GMQ) was recently shown to open ASIC3 at physiological pH. We have investigated the mechanisms underlying this effect and the possibility that GMQ may alter the function of other ASICs besides ASIC3. GMQ shifts the pH dependence of activation to more acidic pH in ASIC1a and ASIC1b, whereas in ASIC3 this shift goes in the opposite direction and is accompanied by a decrease in its steepness. GMQ also induces an acidic shift of the pH dependence of inactivation of ASIC1a, -1b, -2a, and -3. As a consequence, the activation and inactivation curves of ASIC3 but not other ASICs overlap in the presence of GMQ at pH 7.4, thereby creating a window current. At concentrations >1 mM, GMQ decreases maximal peak currents by reducing the unitary current amplitude. Mutation of residue Glu-79 in the palm domain of ASIC3, previously shown to be critical for channel opening by GMQ, disrupted the GMQ effects on inactivation but not activation. This suggests that this residue is involved in the consequences of GMQ binding rather than in the binding interaction itself. This study describes the mechanisms underlying the effects of a novel class of ligands that modulate the function of all ASICs as well as activate ASIC3 at physiological pH.

**Poster: 72****Presenter:** Trendafilov Viktor**Affiliation:** Department of Pharmacology and Toxicology, UNIL, Rue du Bugnon 27, 1005 Lausanne**ASIC3 modulation by extracellular calcium**

Viktor Trendafilov, Omar Alijevic, Stephan Kellenberger

The acid-sensing ion channels (ASICs) are voltage-independent cation-selective channels, which are expressed in the central and the peripheral nervous system and are activated by extracellular acidic pH changes. They play a role in different physiological processes, such as fear-related behaviors, pain sensation, learning and memory, and are also involved in various pathological conditions, namely epilepsy and neurodegeneration after brain ischemia. The activity of ASICs is modulated by several ions and compounds other than protons, as for example the extracellular calcium.

It had been hypothesized that calcium blocks the ASIC3 pore and that this binding is disrupted by acidification, opening thereby the channel. The aim of this project was to determine whether calcium may modulate ASIC3 function not by pore block, but by shifting the pH dependence of activation and desensitization.



We observed strong changes in pH dependence when the extracellular calcium concentration was varied. Calcium may therefore modulate ASIC3 function by affecting its pH dependence. We found however also evidence of a partial pore block by calcium. This indicates that extracellular calcium may have two different binding sites, as well as two different modes of action, namely channel gating or pore block. Identification of the calcium binding site(s) will be necessary to determine the relative importance of these two mechanisms.

In conclusion, extracellular calcium is an important ASIC3 modulator, whose precise mechanism of action has still to be elucidated.

**Poster: 24**

**Presenter:** Morey Czuee

**Affiliation:** DNF

**Role of Munc18c in SNARE-mediated exocytosis**

Czuee Morey, Dirk Fasshauer

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. Munc18c, a close homolog of Munc18a, 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: 42**

**Presenter:** Varoqueaux Frédérique

**Affiliation:** DNF

**Evolution of the neurosecretion machinery in the placozoan *Trichoplax adhaerens***

Frédérique Varoqueaux, Carolyn L. Smith, Maike Kittelmann, Benjamin Cooper, Michael Eitel, Bernd Schierwater, Thomas S. Reese, Dirk Fasshauer

Neurons are highly specialized for fast information transfer, which takes place in the form of vesicular neurotransmitter release at specialized junctions, the chemical synapses. Synapses evolved early in animal evolution, and relatively primitive 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.

We previously found out 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 further investigate the development of the neuronal secretory apparatus by studying the placozoan *Trichoplax adhaerens*, an organism positioned near the root of the animal tree. The animal expresses landmark molecular components of the nervous system of bilaterians, yet lacks typical muscle cells and neurons.

We report a well-organized body plan and two new cell types, lipophils and crystal cells. Lipophils are prevalent cells of the ventral epithelium projecting deep into the interior where they alternate with regularly arrayed star-shaped fiber cells. The sparse crystal cells are distributed at the dorsal fringe of the animal. Gland cells, embedded in the ventral epithelium, express several proteins typical of synapses. A subset of them located at the periphery, also express an FMRamide-like neuropeptide. Together with fiber cells, whose tapered branches connect to most cell types, crystal cells and gland cells could constitute the premise of a nervous system that controls the locomotor and feeding behavior in *Trichoplax*.

