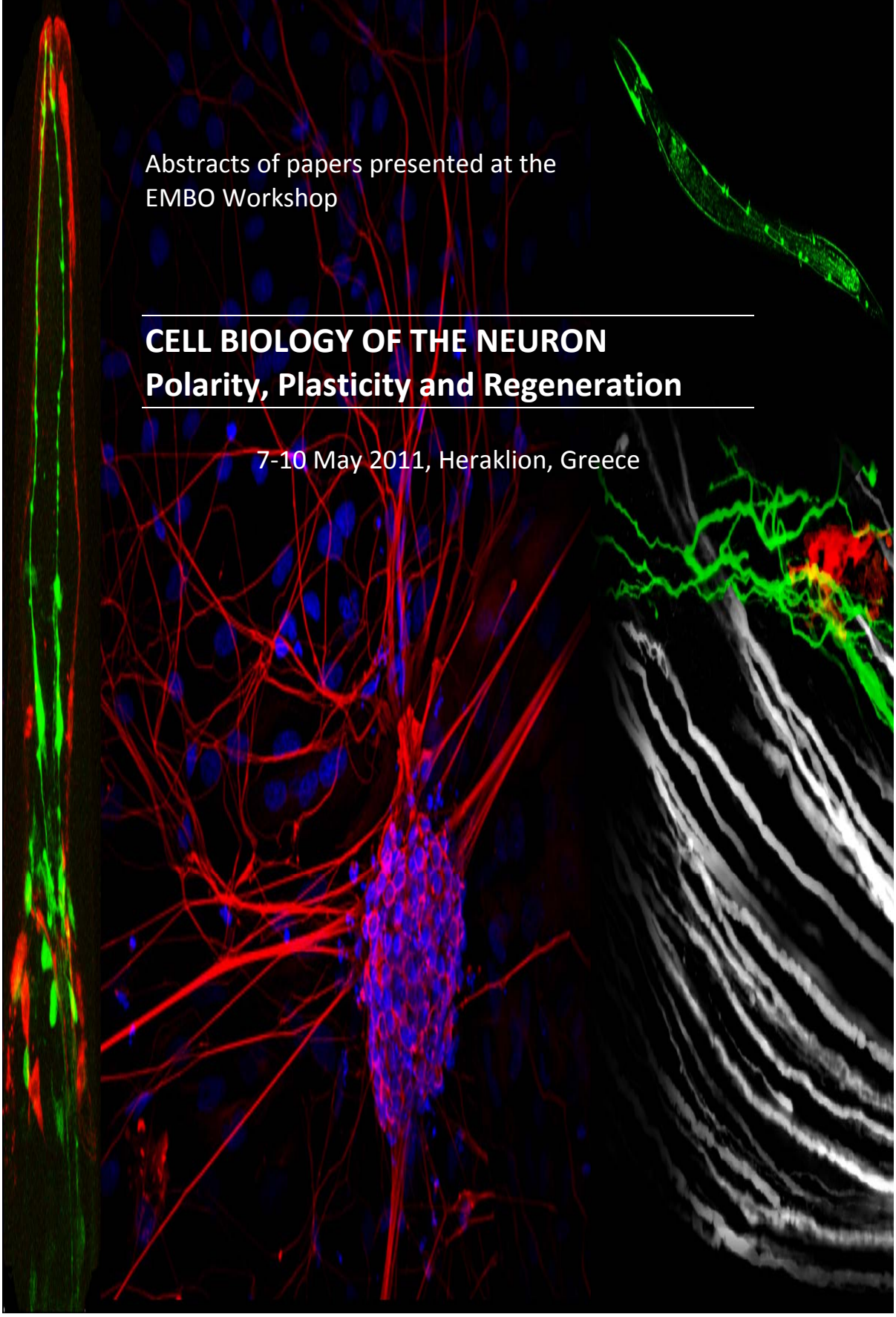

Abstracts of papers presented at the
EMBO Workshop

CELL BIOLOGY OF THE NEURON
Polarity, Plasticity and Regeneration

7-10 May 2011, Heraklion, Greece

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Organizers:

Frank Bradke, Max Planck Institute of Neurobiology, Germany
Casper Hoogenraad, Utrecht University, The Netherlands
Nektarios Tavernarakis, Institute of Molecular Biology and Biotechnology, Greece

The meeting was funded in part by **EMBO** (European Molecular Biology Organization)

CELL BIOLOGY OF THE NEURON: POLARITY, PLASTICITY AND REGENERATION

Saturday, May 7 – Tuesday, May 10, 2011

.. at a glance

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Monday	11.15	**7**	Axon Growth and Regeneration
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Poster Sessions are located in the "Game Room"

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Cell Biology of the Neuron: Polarity, Plasticity and Regeneration

Crete, May 2011

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13.00 - 14.00	*Lunch*
14.00 - 14.15	Opening Remarks
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Chairperson:	**Eckhard Gundelfinger** [Leibniz Institute of Neurobiology, Magdeburg]
14.15 - 14.45	*Molecular Mechanisms of Synapse Stabilization and Disassembly* **Graeme Davis** [University of California, San Francisco]
14.45 - 15.15	*Long Term Dynamics of Inhibitory Synapses* **Ann Marie Craig** [University of British Columbia, Vancouver]
15.15 - 15.45	*Dynamic Regulation of Synaptic Adhesion Complexes by Alternative Splicing* **Peter Scheiffele** [Biozentrum of the University of Basel]
15.45 - 16.00	*Role of Leucine-Rich Repeat Containing Proteins in Excitator Synapse Development* **Joris de Wit** [University of California San Diego]
16.00 - 16.30	*Coffee Break*
SESSION 2	**NEURONAL PLASTICITY – RECEPTOR DYNAMICS**
Chairperson:	**Thomas Schwarz** [Harvard Medical School, Boston]
16.30 - 17.00	*A Nanoscale View into the Dynamic of AMPA Receptor Organization in Synapses* **Daniel Choquet** [University of Bordeaux]
17.00 - 17.30	*Regulation of Neuronal Function and Dysfunction by Protein SUMOylation* **Jeremy Henley** [University of Bristol]
17.30 - 18.00	*Intrecellular Machinery and Signaling for AMPA Receptor Trafficking at Synapses* **Jose Esteban** [Centro de Biologia Molecular, CSIC, Madrid]

18.00 - 18.15 *Non Hyperpolarizing GABAB Receptors Regulate Neuronal Migration, and Axon/Dendrite Growth and Specification by cAMP Pathways*
Laura Cancedda [Italian Institute of Technology, Genova]

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20.00 - 21.00 Keynote Lecture 2:
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Pietro De Camilli [Yale University, New Haven]

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Catherina Becker [University of Edinburgh]

09.30 - 10.00 *Nogo-A is a Negative Regulator of Neurite Growth in the Developing and Adult Nervous System*
Martin Schwab [University of Zurich]

10.00 - 10.30 *Defining the Genetic Program of Axon Regeneration in the CNS*
Zhigang He [Harvard Medical School, Boston]

10.30 - 10.45 *Regeneration of the Adult Zebrafish Brain after Traumatic Lesion: Neurogenic Radial Glial-Type Progenitor Cells make New Neurons*
Michael Brand [Dresden University]

10.45 - 11.15 *Coffee Break*

SESSION 4 **LOCAL mRNA TRANSLATION**

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11.45 - 12.15 *Identification of Localized mRNAs in Hippocampal Neurons*
Eric Schuman [Max Planck Institute of Brain Research, Frankfurt]

12.15 - 12.45	*CYFIP1, a Neuronal elF4E-BP, Links Local Translational Regulation to Spine Remodeling Insights into Mental Retardation and Autism* **Claudia Bagni** [Vesalius Research Center VIB, Leuven]
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09.30 - 10.00	*Molecular Mechanisms Underlying Neuronal Polarization in vivo* **Franck Polleux** [The Scripps Research Institute, La Jolla]

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Kozo Kaibuchi [Nagoya University]

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Kevin Flynn [Max Planck Institute of Neurobiology, Martinsried]

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Frank B. Gertler [Koch Institute for Integrative Cancer Research at MIT]

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James Fawcett [University of Cambridge]

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14.00 - 16.15 *Coffee Break &* Poster Session III – BLUE SESSION

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Yuh-Nung Jan [University of California, San Francisco]

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Eleanor Coffey [Turku Centre for Biotechnology]

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Dietmar Schmucker [Vesalius Research Center VIB, Leuven]

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Mariano Bisbal [Université Joseph Fourier, Grenoble]

18.15 - 18.30 *Characterization of Voltage and Temperature Dependent Gating of Channelrhodopsin 2, and Application in a Dispersed Hippocampal Culture Paradigm*
Thomas Chater [University of Bristol]

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Nikos Kourtis [Institute of Molecular Biology and Biotechnology – FORTH, Heraklion]

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19.15 - 19.30 *A Growth-Inhibitory Proteoglycan Activates Atypical PKC and Modifies Par Complex Function*
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Frederic Saudou [Centre Universitaire, Institut Curie, Orsay]

09.30 - 10.00 *Regulating Mitochondrial Motility*
Thomas Schwarz [Harvard Medical School, Boston]

10.00 - 10.30 *Rapid Trafficking of GABAA Receptors and the Tuning of Inhibitory Transmission*
Josef Kittler [University College of London]

10.30 - 10.45 *From Soma to Synapse: Sorting out Polarized Transport in Neurons*
Lukas Kapitein [Utrecht University, Utrecht]

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11.30 - 12.00	*Homeostatic Plasticity of the Presynapse Extensive Remodeling of the Cytomatrix at the Active Zone upon Prolonged Network Silencing* **Eckart Gundelfinger** [Leibniz Institute of Neurobiology, Magdeburg]
12.00 - 12.30	*Shedding light on the Assembly of Synapse Structure and Function* **Stefan Sigrist** [Freie Universität Berlin]
12.30 - 13.00	*Axon Regeneration in C. elegans* **Yishi Jin** [University of California, San Diego]
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ORAL PRESENTATIONS - ABSTRACTS

Molecular Mechanisms of Synapse Stabilization and Disassembly

Gaeme W. Davis
Department of Biochemistry and Biophysics, University of California, San Francisco

It is well established that the developing nervous system requires the combined activities of synapse formation and elimination (Goda and Davis, 2003; Katz and Shatz, 1996; Luo and O'Leary, 2005; Meyer and Smith, 2006) and there is increasing evidence that this is also true for the maintenance of mature neural circuitry throughout the life of an organism (De Paola et al., 2006; Holtmaat and Svoboda, 2009; Holtmaat et al., 2006; Holtmaat et al., 2005; Trachtenberg et al., 2002; Xu et al., 2009; Yang et al., 2009). The molecular mechanisms that control synapse formation have been studied extensively (Goda and Davis, 2003) and include modulation of the neuronal cytoskeleton (Luo, 2002), target recognition (Ackley and Jin, 2004; Davis et al., 1997; Hughes and Salinas, 1999), synapse assembly (de Wit et al., 2009; Fouquet et al., 2009; Jin and Garner, 2008; Johnson et al., 2009; Linhoff et al., 2009) and stabilization (Datwani et al., 2009; Park et al., 2006; Pielage et al., 2008; Pielage et al., 2005; Schuster et al., 1996; Stellwagen and Shatz, 2002). The opposing mechanisms that disassemble synaptic connections are beginning to emerge and include modulation of growth factor signaling (Eaton and Davis, 2005; Massaro et al., 2009); the submembranous spectrin/ankyrin skeleton (Koch et al., 2008; Pielage et al., 2008; Pielage et al., 2005), cell adhesion (Ackley and Jin, 2004; Dalva et al., 2007; Schuster et al., 1996) and cellular mechanisms that dismantle the neuronal membrane (Luo and O'Leary, 2005; Nikolaev et al., 2009; Pielage et al., 2008; Pielage et al., 2005; Stellwagen and Shatz, 2002; Watts et al., 2003; Watts et al., 2004). Recent evidence in both insect dendrites (Kuo et al., 2006; Williams et al., 2006; Schoenmann et al., 2010) and mammalian neurons (Nikolaev et al., 2009) also provides evidence for activation of effector caspases that drive the destruction of neuronal processes (Nikolaev et al., 2009). Taking advantage of the powerful forward genetic tools of Drosophila, we continue to investigate the molecular mechanisms that are necessary for the stabilization and active disassembly of synaptic connections and neuronal processes. We will present new evidence examining a central role of the spectrin/ankyrin/adducin skeleton coordinating synaptic growth and stability. Data will also be presented for a signaling system, derived from glia, that drives synaptic destabilization and elimination in the neuromuscular system with relevance to both neural development and neurodegenerative disease.

Presented by: **Davis, Graeme**

Long Term Dynamics of Inhibitory Synapses

Ann Marie Craig , Frederick Dobie
University of British Columbia

Dynamics of GABAergic synaptic components have been studied previously over a time range of milliseconds to minutes, revealing mobility of postsynaptic scaffolds and receptors. Here we imaged YFP-Gephyrin inhibitory postsynaptic scaffolds, together with vesicular GABA transporter VGAT, over seconds to days in cultured hippocampal neurons, revealing modes of inhibitory synapse formation and remodeling. Entire synapses exhibited rapid mobility, generally within a confined region. Synapses could translocate >10 microns yet constantly maintain close apposition between Gephyrin and VGAT. Apparent merging and splitting of synaptic clusters was observed, resulting in flux in the density of synaptic puncta over time. New synapse formation was observed primarily on dendrite shafts but also on dendritic protrusions, without apparent interconversion. At nascent synapses, Gephyrin accumulated gradually over several hours, apparently from cytoplasmic pools. At a number of nascent synapses, VGAT accumulated beginning ~ 2 hours before the accumulation of gephyrin. Since VGAT was labeled by active uptake of a luminal domain antibody, these results indicate that recycling vesicles from pre-existing boutons significantly contribute to new synapse formation. Altogether, the long-term imaging of GABAergic synapses reveals complex dynamics and perpetual remodeling contributing to synaptic integration.

Presented by: **Craig, Ann Marie**

Dynamic Regulation of Synaptic Adhesion Complexes by Alternative Splicing

Peter Scheiffele [1], *Takatoshi Iijima* [2], *Karen Wu* [3], *Harald Witte* [2]
[1] *Biozentrum of the University of Basel, Basel, Switzerland and Columbia University, Department of Neuroscience, New York, NY, USA*
[2] *Biozentrum of the University of Basel, Basel, Switzerland*
[3] *Columbia University, Department of Neuroscience, New York, NY, USA*

The assembly of synapses and neuronal circuits relies on an array of molecular recognition events and their modification by neuronal activity. Neurexins are a highly polymorphic family of synaptic receptors diversified by extensive alternative splicing. Several Neurexin splice variants exhibit distinct isoform-specific biochemical interactions and activities in synapse formation and function but the mechanisms governing splice isoform choice are not understood. We demonstrate that Nrxn1 alternative splicing is temporally and spatially controlled in the developing mouse cerebellum. Neuronal activity triggers a calcium and calmodulin-dependent kinase IV-dependent shift in Nrxn1 splice isoform choice. Activity-dependent alternative splicing of Nrxn1 in cerebellar neurons requires the KH-domain RNA binding protein SAM68 which binds intronic splicing silencer elements in the Nrxn1 mRNA. These findings identify activity-dependent alternative splicing as a mechanism for dynamic control of Nrxn1 molecular diversity and uncover SAM68 as a key regulator of this process.

Presented by: **Scheiffele, Peter**

Role of Leucine-Rich Repeat Containing Proteins in Excitatory Synapse Development

Joris De Wit [1], Matthew O'Sullivan [1], Jeff Savas [2], Emily Sylwestrak [1], Davide Comoletti [3], John Yates [2], Anirvan Ghosh [1]
[1] Neurobiology Section, Division of Biology, University of California San Diego
[2] Department of Chemical Physiology, The Scripps Research Institute
[3] Department of Pharmacology, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego

The function of the brain depends on highly specific patterns of connections between neurons. Synaptic adhesion molecules are critical players in organizing connectivity. We have identified the leucine-rich repeat (LRR) containing postsynaptic adhesion molecule LRRTM2 as a key regulator of excitatory synapse development and function. LRRTM2 localizes to excitatory synapses in transfected hippocampal neurons, and knockdown of LRRTM2 leads to a decrease in excitatory synapses without affecting inhibitory synapses. LRRTM2 interacts with PSD95 and regulates surface expression of AMPA receptors, and lentivirus-mediated knockdown of LRRTM2 in vivo decreases the strength of evoked excitatory synaptic currents. Structure-function studies indicate that LRRTM2 induces presynaptic differentiation via the extracellular LRR domain. We identified the presynaptic adhesion molecule neurexin as a receptor for LRRTM2 based on affinity chromatography. LRRTM2 binds to both neurexin1α and neurexin1β, and knockdown of neurexin1 abrogates LRRTM2-induced presynaptic differentiation. These observations indicate that an LRRTM2-neurexin interaction plays a critical role in regulating excitatory synapse development. We are currently investigating the role of another LRRTM family member, LRRTM4, in synapse formation. Overexpression and knockdown experiments show that LRRTM4 has similar effects on excitatory synapse formation as LRRTM2. We are using proteomics to identify the presynaptic binding partner for LRRTM4. LRRTM2 and LRRTM4 display striking differences in expression patterns, suggesting that interactions mediated by these proteins might regulate connectivity between distinct neuronal populations.

Presented by: **De Wit, Joris**

A Nanoscale View into the Dynamic of AMPA Receptor Organization in Synapses

Daniel Choquet
Institute for Interdisciplinary Neuroscience, CNRS Université Bordeaux Segalen

Ionotropic AMPA glutamate receptors (AMPAR) mediate fast excitatory synaptic transmission in the central nervous system. Using a combination of high resolution single molecule imaging techniques and video-microscopy, we have previously established that AMPARs are not stable in the synapse as thought initially, but undergo continuous entry and exit to and from the post-synaptic density through lateral diffusion.

We will present some recent developments in single molecule imaging technologies and their application to track single molecules in live neurons.

We have recently found a new function for this fast diffusion in controlling fast synaptic transmission. Upon consecutive synaptic stimulation at high frequency, synaptic transmission is depressed. This depression shapes the frequency dependent adaptation of individual synapses. AMPAR lateral diffusion allows fast exchange of desensitised receptors with naïve functional ones within or nearby the post-synaptic density. This participates to the recovery from depression in the tens of millisecond time range, in parallel with recovery from desensitization.

In addition, we now show that the Ca2+/Calmodulin-dependent protein kinase II (CaMKII), which is critically required for the synaptic recruitment of AMPA-type glutamate receptors (AMPARs) during both development and plasticity, induces the synaptic trapping of AMPARs diffusing in the membrane. Furthermore, this CaMKII dependent AMPAR immobilization regulates short term plasticity. Thus, NMDA dependent Ca2+ influx in the post-synapse trigger a CaMKII and Stargazin dependent decrease in AMPAR diffusional exchange at synapses that controls synaptic function.

Presented by: **Choquet, Daniel**

Regulation of Neuronal Function and Dysfunction by Protein SUMOylation

Jeremy Henley
University of Bristol

The post-translational modification SUMOylation is a major regulator of protein function that plays an important role in a wide range of cellular processes. SUMOylation involves the covalent attachment of a member of the small ubiquitin-like modifier (SUMO) family of proteins to lysine residues in specific target proteins via an enzymatic cascade analogous to, but distinct from, the ubiquitination pathway. The functional and pathophysiological implications for synaptic protein SUMOylation are far-reaching. I will discuss aspects of our work attempting to identify and functionally characterise SUMO substrates; elucidate the molecular mechanisms regulating, and consequences of, substrate SUMOylation and deSUMOylation; determine the activity-dependence of SUMO and SUMO-specific protease trafficking to synapses; and define how SUMOylation regulates synaptic transmission under basal, stimulated and pathological conditions.

Presented by: **Henley, Jeremy**

Intracellular Machinery and Signaling for AMPA Receptor Trafficking at Synapses

Jose A. Esteban
Centro de Biologia Molecular "Severo Ochoa"

Synaptic connections in the brain are continuously remodeled in response to neuronal activity. This process, known as synaptic plasticity, is widely accepted as the cellular mechanism underlying learning and memory. We now know that an important contributor to synaptic plasticity in the hippocampus and other brain regions is the regulated addition and removal of glutamate receptors at excitatory synapses. In particular, AMPA-type glutamate receptors can be transported in and out of the postsynaptic membrane in a regulated manner, resulting in long-lasting changes in synaptic strength. During this presentation, I will describe our latest results on the intracellular endosomal machinery and signaling mechanisms that control the transport and stability of AMPA receptors at the postsynaptic membrane during plasticity.

Presented by: **Esteban, Jose A.**

Non hyperpolarizing GABAB Receptors Regulate Neuronal Migration, and Axon/Dendrite Growth and Specification by cAMP Pathway

Guillaume Bony , Andrea Contestabile , Laura Cancedda
Italian Institute of Technology

GABA is the principal inhibitory neurotransmitter in the adult brain, acting through both ionotropic chloride-channel GABAA receptors (GABAARs), and metabotropic GABABRs coupled to calcium and potassium channels, and cAMP signaling. Interestingly, during early development GABA is the main neurotransmitter and it is not hyperpolarizing, as GABAARs are depolarizing and mainly excitatory, and GABABRs lack coupling to potassium channels. Despite extensive knowledge on GABAARs as key players in basic processes of neuronal development such as cell migration and morphological maturation, investigation on the role of GABABR receptors in early development remains elusive. Here, we investigated GABABR function during rat cortical development by electroporating in utero pyramidal-neuron progenitors with small-interfering RNA (siRNA) against GABABR. GABABR knockdown impaired neuronal migration and morphological maturation by cAMP signaling. In vitro studies in cell culture confirmed our results in vivo and indicated GABABR signaling as a modulator of neuronal polarization. Furthermore, GABABR activation in vivo triggered cAMP-dependent phosphorylation of LKB1, a kinase involved in neuronal polarization. Finally, field-potential recordings of GABAB siRNA-transfected slices revealed increased basal and induced neuronal-network activity. Thus, non hyperpolarizing GABABRs during early development promote neuronal migration and morphological maturation by cAMP-dependent axon/dendrite specification, leading to cortical circuit rearrangement.

Presented by: **Cancedda, Laura**

Endocytic mechanisms at synapses

Pietro De Camilli
Department of Cell Biology, Howard Hughes Medical Institute, Program in Cellular Neuroscience Neurodegeneration and Repair, Kavli Institute for Neuroscience, Yale University School of Medicine, USA

Endocytosis plays a fundamental role in synaptic function. In axon terminals, the efficient endocytic recycling of synaptic vesicle membranes after exocytosis makes possible the reliable function of synapses even during high frequency stimulation, in spite of their distance from the cell body, where new proteins are synthesized. Postsynaptically, endocytosis plays a key role in the regulation of the number of surface exposed neurotransmitter receptors. While much has been learned about endocytosis, our understanding of this process lags behind the field of exocytosis due in part to the multiplicity of endocytic mechanisms that operate at synapses. We study such mechanisms using a variety of complementary approaches, which include reconstitution experiments with purified endocytic proteins and lipid membranes, broken cell preparations, intact cells, model synapses and genetically modified mice. With these studies we hope not only to improve knowledge of synaptic transmission but also to advance the understanding of fundamental mechanisms in endocytosis. In my talk I will focus on studies of mechanisms underlying membrane deformation and membrane fission at early stages of the endocytic pathway, with emphasis on the role of the GTPase dynamin and its functional partnership with BAR domain containing proteins, endophilin in particular. BAR domains are protein modules that bind the lipid bilayer and have curvature sensing and curvature generating properties. I will also discuss the regulatory role of phosphoinositide metabolism in the progression of membranes along early stations of the endocytic pathway.