**Poster: 59**

**Presenter:** Kienle Nickias

**Affiliation:** DNF

### **Insights into the evolutionary history of NSF**

Nickias Kienle, Tobias H. Klöpfer, Dirk Fasshauer

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 Ca<sup>2+</sup>-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, VCPI, PEX1, PEX6, SPAF, SPAFI, 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: 63**

**Presenter:** Srivastava Nicee

**Affiliation:** DNF

### **Understanding Molecular Evolution of Vesicular Trafficking proteins by using Multiple Sequence Information**

Nicee Srivastava, Nickias Kienle, Tobias H Klöpper, Dirk Fasshauer

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: 75**

**Presenter:** Chen Xiong

**Affiliation:** DNF

### **Speeding up SNARE complex formation through modulation of (t-) SNARE acceptor complexes**

Xiong Chen, Dirk Fasshauer

Assembly of SNARE proteins is thought to be the driving force for fusion of a transport vesicle with its target membrane. Best studied is the exocytosis of synaptic vesicles driven by the SNAREs syntaxin 1, SNAP-25, and synaptobrevin. Neuronal exocytosis happens at a millisecond time scale, whereas SNARE-mediated fusion of liposomes requires hours for completion. In an earlier study, we had found that liposome fusion is drastically accelerated when a syntaxin/SNAP-25 acceptor complex bound to a C-terminal peptide of synaptobrevin is used. Apparently, the modulated acceptor complex prevented the formation of an off-

pathway t-SNARE complex. The speed of exocytosis in yeast is thought to be slower than neurotransmitter release. The involved SNARE complex is composed of the homologous proteins Sso1, Sec9, and Snc2. In vitro, they assemble with comparable speed as the neuronal proteins. During their interaction, a stable t-SNARE complex in 1:1 stoichiometry is formed, but binding of Snc2 is relatively slow. We have now modulated the yeast t-SNARE complex by incorporating a C-terminal Snc2 peptide. We found that the modulated t-SNARE complex accelerated the formation of the ternary SNARE complex drastically, comparable to the effect observed for the neuronal SNAREs. This suggests that modulated t-SNARE complexes may imitate better the structure of the true reaction intermediate during SNARE complex formation.

## TOPIC 3

### NEUROSCIENCE OF ASTROCYTES

**Poster: 4**

**Presenter:** Rocher Anne-Bérengère

**Affiliation:** DNF

**Implication of astrocytes in a mouse model of spontaneous oscillatory neuronal activity**

Anne-Bérengère Rocher, Jean-Yves Chatton

Astrocyte implication in neuronal activity has mostly been evidenced using electrical or pharmacological stimulation to evoke neuronal activity. Whether similar interactions are found in the context of physiological neuronal activity needs to be considered. We implemented an acute slice mouse model displaying spontaneous oscillatory activity of layer 3 entorhinal neurons to study electrophysiological changes in astrocytes arising from this neuronal activity. We simultaneously recorded the membrane potential of individual astrocytes and neuronal network activity using whole-cell patch-clamp and field potential, respectively. Oscillatory neuronal activity consisted in <0.1Hz alternating Up- and Down-states. Astrocytes displayed ~2mV depolarizations during Up-states matching neuronal Up-states in frequency, duration, and amplitude. Both activities were modulated in frequency and amplitude by carbachol and abolished by CNQX or TTX application. The nature of ion movements involved in astrocytic depolarization was then investigated. The contribution of electrogenic glial glutamate uptake was tested by applying the specific inhibitor TFB-TBOA (100nM). Under glutamate transporter inhibition, neuronal Up-states increased in frequency; however synchronized astrocytic depolarizations were maintained. K<sup>+</sup> uptake was then blocked using the astrocytic K<sup>+</sup> inward rectifying channel blocker Ba<sup>2+</sup>. Under these conditions, both neuronal and astrocyte oscillatory activities disappeared. These experiments underline the ability of astrocytes to keep neuronal activity under control by monitoring and regulating the extracellular space homeostasis. The spatial and temporal aspects of these events as well as the extent of glial syncytium participation will be further investigated.

**Poster: 5**

**Presenter:** Rimmele Theresa

**Affiliation:** DNF

**Effect of extracellular potassium changes on glutamate transport induced intracellular potassium responses**

Theresa Rimmele and Jean-Yves Chatton

Astrocytes exert two important mechanisms for the regulation of the extracellular space in the neuronal network: buffering and redistribution of extracellular potassium (K<sub>o</sub>), which significantly increases during the repolarization of neurons, as well as uptake of glutamate, which is released by neurons in the synaptic cleft during synaptic activity. Glutamate

transporter in astrocytes are driven by an Na<sup>+</sup> inward gradient and in each transport cycle for one glutamate three Na<sup>+</sup> and one H<sup>+</sup> are exchanged against one K<sup>+</sup>.

We first investigated the dependency of intracellular potassium concentration (K<sub>i</sub>) levels on K<sub>o</sub> changes as both are expected to be tightly coupled due to the high K<sup>+</sup> conductance of the astrocytic membrane. Because glutamate transport involves the counter-transport of one K<sup>+</sup> from the cytosol, we investigated whether this activity impacted on K<sub>i</sub> as well. In order to evaluate the impact of these two counter directed K<sup>+</sup> ion movements in astrocytes we combined both applications. Therefore we were applying the recently developed K<sup>+</sup> sensitive fluorescent probe Asante Potassium Green-1 (APG-1) allowed us to monitor K<sub>i</sub> changes for the first time using a fluorescent probe.

To investigate the dependency of K<sub>i</sub> levels on K<sub>o</sub> we switched K<sub>o</sub> from 3, to 5.4, 10 and 15mM. We could monitor an increase in K<sub>i</sub>, showing that K<sub>i</sub> is proportionally depending on K<sub>o</sub>. In a next step we could show that glutamate superfusion (200 μM) caused a reversible drop of K<sub>i</sub> that is depending on the glutamate concentration.

Overall the results indicate that APG-1 is a powerful tool to follow K<sub>i</sub> fluctuations in astrocytes, influenced by K<sub>o</sub> changes as well as glutamate transport, which are both modulating together the direction and the intensity of K<sup>+</sup> ion flux.

**Poster: 21**

**Presenter:** Rosafio Katia

**Affiliation:** Département de Physiologie, Rue du Bugnon 7, UNIL, 1005 Lausanne

**Oxygen Tension Controls the Expression of the Monocarboxylate Transporter MCT4 in Cultured Mouse Cortical Astrocytes via a Hypoxia-Inducible Factor-1a-Mediated Transcriptional Regulation**

Katia Rosafio and Luc Pellerin

The monocarboxylate transporter MCT4 is a high capacity carrier important for lactate release from highly glycolytic cells. In the central nervous system, MCT4 is predominantly expressed by astrocytes. Surprisingly, MCT4 expression in cultured astrocytes is low, suggesting that a physiological characteristic, not met in culture conditions, is necessary. Here we demonstrate that reducing oxygen concentration from 21% to either 1 or 0% restored in a concentration-dependent manner the expression of MCT4 at the mRNA and protein levels in cultured astrocytes. This effect was specific for MCT4 since the expression of MCT1, the other astrocytic monocarboxylate transporter present in vitro, was not altered in such conditions. MCT4 expression was shown to be controlled by the transcription factor hypoxia-inducible factor-1 (HIF-1) since under low oxygen levels, transfecting astrocyte cultures with a siRNA targeting HIF-1 largely prevented MCT4 induction. Moreover, the prolyl hydroxylase inhibitor dimethyloxalylglycine (DMOG) induced MCT4 expression in astrocytes cultured in presence of 21% oxygen. In parallel, glycolytic activity was enhanced by exposure to 1% oxygen as demonstrated by the increased lactate release, an effect dependent on MCT4 expression. Finally, MCT4 expression was found to be necessary for astrocyte survival when exposed for a prolonged period to 1% oxygen. These data suggest that a major determinant of astrocyte MCT4 expression in vivo is likely the oxygen tension. This could be relevant in areas of high neuronal activity and oxygen consumption, favoring astrocytic lactate supply to neurons. Moreover, it could also play an important role for neuronal recovery after an ischemic episode. *GLIA* 2014;62:477-490

**Poster: 32**

**Presenter:** Buscemi Lara

**Affiliation:** DNF

**Homer1 proteins and the tuning of astrocytic calcium signaling pathways**

Lara Buscemi, Vanessa Ginet, Francesco Petrelli, Corrado Cali, Paola Spagnuolo, Carlo Sala, Anita Truttmann, Graham Knott, Lorenz Hirt, Julien Puyal and Paola Bezzi

Astrocytes sense and transduce neuronal activity through GPCR-mediated increases in their intracellular Ca<sup>2+</sup> concentration. Although this form of glial excitability has been thoroughly described, its pathophysiological implications are still poorly understood. It has been recently shown that cultured astrocytes possess sub-plasma membrane Ca<sup>2+</sup>-microdomains that control exocytosis of astrocytic glutamate in response to mGluR5 activation and that, in dendritic spines, Homer1b/c (H1b/c) provides a molecular link between IP3Rs on the endoplasmic reticulum and mGluRs on the plasmalemma and controls Ca<sup>2+</sup> signaling. Following these leads, we investigated whether Homer1 proteins could be involved in mGluR-IP3R coupling in astrocytes. We found that astrocytes express H1b/c and negligible levels of Homer1a (H1a), a shorter activity-dependent Homer1 form that competitively prevents mGluR-IP3R interaction. As in glutamatergic spines a stress-induced differential expression of long and short Homer1 forms can significantly alter structural and functional Ca<sup>2+</sup> domains, we used a model of rat neonatal ischemia to study astrocytic Homer1 expression in normal and inflammatory conditions. At 24h post-ischemia we observed decreased H1b/c expression together with increased H1a expression. This shift favoring the short form was more manifest 7 days post-insult, with reactive astrocytes showing very high levels of H1a and mGluR5. Finally, we found that ischemia and H1a overexpression strongly attenuated mGluR5-induced astrocytic Ca<sup>2+</sup> mobilization. The uncoupling of plasmalemmal mGluRs from Ca<sup>2+</sup>-intracellular signaling could be used by astrocytes as a mechanism to tune down the exacerbated glutamatergic response observed after ischemia, thereby protecting the tissue from excessive cell death.