Presented by: **De Camilli, Pietro**

Control of Motor Neuron Generation and Regeneration in Zebrafish

Catherina Becker [1], *Anneliese Norris* [1], *Veronika Kuscha* [1], *Rickie Patani* [2], *Michell Reimer* [1], *Angela Scott* [1], *Zhen Zhong* [1], *Tatyana Dias* [1], *Siddhartan Chandran* [3], *Thomas Becker* [1]
[1] *Centre for Neuroregeneration, University of Edinburgh*
[2] *Cambridge Centre for Brain Repair*
[3] *Euan MacDonald Centre for MND Research, University of Edinburgh*

In adult zebrafish, in contrast to mammals, progenitor cells readily regenerate motor neurons after injury to the spinal cord. Since embryonic signals, such as floor plate derived hedgehog, are often reactivated in regeneration, we used the zebrafish to find novel developmental signals with relevance to adult motor neuron regeneration. Several local signals are known to influence neurogenesis in the vertebrate spinal cord, but whether monoaminergic signals from the brain can regulate spinal neurogenesis is currently unclear. Here we show that signals from the brain, promotes generation of spinal motor neurons during development, and during regeneration of the lesioned adult spinal cord. Using pharmacological, cell ablation, and knock down studies in embryos we define the receptor. Stimulating the same pathway in human embryonic stem cell derived neural progenitor cells also significantly increases the number of differentiating motor neurons, suggesting direct action on progenitor cells. After a spinal lesion in adult zebrafish, the receptor is re-expressed on spinal progenitor cells and ablation of the brain signal decreases, whereas injection of an agonist increases the number of newly-generated spinal motor neurons. Thus our results demonstrate that descending axons influence the generation of spinal neurons during development and adult regeneration. We envision that this newly-discovered signaling mechanism may become relevant for research into promoting neurogenesis after injury or in disease.

Presented by: **Becker, Catherina**

Nogo- A is a Negative Regulator of Neurite Growth in the Developing and Adult Nervous System

Martin Schwab
Dept. of Neuromorphology, Brain Research Institute University of Zurich, Winterthurerstrasse 190, CH-8057 Zürich

During development Nogo-A is present on growth cones and neurites during the main outgrowth faced in many projection neurons including DRG, motoneurons, retinal ganglion cells, pyramidal cells or Purkinje cells. DRG outgrowth of Nogo-A KO mice or wt neurites in the presence of antibodies against Nogo-A, NgR or lingo-1 show strongly fasciculated, long neurite bundles with an almost total absence of branching. Dissociated DRG neurons have less branches and often longer neurites. In vivo, branches of hindlimb and forelimb peripheral nerves are missing in the Nogo-A KO E12.5 – 14.5 embryo and in antibody injected chicken embryos. These results suggest a mutual repulsion between growing neurites by Nogo-A which facilitates branching and decreases fascicle formation. During the final phase of NS maturation, Nogo-A appears in oligodendorcytes while neuronal Nogo-A is down regulated. In vitro binding of active Nogo-A fragments to neurites in chamber cultures lead to rapid internalization and retrograde axonal transport in signaling endosomes; activated RHO- A is associated with the transport vesicles reaching the cell body. There, Creb phosphorylation is down regulated, counter balancing the Creb up regulation by cAMP or neurotrophic factors. The absence of Nogo-A in KO mice leads to higher constitutive levels of in particular cytoskeletal and growth associated proteins and their mRNAs. Antibody injection in intact animals induces transitory sprouting of axonal collaterals e.g. in cerebellum or spinal cord. Antibody application after stroke or spinal cord injuries enhances compensatory fiber growth and regeneration of lesioned axons associated with improved functional recovery of complex sensory motor functions. Similar results were obtained with blockers of NgR or downstream RHO/ROCK signaling and in some lines of Nogo KO mice. A clinical trial with human anti- Nogo-antibodies is currently ongoing in acute spinal cord injured patients.

Presented by: **Schwab, Martin**

Defining the Genetic Pprogram of Axon Regeneration in the CNS

Zhigang He
Harvard Medical School

One of the most exciting and challenging frontiers in neuroscience and medicine is the repair of traumatic injuries to the central nervous system (CNS). A key underlying mechanism for permanent functional deficits is the failure of injured axons to regenerate. The efforts in the past to alleviate the inhibitory influences in the adult CNS environment have failed to promote significant axon regeneration, pointing to the importance of investigating the neuronal intrinsic mechanisms. By using optic nerve injury models, we have systematically analyzed the role of a variety of molecular pathways that control cellular growth in axon regeneration in vivo. As the results, we identified two critical signaling pathways (PTEN- or SOCS3-dependent) and manipulating either or both could result in robust axon regeneration. Our results led to the establishment of the first sets of genetic models with robust CNS axon regeneration and are revealing a genetic program that controls the process of axon regeneration.

Presented by: **He, Zhigang**

Regeneration of the Adult Zebrafish Brain after Traumatic Lesion: Neurogenic Radial Glia-Type Progenitor Cells Make New Neurons

Michael Brand
Dresden University, BIOTEC and CRTD

Severe traumatic injury to the adult mammalian central nervous system (CNS) leads to life-long loss of function and neuronal regeneration is not occurring. In contrast, several non-mammalian vertebrate species, including adult zebrafish, have a remarkable ability to regenerate injured organs, including the CNS. However, the cellular and molecular mechanisms that enable or prevent CNS regeneration are largely unknown. To study brain regeneration mechanisms in adult zebrafish, we developed a traumatic lesion assay, analyzed cellular reactions to injury and show that adult zebrafish can efficiently regenerate brain lesions and lack permanent glial scarring. Using Cre-loxP-based genetic lineage tracing, we demonstrate that her4.1-positive ventricular radial glial progenitor cells react to injury, proliferate and generate neuroblasts that migrate to the lesion site. The newly generated neurons survive for at least 3 months, are decorated with synaptic contacts and express mature neuronal markers. Thus, regeneration after traumatic lesion of the adult zebrafish brain occurs efficiently from radial glia-type stem/progenitor cells.

Presented by: **Brand, Michael**

RNA-Based Control of Visual System Wiring

Christine Holt
University of Cambridge

Axon guidance is a key step in wiring the brain. To establish neural circuits, the axons of differentiating neurons must navigate accurately to distant targets and select precise synaptic partners. We are trying to understand the mechanisms that underlie these highly selective processes in the vertebrate visual system. Emerging evidence points to an increasingly important role for post-transcriptional mechanisms such as RNA localization and local protein synthesis. Sub-cellular profiling shows that axonal growth cones contain a remarkably diverse repertoire of mRNAs that are dynamically regulated with age. In vitro functional assays reveal that directional turning responses of growth cones to some guidance cues depend on local protein synthesis. Our studies support a 'differential translation model' for axon steering in which attractive and repulsive cues induce opposite turning behaviours through eliciting translation of distinct mRNAs. An important component of this cue-induced translation is that it can be controlled with high spatial precision enabling localized sub-cellular responses. A polarised cue, for example, triggers protein synthesis on the near-stimulus side of the 5 micron growth cone that is achieved through co-ordinately regulated asymmetries in receptor activation, mRNA trafficking and translation activation. Our functional studies of specific RNA-binding proteins are beginning to provide in vivo evidence that RNA-based mechanisms play a pre-synaptic role in the establishment and the topographic arrangement of synaptic connections in the visual system.

Presented by: **Holt, Christine**

Identification of Localized mRNAs in Hippocampal Neurons

Erin M. Schuman
Max-Planck Institute for Brain Research, Frankfurt am Main, Germany

It is clear that de novo protein synthesis has an important function in synaptic transmission and plasticity. Many studies shown that mRNA translation in hippocampal neurons is spatially controlled and that dendritic protein synthesis is required for different forms of long-term synaptic plasticity. In spite of this, the populations of mRNAs that are localized to dendrites remain elusive. Recent studies of process-localized transcripts show little overlap in the mRNAs identified, suggesting that there are many more transcripts to be discovered. We have undertaken a transcriptomics approach in order to comprehensively characterize the mRNAs resident in the synaptic neuropil. Analysis of the neuropil dataset yielded a list of 7200 transcripts of which ~2600 are putative process-localized mRNAs. We confirm a subset of these mRNAs as dendritically localized using high-resolution fluorescence in situ hybridization. We also observe that the distribution of some mRNAs in dendrites is regulated by synaptic plasticity. These results demonstrate a previously unappreciated enormous potential for the local protein synthesis machinery to translate proteins in response to intrinsic, synaptic- and volume-transmitted signals.

Presented by: **Schuman, Erin M.**

CYFIP1, a Neuronal eIF4E-BP, Links Local Translational Regulation to Spine Remodeling: Insights into Mental Retardation and Autism

Claudia Bagni, *Silvia De Rubeis*
Vesalius Research Center VIB, Leuven

Fine regulation of mRNA transport and translation at synapses underlies synaptic plasticity and brain development. One of the key molecules implicated in this process is the Fragile X Mental Retardation Protein (FMRP), the protein lost in the most frequent form of inherited mental retardation: the Fragile X Syndrome (FXS). We have demonstrated that FMRP represses translation initiation via its cytoplasmic interacting protein CYFIP1/Sra1, known also as a regulator of actin cytoskeleton. FMRP tethers a specific subset of neuronal mRNAs to CYFIP1, which can in turn bind the translation initiation factor eIF4E, and thus blocks the access of eIF4G. After neuronal stimulation, the CYFIP1-eIF4E complex is released and protein synthesis ensues.

By combining CYFIP1 immunoprecipitation from different subcellular compartments of the neuron and mass spectrometry, we found new interactors of the CYFIP1-FMRP particle assembled in specific molecular complexes according to their subcellular location. Some of these factors are specifically involved in mRNP transport, others in translational regulation and a third class seems to be mainly involved in cytoskeleton remodeling.

We provide novel evidence for an interplay between local translational and cytoskeleton regulation and show how neuronal activity controls this process.

Presented by: **Bagni, Claudia**

CNP/cGMP Signaling Regulates Axon Branching and Growth by Modulating Microtubule Dynamics

Le Ma , Caihong Xia , Zhen Zhao , Zheng Wang
Zilkha Neurogenetic Institute, University of Southern California

The C-type natriuretic peptide (CNP) has been shown recently to positively regulate axon branching, growth, and guidance via activation of cyclic guanosine monophosphate (cGMP) signaling in embryonic dorsal root ganglion (DRG) neurons, but the cellular mechanisms mediating these developmental processes have not been established. In this study, we provide evidence linking CNP/cGMP signaling to microtubule dynamics. First, in embryonic DRG neuronal culture, low doses of the microtubule depolymerization drug nocodazole block CNP/cGMP-dependent axon branching and growth. Second, based on real time imaging of EB3-EGFP labeled growing microtubule ends, we found that global activation of cGMP signaling leads to increased assembly of dynamic microtubules in DRG growth cones and axons, while local application of CNP attracts microtubule assembly and reorients microtubule lattice in the growth cone. To establish the intracellular signaling pathway involved in this regulation, we studied the microtubule regulator CRMP2 and found that its phosphorylation can be relieved by cGMP activation and that the unphosphorylated form of CRMP2 can enhance axon branching and growth. Finally, similar to that of cGMP activation, over-expression of CRMP2 leads to increased microtubule dynamics in both COS cells and DRG neurons, and these activities correlate well with CRMP2 dephosphorylation. Taken together, our study demonstrates a critical role of microtubule dynamics in CNP/cGMP-dependent regulation of axonal development, and provides a model to understand the contribution of dynamic microtubules to axon branching, growth, and guidance.

Presented by: **Ma, Le**

Regulating Synaptic Strength Across the Cleft by N-cadherins

Nathalia Vitureira , Mathieu Letellier , Ian White , Yukiko Goda
MRC LMCB

N-cadherin is a Ca2+-dependent homophilic adhesion protein that plays an important developmental role in guiding and forming synaptic connections, although it remains expressed at mature excitatory synapses. We have investigated the transsynaptic activity of N-cadherin in regulating synaptic efficacy using FM dyes to monitor vesicle turnover in cultured hippocampal neurons. Interfering with N-cadherin expression in isolated postsynaptic neurons reduces basal release probability at synaptic inputs received by the neuron. Surprisingly, this transsynaptic impairment of neurotransmitter release is accompanied by a significant slowing of vesicle endocytosis. In contrast, in neurons postsynaptically impaired for N-cadherin activity, synapses remain capable of homeostatically upregulating release probability. Our findings reveal that regulation of presynaptic efficacy is molecularly dissociable into two components by the requirement for N-cadherin: one for controlling the level of basal presynaptic strength and the other for adjusting the gain.

Presented by: **Goda, Yukiko**

Presynaptic Tenacity: Insights from Live Imaging Experiments

*Ziv, Noam (Faculty of Medicine and Network Biology Research Labs, Technion, Haifa) noamz@netvision.net.*il

The human brain consists of a vast number of neurons interconnected by specialized communication devices known as synapses. It is widely believed that activity-dependent modifications to synaptic connections - synaptic plasticity - represents a fundamental mechanism for altering network function, giving rise to emergent phenomena commonly referred to as learning and memory. This belief also implies, however, that synapses, when not driven to change their properties by physiologically relevant stimuli, should retain these properties over time. Otherwise, physiologically relevant modifications would be gradually lost amidst spurious changes and spontaneous drift. We refer to the expected tendency of synapses to hold onto their properties as "synaptic tenacity".
Imaging studies indicate that many synapses do maintain their location and overall organization over long durations. However, at the same time, other imaging studies reveal that synapses, and in particularly presynaptic compartments, are sites of intense molecular dynamics and membrane trafficking processes. Given the lack of obvious barriers between presynaptic compartments, the neighboring axoplasm and axolemma, and neighboring synapses, the tenacity exhibited by presynaptic sites is by no means an obvious outcome. Yet, to date, not much is known on the principles that govern synaptic tenacity and allow these minute structures to maintain their structure and function in face of the intense molecular dynamics and other "erosive forces" associated with synaptic transmission.
We have begun to examine the relative importance of specific molecules and processes in determining presynaptic tenacity. Specifically we are currently applying imaging technologies combined with molecular and genetic approaches to study the stability of synaptic structures and relationships between synaptic stability and network activity. These approaches and the insights they have provided will be described.

Presented by: **Ziv, Noam**

Class-specific Dendrite Morphology Control by the Actin Bundling Protein Fascin

Julia Negele [1], Caroline Delandre [2], Yun Zhang [1], Friedrich Förstner [3], Adrian Moore [2], Gaia Tavosanis [1]
[1] Dendrite differentiation group, Dept. of Molecular Neurobiology, MPI of Neurobiology, 82152 Munich- Martinsried, Germany
[2] Disease Mechanism Research Core, RIKEN Brain Science Institute, 2-1 Hirosawa, Wako-shi, Saitama, 351-0198, Japan
[3] Dept. of Systems and Computational Neurobiology, MPI of Neurobiology, 82152 Munich- Martinsried, Germany

The branched morphology of dendrites represents a functional hallmark of distinct neuronal types. Indeed, there is a wide range of neuronal class-specific dendrite morphologies. Nonetheless, how distinct branches are generated is not yet understood. In particular, while transcription factor combinations define dendrite patterns, it remains to be elucidated how such programs are executed. We have investigated specific classes of sensory neurons of Drosophila larvae to address the role of the conserved actin bundling molecule fascin. In time-lapse recordings, we found that terminal branchlets of different classes of neurons have distinctive dynamics. Furthermore, they are formed on the basis of molecularly separable mechanisms, since certain neurons require fascin for terminal branching and others do not. Fascin defines the morphological distinction between two classes of neurons as revealed by loss and gain of function experiments. Finally, fascin is a major effector of the transcription factor Cut to define class-specific dendrite morphology. We propose that the distinction between dendrite morphologies requires dedicated molecular mechanisms.

Presented by: **Tavosanis, Gaia**

EB3 Stably links Microtubules to Ankyrin G in the Axon Initial Segment

Christophe Leterrier , Hélène Vacher , Marie-Pierre Fache , Stéphanie Angles d'Ortoli , Francis Castets , Amapola Autillo-Touati , Bénédicte Dargent
INSERM U641

The Axon Initial Segment (AIS) plays a key role in maintaining the molecular and functional polarity of the neuron. The relationship between the AIS architecture and the microtubules (MTs) supporting axonal transport is unknown. Here we identify a direct and specific interaction between the AIS scaffold protein ankyrin G (ankG) and the microtubule plus-end binding (EB) protein EB3. AnkG concentrates and stabilizes EB3 along MTs in the AIS, contrasting with the role of EB3 as a dynamic MT plus-end tracking protein (+TIP). In addition, EB3 participates in AIS stability, and AIS disassembly leads to a cell-wide up-regulation of EB3. Thus, EB3 coordinates a molecular and functional interplay between ankG and the AIS MTs that supports the central role of ankG in the maintenance of neuronal polarity.

Presented by: **Leterrier, Christophe**

Neurogenesis from Glial Cells – Novel Sources for New Neurons in the Adult Brain

Magdalena Götz
Institute of Stem Cell Research, Helmholtz Center Munich and Institute of Physiology, University of Munich, Germany

Radial glial cells, the source of neurons in the developing brain, disappear at later stages in most brain regions of mammals, while they persist in many other vertebrates, such as the zebrafish, into adulthood. In light of this I will discuss adult neurogenesis and glial reaction to injury, lacking scar formation and reactive astrogliosis, in this excellent model of regeneration. I will then discuss glial cell reaction after injury in the mammalian brain and will address the molecular mechanisms for their failure to generate neurons by comparison to the adult neural stem cells. These also possess radial glia hallmarks and persist in few niches of the adult mammalian brain continuing to generate neurons life-long. I will present recent insights into the transcriptome comparison of these adult neural stem cells with other glial cells from the developing and adult brain with and without injury. These data reveal the intriguing concept of lineage priming for adult neural stem cells which seems to be absent in other glial cells from the adult brain. I will close by demonstrating that the neurogenic factors involved in lineage priming of the adult neural stem cells are indeed sufficient to instruct neurogenesis at high efficiency from glia outside the neurogenic niches, even from the adult human patient brain. Therefore, glial cells in the site of brain injury are a novel cell source to elicit repair by local neurogenesis.

Presented by: **Götz, Magdalena**

Establishment of Neuronal Polarity

Carlos G. Dotti
Department of Neuroscience, Centro de Biología Molecular Severo Ochoa, Universidad Autónoma de Madrid, Madrid, Spain
VIB Department of Molecular and Developmental Genetics.K.U.Leuven Department of Human Genetics, Leuven, Belgium

In a recent work we showed that, in *Drosophila* neurons undergoing differentiation *in vivo*, the first membrane deformation occurs at 3.6 minutes after precursor division. Using conventional genetic expression analysis, we observed that adhesion complex components (Bazooka/PAR-3, cadherin-catenin) marks the place where the first deformation later occurs, appearing with a clustered distribution at 2.8 minutes after division (eg. 1 minute before morphological deformation). We also observed that the upstream molecule PIP2, known to be required for cadherin-catenin spatial restriction during epithelia polarization, becomes clustered at the site of future apical neurite formation by 0.7 minutes after division (eg. 3 minutes before morphological deformation). RhoA, whose activity is required for PIP2 generation and cadherin-catenin clustering, is clustered to the pole of future apical neurite from the time of cytokinesis, thus at time 0. To investigate to which extent these concentration differences could be sufficient to determine polarity we used a mathematical modeling approach. In addition to its intrinsic value, this was needed because i) it is not possible to knockdown any of the above molecules in the precursor cell (the consequence is division arrest) and ii) it is not possible to determine the effect of suppression in the newborn neuron due to the rapid onset of polarization. Mathematical modelling allowed to demonstrate: 1) that a polarized domain can form spontaneously in a newborn cell as consequence of a change in the equilibrium (i.e. concentration) state and 2) that occurrence of growth from this pole is enough to trigger the occurrence of a second growth domain at the exact opposite pole. Thus, the *in vivo* data provided insights into the molecular hierarchy accompanying polarization, revealing the existence of conservative mechanisms with the apical-basal polarization of epithelia, and the mathematical modelling proved that any polarized change in the steady-state equilibrium can suffice to determine the site where polarization will occur and the occurrence of growth at the opposite pole.

Presented by: **Dotti, Carlos G.**

Molecular Mechanisms Underlying Neuronal Polarization in vivo

Franck Polleux
The Scripps Research Institute – Dorris Neuroscience Center
La Jolla, CA- 92037-1000 USA

The formation of neuronal circuits during the development of the neocortex involves the coordinated migration of billions of neurons and the establishment of axon and dendrites polarity preceding synaptic formation. Impairment of these processes can lead to socially-devastating neurodevelopmental defects such as autism spectrum disorders or mental retardation. We recently identified one of the extracellular signal underlying axon specification of pyramidal cortical neurons *in vivo* and demonstrated that TGFβ and its receptors are required for axogenesis in vivo (Yi et al. Cell 2010). From an intracellular standpoint, our lab has demonstrated that the serine/threonine kinase LKB1 (a.k.a. STK11/Par4) plays a crucial role in neuron polarization, both in vitro and in vivo, by controlling a kinase pathway responsible for the specification of the axon (Barnes et al., Cell 2007). We showed that the axogenic function of LKB1 requires its ability to activate SAD-A/B kinases which can phosphorylate microtubule-associated proteins such as Tau1. LKB1 is a master kinase phosphorylating and activating at least 11 other serine/threonine kinases including AMP-activated protein kinase (AMPK). I will present recent data showing that AMPK catalytic activity is not required for proper brain development including axon formation but that forms of metabolic stress impairs axon formation through over-activation of AMPK/mTOR signaling. I will also provide evidence showing that two poorly-characterized members of this branch of the kinome (NUAK1/2) are required for axon formation, elongation and branching in vivo downstream of LKB1. Finally, I will review the results of our effort to identify more generally the effectors mediating the downstream function these various LKB1-dependent kinases during axogenesis and axon growth in vivo.

Presented by: **Polleux, Franck**

This work is supported by a NIH grant R01AG031524

Neurotrophins regulate neuronal polarity acting through Ca2+ and CaMKK

Kozo Kaibuchi
Nagoya University, Department of Cell Pharmacology, Nagoya, Japan

Neurons are highly polarized cells that possess axons and dendrites, both of which differentiate from common immature neurites in cultured hippocampal neurons. Various extracellular and intracellular signals have been implicated in axon specification. However, the causal relationship between the intracellular signals and axon specification remains elusive, because live imaging of the signals during axon specification is difficult. We found here that neurotrophins derived from the cultured neurons were required for axon specification. Stimulation of the selected neurite by a local application of neurotrophin-3 (NT-3) induced a rapid increase in Ca2+ in the growth cone followed by neurite outgrowth dependent on an inositol 1, 4, 5-trisphosphate (IP3), and this Ca2+ increase was required for axon specification. Impairment of calmodulin-dependent protein kinase kinase (CaMKK), a Ca2+ effector, prohibited NT-3-induced axon specification both in cultured hippocampal neurons and in cortical neurons in vivo. These results reveal a novel role for Ca2+ signaling in axon specification via CaMKK.

Presented by: **Kaibuchi, Kozo**

ADF/Cofilin Directs Neuritogenesis in the Developing Mammalian Brain

Kevin Flynn [1], Dorothee Neukirchen [1], Sonja Jacob [2], Sabina Tahirovic [1], Boyan Garvalov [1], Roland Wedlich-Söldner [3], J. Victor Small [2], Walter Witke [4], Frank Bradke [1]
[1] *Max Planck Institute of Neurobiology*
[2] *Institute of Molecular Biotechnology, Austrian Academy of Sciences*
[3] *Max Planck Institute of Biochemistry*
[4] *Institute of Genetics, University of Bonn*

Neuritogenesis is the foremost event during neuronal morphogenesis. The underlying intracellular processes and the physiologically relevant players during neuritogenesis have remained largely unidentified. Here, we showed that rapid actin turnover is essential for transforming a spherical neuron into neurite-bearing cell. ADF and cofilin were identified as physiological regulators of neurite formation via brain-specific genetic ablation. ADF/Cofilin (AC) knockout neurons failed to form neurites and showed strong cytoskeletal aberrations including an increase in F-actin levels, disordered actin filament orientation, a reduction in filopodia, and irregular looping of microtubules. Importantly, AC knockout neurons had greatly diminished actin dynamics, including retrograde flow. The F-actin severing activity of AC proteins was pinpointed as the essential activity for neuritogenesis. We propose that AC mediated actin filament severing is the primary driving force underpinning actin turnover in neurons, which may open up intracellular space for the protrusion of bundled microtubules, the backbone of neurites.

Presented by: **Flynn, Kevin**

Lpd Depletion Reveals a Novel SRF-Dependent Function that Specifies Radial versus Tangential Migration of Bipolar Pyramidal Neurons

Elaine M. Pinheiro[1], Zhigang Xie[2], Amy L. Norovich[1,3], Marina Vidaki[1], Li-Huei Tsai[4,5], Frank B. Gertler[1]
[1]Koch Institute for Integrative Cancer Research at MIT, Massachusetts Institute of Technology, Cambridge, MA 02139
[2]Department of Neurosurgery, Boston University School of Medicine, Boston, MA 02118
[3] Current address: Columbia University, New York, NY, 10027
[4]Picower Institute for Learning and Memory. Massachusetts Institute of Technology, Cambridge, MA 02139
[5]Howard Hughes Medical Institute, Cambridge, MA 02139

The six distinct neocortical layers are a hallmark of the mammalian brain essential for many neurological processes. During corticogenesis, pyramidal neurons (which comprise ~80% of the neurons within the adult cortex) arise from a germative region, the ventricular zone (VZ), and migrate along radial glia to their proper position within the cortex. Prior to migration, pyramidal neurons pass through a multipolar stage and then become bipolar and attach to radial glia. As pyramidal neurons migrate radially from their birthplace, they must remain attached to their glial migratory substrate as they pass through the subventricular (SVZ) and intermediate (IZ) zones, regions rich in tangentially migrating interneurons and axon fiber tracts. We examined the role of Lamellipodin (Lpd), a homolog of a key regulator of neuronal migration and polarization in *C.elegans*, in corticogenesis. Lpd depletion caused bipolar pyramidal neurons to adopt a tangential, rather than radial-glial, migration mode without affecting cell fate. Mechanistically, Lpd depletion caused this change in migration mode by reducing the activity of SRF, a transcription factor that can be regulated by changes in the ratio of polymerized to unpolymerized actin. Therefore, Lpd depletion exposes a new role for SRF function that directs pyramidal neurons to select a radial migration pathway along glia rather than a tangential migration mode.

Presented by: **Gertler, Frank B.**

Enhancing the Regenerative Ability of Axons

James Fawcett
University of Cambridge

Most CNS axons have a low intrinsic ability to regenerate, the reasons for which remain to be solved. Three possible reasons are:

Local mRNA translation. Axons in the peripheral nervous system contain many mRNAs, and the machinery to translate these into proteins. This ability is important for axon regeneration, because blocking local translation inhibits axon regeneration. Comparing the mRNAs from embryonic and adult PNS axons there are many changes, including the absence of kinesin mRNAs in adult axons. We find that one of these, kif3C, plays a key role in growth cone regeneration.

Integrins and axon regeneration. In order to grow through the extracellular matrix axons must express appropriate integrins. The main matrix glycoprotein in the damaged CNS is tenascin-C, but tenascin-C binding integrins are lacking. We have transfected alpha9 integrin into neurons, giving them the ability to grow long axons on tenascin in vitro. Transduction of DRG neurons in vivo enhances their ability to regenerate their axons, but only modestly. One problem is that integrin transport into axons is blocked at the axon initial segment. Integrin trafficking relies on Rab11 and Rab coupling protein. Another problem is that CNS inhibitory molecules inactivate integrins.

Presented by: **Fawcett, James**

Signaling Axonal Regeneration in the CNS

Marie T Filbin
Hunter College CUNY

An impediment to axonal regeneration is inhibitors in myelin. One approach to overcome these inhibitors to encourage regeneration is to change the intrinsic state of the axon such that it no longer recognizes these molecules as inhibitory. We showed that if neuronal cAMP is elevated MAG and myelin no longer inhibit axonal growth. This cAMP effect is transcription dependent and we have identified one of the proteins that is up-regulated to be the enzyme Arginase I which is key in synthesis of polyamines. We have shown that the polyamine, putrescine must be converted to spermidine to overcome inhibition and to promote regeneration in vivo. Also, we have shown that spermidine overcomes inhibition by activating the kinase CDK5, though the up-regulation of its activator, p35. Up-regulation of p35 is transcription-independent and translation-dependent and requires the spermidine-induced activation of the eukaryotic initiation factor, eIF5a, by hypusination. Another protein that is up-regulated with cAMP is secretary, leukocyte, protease inhibitor (SLPI). SLPI overcomes inhibition by MAG and myelin. Also, DRG neurons from animals that received SLPI intrathecially for 24 hours are not inhibited by myelin when subsequently cultured. SLPI also promotes optic nerve regeneration when injected intraocularly at the same time as the optic nerve is crushed. We also showed that MAG-induces the phosphorylation of Smad2, which is necessary for inhibition. Interestingly, SLPI enters the neuron and accumulates in the nucleus, where it suppresses expression of Smad2. This in turn decreases the amount of Smad2 that is available for phosphorylation by MAG and so blocks inhibition.

Presented by: **Filbin, Marie T**

The Oriented Emergence of Axons from Retinal Ganglion Cells is Directed by Laminin Contact in vivo

Owen Randlett [1], *Lucia Poggi* [2], *Flavio R Zolessi* [3], *William A Harris* [1]
[1] *Department of Physiology, Development and Neuroscience, University of Cambridge, United Kingdom*
[2] *Institute of Zoology, Heidelberg University, Germany*
[3] *Seccion Biologia Celular, Departamento de Biologia Celular y Molecular, Facultad de Ciencias, Universidad de la Republica, Montevidee, Uruguay*

The site of axon emergence from the cell body of most differentiating neurons is precisely specified during development. For example, cortical pyramidal neurons send out axons apically. How this is accomplished, and the relative importance of intrinsic and extrinsic mechanisms, is not understood. The axons of retinal ganglion cells (RGCs) emerge basally in vivo, yet because RGCs develop from polarized neuroepithelial cells within a polarized environment, disentangling intrinsic and extrinsic influences is a challenge.

We use a combination of in vitro and in vivo time-lapse imaging in zebrafish embryos to demonstrate that Laminin acting directly on RGCs is necessary and sufficient to orient axon emergence in vivo. Laminin contact with the basal processes of newborn RGCs prevents the cells from entering a stochastic Stage 2 phase, directs the rapid accumulation of the early axonal marker Kif5c560-YFP, and leads to the formation of axonal growth cones. These results demonstrate that contact mediated extrinsic cues may be critical for the site of axon emergence, and account for the differences in cellular behavior observed in vitro and in vivo.

Presented by: **Randlett, Owen**

Dendrite Morphogenesis and Functional Implications

Yuh-Nung Jan , HHMI, UCSF

For the past ten years, we have been trying to uncover the rules and the mechanisms that control dendrite morphogenesis by using a group of fly larval sensory neurons known as dendritic arborization (or da) neurons. We have gained some insights about dendrite development including how axons are dendrites are made differently, how a neuron acquires its neuronal type specific morphology, how the dendrites of different neurons are organized, how the size of a dendritic arbor is controlled, and how the pruning and remodeling of dendrites are regulated during development. Of the four different classes of da neurons, the dendrites of class IV da neurons form a regular array that tiles the larval body. Recently we found those neurons actually constitute a novel photoreceptor system using a photo-transduction pathway that is distinct from all the previously known ones. This regular array of photo-sensors enable the larvae to sense light exposure over its entire body to move out of danger.

Previously, we found that the Hippo pathway plays important roles in controlling the dendritic arbor size and tiling of class IV da neurons. In Drosophila, Hippo Kinase regulate Tricorner (Trc) and Warts (Wts), the the only two members of Ser/Thr kinases of the NDR family in fly. Each Kinase then regulates complementary aspects of dendritic arbors: Wts controls maintenance where as Trc controls branching and tiling.

To see whether our findings could be extended to mice, we study the two Trc homologues, NDR1 and NDR2, in mice. They share high sequence homology and both are ubiquitously expressed in the mouse brain throughout development. We find that NDR1/2 function to limit the complexity of the dendritic arbor of mouse hippocampal and cortical neurons, a role analogous to that of their fly and worm homologues. Additionally, we find a new function for NDR1/2: they are required for the proper development of dendritic spines. In order to gain insights concerning how NDR1/2 exert their functions, we set out to identify the substrates of their kinase activity. In collaboration with our colleague Kevan Shokat's lab, we were able to apply their ingenious "chemical genetics and covalent capture method" to identify five NDR1/2 substrates and their phosphorylation sites. Strikingly, four have been implicated in vesicle trafficking. We chose two of them, AAK1 and Rabin8, for further studies. We were able not only to validate both as bona fide NDR1/2 kinase substrates but also found that AAK1 is preferentially required for controlling dentritic arbor complexity whereas Rabin8 is preferentially required for dendritic spine maturation – a finding that paves the way for dissecting the NDR1/2 pathway.

Presented by: **Jan, Yuh-Nung**

The role of Diverse Surface receptor Complexes in Controlling Axonal Branching

Dietmar Schmucker
Vesalius Research Center VIB, Leuven

The proper formation, growth and targeting of axons is essential to build complex and functional nervous systems. In addition to axon guidance, axon branching is an indispensable step in neuronal wiring that enables the innervation of multiple targets and significantly contributes to the exceptional complexity of neuronal networks.

Using forward and reverse genetic approaches, we have identified several key factors that are essential for axonal branching and wiring of Drosophila sensory neurons. Importantly, we identified several surface receptors that either block or enhance axon branch formation (Dscam, RPTPs, Plexins) suggesting that these proteins are involved in receptor-mediated recognition events underlying spatially defined branching positions. We will present an in-depth genetic, developmental and biochemical analysis of the function of these genes in axonal branching. Our goal is identifying conserved signalling pathways controlling axonal branching as well as guidance of distinct axon branches.

Presented by: **Schmucker, Dietmar**

Controlling Axonal Polarization using Micropatterns

Mariano Bisbal [1], *Sophie Roth* [2], *Jacques Brocard* [1], *Ghislain Bugnicourt* [2], *Yasmine Saoudi* [1], *Annie Andrieux* [1], *Sylvie Gory-Fauré* [1], *Catherine Villard* [2]
[1] *Institut des Neurosciences de Grenoble, INSERM, Unité 836, France*
[2] *Institut Néel, CNRS, Grenoble, France*

Neurons are highly polarized cells, which contain a single long axon and several dendrites, dedicated to the transmission and processing of information. Being able to trigger in vitro the axonal fate of one given undifferentiated neurite is an issue faced by neural network engineering that could also improve our knowledge of axonal polarization. Here we described a protocol of neuronal culture that allows the manipulation of axonal differentiation. This achievement resulted from our ability to constraint neuronal shape through its cell body and neurites, with the use of non-specific adhesion and original micropatterns. By controlling neuron polarity of hippocampal neurons we were able to reach more than 85% of differentiation along the predicted direction, providing a simple tool for the design of in vitro networks with a control of the inter-cellular information flow. Beyond their striking control of axonal differentiation, these patterns have proved to be apt tools to explore biological mechanisms associated with the establishment of polarity in neurons. In particular, our results indicated that the centrosome location was not predictive of axonal polarization but rather followed axonal fate. Also the possibility to tune neuritic curvature revealed a role of mechanical tension during axonal differentiation. Finally, pharmacological studies suggested that microtubules, but not actin cytoskeleton, were involved in the triggering of tension-mediated neuronal polarization.

Presented by: **Bisbal, Mariano**

Characterization of Voltage and Temperature Dependent Gating of Channelrhodopsin 2, and Applications in a Dispersed Hippocampal Culture Paradigm

Thomas Chater , Jeremy Henley , Andy Randall , Jon Brown
University of Bristol

Channelrhodopsins are light-activated channels recently adopted as tools for the study of neurobiology. We have performed a detailed investigation of the gating kinetics and voltage-dependence of ChR2 transiently expressed in HEK-293 cells and neuronal culture. Currents were elicited using light pulses of defined duration and intensity generated by a blue LED. Experiments were performed at room temperature (RT~21ºC) and 37ºC. Current responses to light rose rapidly to a peak and then desensitized to a steady-state plateau. When illumination was ceased currents rapidly deactivated. The reversal potential of ChR2 responses was a few mV positive to 0 mV. The peak and plateau phases of ChR2 responses exhibited strong inward rectification with only small outward currents being observed at +55 mV. The rates of ChR2 activation, deactivation and desensitization were ~2 times faster at 37ºC. Activation and deactivation kinetics of ChR2 were significantly slowed by depolarization at both RT and 37ºC. The degree of steady state desensitization was greater at more depolarized potentials. Related to this, desensitization kinetics were not voltage-dependent but recovery from desensitization was slowed by depolarization. We then overexpressed ChR2 in dispersed hippocampal cultures alongside AMPA receptor subunits, and monitored AMPA distribution following LTP and LTD-type light pulses.

Presented by: **Chater, Thomas**

Small Heat Shock Proteins Protect against Neurodegeneration

Nikos Kourtis , Nektarios Tavernarakis
Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology Hellas

Necrotic cell death contributes to the pathogenesis of many neurodegenerative diseases. However, the molecular mechanisms underlying necrosis are not fully understood. We find that activation of the heat shock response pathway by means of heat preconditioning strongly suppresses excitotoxic neuronal death as well as necrotic cell death caused by extreme environmental conditions or hypoxia, in C. elegans. The heat shock response is a highly conserved gene expression program, which is engaged under conditions of stress and coordinates expression of specific genes that protect cells against various stressors. Removal of the heat shock factor 1 (HSF-1), the master transcription regulator which orchestrates the heat shock response, abolishes the protective effect of heat preconditioning. By contrast, overexpression of HSF-1 suppresses neurodegeneration. While screening for potential mediators of the protective effect of heat preconditioning, we found that the small heat shock protein HSP-16.1 is both necessary and sufficient for protection against neurodegeneration. HSP-16.1 exerts its protective effect by modulating calcium release from the Golgi apparatus. Interestingly, the Golgi specific Ca2+ pump pmr-1 is required for heat preconditioning to elicit its protective effect. Loss of pmr-1 function abolishes the capacity of hsp-16.1 overexpression to protect against neurodegeneration. Our findings suggest that intervention strategies based on selective manipulation of the heat shock response may effectively counter excitotoxicity and neurodegeneration.

Presented by: **Kourtis, Nikos**

Identification of the Nogo receptor Family Members NgR1 and NgR3 as CSPG Receptors: Evidence for a Mechanistic Link between CNS Myelin- and CSPG-Mediated Growth Inhibition

Travis L Dickendesher [1], *Katherine T Baldwin* [1], *Yevgeniya A Mironova* [1], *Stephen J Raiker* [1], *Yuntao Duan* [1], *Peter Shrager* [2], *Binhai Zheng* [3], *Larry I Benowitz* [4], *Herbert M Geller* [5], *Roman J Giger* [1]
[1] *University of Michigan Medical School*
[2] *University of Rochester School of Medicine and Dentistry*
[3] *University of California San Diego School of Medicine*
[4] *Harvard Medical School*
[5] *National Institutes of Health*

Following injury to the adult mammalian CNS, severed axons do not regenerate beyond the lesion site. Growth inhibitory molecules in CNS myelin (including Nogo, MAG, OMgp) and chondroitin sulfate proteoglycans (CSPGs) associated with glial scar tissue contribute to this restrictive environment. Here we report on a novel interaction between select members of the Nogo receptor family (NgRs) and CSPGs. NgR1 and NgR3, but not NgR2, bind with high affinity and selectivity to glycosaminoglycan (GAG) chains of neural CSPGs. Soluble NgR1 and NgR3 bind in a chondroitinase ABC lyase (ChaseABC)-sensitive manner to rat brain tissue. They also bind strongly to optic nerve tissue of adult mice subjected to injury and much weaker to control (uninjured) optic nerve. We have mapped the CS-GAG binding motif on NgR1 and show that it is distinct from the binding site of Nogo, MAG, and OMgp. A soluble form of the NgR1 CS-GAG binding motif promotes neurite outgrowth on a mixture of substrate-adsorbed CSPGs. Primary cerebellar granule neurons (CGNs) isolated from NgR123-/- mice grow longer neurites on substrate-adsorbed CSPGs than CGNs isolated from controls. In vivo, loss of all three NgRs leads to significant regeneration of severed retinal ganglion cell (RGC) axons following optic nerve injury compared to controls, an effect that is enhanced in the presence of Zymosan. Our studies suggest that members of the Nogo receptor family are functional receptors for two major classes of growth inhibitory molecules, myelin inhibitors and CSPGs. The identification of shared mechanisms for myelin- and CSPG-mediated inhibition is conceptually novel and provides new insights into the mechanisms that limit neuronal growth and sprouting in the healthy and injured CNS.

Presented by: **Dickendesher, Travis L**

Axonal and Synaptic Degeneration is Regulated by the Hiw E3 Ubiquitin Ligase and Conditioning Lesion

Xin Xiong , Catherine Collins
University of Michigan, Department of Molecular, Developmental and Cellular Biology

Axonal degeneration is a hallmark of many neuropathies and neurodegenerative diseases. Here, using a Drosophila injury model, we find that a specific E3 ubiquitin ligase, Highwire (Hiw), promotes axonal degeneration by regulating the levels of the Wallenda/DLK kinase and a downstream nuclear signaling cascade. Mutations in hiw dramatically inhibit the initiation of Wallerian degeneration after injury in multiple neuron types and developmental stages. Because Hiw has recently been found to regulate an injury response pathway in neurons, we tested whether this pathway mediates an adaptive response to injury. Using a conditioning lesion assay in Drosophila motoneurons, we've found that neurons which have been injured once have an increased resiliency to degeneration after a second injury, and that this requires cell autonomous activation of the Hiw-regulated Wnd signaling pathway. These findings implicate conserved axonal signaling molecules in a mechanism that controls remarkable plasticity in the axonal degeneration process, allowing neurons to adapt to axonal stress.

Presented by: **Collins, Catherine**

A Growth-Inhibitory Proteoglycan Activates Atypical PKC and Modifies Par Complex Function

Joel Levine , Seong il Lee , Weibing Zhang
Dept. of Neurobiology and Behavior, SUNY, Stonybrook, NY USA

Growing axons are guided to their targets by attractive and repulsive substrate bound cues. Prominent among the repulsive cues are the chondroitin sulfate proteoglycans (CSPGs), but little is known about how these molecules affect growth cone function. Here, we studied the role of atypical protein kinase Cζ (aPKC) and the polarity complex in axon growth inhibition mediated by the NG2 CSPG. NG2 is a major component of the glial scars that form at CNS injury sites and inhibits axon growth and induces growth cone collapse in vitro.

Pharmacological inhibition of aPKC and siRNA knock-down reversed NG2-mediated axon growth inhibition of newborn rat cerebellar granule neurons (CGNs). Short-term treatment of either CGNs or HT22 neuronal cells with NG2 activated aPKC as shown by in vitro kinase assays, an increase in T410 phosphorylation on immunoblots and the translocation of aPKC from the cytoplasm to the membrane. aPKC is part of the polarity complex and is activated by CDC42 binding to par 6. Transfection of CGNs with either dominant-negative CDC42, mutant forms of par6 that are unable to bind to CDC42 or a fragment of aPKC that competes with the whole enzyme for par6 binding reversed NG2-mediated growth inhibition and prevented the biochemical activation of aPKC by NG2. NG2 treatment also increased the association of par6 with aPKC and perturbed the normal cellular distribution of par 3.

These results show that molecules associated with glial scars negatively affect axon growth, in part, by activating aPKC and perturbing the structure of the Par complex. Atypical PKC is a new target for therapeutic interventions to promote axon regeneration.

Presented by: **Levine, Joel**

MyosinV-Dependent Transport of PTEN Regulates PI3K Signalling and Neuronal Morphogenesis

Britta Eickholt [1], Tomas Perez [1], Michiel van Diepen [1], Maddy Parsons [2], Nick Leslie [3]
[1] King's College London, MRC Centre for Developmental Neurobiology, King's College London, United Kingdom
[2] The Randall Division of Cell and Molecular Biophysics, King's College London, United Kingdom
[3] University of Dundee, Dundee, Scotland.

We recently demonstrated a requirement for functional actin-based motor proteins in the control of PI3K signalling, a mechanism involving a previously unknown association between PTEN and class V Myosins (van Diepen et al., 2009, Nat Cell Biol 11, 1191-6). FRET measurements revealed that PTEN interacts directly with MyosinV at discreet cellular sites dependent on PTEN phosphorylation. In addition, inactivation of MyosinV-transport function in CNS neurons affects neuronal soma size in vitro and in vivo, which – in line with known attributes of PTEN-loss - required PI3K and mTor.

Here we will present our current work testing the functional significance of the PTEN:MyosinV interaction in regulating PI3K signalling and neuronal morphogenesis in response to growth factors and axon guidance cues.

Presented by: **Eickholt, Britta**

Huntington's Disease: Huntingtin and the Control of Cellular Dynamics

Frederic Saudou
Institute Curie - CNRS UMR 3306 - INSERM U1005

Huntington's disease (HD) is a fatal neurodegenerative disorder characterized by neuronal dysfunction and the selective death of striatal neurons in the brain. The mutation that causes disease is an abnormal expansion of a polyglutamine (polyQ) stretch in the N-terminus of the 350 kD protein huntingtin. The mechanisms by which huntingtin induces dysfunction and death of neurons in the brain are not clearly understood but they involve in part the loss of the protective properties of wild-type huntingtin.