## TOPIC 4

### DEVELOPMENTAL AND STEM CELL NEUROSCIENCE

**Poster: 2 - Poster selected for a Blitz oral presentation**

**Presenter:** Stoppini Luc

**Affiliation:** Hepia, University of Applied Sciences, 4, rue de la Prairie, 1202 Genève (1)  
University of Geneva (2)  
Swiss Center of Applied Human Toxicology (SCAHT) (3)  
Département de Physiologie, UNIL (4)

**3D engineered nervous tissues derived from human embryonic stem cells and iPS cells as in vitro models for neurotoxicity studies**

L. Stoppini(1,3), Adrien Roux (1,3), A. Sandoz (1), I. Charvet (1,2); KH Krause (2), Jenny Sandstrom (3,4); Florianne Tschudi-Monnet (3,4).

The aim of our work is to develop 3D stable and long term in vitro neural cultures that may be used as a model for in vitro neurotoxicity testing. Human stem cells (H9, CH6, iP), were differentiated to generate neuroprogenitor cells. Functional 3D neural networks were examined after up to 2 months in culture. To characterize the models we measured changes in gene- and protein expression of neural markers by real-time PCR and western blotting, as well as morphological analyses and extracellular recordings of electrophysiological activities. 3D cultures expressed markers of both mature neurons and astrocytes, and intricate neurite networks were observed in 2D cultures. Preliminary experiments on two-month old human 3D histotypic tissues, treated for 24-48 hours with either methylmercury or trimethyltin chloride (10 nM-20 microM for both molecules), showed decreased gene expression of the neuronal markers, beta-3 tubulin, synapsin-1, NeuN, as well as increased level of the glial marker GFAP. This is indicative for neuronal failure and astrogliosis. Further developing of these human in vitro models could enable screenings of toxicological profiles of chemicals or of new drug candidates.

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**Poster: 25 - Poster selected for a Blitz oral presentation**

**Presenter:** Bariselli Sebastiano

**Affiliation:** Department of Basic Neurosciences, Medical Faculty, University of Geneva, 1 rue Michel Servet, 1211 Geneva

**Down-regulation of Shank3 in the VTA impairs postnatal maturation of excitatory synapses onto DA neurons.**

Sebastiano Bariselli, Eoin Cornelius O'Connor, Chiara Verpelli, Carlo Sala, Christian Lüscher, Camilla Bellone

Shank3 is a scaffolding protein of the postsynaptic density that plays a critical role in orchestrating glutamatergic receptors at the synapse. Functionally, Shank3 links group I mGluRs to NMDARs and AMPARs through its interaction with Homer proteins and it is important in regulating synaptic transmission and plasticity. In the ventral tegmental area

(VTA), mGluR1 function is required for driving postnatal maturation of AMPARs and NMDARs but the role of Shank3 is unknown. Here we show that in vitro manipulation of Shank-Homer interaction impairs mGluR1-driven switch of NMDAR subunit composition at excitatory synapses onto dopamine (DA) neurons of the VTA. In order to evaluate the role of Shank3 in the VTA in vivo, we knocked down all the major isoforms of the gene in neonatal mice. We performed whole cell patch clamp technique of DA neurons on acute midbrain slices and characterized excitatory transmission at juvenile synapses. We observed that the absence of Shank3 during critical period of development disrupts postnatal maturation of AMPARs and NMDARs. Haploinsufficiency of Shank3 in human is one of the most prevalent causes of autism spectrum disorders (ASDs), neurodevelopmental pathologies characterized by impaired social interactions. Since knocked down of Shank3 specifically perturbs the postnatal development of DA neurons, our manipulation would possibly lead to impairment in social behaviours.

**Poster: 11**

**Presenter:** Sultan Sebastien

**Affiliation:** DNF

**Astrocytes locally control the formation of dendritic spines on adult-born hippocampal neurons**

Sebastien Sultan, Liyi Li, Jonathan Moss, Francesco Petrelli, Elias Gebara, Paola Bezzi, Josef Bischofberger, Nicolas Toni

Adult hippocampal neurogenesis results in the continuous formation of neurons that functionally integrate into pre-existing networks and participate to mechanisms of learning. The mechanisms regulating the synaptic integration of adult-born neurons are still poorly known. Here, we show that blocking exocytosis from astrocytes in the adult brain inhibited the formation, maturation and function of spine synapses on adult-born hippocampal neurons, without interfering with pre-existing synapses. Interestingly, the role of astrocytes is limited to their territory, since dendritic segments that extended outside blocked astrocytes formed normal spines. This effect may be mediated by D-Serine, as extracellular D-Serine concentration was reduced in animals with impaired astrocytic function and D-Serine administration restored dendritic spine density on new neurons. Thus, astrocytes locally control synapse formation on adult-born neurons in their territories. These results reveal a novel role for astrocytes in the adult brain, relevant to adult neurogenesis and mechanisms of neuronal network formation.