We previously reported that huntingtin is subjected to phosphorylations such as S421 by the IGF-1/Akt pathway that modify its toxicity, suggesting that protein context, and thereby huntingtin function is a crucial regulator of the toxicity elicited by the polyQ expansion. In support, we demonstrated that huntingtin controls the microtubule-based transport of neurotrophic factors such as BDNF. This function is altered in disease, leading to a decrease in neurotrophic support and death of striatal neurons.

We recently demonstrated that huntingtin phosphorylation at S421 by the IGF-1/Akt pathway restores huntingtin ability to transport vesicles along microtubules in HD. We also analyzed the function of this phosphorylation on wild type huntingtin and found an unexpected role in transport directionality. This further demonstrates the important role of htt as a key regulator of axonal transport in health and disease. Here, we will extend the function of huntingtin as a critical protein that control dynamic processes and how these processes are altered in normal and pathological conditions.

Presented by: **Saudou, Frederic**

Regulating Mitochondrial Motility

Thomas Schwarz
Children's Hospital, Boston and Harvard Medical School

Mitochondrial movement makes possible their fission and fusion and also distributes them along axons and dendrites. Neurons regulate mitochondrial motility to meet changing energy needs and Ca2+-buffering requirements. This study aimed to elucidate regulatory mechanisms that govern mitochondrial motility. Video microscopic, genetic, and biochemical studies were undertaken in Drosophila and mammalian cells. The milton/Miro mediates several pathways that regulate mitochondrial motility. In one regulatory mechanism, elevated cytosolic Ca2+, which can potentially signal a local need for ATP and Ca2+ buffering, arrests the movement of mitochondria. This is accomplished by Ca2+ binding to the EF hands of Miro, which permits Miro to interact directly with the KHC motor domain and thereby inhibit its association with microtubules. Normally, all axonal mitochondria have bound kinesin, whether moving anterograde, retrograde, or stationary and this is not altered by Ca2+. A second regulatory pathway, however, uncouples the motor from the mitochondrion via the proteosome-based degradation of Miro. This pathway is mediated by two proteins known to be mutated in forms of Parkinson's Disease: the kinase PINK1 and the E3 ubiquitin ligase Parkin. PINK1 phosphorylation of Miro is upstream of Parkin in triggering Miro degradation. Youle and colleagues have shown that PINK1 and Parkin function in a likely quality control pathway by which damaged mitochondria are targeted for clearance by a still unknown mechanism. The arrest of their motility by Miro degradation may be a first step in isolating mitochondria for subsequent clearance.

Presented by: **Schwarz, Thomas**

Trafficking of GABAARs to Synapses is Mediated by a HAP1-KIF5 Protein Complex and is Disrupted by Mutant Huntingtin

Josef Kittler
Department of Neuroscience, Physiology, and Pharmacology, University College London, Gower Street, London, WC1E 6BT, UK

The density of GABA type A receptors (GABAARs) at synapses can regulate inhibitory synapse strength with important implications for information processing and nervous system plasticity and pathology. Currently, however, the machinery that delivers and removes GABAARs from inhibitory synapses remains poorly characterised. We demonstrate that GABAARs are trafficked to synapses by the kinesin family motor protein 5 (KIF5) and identify the adaptor linking the receptors to KIF5 as the huntingtin-associated protein 1 (HAP1). Disrupting the HAP1-KIF5 complex decreases synaptic GABAAR number and reduces the amplitude of inhibitory postsynaptic currents. Altered inhibition may also contribute to Huntington's disease, which is caused by a polyglutamine repeat in the protein huntingtin, a key binding partner of HAP1. When huntingtin is mutated, as in Huntington's disease, we find that GABAAR transport and inhibitory synaptic currents are reduced. Thus, HAP1-KIF5-dependent GABAAR trafficking is a fundamental mechanism controlling the strength of synaptic inhibition in the brain and may also be a target for disrupted inhibition in Huntington's disease.

Presented by: **Kittler, Josef**

From Soma to Synapse: Sorting out Polarized Transport in Neurons

Lukas Kapitein [1], *Max Schlager* [1], *Marijn Kuijpers* [1], *Phebe Wulf* [1], *Myrrhe Van Spronsen* [1], *Fred MacKintosh* [2], *Casper Hoogenraad* [1]
[1] *Utrecht University*
[2] *VU University Amsterdam*

To establish and maintain their polarized morphology, neurons employ active transport driven by molecular motors to sort cargo between axons and dendrites. However, the basic traffic rules governing polarized transport on neuronal microtubule and actin arrays are unclear. To directly examine how cytoskeletal motors contribute to polarized dendritic transport, we established a trafficking assay in hippocampal neurons to selectively probe specific motor protein activity. This revealed that, unlike kinesins, microtubule minus-end directed dynein motors drive cargo selectively into dendrites, governed by their mixed microtubule array. Furthermore, several myosin family members were found capable of short-range cargo delivery into spines, rather than driving long-range transport. These results demonstrate a powerful approach to study specific motor protein activity inside living cells and imply a key role for dynein in dendritic transport. We propose that dynein establishes the initial sorting of dendritic cargo while kinesins and myosins assist in subsequent delivery.

Presented by: **Kapitein, Lukas**

Ion-Flux Independent NMDA Receptor Function is Required for Aβ-Induced Synaptic Depression

Helmut Kessels [1], *Sadegh Nabavi* [2], *Roberto Malinow* [2]
[1] *N.I.N.*
[2] *UCSD*

The mechanisms by which oligomeric beta amyloid (Aβ), a peptide complex believed to contribute to Alzheimer's disease, disrupts synaptic function are not known. Here we show that Aβ-mediated synaptic depression is blocked by NMDA receptor (-R) inhibitors that target the NR2B-subunit; inhibitors targeting the NR2A-subunit, NR1-subunit or ion channel of NMDA-Rs are ineffective. Our results suggest that oligomeric Aβ employs non-ionic NMDA-R signaling requiring NR2B function to produce synaptic deficits.

Presented by: **Kessels, Helmut**

Homeostatic Plasticity of the Presynapse: Extensive Remodeling of the Cytomatrix at the Active Zone upon Prolonged Network Silencing

Eckart D. Gundelfinger [1], *Vesna Lazarevic* [2], *Cornelia Schöne* [3], *Martin Heine* [1], *Anna Fejtova* [1]
[1] *Leibniz Institute for Neurobiology (LIN) Magdeburg, Germany*
[2] *Leibniz Institute for Neurobiology (LIN) & German Center for Neurodegenerative Disorders (DZNE) Magdeburg*
[3] *Leibniz Institute for Neurobiology (LIN) & Dept. Pharmacology, University of Cambridge (UK)*

Homeostatic synaptic scaling is a form of synaptic plasticity that warrants functioning of neuronal networks by tuning their activity to physiologically meaningful levels. While a general sketch of cellular and local postsynaptic mechanisms of homeostatic plasticity is emerging (Turrigiano, 2008, Cell 135, 422ff; Pozo & Goda, 2010, Neuron 66, 338ff) very little is known about presynaptic homeostatic adaptations. Physiological studies, however, suggest a significant contribution of the presynapse, at least at later stages of network development. Using matured primary cultures of cortical neurons we explored the hypothesis that adaptive homeostatic tuning involves molecular remodeling of the presynapse including the cytomatrix at the active zone (CAZ) that spatially and temporally organizes neurotransmitter release. A significant down-regulation of various core components of the CAZ including Piccolo, Bassoon, RIM, CAST/ELKS, Munc13, liprin-alpha and synapsin was observed upon prolonged global synaptic silencing. Analyses at individual synapse levels revealed a particular regulation for RIM, which despite its global down-regulation is up-regulated at a particular subset of synapses. Global silencing also induced the enrichment of potential regulators of presynaptic release probability such as synaptotagmin-1, SV2B or P/Q-type calcium channels. These mechanisms may contribute to the observed up-regulation of synaptic activity upon prolonged network silencing.

Presented by: **Gundelfinger, Eckart D.**

Shedding Light on the Assembly of Synapse Structure and Function

Stephan Sigrist
Freie Universität Berlin

The majority of rapid cell-to-cell communication mechanisms and information processing within the nervous system makes use of chemical synapses. Thereby, the molecular organization of presynaptic active zones, the places where neurotransmitter filled synaptic vesicles get released, is a focus of intense investigation. We recently showed that Bruchpilot (BRP) of Drosophila melanogaster, which features homologies to the mammalian CAST/ERC family, is essential for structural organization and efficient neurotransmitter release at active zones(Kittel et al., 2006; Wagh et al., 2006).

Our group established protocols to directly visualize protein dynamics during synapse assembly and plasticity in living animals using confocal microscopy(Fuger et al., 2007; Rasse et al., 2005; Schmid et al., 2008). However, light microscopic inspection of synaptic organization is often restricted by the limited resolution of conventional light microcopy due to diffraction. Thus, we adapted a recent advance in high-resolution light microscopy (stimulated emission depletion microscopy, STED) for the analysis of synapse substructures. STED breaks the diffraction barrier and allows localization of proteins well below 100 nm. Using STED, we showed that BRP shapes the presynaptic active zone architecture by adopting an extended conformation, shining first light on the underlying macromolecular organization of active zones(Fouquet et al., 2009; Kittel et al., 2006; Owald et al. 2010). Now, we are combining STED sub-diffraction resolution with in vivo visualization of macromolecular organization.

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Presented by: **Sigrist, Stephan**

Axon Regeneration in C. elegans

Yishi Jin
Nagoya University

The mechanisms underlying the ability of axons to regrow after injury remain little explored at the molecular genetic level. We use a recently established laser injury model in Caenorhabditis elegans mechanosensory neurons to screen >500 conserved genes for novel regulators of axonal regrowth. We uncover several unexpected functional clusters of genes required for efficient regrowth, including genes classically known to affect membrane excitability, neurotransmission, and synaptic vesicle endocytosis. We also show that multiple extracellular factors act to inhibit the elongation of regrowing axons. We find that a conserved Guanine nucleotide Exchange Factor (GEF) acts as an intrinsic inhibitor of regrowth, in part via regulating microtubule dynamics. Extensive genetic analysis further shows that several newly identified factors act upstream of the DLK-1 MAP kinase pathway. Identification of these pathways significantly expands our understanding of the genetic basis of axonal injury responses and repair.

Presented by: **Jin, Yishi**

POSTER PRESENTATIONS - ABSTRACTS

COUP-TFI Promotes the Acquisition of Proper Final Morphology by Callosal Natalie Projection Neurons through Negative Regulation of Rnd2 Expression during Neuron Radial Migration

Christian Alfano [1], Luigi Viola [2], Julian Heng [3], Mmarinella Pirozzi [2], Michael Clarkson [1], Ggemma Fflore [2], Antonia Demaio [2], Andreas Schedl [1], Francois Guillemot [4], Michele Sstuder [1]
[1] *INSERM U636*
[2] *TIGEM-Telethon Institute of Genetics and Medicine*
[3] *ARMI-Australian Regenerative Medicine Institute*
[4] *NIMR-National Institute for Medical Research*

During late stages of corticogenesis upper layer neurons migrate to their final laminar position and send axons both to their contralateral and ispilateral targets. Although many studies suggest a correlation between correct radial migration of late-born neurons and their proper final differentiation, direct evidences of a link between these two processes is still lacking. Callosal cell population is one of the major components of upper layers and it was previously shown that, in the absence of the orphan nuclear receptor COUP-TFI, corpus callosum formation is strongly impaired. In our study we show that COUP-TFI controls migratory properties and final morphology of callosal projection neurons by modulating Rnd2 expression levels along the rostro-caudal axis of the developing neocortex. Our data clearly show that in COUP-TFI KO mice late-born neurons are correctly specified, but are delayed in their migration and show a defective transition from multipolar to bipolar shape. Interestingly, the levels of the small Rho-GTPase Rnd2, a crucial modulator of neuron radial migration, are increased in COUP-TFI KO brains. In this study we demonstrate that COUP-TFI directly represses Rnd2 expression promoting neuron radial migration. Indeed, by lowering Rnd2 levels in COUP-TFI KO brains, radial migration of callosal neurons and their morphological transition are restored in a cell-autonomous manner. Moreover, we show that in the absence of COUP-TFI function both innervation of the contralateral cortex and dendritogenesis of mutant callosal neurons are strongly impaired. These defects are remarkably rescued by restoring callosal neuron migratory properties. Thus, our data demonstrate that COUP-TFI promotes the acquisition of proper final morphology by callosal projection neurons through fine regulation of their radial migration.

Presented by: **Alfano, Christian**

Poster No 001
Red Session

Activity-Driven AMPA Receptor Remodelling in Hippocampus Mediated by RNA Editing

Ales Balik [1], *Andrew Penn, Christian Wozny, Ingo Greger*
[1] *MRC-LMB, Cambridge, UK*

Signalling at excitatory synapses is determined by the composition of the AMPAR complex, which differs during development and depends upon the history of synaptic excitation. The rules controlling AMPAR assembly in the endoplasmic reticulum are not well established. Alternative splicing (flip/flop exons) and RNA editing (R/G site) in the ligand-binding domain (LBD) are implicated in subunit assembly of recombinant AMPARs. Both alternative processed sites strategically located on the interface of LBD modulate receptor tetramer. Here we reveal a dynamic RNA reprogramming at these sites in response to altered neuronal activity within hippocampus. RNA processing is bidirectional depending on the sign of activity and selectively occurs in the CA1 subfield. Observed global changes in RNA processing have been also detected at the level of individual pyramidal neurons. Level of R/G site editing evidently correlates with expression and self-editing of the editase enzyme ADAR2. Using different electrophysiology approaches we found altered functional properties of AMPARs in activity deprived cells. The subunit composition of surface-expressed AMPARs was unchanged. However, high-resolution recordings indicate that changes observed at the level of recoded mRNA (proportion of edited/unedited flip form) have the capacity to translate into remodelled AMPARs. We integrate our findings into an assembly model that could account for the differences between AMPAR splice variant expression and the pharmacological and functional properties of synapticaly-expressed receptors. Our results suggest that RNA editing provides a dynamic mechanism capable of adjusting response properties in neuronal circuits by AMPAR subunit remodelling.

Presented by: **Balik, Ales**

Poster No 002
Green Session

The Function of the Zinc-Finger Factor Insm1 in Postnatal Neurogenesis

Kira Balueva [1], *Camille Boutin* [2], *Harold Cremer* [2], *Carmen Birchmeier* [1]
[1] *Max-Delbrueck-Centrum for Molecular Medicine, MDC*
[2] *Developmental Biology Institute of Marseille-Luminy, IBDML*

The Insulinoma-associated 1 (Insm1) gene encodes a putative transcription factor. Insm1 is expressed in neuroendocrine tumors, in developing and adult endocrine cells, and in the nervous system. Interestingly, Insm1 is expressed transiently during differentiation of all neuronal cell types.

In the postnatal and adult nervous system, Insm1 expression is restricted to neurogenic regions in the forebrain. Expression is detected in the subventricular zone of the lateral ventricles, in the rostral migratory stream and the dentate gyrus of the hippocampus. To define the role of Insm1 in adult neurogenesis, we used Cre-mediated conditional recombination of Insm1Floxed allele to ablate Insm1 expression in the subventricular zone of the forebrain. Cre was either expressed using transgenic technology i.e. the Brn4Cre allele, or it was delivered by electroporation. We found that the lack of Insm1 cell-autonomously affected development of neuronal progenitors in the postnatal olfactory system. In particular, mutation of Insm1 caused prolonged expression of Mash1, and delayed neuronal differentiation: neuronal migration was slower, and the numbers of mature granule neurons of the olfactory bulb generated within a defined time window were reduced. Thus, the lack of Insm1 interfered with neuronal differentiation. However, it did not block differentiation completely. The delay in differentiation was accompanied by an increase in the numbers of neuronal progenitors and neuroblasts in the subventricular zone and the rostral migratory stream of Insm1 mutant mice. Our data indicate that Insm1 is essential for the exit from the amplifying progenitor state, and that its mutation leads to prolonged replication of postnatal neuronal progenitors.

Presented by: **Balueva, Kira**

Poster No 003
Blue Session

Defining Roles of PKMzeta in AMPA Receptor Trafficking

Ellen Barker [1], *Jeremy Henley*
[1] *University of Bristol*

Synaptic plasticity, the modulation of communication efficacy at synapses shapes the neural networks thought to encode memories. Long term potentiation (LTP), the persistent enhancement in synaptic communication, is one such type of plasticity. LTP has two main mechanistically distinct phases, an induction (early) and a maintenance (late) phase (E-LTP, L-LTP) thought to be analogous to the processes of initial learning and long-term memory storage. AMPA receptors (AMPAR) mediate the majority of fast excitatory transmission in the brain, so changes in their expression at synapses underlie synaptic plasticity, with an increase in AMPAR number or conductivity leading to LTP. A brain-specific atypical Protein kinase C isoform, PKMζ has been shown to be both necessary and sufficient for L-LTP, but not E-LTP. Perfusion of ZIP, a specific inhibitor of PKMζ into hippocampi of live rats prevents memories of a task persisting beyond about 10 minutes, and completely erases pre-established memories. As well as showing PKMζ is a key factor in memory formation; these studies provide compelling evidence for links between the biphasic models of LTP and of memory formation. I am interested in the mechanisms of PKMzeta-dependent AMPAR trafficking on a molecular level using cultured neurons. I show that ZIP decreases surface expression of GluR2 but not GluR1, although rates of endocytosis of both are increased. Since GluR1 and GluR2 subunits have distinct roles and trafficking patterns in E-LTP and L-LTP, I hope to better define the roles of PKMζ in the context of these phases by characterising endo- and exocytic events, as well as identifying interactions with other proteins involved.

Presented by: **Barker, Ellen**

Poster No 004
Red Session

A Role for STRAD Pseudokinases in Cerebral Cortex Development

Anthony Barnes, Biliana Veleva-Rotse, Alexandria Harrold, Lillian Welch, Gaby Haddock, Adiba Ali, Karen Thiebes
Oregon Health and Science Univ

Approximately 10% of the mammalian kinome encodes proteins that lack critical catalytic residues, but it remains unclear how these functionally inactive kinases contribute to cellular physiology. Ste20-related adapter proteins α and β (STRADα and STRADβ) are two such pseudokinases, and to date the only known function of STRADs are as allosteric activators of the protein kinase LKB1. It has been recently shown that STRADα can also serve to regulate the nucleo-cytoplasmic trafficking of LKB1 as well. We have previously demonstrated a requisite role for LKB1 in the establishment of neuronal polarity during cortical development. A recent report has identified a human pediatric syndrome resulting from a partial deletion of the STRADα gene, Polyhydramnios, Megalencephaly, and Syndromic Epilepsy (PMSE). PMSE patients exhibit several CNS symptoms including cell migration defects, severe cognitive delay, and infantile onset epilepsy. To better understand PMSE, we have characterized STRADα expression during mouse brain development at both the mRNA and protein levels in the mouse cortex and we find significant levels of STRADα and STRADβ expression in the developing and adult brain. STRADα is observed in both progenitors and post-mitotic neurons in the developing cortex. We find multiple STRAD isoforms expressed in brain, and two splice forms of STRADα and STRADβ expressed in neurons. Recent work suggests a role for STRADα in cortical migration and we are currently working to establish which isoforms of STRADα are required for this activity. Together our results reveal a complex expression pattern for STRADα and suggest critical roles for these pseudokinases during brain development.

Presented by: **Barnes, Anthony**

Poster No 005
Green Session

Reelin Regulates the Dendritic Targeting of the Ion Channel HCN1 in the Postnatal Hippocampus

Justin Barry [1], *Rebecca Piskorowski* [2], *Vivien Chevaleyre* [2], *Steve Siegelbaum* [2]
[1] *Columbia University, Dept. of Biological Sciences*
[2] *Columbia University, Neurobiology and Behavior*

Reelin regulates the dendritic targeting of the ion channel HCN1 in the postnatal hippocampus

The HCN1 hyperpolarization-activated, cyclic nucleotide gated cation channel is highly expressed in the distal apical dendrites of hippocampal CA1 pyramidal neurons, where the channels regulate dendritic excitability and synaptic integration. This region of the dendrite, in stratum lacunosum moleculare (SLM), receives input from entorhinal cortex whereas more proximal dendrites, in stratum radiatum (SR), receive input from neighboring CA3 neurons. The specificity of inputs and composition of ion channels divides the apical dendrites into discrete compartments that play distinct roles in hippocampal function. Here we investigate the mechanisms by which HCN1 is selectively targeted to distal dendrites in SLM.

One intriguing candidate is the extracellular matrix protein reelin, which is required during mammalian embryonic development for the control of radial neuronal migration and the formation of cellular layers in the cortex and the cerebellum. High levels of reelin persist after birth, but their function is less clear. Although reelin controls the surface expression of NMDA receptors and AMPA receptors via phosphorlyation by SRC family kinases (SFKs) and phosphoinositide-3' kinase (PI3K), respectively, it is not known if reelin regulates the expression of voltage-gated channels. We hypothesized that reelin might control the expression and targeting of HCN1 as reelin is highly expressed by cells in SLM. In addition, HCN channel activity is regulated by SFKs, suggesting a mechanism by which reelin might act.

To test this hypothesis, we examined the effects of application of a reelin-function-blocking antibody (CR-50) on HCN1 distribution in organotypic slice cultures from postnatal rat hippocampus. To our surprise, reelin blockade increased the HCN1 signal in the SLM of CA1 neurons, as measured with immunohistochemistry. This effect appeared to represent a specific increase in expression in the distal dendrites, as there was little change in HCN1 signal in SR. Reelin caused an even more striking increase in HCN1 in the distal dendrites of CA2 neurons, where HCN1 levels are usually quite low. Whole-cell recordings of CA1 and CA2 cells revealed that reelin blockade increased the membrane voltage sag upon hyperpolarization, indicative of increased HCN current. Changes in gross neuronal morphology after CR50 treatment were insignificant and could not account for the marked change in HCN1 density.

In summary, we find that reelin is a negative regulator of the dendritic targeting of the HCN1 voltage-gated ion channel. We are currently exploring the mechanism by which reelin regulates HCN1 and examining other candidate proteins, focusing on those that interact with reelin, that may play a role in targeting HCN1 to the distal dendrite.

Presented by: **Barry, Justin**

Poster No 006
Blue Session

The Absence of TAG-1 Results in Perturbation of Olfactory Bulb Organization and Function

George Bastakis, Maria Savvaki, Domna Karagogeos
IMBB FORTH/Medical school University of Crete

The immunoglobulin superfamily member TAG-1 plays an important role in neurite outgrowth, fasciculation, neuronal migration and axon guidance during development. The main projecting neurons of the olfactory bulb (OB) (mitral and tufted cells) express TAG-1 during development but the exact role of this molecule in olfactory system has not yet been investigated. The aim of the current project is to elucidate the role of TAG-1 in the development and organization of the olfactory system by using mice deficient for TAG-1 (Tag-1 ko) as a tool.

In adult and P0 (newborn) TAG-1 deficient mice we have observed significantly decreased numbers of mitral cells in the olfactory bulb mitral cell layer (MCL) compared to control animals. These results suggest that the defect in mitral cell number can be attributed (i) to apoptosis (ii) to deficits during the development of the olfactory bulb.

We examined the first case with IHC experiments in OB cryosections at various developmental stages (E13,5-E18,5). We didn't find any difference in cell apoptosis (caspase3, TUNEL assays). These data indicate that the reduction of mitral cells in adult mice was not an outcome of cell death. Subsequently, we began searching the possible effect of TAG-1 absence that causes defects during olfactory system development. By using a specific marker for projecting neuron precursors (in OB) we found that there is no difference in their numbers between Tag-1 ko and wt at E14,5, during their migration toward the MCL.

We further investigate the possible role of TAG-1 in mitral cell migration/ guidance with the use of a number of specific markers for projecting neurons, guiding molecules and proliferation markers in-vitro and ex-vivo.

In addition behavioral analysis on Tag-1 deficient animals have shown no olfactory learning impairments, but a probable olfactory memory deficit. We plan to subject the animals in extensive olfactory behavioral trials, to elucidate the importance of mitral cell layer organization in the olfactory system function.

Presented by: **Bastakis, George**

Poster No 007
Red Session

Analysis of Synaptic Connectivity in Neuroligin-3 Mutant Mice

Stephane Baudouin [1], *Stephan Gerharz* [1], *Hatstatt Laetitia* [1], *Kenji Tanaka* [2], *Kaspar Vogt* [1], *Peter Scheiffele* [1]
[1] *Biozentrum, University of Basel, Department of Cell Biology, Basel, Switzerland*
[2] *2Division of Neurobiology and Bioinformatics, National Institute for Physiological Sciences, Okazaki, Japan.*

Purkinje cells in the cerebellum integrate information relayed via the mossy fiber granule cell pathway and climbing fiber input received from the inferior olive. Parallel fiber formation and stability are mediated in part through the neurexin – cerebellin – GluRdelta2 complex. However, even in the absence of GluRdelta2 a substantial number of parallel fiber (PF) synapses are assembled. We examined the function of the neuroligin adhesion protein family, a second class of postsynaptic partners for the presynaptic neurexins in parallel fiber synaptogenesis. We found that in PCs, NL3 can be detected exclusively at PF synapses. Loss of NL3 results in a decrease in the staining intensity for the presynaptic vesicular glutamate transporter 1 (vGlut1) supporting an important function for NL3 in PF synapse assembly. Postsynaptically, PC spines exhibit no change in GluRdelta2 concentration but a selective increase in the synaptic concentration of the type 1 metabotropic glutamate receptor. During development, parallel fiber (PF) and climbing fiber (CF) excitatory synapses compete to acquire a specific and non-overlapping territory of innervation on PCs. In NL3 knock-out mice, the territory of CF innervation is increased and CF EPSCs are increased. Our data suggest that NL3 contributes to the trans-synaptic organization of PF synapses and is essential for the regulation of the PF-CF synaptic competition during development. Considering that mutations in NLGN genes are associated with autism-spectrum disorders these findings also provide important insights into NL3-related disease phenotypes.

Presented by: **Baudouin, Stephane**

Poster No 008
Green Session

Xlr Genes Control Synaptogenesis Processes

Beatriz Cubelos
Universidad Autonoma de Madrid

Turner Sybdrome (TS) is caused by a chromosomal anomaly characterized by the presence of a single X chromosome but have no Y chromosome, i.e. the individuals with TS are X0 and have a female phenotype. Among other symptoms, women with TS have a number of cognitive disorders such as autism, dementia and mental retardation. A mouse model of TS (39X0) was used to identify genes with altered expression in brain, candidates to cause the cognitive defects of TS. The most likely candidate genes were the Xlr3b and Xlr4b genes, which are overexpressed 5-6 fold compared to normal mice. During my last postdoctoral stage, I identified the Xlr3b and Xlr4b genes as downstream mediators of Cux genes in synaptogenesis. As an independent researcher under the Ramón y Cajal Program, I propose now to study the function of the Xlr genes in formation and maturation of the dendritic spines and synapses. The underlying hypothesis is that the overexpression of the Xlr genes abrogates the formation of mature spines that subsequently cause a defect in the formation and stability of the excitatory synapses. My preliminary results in wild type mice electroporated in utero and which overexpress Xlr4b in a few cortical neurons show abnormal, immature dendritic spines such as those found in X0 mice. In this project I propose to begin an independent new research group dedicated to the study of the effect of Xlr gene overexpression in synaptogenesis in different areas of the brain and its final consequences in the formation of neuronal circuits and cognitive processes. To this end, I propose the generation of transgenic mice with different levels of overexpression of Xlr3b and Xlr4b in all or part of the brain. I will use knockdown experiments with shRNA constructs to try to revert the effects of overexpression. In addition, in utero electroporation will serve to back up the results generated in transgenic mice in conditions where a few neurons overexpress Xlr within a wild type cell context. The transgenic mice will be studied at a immunohistochemical, biochemical, functional (electrophysiology) level and also in behavioural tests. Finally, I will carry out comparative gene expression studies to identify possible target genes regulated by Xlr overexpression that could mediate their effect in dendrite spine formation and synaptogenesis.

Presented by: **Cubelos, Beatriz**

Poster No 009
Blue Session

The Potential Role of the CCT Complex in the Regulation of Long Range Axonal Transport

Kinga Bercsenyi, Guillermo Menendez, Bradley Spencer-Dene, Raphael Chaleil, Giampietro Schiavo
Cancer Research UK, London Research Institute

As a motoneuron spans the whole size of an organism it must find effective ways to maintain efficient communication between its different parts, ensuring the integration of different inputs, the regulation of synaptic transmission and the maintenance of neuronal homeostasis. Fast axonal transport constitutes the backbone of long-distance communication in neurons, functionally connecting distal areas with the soma and vice versa. In spite of its relevance, characterisation of the mechanisms controlling the targeting of specific ligands, such as growth factors and their receptors, to specific axonal transport routes remains in its infancy.

Several subunits of the CCT chaperonin complex were found to be associated with long-range axonal retrograde transport organelles in a proteomic screen. These organelles carry neurotrophins and their receptors from the neuromuscular junction to the cell soma.

The CCT complex is a multisubunit chaperonin which binds to proteins belonging to different functional groups, including endocytosis, cytoskeleton, chromatin remodelling and the nuclear pore complex. Mutations in CCT subunits cause severe neurodegenerative disorders. Patients suffering from mutilating sensory neuropathy all shared a mutation in CCT5. CCT also interacts with polyQ-expanded huntingtin protein and inhibits its aggregation, suggesting that this complex may have a role in the clearance of protein aggregates, the formations of which have been associated with several neurodegenerative diseases.

The goal of my project is to investigate the role of CCT in the regulation of the biogenesis and transport of signalling endosomes. By characterizing the function(s) of CCT, I plan to shed light on how alterations of CCT activity lead to neurodegenerative disorders connected to long-range axonal transport.

Presented by: **Bercsenyi, Kinga**

Poster No 010
Red Session

Otx2-glycosaminoglycan interactions for Otx2 transfer in the visual cortex

Clémence Bernard, Julien Spatazza, Ariel Di Nardo and Alain Prochiantz
CNRS UMR 7233, Collège de France, Paris, France

Otx2 homeoprotein activates a critical period during which the visual cortex is plastic. This signalling requires an activity-dependent intercellular transfer, as Otx2 is not expressed in the visual cortex. We have shown that during postnatal development, Otx2 is transferred specifically into parvalbumin (PV) GABAergic interneurons and induces their maturation.

The specific internalization of Otx2 by PV-cells led us to study whether glycosaminoglycans (GAG) present at the surface of PV cells, called perineuronal nets (PNNs), are involved in Otx2 recognition. We find that PNN hydrolysis by a chondroitinase, which reopens ocular dominance plasticity in adulthood, reduces the number of Otx2-positive cells in the visual cortex. A consensus GAG-binding motif containing an arginine-lysis (RK) doublet has been identified within Otx2 primary sequence. This motif has a high affinity for chondroitin sulfates, antagonizes Otx2 specific internalization by PV cells in vivo, and reopens a window of plasticity in the adult visual cortex. These results are not observed when the RK doublet is replaced by 2 alanines.

We introduced this RK→AA mutation in a transgenic knock-in Otx2-AA mouse. The preliminary immunohistochemical studies of these mice suggest that this mutation disrupts the interaction between Otx2 and PNNs and impacts visual circuits development. Indeed, in these mice, we observed dose-dependent changes of PV and PNN staining intensity, which suggests a defect in PV interneuron maturation. This will be confirmed by an electrophysiological study of these Otx2-AA mice.

Presented by: **Bernard, Clémence**

Poster No 011
Green Session

d-IMP, the Drosophila Ortholog of the Mrna Transport Factor ZBP1 Controls Axon Growth and Branching in vivo

Caroline Medioni [1], Anne Ephrussi [2], Florence Besse [1]
[1] Institute of Developmental Biology and Cancer, Parc Valrose, 06108 Nice Cedex2, France
[2] EMBL, Meyerhofstrasse 1, 69117 Heidelberg, Germany

In vivo, developing neurons extend axonal processes through a complex environment to find their targets. Studies performed in cultured neurons have shown that recruitment of specific mRNAs to axon growth cones, and local axonal translation of these mRNAs is very important for the growth cone to sense extracellular cues and translate them into polarized growth. Although it has been shown that the RNA-binding protein ZBP1, a conserved factor involved in mRNA targeting, is dynamically recruited to vertebrate axon growth cones, and that its binding to b-actin mRNA is required for axon turning in vitro (Leung et al. 2006; Yao et al. 2006), the biological role of this post-transcriptional regulatory process still remains to be tested in vivo. Furthermore, the molecular mechanisms controlling axonal mRNA transport and translation largely remain to be explored.

To address these questions, we are using a population of neurons located in the Drosophila brain as a genetically tractable model system. In this system, we have discovered that d-IMP, the Drosophila ortholog of ZBP1, is required for polarized axon growth and branching. Using a live-imaging protocol we have recently developed, we have further shown that d-IMP is actively transported to growing axons in vivo, consistent with a function in active transport of selected mRNAs. To identify d-IMP mRNA targets, we have combined candidate-based and genome-wide approaches, and have so far focused on a target found in axons and encoding a regulator of the actin cytoskeleton. We have shown that this regulator is essential for the polarized growth of axons, is required genetically downstream of d-IMP, and has a conserved 3'UTR bound by d-IMP. Results from experiments aiming at analyzing the distribution of this target using in vivo reporters will be presented.

Presented by: **Besse, Florence**

Poster No 012
Blue Session

Identification of Novel Growth Associated Isoforms: A Deep Sequencing Approach

John Bixby, Jessica Lerch-Haner, Dario Motti, Frank Kuo, Dinara Strikis, Vance Lemmon
University of Miami Miller School of Medicine

Dorsal root ganglion (DRG) neurons regenerate axons after injury, unlike mature central nervous system (CNS) neurons. Additionally, extrinsic inhibitory cues limit regenerative responses in most neurons. Microarray studies on different neuronal types have identified single genes important for regenerative responses, but these are limited in gene coverage, dynamic range, and ability to distinguish gene isoforms. We used deep sequencing technology to profile the transcriptomes of dorsal root ganglion (DRG) neurons and CNS neurons grown on both permissive and inhibitory substrates. RNA from each neuronal type was sequenced, generating over 40 million sequence reads per sample. The reads were mapped to a reference genome and transcript abundance and isoform information was generated (Trapnell et al, Bioinform. 25: 1105, 2009; Biotechnol. 28: 511, 2010). Comparison of transcript abundance, calculated as reads per kilobase of exon per million mapped reads, indicates high reproducibility between biological replicates ($R2 \geq 0.98$). While 30% of all transcripts corresponding to the genome annotation are associated with known genes, almost 70% are associated with novel gene isoforms. Isoform abundance varies with neurons in different states. For genes known to be important for regenerative responses, such as the signal transducer and activator of transcription family, the Krüppel-like factor family, the activating transcription factor family, and phosphatase and tensin homolog, we identified multiple isoforms, including novel isoforms. By studying the patterns of isoform expression and their functional consequences we will obtain novel insights into the mechanisms governing gene regulation of neuronal growth.

Presented by: **Bixby, John**

Poster No 013
Red Session

Wnts Locally Activate CaMKII at Dendritic Spines to Promote Spine Growth and Synaptic Strength

Kieran Boyle, Lorenza Ciani, Ellen Dickins, Derek Anane, Macarena Sahores, Douglas Lopes, Alasdair Gibb, Patricia Salinas
University College London

Wnt signalling molecules are well established as presynaptic organizers in the central nervous system, but their role at the postsynaptic side of central synapses remains unclear. Here we demonstrate that Wnt7a, signalling through Dishevelled-1 (Dvl1), promotes postsynaptic development at hippocampal excitatory synapses by increasing dendritic spine number and size, clustering of PSD-95 and AMPA receptors, and excitatory synaptic strength. Crucially, spine number and size is diminished in vivo in the hippocampus of mutant mice lacking Wnt7a and Dvl1, and excitatory synaptic transmission is also disrupted in acute slices from these mice.

Dendritic expression of Dvl1 increases spine size and mEPSC frequency and amplitude, demonstrating that Wnt signalling directly regulates excitatory postsynaptic structure and function. To probe the intracellular pathway responsible for this effect, we expressed PSD-95-Vim-CFP, a synaptic reporter of Ca2+/Calmodulin-dependent protein kinase II (CaMKII) activity. Wnt7a rapidly increases CaMKII activation in spines, and CaMKII inhibition abolishes Wnt7a-mediated spine growth and increased synaptic strength.

Our results demonstrate that Wnt7a locally activates CaMKII at dendritic spines to promote spine growth and increase synaptic strength at central excitatory synapses. This not only extends our understanding of the role of Wnt signalling in normal synaptic development, but also suggests Wnt signalling could play a role in neurological conditions in which spine structure is disrupted, such as Fragile X, Down and Rett syndromes.

Presented by: **Boyle, Kieran**

Poster No 014
Green Session

RNA Localization and Local Translation Contribute to the Ability of cAMP to Promote Axonal Regeneration

Christine Cain [1], *Rathna Chaudhry* [1], *Jonathan Davila* [2], *Mavis Swerdel* [2], *Noo-Li Jeon* [3], *Ronald Hart* [2], *Marie Filbin* [1]
[1] *Hunter College in the City of New York*
[2] *Rutgers University*
[3] *University of California at Irvine*

Inhibition by myelin is a significant obstacle in the ability of CNS neurons to regenerate. Neurons can overcome myelin inhibition if levels of cAMP are elevated. However, the global effects of cAMP make it unfeasible for use as a therapy to treat spinal cord injury. Here, we have identified novel downstream effectors of cAMP in the form of RNAs localized to axons. In studies employing translational inhibition, we asked if cAMP-dependent local translation contributes to ability of axons to grow after injury. To identify the pool of cAMP-responsive RNAs in axons, we compartmentalized DRG neurons in microfluidic chambers, treated soma with cAMP and subjected axonal RNA samples to microarray and Solid sequencing analysis. To examine the significance of local translation in the cAMP effect, we elevated cAMP in soma through the use of microfluidics or peripheral lesion and applied translation inhibitors to axons. We found 49 specific mRNAs regulated more than 1.5-fold and hundreds of microRNAs regulated more than 2-fold in axons. Many mRNAs contained CRE sites, affirming their positions in the cAMP/PKA/CREB pathway, as well as binding sites for several of the identified axonal microRNAs. FISH and qPCR experiments validated our screens. Overexpression of microRNAs and subsequent neurite outgrowth assays revealed miR-151 as a specific cAMP effector. Moreover, in vitro and in vivo studies demonstrated a clear reversal of the cAMP effect when translation inhibitors were applied to axons under inhibitory conditions. Our results link local translation to the cAMP effect and identify novel molecules for potential therapeutic development.

Presented by: **Cain, Christine**

Poster No 015
Blue Session

Neurotrophins/p75NTR Signaling Specifies Axons during Cortical Development and Adult Neurogenesis

Marco Canossa, Emanuela Zuccaro, Matteo Bergami, Beatrice Vignoli, Guillaume Bony, Spartaco Santi, Laura Cancedda
Italian Institute of Technology

Introduction. How a newly generated neuron initiates polarizing signals that specify a single axon and multiple dendrites is critical for patterning neuronal circuits "in vivo". Here we report that the pan-neurotrophin receptor (p75NTR) is a polarity regulator that localizes asymmetrically in developing neurons and initiates neurotrophins signals for specification of the future axon. Results. (i) In cultured neurons local exposure to neurotrophins recruits p75NTR and mPar-3 into one undifferentiated neurite, determining a complex essential for axogenesis. (ii) Disruption of p75NTR sub-cellular localization, by knockdown or ectopic expression, prevents from asymmetric accumulation/activation of conserved cell polarity signalling pathways, and neurons fail to initiate axons. (iii) "In vivo" studies revealed that p75NTR governs newborn neurons polarity in the developing cortex and adult hippocampus, which overrides the pattern and assembly of neuronal circuits in these brain areas. Conclusion. Neurotrophins/p75NTR signaling initiate polarity programs resulting in stable signals for axogenesis and neuronal circuits formation.

Presented by: **Canossa, Marco**

Poster No 016
Red Session

Evidence for a Mechanical Coupling between N-Cadherin Adhesions and F-Actin in Shaping Dendritic Spines

Anaël Chazeau, Katalin Czondor, Mikael Garcia, Grégory Giannone, Olivier Thoumine
Interdisciplinary Institute for NeuroScience UMR 5297

The dynamic morphology of dendritic spines is controlled by both the actin cytoskeleton and adhesive molecules such as cadherins. We tested here the hypothesis that actin remodeling in dendritic spines could be regulated by N-cadherin adhesion and myosin contractility.

We first examined the localization of N-cadherin, actin and myosin in primary hippocampal neurons by STED microscopy. Actin-GFP and N-cadherin-DsRed co-localized mainly at the head of dendritic spines, suggesting a tight association between the two proteins. In contrast, MLC (myosin light chain)-GFP was more localized at the spine base, suggesting a role for myosin II in pulling actin filaments rearward.

We then characterized, the basal motility index of dendritic spines using time lapse imaging of actin-GFP, upon treatment to increase myosin II activity, or expression of an N-cadherin mutant. N-Cadherin mutants' dendritic spines had a higher motility index than N-Cadherin-WT spines, suggesting that the mechanical connection between post-synaptic N-cadherin and actin is stabilizing dendritic spines. Activation of myosin II induced a retraction of actin-GFP spots from the tip to the base of the spine, suggesting a role for myosin II in generating retrograde F-actin flow in dendritic spines. Furthermore, for N-Cadherin mutants the proportion of spines retracting was higher than for N-Cadherin-WT spines. To refine our understanding of actin flow and motility, we are performing single molecule experiments (actin-mEos) using PALM.

Taken together, these data show that actin remodeling and flow in dendritic spines is regulated by N-cadherin adhesion and myosin contractility, suggesting a tight mechanical coupling between these three molecules.

Presented by: **Chazeau, Anaël**

Poster No 017
Green Session

Unraveling the Regulation of Intracellular Trafficking During the Establishment and Maintenance of Neuronal Polarity

Joanna Chiang, Andreas W. Püschel
Institut für Molekulare Zellbiologie, Schlossplatz 5 Westfälishe Wilhelm-Universität Münster, Germany

The establishment of a polarized cell structure is essential for the differentiation, maturation and function of a neuron. The interplay of different signaling pathways and cell structures orchestrates the initiation of polarity in a neuron. It has been shown that increased bulk cytoplasmic flow into a neurite precedes its extension as the single axon. This observation highlights the importance of the transport machinery in neuronal polarity but little is known about how polarized transport into the nascent axon is initiated.

Intracellular transport is also essential for the maintenance of polarity through its role in sorting proteins to axons and dendrites. Here we investigate members of the Rab family of GTPases that function as regulators of vesicular trafficking for their role in neuronal polarity. To identify Rabs potentially involved in neuronal polarity we screened a set of Rabs by expressing constitutively active and dominant-negative mutants in rat hippocampal neurons and analyzing the resulting phenotypes. We could identify several Rab GTPases that induce the formation of multiple axons when expressed in already polarized neurons. This analysis suggest a role for vesicle trafficking in the negative feedback that blocks the formation of supernumerary axons after polarity is established. By identifying Rab GTPases that act as regulators of endocytosis and vesicle trafficking, we hope to decipher how polarized transport is established, how it contributes to the maintenance of neuronal polarity. In parallel, we are investigating effectors downstream of these GTPases to unravel pathways that are linked to them. Together these experiments will lead to a better understanding of polarized transport and its regulation in neurons.

Presented by: **Chiang, Joanna**

Poster No 018
Blue Session

Cellular Models to Explore Human Synaptogenesis

Laurie Chicha [1], *Frederic Knoflach* [1], *Rachel Haab* [1], *Josef Bischofberger* [2], *Roberto Iacone* [3], *Bernd Bohrmann* [1], *Martin Graf* [3], *Sannah Jensen* [3], *Ravi Jagasia* [1]
[1] *F. Hoffmann-La Roche Ltd - CNS Discovery*
[2] *Institute for Physiology, University Basel*
[3] *F. Hoffmann-La Roche Ltd - Discovery Technology*

A major challenge in CNS research is the establishment of cellular assays that accurately recapitulate normal and diseased neurophysiology in vitro. Synapses are fundamental structures in neuronal communication and the formation, remodeling and elimination of synapses are continual physiological processes. The relevance of synaptic homeostasis is highlighted by the fact that mutations in human post-synaptic density (PSD) proteins occur in over 100 neurological and psychiatric disorders. Here we aim to model human synaptogenesis in vitro using pluripotent stem cells. To this end, we characterize the physiologic and synaptic properties of two different neuronal subtypes differentiated from human embryonic stem cells, enriched in GABAergic or dopaminergic features. We describe the establishment of several assays to characterize synaptic properties including specific immunolabeling, electrophysiology and calcium imaging. We envision that such cellular models will be instrumental in the elucidation of mechanisms regulating human synaptic functions and in the future, we plan to extend our work to the modeling of pathophysiological mechanisms of synaptic dysfunction occurring in CNS diseases.

Presented by: **Chicha, Laurie**

Poster No 019
Red Session

The Effect of α-Synuclein and β-Amyloid on the Growth and Retraction of Neurites in Cultured Hippocampal Neurons

Hanako Tsushima, Francesco Difato, Axel Blau, Evelina Chieregatti
Dept. of Neuroscience and Brain Technologies, Italian Institute of Technology, Genoa, Italy

Alpha-synuclein (α-syn) and beta–amyloid (Aβ) are the major components of fibrillary lesions such as Lewy bodies and amyloid plaques that are the hallmarks of neurodegenerative disorders, such as Parkinson's disease (PD) and Alzheimer's disease (AD). To date, the physiological functions of α-syn and Aβ are not well-defined. Overexpression, mutation or aggregation of these proteins can induce alterations in neuronal function that subsequently leads to neuronal degeneration.

We demonstrate that incubation of hippocampal neurons at early days in culture with Aβ1-42 peptide increases lamellipodia formation. In addition, we show that Aβ increases the amount of polymerized actin in the presence of drugs that depolymerize or stabilize F-actin structure, such as cytochalasin, latrunculin and jasplakinolide.