**Poster: 12**

**Presenter:** Gebara Elias

**Affiliation:** DNF

**Adult hippocampal neurogenesis inversely correlates with microglia in physiological conditions and Taurine treatment**

Elias Gebara, Sebastien Sultan\*, Florian Udry\*, Jacqueline Kocher-Braissant and Nicolas Toni

\*These authors contributed equally to this work

Adult hippocampal neurogenesis results in the formation of new neurons and is a process of brain plasticity involved in learning and memory. The proliferation of adult neural stem or progenitor cells is regulated by several extrinsic factors such as experience, disease or aging and intrinsic factors originating from the neurogenic niche. Microglia is very abundant in the dentate gyrus (DG) and increasing evidence indicates that these cells mediate the inflammation-induced reduction in neurogenesis. However, the role of microglia in neurogenesis in physiological conditions remains poorly understood. In this study, we monitored microglia and the proliferation of adult hippocampal stem/progenitor cells in physiological conditions known to increase or decrease adult neurogenesis, voluntary running and aging respectively. We found that the number of microglia in the DG was strongly inversely correlated with the number of stem/progenitor cells and cell proliferation in the granule cell layer. Accordingly, co-cultures of decreasing neural progenitor/glia ratio showed that microglia but not astroglia reduced the number of progenitor cells. Moreover, Taurine treatment known to prevent inflammation showed an increase of adult neurogenesis on old mice. Together, these results suggest that microglia inhibits the proliferation of neural stem/progenitor cells. Thus, inhibiting microglia leads to an increase of adult neurogenesis.

**Poster: 28**

**Presenter:** Moss Jonathan

**Affiliation:** DNF

### **The ultrastructure of adult neural stem cells in the hippocampal neurogenic niche**

Jonathan Moss and Nicolas Toni

Deep in the dentate gyrus lie the radial glia-like (RGL) stem cells; the cornerstone of a production line that supplies the hippocampus with new neurons throughout adult life. The bodies of these self-renewing stem cells sit in the subgranular zone of the dentate gyrus, but their processes stretch across the granule cell layer, squeezing through the tight gaps between mature granule cells. Once clear of the granule cell layer these processes split into finer and finer threads, forming dense webs across the molecular layer. Upon activation, RGL stem cells can divide and differentiate into new neurons, but before they do, they retract their complex processes, begging the question: what purpose do these processes serve?

We sought to answer this question by first examining the ultrastructure of RGL stem cell processes using light and electron microscopy. RGL stem cells, identified by their expression of Nestin and their distinctive morphology, were examined in Nestin-GFP transgenic mice. Their processes were then labelled using either an immunogold or an immunoperoxidase protocol. Subsequent light and electron microscopic analyses revealed that the fine processes took the form of long beaded strings, displaying regular varicosities. Branching from these varicosities were yet finer processes that wrapped around synapses in their immediate vicinity, in a similar fashion to the processes of astrocytes. Processes also appeared to wrap around nearby blood capillaries, with a pronounced thickening and higher numbers of cytosolic mitochondria at the points of their intersection.

These findings introduce the possibility that stem cell function might be controlled by an interaction between neuronal or vascular networks and the processes of the stem cell.

**Poster: 47****Presenter:** Krzisch Marine**Affiliation:** DNF**Effects of altered expression of SynCAM1, Neuroligin-1B and Neuroligin-2A on adult-born neuron integration and survival**

Marine Krzisch, Laura Jabinet, Jan Armida, Nicolas Toni

Hippocampal adult neurogenesis results in the formation of new neurons in the adult hippocampus and participates to learning. The maturation and survival of new neurons is regulated by their activity during the first month after division. Here, we tested whether enhancing synaptogenesis may increase new neurons' survival and maturation during this critical developmental phase.

We tested the effect on the integration and survival of three cell adhesion molecules, SynCAM1, Neuroligin-1B (NL1B) and Neuroligin-2A (NL2A). SynCAM1 is known to increase excitatory synaptic efficiency, whereas NL1B increases the formation of excitatory synapses. NL2A increases the formation of both excitatory and inhibitory synapses. We used a viral-mediated cell specific gene delivery approach to selectively overexpress SynCAM1, SynCAM1 dominant negative isoform (dnSynCAM1), NL1B or NL2A in adult-born hippocampal neurons in wild-type mice. We then assessed changes in neuronal survival and maturation.

SynCAM1 increased dendritic spine maturation, whereas NL2A and NL1B increased dendritic spine formation. dnSynCAM1 induced the opposite effects as SynCAM1 on spine maturation. The overexpression of SynCAM1 and NL1B had no effect on neuronal survival, whereas the overexpression of dnSynCAM1 decreased neuronal survival, suggesting that the maturation of excitatory synapses is crucial for adult-born neuron survival, but that enhancing the formation or the maturation of excitatory synapses is not sufficient to increase neuronal survival. The overexpression of NL2A increased neuronal survival, suggesting that inhibitory synaptogenesis is crucial for neuronal survival.