Aβ has been found to interact with α-syn, enhancing their properties to form aggregates. In addition we showed that α-syn interacts with actin and plays a major role in actin dynamics. Therefore we investigated the possible enhancement or rescue of the Aβ's effect by α-syn, by comparing the phenotype of wild-type mice neurons against that of neurons from a mice strain carrying a deletion of α–syn gene, as well as that of neurons reconstituted by electroporation of α-syn. We show that the effect of Aβ reduces the number of neurites of developing neurons at early days in culture and that this effect is dependent on α-syn presence.

Drugs that perturb actin dynamics also inhibit membrane retrieval that is indirectly compensated by Golgi-derived –constitutive exocytosis. These membrane processes have been implicated in neurite retraction and regrowth. The cytoskeletal effect and the neurite reduction we saw upon application of Aβ, points to the importance of studying the role of these proteins on cytoskeletal-driven processes. Actin has a key role in neurite regeneration after injury and in growth cone turning. We observed that actin dynamics and the formation of actin waves were altered during regeneration of neurites after lesion. Thus we are currently studying the effect of α-syn and Aβ on these processes by laser-assisted dissection of the axon of hippocampal neurons in culture and by the use of optically-trapped microspheres coated with α-syn and with Aβ.

Presented by: **Tsushima, Hanako**

Poster No 020
Green Session

Protein Kinase Substrate Screen Reveals Dominant Role for JNK in Regulating Morphology Changes During Brain Development

Eleanor Coffey [1], *Justyna Zdrojewska* [1], *Nina Westerlund* [1], *Benny Bjorkblom* [2], *Emilia Komulainen* [3]
[1] *Turku Centre for Biotechnology, Åbo Akademi & University of Turku*
[2] *University of Stavanger, Stavanger, Norway*
[3] *benny.bjorkblom@gmail.com*

Protein phosphorylation is the most common post-translational modification used by cells to control function. It is no surprise therefore that protein kinases contribute to the morphological changes associated with neuronal development. c-Jun N-terminal kinases (JNKs) are known to be essential for brain development as mice lacking JNKs 1 and 2 fail to complete neural tube closure. The substrates of JNK mediating this event have remained elusive. To better understand the function of JNK in embryonic brain we developed a proteomic screen to identify kinase substrates. Interestingly, a large proportion of JNK substrates were cytoskeletal regulatory proteins, the identity of which prompted us to investigate JNK function in neuronal migration, dendrite architecture and spine morphology regulation. The data to be presented demonstrates that JNK and its targets SCG10 and MAP2 play critical roles in regulating radial migration and multipolar stage exit1 during formation of the cortex and subsequent to this, JNK controls dendrite architecture and spine morphology.

1Westerlund, N. Zdrojewska, J., Padzik, A., Komulainen, E., Björkblom, B., Rannikko, E., Tararuk, T., Garcia-Frigola, C., Sandholm, J., Nguyen, L., Kallunki, T., Courtney M., Coffey, E. Phosphorylation of SCG10/stathmin-2 determines multipolar stage exit and neuronal migration rate. Nature Neuroscience, 2011 in press

Presented by: **Coffey, Eleanor**

Poster No 021
Blue Session

Roles of the AMPK-Related Kinases NUAK1 and NUAK2 Downstream of LKB1 during Cortical Development

Julien Courchet [1], *Mariko Hirano* [2], *Shinichi Aizawa* [2], *Franck Polleux* [1]
[1] *The Scripps Research Institute, La Jolla, CA*
[2] *Center for Developmental Institute, RIKEN, Kobe*

Cortical development involves the coordinated migration of billions of neurons and the establishment of axon/dendrite polarity underlying the the proper flow of information transfer during the formation and function of neuronal circuits. Impairment of these processes can lead to socially-devastating neurodevelopmental defects such as autism spectrum disorders or mental retardation. Our lab recently demonstrated that the activity of the polarity kinase LKB1/STK11/Par4 is required and sufficient to induce neuron polarization and axon specification, both in vitro and in vivo in part through its ability to activate two members of the AMPK kinase family called SAD-A/B kinases (also called BRSK1/2) (Barnes et al., Cell 2007). We now identified additional roles for LKB1 involving two other distinct and poorly characterized downstream targets of the AMPK-related kinase family, NUAK1 (ARK5/OMPHK1) and NUAK2 (SNARK/OMPHK2). NUAK1/2 kinases are highly enriched in the brain during development and display specific, yet complimentary expression patterns in the embryonic cortex. Simultaneous knockout of Nuak genes lead to severe developmental abnormalities including exencephaly as well as impaired neurogenesis and cell survival, indicating that NUAKs function is essential for brain development. Using both in vitro and in vivo assays, we found that NUAK kinases play a limited role downstream of LKB1 in the process of axon specification, but are required later for proper axonal elongation and branching. Finally, we obtained evidence suggesting that this new LKB1/NUAK pathway mediate their function in axon growth in part by activating proteins required for endocytosis, vesicular trafficking and autophagy. Overall, our results identify a previously uncharacterized role of LKB1 in axon elongation and uncover a new signaling pathway involving multiple kinases and potentially linking polarity complexes to the regulation of axonal trafficking.

Presented by: **Courchet, Julien**

Poster No 022
Red Session

AMPA and Kainate Receptors are Differentially Regulated by CaMKII Phosphorylation

Françoise Coussen [1], *Mario Carta, Patrizio Opazo, Daniel Choquet, Christophe Mulle*
[1] *CNRS*

Ca2+/Calmodulin-dependent protein kinase II (CaMKII) is critically required for the synaptic recruitment of AMPA-type glutamate receptors (AMPARs). However very little is known about the role of CaMKII on the trafficking of kainate-type glutamate receptors (KARs). KARs expressed in cultured hippocampal neurons can be modulated by calcineurin and CaMKII. Ca2+ influx through NMDARs resulted in a transient depression of KAR current. The recovery of the current amplitude is dependent on CaMKII activity. Postsynaptic KARs expressed at hippocampal mossy fiber synapses are likely composed of GluK2/GluK5 subunits. In these heteromers, only GluK5 contains potential phosphorylation sites for CaMKII.

We have studied the role of CaMKII phosphorylation in the dynamics of GluK2/GluK5 KARs. In particular we examined how the interaction between phosphorylated K5 with PSD95 controls these processes. Finally, we compared the regulation of the mobility of AMPARs and KARs by CaMKII.

Presented by: **Coussen, Françoise**

Poster No 023
Green Session

The srGAP Family Proteins Play Distinct Roles in Neuronal Development

Jaeda Coutinho-Budd [1], *Takayuki Sassa* [1], *Vladimir Ghukasyan* [1], *Sabrice Guerrier* [1], *Franck Polleux* [2]
[1] *University of North Carolina at Chapel Hill*
[2] *The Scripps Research Institute*

During cortical development, glutamatergic neurons migrate to the outermost layer of the cortex, and subsequently form a single axon and a complex dendritic arbor in order to establish proper connections. Both neuronal migration and morphogenesis require extensive coordination of membrane remodeling and cytoskeletal dynamics. Most studies of the mechanisms underlying neuronal migration and differentiation have focused on signaling pathways regulating the cytoskeleton. However, over the past decade, a paradigm shift has occurred that strongly suggests that the actin and microtubule cytoskeleton are not playing an instructive role with regard to membrane deformation involved in cell shape changes. Rather membrane-deforming proteins of the BAR-superfamily (subdivided into BAR/N-BAR, F-BAR, and I-BAR) have been shown to interface membrane deformation with cytoskeletal dynamics, though little is known about the importance of these proteins during brain development. We have previously shown that srGAP2 acts as a non-canonical F-BAR domain to induce filopodia-like protrusions, rather than membrane invaginations. In this study, we have conducted a deeper investigation into this non-canonical family of F-BAR proteins. Our results explore the molecular mechanisms underlying the unexpected degree of functional diversity among members of the srGAP protein family.

Presented by: **Coutinho-Budd, Jaeda**

Poster No 024
Blue Session

A General Quantitative Model of AMPA Receptor Trafficking at Synapses

Katalin Czondor [1], Magali Mondin [2], Mikael Garcia [3], Martin Heine [4], Renato Frischknecht [4], Jean-Baptiste Sibarita [2], Daniel Choquet [2], Olivier Thoumine [2]
[1] Interdisciplinary Institute for Neuroscience (UMR 5297), Centre National de la Recherche Scientifique,Bordeaux, France
[2] Interdisciplinary Institute for Neuroscience (UMR 5297), Centre National de la Recherche Scientifique, Bordeaux, France
[3] 1. Interdisciplinary Institute for Neuroscience (UMR 5297), Centre National de la Recherche Scientifique, Bordeaux, France
[4] Leibniz Institute, Magdeburg, Germany

Membrane trafficking of AMPA receptors (AMPARs) plays a pivotal role in controlling the strength of excitatory synaptic transmission. The number of AMPARs at synapses is regulated by at least two parallel processes: 1/ membrane trafficking by endocytosis and exocytosis, and 2/ trapping of membrane-diffusing receptors by scaffold molecules. In order to better understand the interplay of these mechanisms, we developed a quantitative framework integrating diffusion/trapping and internal recycling mechanisms. A computer algorithm was built to simulate the behavior of individual AMPARs. Single nano-particle tracking and FRAP experiments measuring AMPAR dynamics in primary hippocampal neurons were used to verify the developed framework. In agreement with the parameters measured in vitro, the results of the simulations showed correlation between increased synaptic density and the drop in diffusion coefficient. The mean and variance of AMPAR number at synapses and the influence of endocytosis/exocytosis on AMPAR distribution was also described. Importantly, the comparison between experimental data and theoretical curves allowed the predictions of the binding rate of AMPARs to their scaffold. According to the model, the increase in AMPAR number at synapses induced by potentiation protocols relies on a fine tuning between local exocytosis and increased binding affinity of AMPARs to their scaffold. Inversely, the model predicts that synaptic depression relies on the locus and rate of AMPARs internalization. This framework thus allows estimation of key parameters controlling AMPAR trafficking in the post-synaptic membrane and opens the way to a general quantitative description of synaptic strength and plasticity.

Presented by: **Czondor, Katalin**

Poster No 025
Red Session

Mutations in Human RAB39B Gene are Responsible for X-Linked Non-Specific Mental Retardation

Patrizia D'Adamo [1], *Maila Giannandrea* [1], *Maria Lidia Mignogna* [1], *Maria Passafaro* [2]

[1] *Dulbecco Telethon Institute, Dibit - San Raffaele Scientific Institute, Milano, Italy*

[2] *Dulbecco Telethon Institute, CNR, Milano, Italy*

Recently we identified two independent loss of function mutations in RAB39B gene in two MRX families. Patients of both families present a moderate to severe mental retardation and some of them present also additional features such as epileptic seizures, macrocephaly and autism spectrum disorder. RAB39B is one of the RAB proteins with unknown function and we first characterized its expression profile and its cellular localisation. By using different techniques, we determined that RAB39B is predominantly expressed in brain, particularly in neurons and the co-localization with markers of Golgi compartments, suggested an involvement in the Golgi-related traffic.

In order to understand how the lack of RAB39B could be involved in the pathology of XLMR, we down-regulated Rab39b on hippocampal neurons by shRNA technology. We showed that the 70% reduction of RAB39B did not affect the dendrites and axons formation. In contrast, at 7 days in vitro (DIV), we found a reduction in SYNAPSIN I positive puncta at the pre-synaptic sites, suggesting a decrease in the mean number of synapses. This data was validated by western blot analysis, where different synaptic proteins were decreased in their total amount at 7 DIV and 14 DIV, suggesting that RAB39B is important for synapses formation and/or maintenance.

In order to understand the molecular mechanisms altered by RAB39B absence, two-hybrid screening allowed us to identify PICK1 (Protein interacting with C kinase 1) as the major RAB39B interacting protein, confirmed by GST-pull down and co-immunoprecipitation.

The discovery of PICK1 as an interactor of RAB39B, led us to focus our attention on neuronal receptors. Actually, PICK1 is involved in the clustering of various receptors (AMPAR and mGluRs), possibly by acting both pre- and post-synaptically, at the receptor internalization level, thus affecting LTD and LTP formation.

Presented by: **D'Adamo, Patrizia**

Poster No 026
Green Session

Mechanisms of Neuronal Remodeling by BDNF

Katrin Deinhardt [1], *Barbara Hempstead* [2], *Moses Chao* [1]
[1] *Skirball Institute, NYU SoM*
[2] *Weill Cornell Medical College*

The establishment and refinement of neural circuits during development depends upon a dynamic process of outgrowth and branching of axons and dendrites that leads to synapse formation and connectivity. However, the mechanisms that translate extracellular signals into axonal branch formation are incompletely understood, as are the mechanisms that negatively regulate the arborization of processes.

The neurotrophin BDNF plays an essential role in the outgrowth and activity-dependent remodeling of axonal arbors in vivo. We recently reported that the MAP kinase phosphatase-1 (MKP-1) controls BDNF-dependent axon branching. MKP-1 expression induced by BDNF signaling exerts spatio-temporal deactivation of JNK, which negatively regulates the phosphorylation of JNK substrates that impinge upon microtubule destabilization. Indeed, neurons from mkp-1 null mice were unable to produce axon branches in response to BDNF.

On the other hand, dendritic growth is affected negatively by the BDNF precursor, proBDNF. Mice impaired in proBDNF processing display markedly reduced dendritic complexity in vivo, suggesting that proneurotrophins negatively influence neuronal morphology. Both proBDNF and proNGF acutely cause collapse of growth cones in vitro, a retraction that is initiated by a novel interaction between p75NTR and the sortilin family member, SorCS2. Downstream, two discrete signaling pathways involving the inactivation of the actin-bundling protein fascin and the dissociation of the Rac activator Trio from p75NTR/SorCS2 and subsequent inactivation of Rac, induce growth cone collapse. These results identify a bifunctional signaling mechanism through which proneurotrophins and their mature forms differentially modulate neuronal morphology in the nervous system.

Presented by: **Deinhardt, Katrin**

Poster No 027
Blue Session

Regeneration of Axons in Injured Spinal Cord by Activation of BMP/Smad1 Signaling Pathway in Adult Neurons

Hongyan Jenny Zou [1], *Pranav Parikh* [2], *Yuhan Hao* [2], *Mohsen Hosseinkhani* [2], *Shekhar Patil* [2], *George Huntley* [2], *Marc Tessier-Lavigne* [3]
[1] *Mount Sinai School of Medicine, Dept of Neurosciences*
[2] *Mount Sinai School of Medicine*
[3] *Genentech/Rockefeller University*

Axon growth potential is highest in young neurons, but diminishes with age, thus becoming a significant obstacle to axonal regeneration following injury in maturity. The mechanism for the decline is incompletely understood, and no treatment is available to rekindle innate growth capability. Here, we show that Smad1-dependent BMP signaling is developmentally regulated and governs axonal growth in dorsal root ganglion (DRG) neurons. Down-regulation of the pathway contributes to the age-related decline of the axon growth potential. Re-activating Smad1 selectively in adult DRG neurons results in robust sensory axon regeneration in a mouse model of spinal cord injury (SCI). Smad1 signaling can be effectively manipulated by an AAV vector encoding BMP4 delivered by a clinically applicable and minimally invasive technique, an approach devoid of unwanted abnormalities in mechanosensation or pain perception. Importantly, transected axons are able to regenerate even when the AAV treatment is delivered after SCI, thus mimicking a clinically relevant scenario. Together, our results identify a new therapeutic target to promote axonal regeneration after SCI.

Presented by: **Zou, Hongyan Jenny**

Poster No 028
Red Session

Analysis of the Mechanisms by which Citron Proteins and TTC3 Modulate Early Differentiation in Primary Hippocampal Neurons

Gaia Berto [1], Paola Camera [1], Cristina Iobbi [1], Elena Scarpa [1], Ylenia Bosio [1], Federico Bianchi [1], Carlos Dotti [2], Ferdinando Di Cunto [1]
[1] Molecular Biotechnology Center, University of Torino, Italy
[2] VIB Department of Molecular and Developmental Genetics, University of Leuven, Belgium

Wiring of the central nervous system (CNS) during development depends on the sprouting and elongation of axons and dendrites, at the right time and in the right direction. Even subtle abnormalities affecting these events can produce devastating consequences, such as epilepsy and mental retardation. Among the cell-autonomous factors that contribute to neuronal differentiation, the dynamic crosstalk between membrane trafficking events and cytoskeletal reorganization certainly plays a major role. The proteins encoded by the Citron gene are binding partners of the small GTPase RhoA, known for locally regulating actin organization in proliferating neuronal precursors as well as in differentiating and differentiated neurons. We have recently discovered that Citron proteins are capable to interact with TTC3, a protein encoded by one of the genes of the Down syndrome critical region. Moreover, we have shown that Citron proteins and TTC3 may cooperate to regulate neuronal differentiation. In this presentation we will show the results of our most recent research about the mechanisms by which Citron proteins and TTC3 regulate neuronal differentiation in rat primary hippocampal neurons. Our results will provide new insight about how Golgi membranes and actin cytoskeleton influence axonal growth during early differentiation.

Presented by: **Di Cunto, Ferdinando**

Poster No 29
Green Session

Paracrine Pax6 Activity Regulates Oligodendrocyte Precursor Cell Migration in the Chick Embryonic Neural Tube

Elizabeth Di Lullo [1], *Celine Haton* [2], *Michel Volovitch* [1], *Jean-Leaon Thomas* [2], *Alain Prochiantz* [1]
[1] *College de France*
[2] *Brain and Spinal Cord Institute*

Homeoprotein transcription factors play fundamental roles in development, ranging from embryonic polarity to cell differentiation and migration. Research in recent years has underscored the physiological importance of homeoprotein intercellular transfer in eye field development, axon guidance and retino-tectal patterning and visual cortex plasticity. In this study, we investigate a possible role for homeoprotein Pax6 transfer in oligodendrocyte precursor cell migration. Using the chick neural tube as a model, we define the extracellular expression of Pax6 and the effects of gain and loss of extracellular Pax6 activity. Two models are used, open book culture of neural tubes with recombinant Pax6 protein or Pax6 blocking antibodies, and in vivo gene transfer experiments involving expression of secreted Pax6 protein or secreted Pax6 antibodies. Our data provide converging evidence showing that oligodendrocyte precursor cell migration is promoted by extracellular Pax6. The paracrine effect of Pax6 on oligodendrocyte precursor cell migration is thus a new example of non-cell autonomous homeoprotein activity

Presented by: **Di Lullo, Elizabeth**

Poster No 030
Blue Session

Identification of the Nogo Receptor Family Members NgR1 and NgR3 as CSPG Receptors: Evidence for a Mechanistic Link between CNS Myelin- and CSPG-Mediated Growth Inhibition

Travis L Dickendesher [1], Katherine T Baldwin [1], Yevgeniya A Mironova [1], Stephen J Raiker [1], Yuntao Duan [1], Peter Shrager [2], Binhai Zheng [3], Larry I Benowitz [4], Herbert M Geller [5], Roman J Giger [1]
[1] *University of Michigan Medical School*
[2] *University of Rochester School of Medicine and Dentistry*
[3] *University of California San Diego School of Medicine*
[4] *Harvard Medical School*
[5] *National Institutes of Health*

Following injury to the adult mammalian CNS, severed axons do not regenerate beyond the lesion site. Growth inhibitory molecules in CNS myelin (including Nogo, MAG, OMgp) and chondroitin sulfate proteoglycans (CSPGs) associated with glial scar tissue contribute to this restrictive environment. Here we report on a novel interaction between select members of the Nogo receptor family (NgRs) and CSPGs. NgR1 and NgR3, but not NgR2, bind with high affinity and selectivity to glycosaminoglycan (GAG) chains of neural CSPGs. Soluble NgR1 and NgR3 bind in a chondroitinase ABC lyase (ChaseABC)-sensitive manner to rat brain tissue. They also bind strongly to optic nerve tissue of adult mice subjected to injury and much weaker to control (uninjured) optic nerve. We have mapped the CS-GAG binding motif on NgR1 and show that it is distinct from the binding site of Nogo, MAG, and OMgp. A soluble form of the NgR1 CS-GAG binding motif promotes neurite outgrowth on a mixture of substrate-adsorbed CSPGs. Primary cerebellar granule neurons (CGNs) isolated from NgR123-/- mice grow longer neurites on substrate-adsorbed CSPGs than CGNs isolated from controls. In vivo, loss of all three NgRs leads to significant regeneration of severed retinal ganglion cell (RGC) axons following optic nerve injury compared to controls, an effect that is enhanced in the presence of Zymosan. Our studies suggest that members of the Nogo receptor family are functional receptors for two major classes of growth inhibitory molecules, myelin inhibitors and CSPGs. The identification of shared mechanisms for myelin- and CSPG-mediated inhibition is conceptually novel and provides new insights into the mechanisms that limit neuronal growth and sprouting in the healthy and injured CNS.
Presented by: **Dickendesher, Travis L**

Poster No 031
Red Session

Establishment and Characterization of a Human In Vitro Model System of Neurodegeneration

Liudmila Efremova [1], *Marcel Leist*
[1] *University of Konstanz*

Neurite degeneration is a process occurring during many circumstances, such as chemotherapeutic drug treatment or accidental poisoning, in neurodegenerative diseases and also during the development of the nervous system. It seems that this process has its own local self-destruction program which is distinct from that of mediating apoptosis. But the complete mechanisms are not well studied yet.

We focus on in vitro models of neurodegeneration by using a number of different approaches. We are interested in human effects of the Wlds protein. This fusion protein arose in a mouse by gene translocation and is known to delay Wallerian degeneration of neurites by up to 2-3 weeks. After cloning of the corresponding cDNA, the effects of the protein have been tested in other models. However, all investigations have so far used to rats, mice, flies and fish, but not human cells. If the phenotype could be reproduced in man, important drug targets could be derived from it. We have expressed variants of the protein in human neurons and found a very peculiar nuclear accumulation in a defined subnuclear domain. To define the start point of degeneration in the cell body or in neurites, and to investigate which of the processes is affected by wld(s), we constructed microfluidics chambers which allow separation the somata and neurites. We optimize this device to allow physical and chemical treatments to axons and somata. As additional approach to facilitate the study of neurite toxicity in vitro, we have established techniques which allow the growth of neuronal cells in defined patterns.

Data will be presented on the characterization of the human neuronal model and on approaches to trigger selective degeneration of neurites to study molecular pathways involved in this process.

Presented by: **Efremova, Liudmila**

Poster No 032
Green Session

Phosphorylation of SCG10/stathmin-2 Determines Multipolar Stage Exit and Neuronal Migration Rate

Justyna Zdrojewska [1], *Nina Westerlund* [1], *Artur Padzik* [1], *Emilia Komulainen* [1], *Benny Bjorkblom* [2], *Emmy Rannikko* [1], *Laurent Nguyen* [3], *Tuula Kallunki* [4], *Michael Courtney* [5], *Eleanor Coffey* [1]

[1] *Turku Centre for Biotechnology Åbo Akademi University and University of Turku*

[2] *The Norwegian Centre for Movement Disorders, Stavanger, Norway*

[3] *GIGA-Neurosciences, University of Liege, Belgium*

[4] *Danish Cancer Society, Copenhagen, Denmark*

[5] *University of Eastern Finland Kuopio Campus*

Cell migration is a consequence of the sum of positive and negative regulatory mechanisms. Although appriopriate migration of neurons is a principal feature of brain development, the negative regulatory mechanisms remain obscure. We found that JNK1 was highly active in developing cortex and that selective inhibition of JNK in the cytoplasm markedly increased both the frequency of exit from the multipolar stage and radial migration rate and ultimately led to an ill-defined cellular organization. Moreover, regulation of multipolar- stage exit and radial migration in Jnk1-/- (also known as Mapk8) mice, resulted from consequential changes in phosphorylation of the microtubule regulator SCG10 (also called stathmin-2). Expression of an SCG10 mutant that mimics the JNK1-phopshorylated form restored normal migration in the brains of Jnk1-/- mouse embryos. These findings indicate that the phosphorylation of SCG10 by JNK1 is a fundamental mechanism that governs the transition from the multipolar stage and the rate of neuronal cell movement during cortical development.

Presented by: **Zdrojewska, Justyna**

Poster No 033
Blue Session

Regulation of Neurotransmitter Receptor Trafficking and Degradation During Synaptic Plasticity

Monica Fernandez-Monreal, Jose A. Esteban
CBMSO/CSIC

The strength of synaptic transmission depends partly on the number of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR) in the surface and, thus, can be modulated by postynaptic membrane trafficking events. These processes are critical for some forms of synaptic plasticity such as long-term potentiation (LTP) and long-term depression (LTD). In the case of LTD, AMPARs are internalized in response to synaptic activity. AMPAR endocytosis is triggered by NMDAR activation, is Ca2+-dependent and requires protein phosphatases (Mulkay et al., Science, 1993 and Nature, 1994; Ehlers, Neuron, 2000) and the small GTP-ase Rab5 (Brown et al., Neuron, 2005). However, the fate of the internalized subunits upon LTD induction is still matter of debate. Here we studied the degradation pathway of the GluA1 subunit of AMPAR that follows LTD. To this aim, we used biochemical methods, electrophysiological recording and video-microscopy. We observed that GluA1 is degraded in lysosomes during LTD, but this degradation was not involved in the expression of LTD. However, trafficking of AMPAR to lysosomes is an important event for the LTD. Altogether, we contribute with more evidences to trace the fate of AMPARs during LTD.

Presented by: **Fernandez-Monreal, Monica**

Poster No 034
Red Session

Role of GluN2B in the Synaptic Accumulation of NMDA Receptors

Joana Ferreira [1], *Kevin She* [2], *Amanda Rooyakkers* [2], *Ann Marie Craig* [2], *Ana Luísa Carvalho* [1]
[1] *Center for Neuroscience and Cell Biology & Department of Life Sciences, University of Coimbra, 3004-517 Coimbra, Portugal*
[2] *Brain Research Centre & Department of Psychiatry, University of British Columbia,Vancouver BC, Canada*

The N-methyl-D-aspartate receptor (NMDAR) plays a crucial role in shaping the strength of synaptic connections, which underlies some forms of learning and memory formation in the central nervous system. This type of ionotropic glutamate receptors may be formed by multiple combinations of an obligatory GluN1 subunit with GluN2 (A to D) or GluN3 (A to B) subunits. In the hippocampus and the neocortex, the major GluN2 subunits are GluN2A and GluN2B, which are differentially expressed during development. The contribution of each subunit to the synaptic traffic of NMDARs and therefore for synaptic plasticity is still controversional.

To better understand the specific contribution of the GluN2 subunits to the synaptic expression of NMDARs we used neuronal cultures from GluN2A(-/-) and GluN2B(-/-) mice, and found that, whereas the synaptic expression of NMDARs is normal in GluN2A(-/-) hippocampal neurons, there is a dramatic decrease on the number, intensity and area of synaptic GluN1 clusters in GluN2B(-/-) hippocampal neurons, when compared with GluN2B (+/+) neurons. We also found that chronic activity blockade, which increases the synaptic clustering of NMDA receptors in GluN2B(+/+) neurons, does not have an effect on GluN2B (-/-) neurons. Furthermore, in cortical GluN2B (-/-) neurons we observed a significant decrease of GluN1 and GluN2A protein expression levels, although no changes on mRNA relative levels were detected by real-time PCR. We assayed the total surface expression of NMDARs by biotinylation assays and observed that the dramatic effect observed on the total levels of NMDARs on GluN2B (-/-) neurons reflects on the cell surface levels of these proteins. Taken together, these results suggest the GluN2B-containing NMDAR may be responsible for organizing a synaptic scaffold required for synaptic delivery and/or stabilization of NMDARs.

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Presented by: **Ferreira, Joana**

Poster No 035
Green Session

Proteomics Reveals the Protein Degradation Pathways Present in the Radial Nerve Cord of the Sea Star Wound Healing Events and eEarly Stages of Arm Regeneration

Catarina Franco [1], *Romana Santos* [2], *Ana Varela* [1]
[1] *Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Oeiras, Portugal*
[2] *Unidade de Investigação em Ciências Orais e Biomédicas, Faculdade de Medicina Dentária, Universidade de Lisboa, Portugal*

Thanks to their spectacular regenerative capabilities, echinoderms were recently considered as important models to explore the basic mechanisms of the regeneration phenomenon and its molecular aspects. The common sea star species Marthasterias glacialis, a close relative of Asterias rubens, is also capable of survival and complete regeneration if up to one fifth of the central body remains attached to an arm and for this reason it was selected as a model echinoderm to study the regeneration events of the radial nerve cord. Sea stars were collected at low tide and subsequently divided in 6 experimental groups: 3 regenerating and 3 control groups. After 48h; 13 days and 10 weeks post arm tip ablation, the radial nerve cords from the injured arms were colected in order to study different regeneration phases: wound healing, early regeneration and tissue re-growth.

In order to find protein candidates actively involved in these two events of sea star radial nerve cord regeneration the experimental approach of 2-D Fluorescence Difference Gel Electrophoresis (2D-DIGE) was employed. Nerve cord proteins that showed a differential expression pattern between the control groups and the wounded groups were selected and identified.

Several calpain-preferred substracts (i.e. α-spectrin) were identified as proteolytic products indicating that an activation of this calcium dependent protease is most likely to be occurring at the selected time point of arm regeneration. It has been described for several invertebrate/vertebrate models that protein degradation mediated by calpain activation is required for recreation of the axon growth cone and axon regeneration after injury. Therefore, to our knowledge this study presents the first evidence that this specific protein degradation has also a main role in the early events of echinoderm nerve cord regeneration.

Presented by: **Franco, Catarina**

Poster No 036
Blue Session

Neuronal Mechanosensitivity in Development and Regeneration

Kristian Franze [1], *Hanno Svoboda* [1], *Andreas F. Christ* [1], *Pouria Moshayedi* [1], *Luciano da F. Costa* [2], *James Fawcett* [1], *Christine E. Holt* [1], *Jochen Guck* [1]
[1] *University of Cambridge, UK*
[2] *University of Sao Paulo, Brazil*

While our understanding of biochemical cell signaling is increasing rapidly, the current knowledge about cellular responses to physical stimuli is very limited. Here we show that neurons respond to mechanical cues in their environment. Morphology, growth rate, and fasciculation of outgrowing Xenopus retinal ganglion cell axons significantly depended on the mechanical properties of their substrate. On softer substrates, axons fasciculated more and preferentially grew in a common direction, similar as in vivo, where these axons build the optic nerve. We used traction force microscopy and scanning force microscopy in combination with calcium imaging to suggest a possible model for neuronal mechanosensing. Using culture substrates incorporating gradients of mechanical properties, we found that neuronal axons are repelled by stiff substrates while activated glial cells are attracted toward them. Applying a modified scanning force microscopy technique, we found mechanical gradients in nerve tissue along which neurons grow in vivo. Hence, our data suggest that CNS cell growth and migration are not only guided by chemical signals - as it is currently assumed - but also by the nerve tissue's mechanical properties. Furthermore, mechanics could also be involved in the inhibition of neuronal regeneration in the mammalian CNS. After traumatic nerve tissue injury, reactive gliosis leads to the development of an inhibitory (stiff) glial scar. While the search for the initial molecular inducer of the gliosis continues, our data suggest that gliosis is triggered by mechanical cues. Interfering with CNS cell mechanosensitivity could ultimately avoid gliosis and neuronal repulsion, leading to the successful treatment of nerve tissue injuries.

Presented by: **Franze, Kristian**

Poster No 037
Red Session

JNK-Induced Changes in Axonal Transport are mediated by the Bidirectional Co-regulator JIP1

Meng-meng Fu, Erika Holzbaur
University of Pennsylvania

The polarized morphology of the neuron requires the delivery of synaptic proteins, the clearance of damaged or misfolded proteins and the relay of neurotrophic as well as stress signals along the axon. The regulation of axonal transport, however, is poorly understood. We propose that intracellular JNK (c-jun N-terminal kinase) signaling can modulate changes in organelle transport via its scaffolding protein JIP1 (JNK-interacting protein). In neuronal cultures, pharmacological inhibition of JNK reversibly arrests dense phase vesicle transport in both directions while activation of JNK increases the speed of retrograde transport. Live-cell imaging of neurons transfected with fluorescently tagged vesicular markers indicates that cargos affected by JNK inhibition include APP-positive and Rab7-positive vesicles. Targeted siRNA knockdown of JIP1 also decreases the velocity of transport of APP-positive and Lysotracker-positive vesicles, implicating JIP1 as a candidate mediator of JNK-dependent changes in axonal transport. JIP1 is known to associate with JNK, with vesicular transmembrane proteins and with kinesin light chain (KLC). We now show in coimmunoprecipitations that JIP1 additionally binds to the C-terminus of p150Glued, a subunit of dynactin. KLC and p150Glued have distinct binding sites within JIP1 and truncated JIP1 lacking the KLC-binding domain retains its ability to bind to p150Glued. These observations support the role of JNK in regulating transport at the cargo level via its scaffolding protein JIP1, which acts as a bidirectional co-regulator of transport by binding to both anterograde and retrograde motors.

Presented by: **Fu, Meng-meng**

Poster No 038
Green Session

N-Cadherin Specifies First Asymmetry in Developing Neurons

Annette Gaertner [1], Eugenio Fornasiero [2], Sebastian Munck [3], Kristl Vennekens [3], Flavia Valtorta [2], Carlos Dotti [3]
[1] Laboratory of Neuronal Differentiation VIB Department of Molecular and Developmental Genetics KULeuven - Center for Human Genetics, Belgium
[2] S. Raffaele Scientific Institute/ Vita-Salute University, Milano, Italy
[3] Laboratory of Neuronal Differentiation VIB Department of Molecular and Developmental Genetics KULeuven - Center for Human Genetics

Neuronal polarization starts with the breakage of membrane symmetry to entail the growth of the first neurite, which determines the position of a second neurite from the opposite pole. This bipolar architecture supports migration and subsequent axonal dendritic polarisation, and thus brain organization primarily depends on the mechanisms that regulate the earliest polarized events and thus orient the neuronal axis of growth. Here we demonstrate that the orientation of this early established polarity axis is regulated by extrinsic signals, impinging on cell-autonomous mechanisms.

In detail, we show that neurite formation precedes cytoplasmatic polarisation, i.e. translocation of the Golgi and centrosome to the neurite pole. Moreover, both neurite formation and subsequent translocation of organelles is defined, in time and space, by the polarized signalling of N-cadherin but not of other adhesive ligands. Lack of functional N-cadherin led to neuronal misalignment in vivo. Our data show the fundamental role N-cadherin signals have for the establishment of a properly oriented polarity axis in the developing cortex.

Presented by: **Gaertner, Annette**

Poster No 039
Blue Session

Nanoscale Mechanical Coupling between N-Cadherin Adhesion and Actin Dynamics in Growth Cone Motility

Mikael Garcia [1], *Lucie Bard* [1], *Amélie Argento* [2], *Olivier Thoumine* [1]
[1] *(1) Interdisciplinary Institute for Neuroscience UMR 5297, CNRS, University of Bordeaux, France*
[2] *CYTOO SA, Minatec, Grenoble, France*

The adhesion molecule N-cadherin stimulates axon outgrowth during nervous system development, but the underlying mechanisms are unclear. One model, the so-called molecular clutch (Giannone et al., Trends Cell Biol, 2009), could involve a direct mechanical linkage between N-cadherin adhesion and actin-based motility in growth cones. Using live imaging of primary hippocampal neurons, we demonstrated a correlation between growth cone migration rate on N-cadherin coated substrates, and the mechanical coupling between N-cadherin coated beads and the retrograde actin flow probed by optical tweezers. This relationship holds by varying ligand density and expressing mutated N-cadherin receptors or siRNAs against α-catenin. By restraining microsphere motion using the optical trap or a micro-needle, we further show slippage of cadherin-cytoskeleton bonds at low forces, and local F-actin accumulation at higher forces. These data support a direct transmission of actin-based traction forces to N-cadherin adhesions, through catenin partners, driving growth cone advance (Bard et al., J Neurosci, 2008). We now develop new methodologies to explore these mechanisms at the nanoscale level on the ventral growth cone surface. We are plating neurons on micro-patterned substrates coated with N-cadherin, and use Photo-Activation Localization Microscopy combined with single molecule tracking (SPT/PALM), to map at high resolution the flows of the molecules involved in the molecular cluch (N-cadherin, α-catenin, vinculin, actin) at N-cadherin adhesions. To probe the mechanisms of force transduction, we investigate the effect of drugs against myosin II and N-cadherin mutants on the redistribution of these molecules.

Presented by: **Garcia, Mikael**

Poster No 040
Red Session

Dynamin-Independent Cycling of AMPA Receptors Maintains Synaptic Transmission

Oleg Glebov [1], *Cezar Tigaret* [2], *Jack Mellor* [2], *Jeremy Henley* [1]
[1] *MRC Centre for Synaptic Plasticity, School of Biochemistry, University of Bristol*
[2] *MRC Centre for Synaptic Plasticity, School of Physiology&Pharmacology, University of Bristol*

AMPA-type glutamate receptors (AMPAR) carry out the majority of fast excitatory transmission in the CNS and are implicated in storage of information. While the changes in synaptic properties encoded by plasticity of AMPAR can be extremely stable, lasting for years, the surface population of synaptic AMPARs undergoes constant turnover with a half-life of circa 30 hours. The mechanisms that underlie the functional dichotomy between activity-dependent AMPAR trafficking in synaptic plasticity and basal turnover of synaptic AMPARs are poorly understood. Here, we show that local constitutive cycling of synaptic AMPARs operates through an unconventional endocytic mechanism that is independent of dynamin, clathrin, and early endosomes. In contrast, both the long-term depression (LTD) form of synaptic plasticity and agonist-induced AMPAR internalization require the function of dynamin and involve early endosomes. Thus, functional segregation between the constitutive AMPAR cycling and synaptic plasticity is realized via distinct mechanisms of membrane trafficking.

Presented by: **Glebov, Oleg**

Poster No 041
Green Session

Mechanisms Controlling the Number and Location of Synaptic Kainate Receptors and their Implication in Synaptic Maintenance

Inmaculada M González-González [1], *Milos Petrovic* [1], *Jose Antonio Esteban* [2], *Jeremy M Henley* [1]
[1] *MRC Centre of Synaptic plasticity, University of Bristol. Bristol. UK*
[2] *Centro de Biologia Molecular Severo Ochoa (CBMSO)*

Kainate receptors (KAr) are ionotropic glutamate receptors made up of different subunit combinations and implicated in synaptic plasticity in the hippocampus. KAr are targeted to specific cell surface compartments (including pre and postsynaptic membranes) where they perform distinct functional roles. Thus, the processes regulating the constitutive and activity-dependent trafficking of kainate receptors KARs are highly intricate. The proteins interacting with KARs are inextricably involved in these processes because, in addition to organizing receptor expression, they also connect receptors to anchoring and signaling pathways that modulate the trafficking of synaptic receptors and motility of axonal filopodia. There are a number of post-translational modifications, interacting proteins and kinases which have been shown to be involved in KAr trafficking. Phosphorylation of KAr by PKC has been shown to enhance and depress KAr mediated EPSCs by modulating the surface delivery of KAr. In addition, surface expression of KAr at the plasma membrane seems to depend on C-terminal domain, which carries the phosphorylation site S868. Recently, we have made a significant progress towards defining and understanding the mechanisms underlying the dynamics of activity-dependent KARs trafficking into the spine and their contribution to the modulation of synaptogenesis and synapses maintenance in pathological and physiological conditions.

Presented by: **González-González, Inmaculada M**

Poster No 042
Blue Session

Investigating the Role of Nogo-A in the Maturation of the Visual System

Anna Guzik-Kornacka, Vincent Pernet, Martin Schwab
Brain Research Institute, ETH and University Zurich, Switzerland

Nogo-A is well known as a myelin-associated protein which inhibits neuronal regeneration and growth after injury. Apart from the expression in oligodendrocytes, Nogo-A is also expressed in many central and peripheral neurons during development, and in some populations of adult neurons. Not much is currently known on the role of Nogo-A in neurons. Regulatory functions for precursor migration and fiber growth, fasciculation and branching have been shown, but neuronal Nogo-A functions at later stages are largely unclear. In the cerebellum, Nogo-A expression is down-regulated in Purkinje cells at the time of the establishment of synaptic contact with the parallel fibers and with neurons of the deep cerebellar nucleus. Retinal ganglion cells (RGCs) also express high level of Nogo-A during embryonic and postnatal development, whereas in adult RGCs the expression is strongly diminished. The developmental downregulation of Nogo-A in RGCs coincides with the maturation of the visual system. At birth, retinal ganglion cell terminals from both eyes innervate overlapping territories in the lateral geniculate nucleus (LGN), but segregate into distinct eye-specific domains by postnatal day 8 (P8). Nogo-A/B and Nogo-66 receptor (NgR) have been suggested to restrict visual cortex plasticity after the so-called critical period. This is related in time to the myelination of cortical axons, suggesting that oligodendrocyte-derived Nogo-A stabilizes these synaptic contacts. On the other hand, a role of neuronal Nogo-A has not been directly studied, neither in the visual cortex nor for the refinement of the retino-geniculate projections. We hypothesize that neuronal Nogo-A may participate in the refinement of the eye-specific retinal projections to the LGN. To test this hypothesis we investigated the influence of systemic deletion or acute blockade of Nogo-A with a function-blocking antibody on the maturation of retinogeniculate projections.

Presented by: **Guzik-Kornacka, Anna**

Poster No 043
Red Session

Involvement of PI 3-K/Akt and MEK/MAPK Signaling Pathways in γ-enolase-mediated Neurite Outgrowth and Survival

Anja Hafner [1], *Nataša Obermajer* [2], *Janko Kos* [2]
[1] *Faculty of Pharmacy, Department of Pharmaceutical Biology, University of Ljubljana, Ljubljana, Slovenia*
[2] *Faculty of Pharmacy, Department of Pharmaceutical Biology, University of Ljubljana, Ljubljana, Slovenia; Jožef Stefan Institute, Department of Biotechnology, Ljubljana, Slovenia*

γ- Enolase, a gylcolytic enzyme, is specifically expressed in neurons. It exerts activity similar to neurotrophic factors, and was suggested to play an important role in growth, differentiation, survival and regeneration of neurons. In our previous studies (Obermajer et al., 2009, Hafner et al., 2011), we reported cathepsin X and γ1-syntrophin as regulators of γ-enolase neurotrophic activity. In the present study, we investigated the involvement of γ-enolase in two signaling pathways, PI 3-K/Akt and MEK/MAPK, which are major effectors triggered by neurotrophic factors. Both pathways were activated in SH-SY5Y cells in response to γ-enolase stimulus. However, PI 3-K/Akt rather than MEK/MAPK pathway is involved in γ-enolase-enhanced cell survival. On the other hand, for γ-enolase stimulated neurite outgrowth the activation of both pathways is required. We demonstrated the activation of ERK1/2, leading to expression of GAP-43 protein, specific for growth cones, as well as activation of PI 3-K is necessary for γ-enolase-induced neurite outgrowth. This was confirmed by using MEK and PI 3-K inhibitors, which blocked or attenuated this molecular event. Dynamic remodelling of the actin-based cytoskeleton is fundamental for neurite outgrowth; therefore γ-enolase might have a role in this process as well. Indeed, we found that γ-enolase through activation of PI 3-K regulates RhoA kinase, a key regulator of actin cytoskeleton organization. Moreover, an inhibition of RhoA down-stream effector ROCK by Y27632 results in enhanced γ-enolase induced neurite outgrowth, accompanied by increased β-tubulin expression, actin polymerization and its redistribution to growth cones. We propose that γ-enolase, by activating MEK/MAPK and PI 3-k/Akt signaling pathways, plays an important role in neuronal differentiation and neurite regeneration.

Presented by: **Hafner, Anja**

Poster No 044
Green Session

Molecular Dissection of EphA4-Mediated Signaling

Christine Hassler, Rüdiger Klein
Max-Planck-Institute for Neurobiology

In the developing nervous system, Eph receptor tyrosine kinases (Ephs) and their ephrin ligands act as a contact-dependent and bidirectional signaling system, since both receptors and ligands are cell-surface tethered and can signal into their respective cells. The Eph-ephrin system plays prominent roles during axon guidance ranging from the regulation of midline crossing decisions to the establishment of topographic maps.

We are currently analyzing, under physiological conditions, the signaling requirements of two non-catalytic modules in the cytoplasmic region of EphA4, the SAM domain and PDZ-binding-motif (PBM). No in vivo functions have so far been assigned to these modules, although they are highly conserved among Ephs across phyla. We have established a knock-in mouse line expressing an EphA4 isoform lacking SAM+PBM (EphA4 ΔSP), but retaining full kinase activity, and will be comparing EphA4 ΔSP mice to existing lines expressing wild-type EphA4 or kinase-inactive EphA4 containing SP. Our main questions are: Are the SP modules required for, or modulating, EphA4 signaling in some developmental contexts, but not others? What is the effect of SP depletion on the cell biology of neurons? In particular, is this region required for various EphA4-dependent axon guidance decisions?

So far, our analysis revealed two potentially interesting results: First, we find that EphA4 ΔSP mice do not display defects in axon guidance of the thalamocortical system. This suggests that the EphA4 ΔSP isoform can still respond to a gradient of EphrinA5 in the cortex and mediate the development of a normal topography. Second, EphA4 ΔSP mice display defects in formation of the dorsal funiculus, a white matter structure in the spinal cord containing corticospinal tract (CST) axons. CST axon guidance has previously been shown by us and others to require functional EphA4 receptors. Although the exact role of EphA4 in formation of the dorsal funiculus is unknown, it is likely that EphA4 controls wiring across the midline which expresses ephrins. We speculate that EphA4's SP region is not required for EphA4-dependent topographic mapping, but may modulate the behavior of cells and their axons towards the spinal cord midline.