**Poster: 29****Presenter:** Venturini Giulia**Affiliation:** Institut de Recherche en Ophtalmologie, Sion

Hôpital Ophtalmique Jules-Gonin, Université de Lausanne, Lausanne

**Onset and progression of the retinal phenotype in the rd7 mouse model of inherited retinal degeneration**

Giulia Venturini, Désirée von Alpen and Pascal Escher

Background: The rd7 is a spontaneous mouse model of retinal degeneration caused by inactivation of the photoreceptor-specific nuclear receptor NR2E3. It manifests as retinal folding, retinal spots and late-onset retinal degeneration. Interestingly, it shows similar clinical features to a group of allelic disorders in humans, namely enhanced S-cone syndrome (ESCS) and Goldmann-Favre syndrome (GFS), whose genetic cause are recessive mutations in the NR2E3 gene. Our aim was to further characterize the onset and progression of retinal degeneration in this mouse model.

**Materials and Methods:** Nr2e3rd7/rd7 mice were mated to C57BL/6J mice. C57BL/6J Nr2e3rd7/rd7 mice were clinically examined by indirect ophthalmoscopy. Eyes were enucleated and sectioned for staining with hematoxylin and eosin.

**Results:** Fundus photography of C57BL/6J Nr2e3rd7/rd7 mice revealed that retinal spots are already present at P12, when the eyes are still closed. Histological examination of C57BL/6J Nr2e3rd7/rd7 mice at P12, P13, P18, P21 and P28 showed that retinal folding is refined to the mid-peripheral retina, but its distribution is not homogeneous between the dorsal and ventral retina. Waves and rosettes appear at P12, in concordance with the appearance of retinal spots, and reach their maximum expansion at P21.

**Conclusions:** C57BL/6J Nr2e3rd7/rd7 mice provide a good model for ESCS/GFS, showing a mid-peripheral distribution of the retinal folding and a late-onset retinal degeneration as observed in the patients.

**Poster: 33**

**Presenter:** Escher Pascal

**Affiliation:** Institut de Recherche en Ophtalmologie, Sion

Hôpital Ophtalmique Jules-Gonin, Université de Lausanne, Lausanne

**Dual function of the photoreceptor-specific nuclear receptor PNR/NR2E3 in photoreceptor development and maintenance**

Arnaud Boulling, Giulia Venturini, Désirée von Alpen and Pascal Escher

The photoreceptor-specific nuclear receptor PNR/NR2E3 is necessary for proper rod versus cone photoreceptor specification. In the murine retina, PNR/NR2E3 starts to be expressed during late embryonic development, its expression peaks at postnatal days 6-7, when rod generation is at its highest levels, and is then maintained at lower levels in adult photoreceptors. In heterologous transactivation assays, PNR/NR2E3 acted as a repressor of several cone-specific genes, but potentiated the activation of rhodopsin expression by the photoreceptor-specific transcription factors CRX (cone rod homeobox) and NRL (neural retina leucine zipper). This dual activity of PNR/NR2E3 was further assessed by ex vivo electroporations of mouse retinas. Activity of a cone- and a rod-specific promoter was assessed at different time-points in wild-type C57BL/6J retinas, and compared to that of C57BL/6J Nr2e3rd7/rd7 retinas, where PNR/NR2E3 is not expressed.

We discuss these findings in the context of a dominant and the recessive mutations in the photoreceptor-specific nuclear receptor PNR/NR2E3 that cause respectively severe retinitis pigmentosa and the clinically milder Goldmann-Favre syndrome (enhanced S-cone syndrome).

**Poster: 38**

**Presenter:** Fülling Christine

**Affiliation:** DNF

**Influence of 5HT1a-receptor on NR2B containing NMDA receptors at the synapse**

Christine Fülling, Alexandre Pinault, Anita Lüthi, Jean-Pierre Hornung

Serotonin 1a receptor knockout (5HT1aR-KO) mice are well-known for increased expression of anxiety-like behavior, a feature also found in humans exhibiting a *htr1a* promoter polymorphism. Moreover, converging evidence suggests that 5HT1aR-KO mice are also impaired in hippocampal-dependent learning tasks. In this respect, we found that the hippocampal NR2A:NR2B subunit-ratio, which is central to cognitive integrity, is subject to intact 5HT1aR expression. In organotypic hippocampal cultures inhibition of 5HT1aR by WAY100635 increased arborization in the stratum radiatum, a similar phenotype was found in slices obtained from 5HT1aR-KO mice in comparison to wild-type (WT) mice. Interestingly, inhibition of the NR2B subunit reversed the enhanced arborization of 5HT1aR-KO cultures, suggesting that 5HT1aR affects hippocampal arborization through regulation of NR2B. WT-mice initially have high levels of NR2B vs. NR2A and this subunit-ratio typically switches in favor of NR2A during the first postnatal weeks. However, we observed decreased NR2A:NR2B subunit-ratios around p30 using field potential recordings from hippocampal Schaffer collateral synapses in 5HT1aR-KO. Thus, the subunit-switch could be altered in 5HT1aR-KO, which is further supported by the overlap of the NR2A:NR2B subunit-switch and the critical period of 5HT1aR expression. During this period, 5HT1aR expression is required to prevent exaggerated anxiety-like behavior and may also underlie impaired cognitive functions. We hypothesize that the NR2A:NR2B subunit-ratio of 5HT1aR-KO mice is fundamental for its behavioral phenotype. Hence, we are exploring the mechanism by which 5HT1aR regulates cerebral NR2A:NR2B subunit-ratios leading to long-lasting changes in connectivity affecting cognitive and emotional behavior.

**Poster: 69**

**Presenter:** Pinault Alexandre

**Affiliation:** DNF

**Estrogen modulates CA1 pyramidal morphology through NR2B NMDA subunit: its contribution in the female 5HT1A-deficient mouse phenotype.**

Alexandre Pinault, Alessandro Cumbo, Silvia Pedrani, Sophie Mutel, Jean-Pierre Hornung

Our group has reported exuberant oblique dendrite growth and NR2B subunit overexpression in CA1 pyramidal neurons (CA1-PN) of in the juvenile and adult serotonin 1A receptor-deficient mice (*Htr1a*KO). In the present study, we show that this morphological phenotype is gender specific and developmentally regulated. Indeed, analyzing dendritic morphology development from P10 to P60 in wild type and *Htr1a*KO mice, the dendritic exuberance is observed only in *Htr1a*KO males whereas an adjustment of the number of branches occurs during puberty of *Htr1a*KO females. Our working hypothesis is that estrogen receptors (ERs) contribute during critical period to the morphologic phenotype observed in the *Htr1a*KO female mice and could interact with NR2B.

Estrogen modulates dendritic growth of CA1-PN in vitro in organotypic slices. This modulation occurs via GPR30, a specific ER coupled to a G-protein. Moreover, downstream inhibition of DAPK1 by GPR30, reduces the membrane attachment of NR2B subunit and thus decreases the number of dendritic branches in vitro. Since testosterone, transformed in estrogen via CYP19 aromatase, regulates brain masculinization during early postnatal development via ER, we investigate in vivo and in vitro if testosterone is involved in these morphological changes. Peripheral injection of testosterone in newborn female *Htr1a*KO

mice resulted in masculinization of BNST, but failed changing the dendritic morphology of CA1. On the contrary, subcutaneous injection of the GPR30 agonist G-1 to newborn male Htr1aKO mice reduces the dendritic morphology of CA1 pyramidal neurons in adulthood to levels found in female mice. Our current data suggest that estrogen rise occurring first at puberty in females compensate in vivo the exuberance of CA1 oblique dendrites.

**Poster: 41**

**Presenter:** Azarias Guillaume

**Affiliation:** Department of Biomedicine, University of Basel, Mattenstrasse 28, 4058 Basel

**Dissecting a spatio-temporal Rho GTPase signaling network regulating neuronal growth cone extension**

Guillaume Azarias, Maria Bagonis, Ludovico Fusco, Gaudenz Danuser and Olivier Pertz

Neuronal outgrowth determines the brain connectivity and requires a tight spatio-temporal signaling to the cytoskeleton through the Rho family GTPases. Classical studies have suggested that RhoA only activates neurite contraction, whereas Rac1 and Cdc42 allow neurite extension. However, recent live cell imaging experiments using biosensors have indicated that Rho GTPase activation occurs in transient micrometer-sized subcellular domains correlating with cell edge morphodynamics.

Using real-time fluorescence imaging of FRET-based Rho GTPase biosensors, we quantified the spatio-temporal Rho GTPase activation dynamics within neuronal growth cones at micrometer length and second time scale. Unexpectedly, we observed that all three canonical Rho GTPases are activated in the advancing growth cone in specific micrometer-sized subcellular zones. RhoA, Cdc42 and Rac1 were found activated at the tip of filopodia. Cdc42 and Rac1 activity patterns were also found in the growth cone body and at the basis of filopodia. Thus, the three canonical Rho GTPases might collaborate to fine tune growth cone cytoskeletal dynamics by placing specific effector pathway in time and space. The challenge is now to measure how the activation of the three GTPases fluctuate during the filopodium protrusion and retraction cycles that allow growth cone advance. We report on a novel computer vision approach that allows automated filopodium dynamics segmentation for quantitative analysis of our dynamic timelapse datasets. This will be used to produce a multiplexed model of the activation dynamics of the three Rho GTPases. Thus, our multidisciplinary methodology will allow resolving a complex spatio-temporal signaling network at the time and length scale on which growth cone cytoskeleton operates.

**Poster: 76**

**Presenter:** Lüchtenborg Anne-Marie

**Affiliation:** Department of Pharmacology and Toxicology, UNIL, Rue du Bugnon 27, 1005 Lausanne

**Heterotrimeric Go links Wntless signaling with Ankyrin in neurons**

Anne-Marie Lüchtenborg, Gonzalo P. Solis, Diane Egger-Adam, Chen Lin, Alexey Koval, Maxime G. Blanchard, Stephan Kellenberger, Vladimir L. Katanaev



The mechanisms of synaptic remodeling are to date only partially understood. Amongst others, wingless (Wg, Drosophila Wnt) signaling has been demonstrated to be involved in several aspects of this process. We use the advantages of the glutamatergic Drosophila neuromuscular junction (NMJ) system to gain molecular insights into the function of Wg signaling in synapse formation and stability. We identified the heterotrimeric G-protein Go as a new player in correct synapse formation, transducing the Wg-Frizzled2 signal in the presynaptic cell. Furthermore, we identified the neuronal protein Ankyrin2 (Ank2) as a direct interaction partner of the alpha subunit of Go in both biochemical and genetic assays. Giant isoforms of Ank2 have been shown to be implicated in NMJ formation but have so far been believed to be a structural component of the synapse linking cytoskeleton and plasma membrane. Here, we demonstrate that Ank2 is a direct downstream target of G $\alpha$  in the Wg signaling cascade. Our findings in Drosophila are corroborated by our study in mammalian neuronal cells which show that G $\alpha$  dependent neurite outgrowth depends on AnkyrinB and AnkyrinG. Thus, the interaction between G $\alpha$  and Ankyrin is conserved from insects to mammals and plays an important role in neurite growth and synapse formation.

**Poster: 79**

**Presenter:** Michel Kielar

**Affiliation:** Hôpital Nestlé CHUV, DNC, Service de Neuroréhabilitation et de Neuropsychologie, Av. Pierre-Decker 5, 1011 Lausanne

**Mutations in Eml1 lead to ectopic progenitors and neuronal heterotopia in mouse and human.**

Michel Kielar\*, Françoise Phan Dinh Tuy\*, Sara Bizzotto &, Cécile Lebrand &, Camino de Juan, Karine Poirier, Renske Oegema, Grazia Maria Mancini, Nadia Bahi-Buisson, Victor Borrell, Egbert Welker, Jamel Chelly, Alexandre Croquelois#, Fiona Francis#

# shared senior authorship

Neuronal migration disorders such as lissencephaly and subcortical band heterotopia are associated with epilepsy and intellectual disability. DCX, LIS1 and TUBA1A are mutated in these disorders, however corresponding mouse mutants do not show heterotopic neurons in the neocortex. On the other hand, spontaneously arisen HeCo mice display this phenotype and our study reveals that misplaced apical progenitors contribute to heterotopia formation. While HeCo neurons migrate at the same speed as wild-type, abnormally distributed dividing progenitors were found throughout the cortical wall from E13. We identified Eml1, coding for a microtubule-associated protein, as the mutant gene in HeCo mice. No full-length transcripts were identified due to a retrotransposon insertion in an intron. Eml1 knock-down mimics the HeCo progenitor phenotype, and re-expression rescues it. We further show that EML1 is mutated in ribbon-like heterotopia in human. Our data link abnormal spindle orientations, ectopic progenitors and severe heterotopia in mouse and human.

## TOPIC 5 NEUROSCIENCE COMMUNICATION

**Poster: 14**

**Presenter:** Bamba-Charrière Nonny

**Affiliation:** Freelance Scientific Translator, 1897 Le Bouveret

**The Nervous System our main translation tool**

Nonny Bamba-Charrière

A comic analogy between the Nervous System and the translator, so a non formal, out of frame poster like a veiled reference to one of the role of the NS in our body.

**Poster: 80**

**Presenter :** Romain-Daniel Gosselin

**Affiliation:** Biotelligences LLC, Av. Gratta-Paille 2, 1018 Lausanne

**Misuse of statistics in experimental neuroscience. A critical evaluation of six periodicals.**

Sara Sadozai-Slama; Romain-Daniel Gosselin

Biostatistics are inherent to biomedical research. Their correct use minimizes the incidence of false positive or false negative results and ensures experimental reproducibility. It is well described that researchers in life sciences misuse and misunderstand the fundamental concepts in biostatistics, hence a mounting concern about the rate of erroneous conclusions and irreproducible data. In this study we quantified the articles that failed to comply with official statistical guidelines in six leading neuroscience periodicals in 2014. The publications we analysed were invariably studded with statistical flaws, regardless of journal impact factor. We found that inappropriate design or analytic procedures were frequent, including failure to correct for multiple comparisons (11/46 articles; 24%), sole reliance on p-value to conclude (36/48 articles; 75%), underpowered or unknown sample size (27/48 articles; 56%) and failure to respect the assumptions for parametric tests (25/44 articles; 57%). Other frequent flaws included unknown or erroneous error bars (39/48 articles; 81%), absence of statistical paragraph (4/48 articles; 8%) and no exact p-values given (29/48 articles; 60%). These results show that neurobiologists misuse biostatistics and suggest a high occurrence of false positive and false negative results in publications. We believe that most of these flaws could be overcome by simply improving the understanding of basic notions of biostatistics in the scientific community.

Piled Higher and Deeper by Jorge Cham

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