Presented by: **Hassler, Christine**

Poster No 045
Blue Session

KCNQ5, a Synaptic Member of the Voltage-Gated KCNQ Potassium Channel Family Mediates a Component of the Afterhyperpolarization Current in Mouse Hippocampus

Matthias Heidenreich [1], *Anastasios Tzingounis* [2], *Tatjana Kharkovets* [3], *Guillermo Spitzmaul* [1], *Henrik Jensen* [4], *Roger Nicoll* [2], *Thomas Jentsch* [1]
[1] *Leibniz-Institut für Molekulare Pharmakologie (FMP) and Max-Delbrück-Centrum für Molekulare Medizin (MDC), Berlin*
[2] *Department of Cellular and Molecular Pharmacology and Department of Physiology, University of California San Francisco*
[3] *Zentrum für Molekulare Neurobiologie (ZMNH), Unversität Hamburg*
[4] *Department of Medical Physiology, The Panum Institute, University of Copenhagen*

Mutations in KCNQ2 and -3 voltage-gated potassium channels lead to neonatal epilepsy as a consequence of their key role in regulating neuronal excitability. Previous studies in the brain have primarily focused on these KCNQ family members, which contribute to M-currents and afterhyperpolarization conductances in multiple brain areas. In contrast, the function of KCNQ5 (Kv7.5), which also displays widespread expression in the brain, is entirely unknown. Here, we developed mice that carry a dominant negative mutation in the KCNQ5 pore to probe whether it has a similar function as other KCNQ channels. This mutation renders KCNQ5dn-containing homomeric and heteromeric channels non-functional. We find that Kcnq5dn/dn mice are viable and have normal brain morphology. Furthermore, expression and neuronal localization of KCNQ2 and KCNQ3 subunits at axon initial segments is unchanged. In contrast to KCNQ2 and KCNQ3, KCNQ5 is localized to a subset of synaptic terminals throughout the brain. In the CA3 area of hippocampus, a region that highly expresses KCNQ5 channels, the medium and slow afterhyperpolarization currents are significantly reduced. In contrast, neither current is affected in the CA1 area of the hippocampus, a region with low KCNQ5 expression. Our results demonstrate that KCNQ5 is a synaptic member of the KCNQ family and contributes to the afterhyperpolarization currents in hippocampus in a cell-type specific manner.

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Presented by: **Heidenreich, Matthias**

Poster No 046
Red Session

Microtubule Stabilization Reduces Scarring and Causes Axon Regeneration after Spinal Cord Injury

Farida Hellal, Andres Hurtado, Joerg Ruschel, Kevin Flynn, Claudia Laskowski, Martina Umlauf, Lukas Kapitein, Dinara Strikis, Vance Lemmon, John Bixby, Casper Hoogenraad, Frank Bradke

Hypertrophic scarring and poor intrinsic axon growth capacity constitute major obstacles for spinal cord repair. These processes are tightly regulated by microtubule dynamics. Here, moderate microtubule stabilization decreased scar formation after spinal cord injury in rodents via various cellular mechanisms, including dampening of transforming growth factor-β signalling. It prevented accumulation of chondroitin sulfate proteoglycans and rendered the lesion site permissive for axon regeneration of growth competent sensory neurons. Microtubule stabilization also promoted growth of central nervous system axons of the Raphe-spinal tract and led to functional improvement. Thus, microtubule stabilization reduces fibrotic scarring and enhances the capacity of axons to grow.

Presented by: **Hellal, Farida**

Poster No 047
Green Session

Scribble1 Deletion in the Principal Neurons of the Brain Alters Hippocampal LTD and AMPAR Endocytosis

Muna Hilal [1], Nicolas Piguel [1], Maité Moreau [1], Yah-se Abada, Thomas Papouin [3], Elodie Richard, D.Nora Abrous [4], Stéphane H.R Oliet [3], Mireille Montcouquiol [1], Nathalie Sans [1]

[1] Planar polarity and plasticity group, INSERM Neurocentre Magendie, Bordeaux

[3] Glia-Neuron Interactions Group, INSERM Neurocentre Magendie, Bordeaux

[4] Neurogenesis and Pathophysiology Group, INSERM Neurocentre Magendie, Bordeaux

Scaffold proteins at the post-synaptic density regulate the strength of synaptic activity by organizing neurotransmitter receptors and other associated signaling proteins, which are required for normal learning and memory. Scribble1 (Scrib1) is a scaffold protein that belongs to the LAP (leucine-rich repeats and PDZ domains) protein family, with 16 leucine rich repeats and 4 PDZ (PSD-95/Dlg/ZO-1) domains. Our previous work with a spontaneous mouse model for Scrib1 has identified an important role for the protein in synaptogenesis and actin polymerization. Here we used conditional knock-out mice presenting a specific loss of Scrib1 in the forebrain to extend and complete our understanding of Scrib1 role(s) in the central nervous sytem. Mice lacking Scrib1 in principal neurons exhibited impairment in spatial learning and memory in the Morris water maze test compared to wild type ones. We also observed a decrease in spine density and altered dendritic morphology of hippocampal CA1 pyramidal neurons. Additionally, these neurons had normal presynaptic parameters but an overall decrease in basal synaptic transmission. When tested for synaptic plasticity, CA1 pyramidal neurons revealed an altered synaptic depotentiation whereas long-term potentiation was normal. Our in vitro results show a decrease in AMPAR endocytosis in neurons in which Scrib1 was downregulated, which could explain these electrophysiological characteristics. Importantly, this puts forward a functional link between synaptic depotentiation and learning and memory processes. Finally, these results suggest an original and critical role for Scrib1 in AMPAR endocytosis that affects synaptic function and plasticity, dendritic morphology and higher cognitive functions.

Presented by: **Hilal, Muna**

Poster No 048
Blue Session

Trafficking and Polarity of Cannabinoid Receptor Type 1

Keri Hildick, Nadia Jafaari, Jeremy Henley
MRC Centre for Synaptic Plasticity, University of Bristol

The cannabinoid receptor type 1 (CB1) is one of the most abundantly expressed G-protein coupled receptors (GPCRs) in the central nervous system (CNS). CB1Rs play a primary presynaptic role in mediating retrograde synaptic plasticity at both excitatory and inhibitory synapses. Receptor trafficking underlies desensitization and down-regulation in response to pharmacological stimulation. In addition, constitutive endocytosis of CB1 receptors has been evidenced to play a role in axonal targeting and the polarized surface distribution of CB1 in hippocampal neurons, although how the activation state of the receptor and putative regulatory proteins are involved, remains controversial. To examine the trafficking of CB1 we have engineered a CB1R clone – tagged with a pH sensitive fluorophore (SEP-CB1) – thereby enabling the trafficking of surface CB1 to be studied using real-time live cell imaging in primary hippocampal neurons. With the use of fluorescence recovery after photobleaching (FRAP), we aim to quantitatively analyze the diffusion kinetics of CB1R, complimented by characterization of the endocytotic sorting pathway by immunocytochemistry. In addition, we have conducted a proteomics screen, using GST-tagged C-terminal fragments of the CB1R as bait, to pull down novel C-terminal interactors, which may regulate CB1R trafficking. Herein, we present the validation of these approaches and discuss the progression of the CB1R trafficking pathway characterization.

Presented by: **Hildick, Keri**

Poster No 049
Red Session

RNA-Binding Protein Hermes Regulates Axon Guidance and Branching in the Developing Visual System

Hanna Hornberg, Daniel Maurus, Francis Wollerton-van Horck, Maarten Zwart, William Harris, Christine Holt
Department of Physiology, Development and Neuroscience, University of Cambridge, Downing St, CB2 3DY, Cambridge, UK

In the developing visual system, retinal ganglion cells (RGCs) project topographically along the anterior-posterior (A-P) and dorsal-ventral (D-V) axes to their target area in the optic tectum, where they arborize and form synaptic connections. The formation of this circuit is tightly regulated both by extracellular guidance cues in the environment, and by intracellular processes in the RGC axons such as RNA localization and translation. Here, we reveal the functional role of the RNA-binding protein Hermes (also known as RBPMS) in retinal axon navigation in vivo. Hermes is expressed exclusively in RGCs in the CNS and is present in their growing axons and growth cones where it localises in granules. Antisense morpholino knockdown of Hermes in embryonic zebrafish caused axons along the D-V axis to make positional errors in the optic tract, and to enter the tectum through an indirect route . Axons also failed to map correctly along the A-P axis. Furthermore, Hermes knockdown in both zebrafish and Xenopus significantly impaired retinal axon branching in the tectum. Overexpression of a dominant negative Hermes protein defective in a domain required for granular localisation in Xenopus also impaired branching, suggesting that proper localization of Hermes is critical to its function. These results show that Hermes contributes to both mapping and arborisation and suggests an important role in several aspects of circuit formation.

Presented by: **Hornberg, Hanna**

Poster No 050
Green Session

Negative Feedback Enhances Robustness during Polarity Establishment

Audrey Howell [1], *Chi-Fang Wu* [1], *Meng Jin* [2], *Timothy Elston* [2], *Daniel Lew* [1]
[1] *Duke University, Durham, NC 27710*
[2] *University of North Carolina at Chapel Hill, Chapel Hill, NC 27599*

Although polarized cells often follow positional cues when establishing an axis of polarity, many cells are able to establish a unique, if randomly oriented, axis in the absence of such cues. The Rho-type GTPase Cdc42p is an essential polarity factor in many organisms that becomes locally activated at the "front" of the cell. In budding yeast it has been proposed that stochastically arising clusters of active Cdc42p are amplified by a positive feedback loop utilizing a complex containing a Cdc42p effector and the Cdc42p guanine nucleotide exchange factor. Although this positive feedback mechanism can explain how a local cluster of active Cdc42p grows, it does not address why only a single cluster is present at bud emergence. It is possible that only a single Cdc42p cluster is amplified, or that multiple Cdc42p clusters compete for some limiting factor so that only a single bud site remains. In order to distinguish between these hypotheses, we performed high-speed timelapse microscopy on cells establishing polarity. We found that multiple clusters of polarity factors formed, and that they appeared to compete so that only a single site remained. We also observed unexpected oscillatory localization of polarity factors at the bud site suggesting the presence of negative feedback during polarity establishment. Mathematical modeling suggested that the addition of negative feedback to the polarity establishment circuit could produce the dampened oscillations of polarity factor concentration seen in vivo. Furthermore, the models suggested that negative feedback would increase the robustness of the polarity establishment circuit and we found that polarity establishment in yeast was in fact robust to increases in polarity factor concentration.

Presented by: **Howell, Audrey**

Poster No 051
Blue Session

Development of a New Strategy to Control Protein Function in the Developing Mouse CNS

Rachel Jackson [1], Wenlin An [1], Laura Ward [1], Michiel Van Diepen [1], Luke Whiley [2], Cristina Legido-Quigley [2], Thomas Wandless [3], Karen Liu [4], Britta Eickholt [1]
[1] MRC Centre for Developmental Neurobiology, King's College London
[2] Pharmaceutical Science Division, King's College London
[3] Department of Chemical and Systems Biology, Stanford University
[4] Department of Craniofacial Development, King's College London

To study a protein in vivo it is desirable to perturb its normal function in a spatially and temporally restricted fashion. We are exploiting a recently developed approach to achieve this, based on a strategy that allows protein function to be regulated in a rapid, reversible and tuneable manner. In this technique, a destabilising domain derived from E. coli dihydrofolate reductase (DHFR*) is fused to a protein of interest, causing its efficient degradation. Presence of a DHFR* ligand, the antimicrobial drug trimethoprim (TMP), stabilises the protein and confers biological activity. TMP is a cost-effective drug widely used in veterinary and medical applications, which passes the blood-brain barrier making it an ideal tool to manipulate proteins in the CNS.

We combine in utero electroporation of DHFR* tagged constructs into the cortex of mouse embryos with subsequent stabilisation by systemic application of TMP. We have tested methods to deliver the synthetic ligand, analysing its ability to cross the blood-brain barrier, and investigated if DHFR* fusion proteins can be stabilised rapidly and reversibly. These characteristics will introduce a new layer of control to inducible gene expression systems currently used in mice.

Using this technique we aim to study the interaction of two pathways with multiple roles in brain development, the Semaphorins and PTEN. We have generated DHFR*-Semaphorin ligands and DHFR*-Cre, which will be used in combination with floxed-PTEN alleles. This system allows for combinatorial control of two or more DHFR* fusion proteins, enabling the investigation of possible interactions between these two pathways in mice.

Presented by: **Jackson, Rachel**

Poster No 052
Red Session

Interplay between Polyglutamylases and Deglutamylases Regulates Polyglutamylation Levels of Neuronal Microtubules

Carsten Janke [1], *Krzysztof Rogowski, Juliette van Dijk, Maria M. Magiera, Christophe Bosc, Jean-Christophe Deloulme, Anouk Bosson, Max Holzer, Annie Andrieux, Marie-Jo Moutin*
[1] *Institut Curie*

Polyglutamylation is a posttranslational modification that is enriched on the neuronal microtubule cytoskeleton. The modification generates glutamate side chains of variable lengths on the carboxy-terminus of tubulin, which is also an important interaction site for MAPs and molecular motors. Thus, polyglutamylation is expected to regulate the dynamics of microtubules as well as the motor traffic in neurons.

We have discovered the enzymes that generate polyglutamylation (polyglutamylases) and recently also the reverse enzymes (deglutamylases). Polyglutamylases, which are members of the Tubulin Tyrosine Ligase Like protein family (TTLL), are characterized by particular substrate and reaction specificities. Deglutamylases are members of the Cytosolic CarboxyPeptidase (CCP) family, and are also subdivided into enzymes with different specificities. The specialisation of the modifying enzymes suggests multi-enzyme mechanisms in which the final pattern of polyglutamylation is determined by the types of enzymes that are involved in the generation and the removal of the modification.

We have analyzed Purkinje cell degeneration (pcd) mice that lack one of the key deglutamylases and demonstrated a strong increase in microtubule polyglutamylation in brain regions that degenerate in the adult animals. Strikingly, Purkinje cells that are completely degenerated after six weeks were partially protected from degeneration after depletion of the major neuronal polyglutamylase. This demonstrates that controlling the length of the glutamate side chains on tubulin is critical for neuronal survival, and suggests an important role of polyglutamylation in the regulation of the neuronal microtubule network.

Presented by: **Janke, Carsten**

Poster No 053
Green Session

IQGAP1: Role in Spines Morphogenesis

Ignacio Jausoro, Alfredo Cáceres
Instituto Mercedes y Martin Ferreyra. Córdoba, Argentina.

IQGAP1 is a scaffold protein that interacts with many proteins involved in cytoskeletal dynamics. In our laboratory we explored how IQGAP1 regulates cytoskeletal dynamics and its function in neurons. In this study we examined the subcellular distribution of IQGAP1 and its function in relation to cytoskeletal regulation, especially focused on actin cytoskeleton. Due to the great influence of IQGAP1 on actin cytoskeleton organization, we analyzed the involvement of this protein in spine morphogenesis. Our observations show the localization of IQGAP1 in dendritic spines, as well as an active participation in spine morphogenesis. We demostrated that the interaccion domain with microfilaments is essential for the formation of spine head and the interaction of IQGAP1 with small GTPases is necessary for the generation of spine neck. Finally, we have shown that the carboxy-terminal domain of IQGAP1 has effect on spines length. Taken together,our results demostrate the involvement of the different domains of IQGAP1 in physiological processes, and dendritic spines morphogenesis.

Presented by: **Jausoro, Ignacio**

Poster No 054
Blue Session

RNA Binding Protein Vg1RBP (ZBP1) Regulates Terminal Arbor Formation but not Pathfinding in the Developing Visual System In Vivo

Adrianna Kalous [1], *Joel Yisraeli* [2], *James Stake* [1], *Christine Holt* [1]
[1] *Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, UK*
[2] *Department of Anatomy and Cell Biology, Institute for Medical Research, Hebrew University, Jerusalem, Israel*

The VICKZ proteins comprise a family of highly conserved RNA binding proteins that regulate mRNA localization and play roles in cellular motility and polarity (1). In embryonic axons, the Xenopus laevis (Vg1RBP) and chick (ZBP1) homologs localise β-actin mRNA to growth cones in response to specific guidance cues in vitro (2-4). Interaction of ZBP1 with β-actin mRNA, and local synthesis of β-actin in the growth cone, are required for chemotropic responses to guidance cues in vitro (2,3). These findings suggest that Vg1RBP/ZBP1 may play a critical role in axon guidance in vivo. Here we have tested the requirement for Vg1RBP in axon pathfinding (long-range guidance) and terminal arbor formation (short-range guidance) in the developing Xenopus visual system. Vg1RBP function was disrupted in retinal ganglion cells by antisense morpholino knockdown and by expression of a dominant-negative form of Vg1RBP that blocks mRNA binding. Retinal axon trajectories were analysed for long- range pathfinding errors (retina to tectum) and short-range guidance defects within the tectum, such as branching and topographic mapping defects. Disruption of Vg1RBP function did not affect long-range guidance of axons, but caused defects in within the tectum, including: 1) overshooting the tectum, 2) reduced branching, and 3) topographic mapping errors. Terminal field defects were not due to a developmental delay, as axons reached the tectum at the appropriate stage of development. The results reveal that Vg1RBP has a specific role in terminal arbor formation and indicate that mRNA localization and translation contribute to the regulation of specific aspects of guidance in vivo.

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Presented by: **Kalous, Adrianna**

Poster No 055
Red Session

The Actin Nucleator Cobl Plays a Role in Neuronal Differentiation and Cerebellar Development and Relies on Association with Abp1

Natja Haag [1], Lukas Schwintzer [1], Rashmi Ahuja [1], Julia Grimm [1], Nicole Koch [1], Heike Heuer [2], Britta Qualmann [1], Michael Kessels [1]
[1] Institute for Biochemistry I, University Hospital Jena, Friedrich-Schiller-University Jena
[2] Research Group Neuroendocrinology; Leibniz Institute for Age Research

Neuromorphogenesis relies on actin cytoskeletal forces to initiate, promote and maintain the underlying cell shape changes. This requires a careful control of actin nucleation at the cell cortex in time and space. Here we describe that the F-actin binding protein Abp1, interacts with the novel actin nucleator Cobl. Both heterologous and endogenous coimmunoprecipitations from rat brain demonstrate that Abp1/Cobl interactions exist in vivo. Further analyses revealed that Abp1/Cobl complexes form by the thus far uncharacterized Cobl-Homology domain and are associated with the cell cortex.

Immunohistochemistry revealed that Cobl is especially highly expressed in Purkinje cells – cells that constitute the sole source for all motor coordination in the cerebellar cortex. Gene gun experiments with developing cerebellar slices showed that Cobl-deficient Purkinje cells have a strongly impaired arborization. Lack of Abp1 caused a similar phenotype. Thus both Cobl and Abp1 are crucial for proper development of the elaborate dendritic arbor of Purkinje cells. To explicitly prove that Cobl and Abp1 play important roles in dendritogenesis in form of Abp1/Cobl complexes, we demonstrated that Abp1 RNAi suppressed the effects of Cobl on dendritic arborization. Likewise, interfering with Cobl/Abp1 complex formation suppressed Cobl-mediated effects. Thus, Abp1 is an integral part of the Cobl-actin nucleation machinery and together Abp1 and Cobl are important for proper neuromorphogenesis.

Presented by: **Kessels, Michael**

Poster No 056
Green Session

Proper Synaptic Vesicle Formation and Neuronal Network Activity Critically Rely on Syndapin I

Dennis Koch [1], Isabella Spiwoks-Becker [2], Victor Sabanov [3], Tamar Dugladze [4], Anne Stellmacher [5], Rashmi Ahuja [6], Julia Grimm [1], Susann Schüler [1], Anke Müller [7], Frank Angenstein [8]

[1] Institute for Biochemistry I, University Hospital Jena - Friedrich Schiller University of Jena, 07743 Jena, Germany

[2] Institute for Microanatomy and Neurobiology, Johannes Gutenberg-University of Mainz, 55128 Mainz, Germany

[3] Laboratory of Biological Psychology, Department of Psychology, Katholieke Universiteit Leuven, Leuven, 3000, Belgium

[4] Cellular and Network Physiology Group, Institute of Neurophysiology, Charité Universitätsmedizin Berlin, 10117 Berlin, Germany

[5] Dpt. of Neurochemistry, Leibniz-Institut for Neurobiology, Brenneckestr. 6, 39118 Magdeburg, Germany

[6] Massachusetts General Hospital Cancer Ctr 149-7205 149 13th Street Charlestown, MA 02129

[7] RG Neuralomics, Leibniz-Institut for Neurobiology, Brenneckestr. 6, 39118 Magdeburg, Germany

[8] Non-invasive brain imaging, Leibniz Institute for Neurobiology, 39118 Magdeburg, Germany

Synaptic transmission relies on effective and accurate compensatory endocytosis. F-BAR proteins are predestined to serve as membrane curvature sensors and/or inducers and may thereby support membrane remodeling processes, yet, their in vivo functions urgently await disclosure. We demonstrate that the F-BAR protein syndapin I is crucial for proper brain function. Syndapin I knock-out mice suffer from seizures, a phenotype consistent with excessive hippocampal network activity. Loss of syndapin I causes defects in presynaptic membrane trafficking processes evident by loss of synaptic vesicle size control and defects in synaptic activity. Upon high-capacity retrieval accumulation of endocytic intermediates are observed in the ribbon synapses of the retina. Detailed molecular analyses demonstrate that syndapin I plays an important role in the recruitment of all dynamin isoforms, central players in vesicle fission reactions, to the membrane. Consistently, syndapin I KO mice share phenotypes with dynamin I KO mice, whereas their seizure phenotype is very reminiscent of fitful mice expressing a mutant dynamin. Syndapin I thus acts as pivotal membrane anchoring factor for dynamins during regeneration of synaptic vesicles.

Presented by: **Koch, Dennis**

Poster No 057
Blue Session

Dynein Light Chain LC8 is a Dimerization Hub for the Memory-Related KIBRA Protein

Joachim Kremerskothen, Kerstin Duning, Bernd Ribbrock, Hermann Pavenstaedt
Department for Molecular Nephrology, University Hospital Muenster, Muenster, Germany

Memory formation in the mammalian brain is based on a dynamic remodelling of synaptic contacts in concert with the constant turnover of neuroreceptors at postsynaptic sites. These processes are achieved through highly regulated intra- and interneuronal transport mechanisms and a dynamic cytoskeleton network in axons and dendrites. Recent studies have shown that the expression of the KIBRA scaffolding protein is crucial for memory formation and the development of neurodegenerative diseases. In neurons, KIBRA is enriched at postsynaptic sites where it interacts with the actin-binding proteins Synaptopodin and Dendrin. Additional KIBRA binding partners include protein kinase M (PKM) zeta as well as the dynein light chain LC8. LC8 was initially discovered as part of the dynein motor complex and was thought to act mainly as a cargo adapter. However, recent findings suggest that LC8 is also able to stabilize multiprotein complexes by promoting dimerization of its binding partners. Because the intracellular transport of KIBRA is mainly dynein-independent, we examined the functional role of the interaction with LC8 in more detail. Using in vitro binding assays and yeast two-hybrid experiments we were able to map two distinct LC8 binding motifs within the KIBRA molecule. Mutation analysis revealed that only dimeric LC8 is able to associate with KIBRA and that this association is controlled by LC8 phosphorylation. Interestingly, binding of LC8 facilitates KIBRA dimerization and regulates the interaction with its other binding proteins. In summary, our data indicate that LC8 is a molecular hub for KIBRA dimerization. Thus, LC8 could serve as a regulatory element in neurons mediating assembly/disassembly of structural protein networks containing the KIBRA dimer.

Presented by: **Kremerskothen, Joachim**

Poster No 058
Red Session

The Amyotrophic Lateral Sclerosis 8 Protein VAPB Disrupts the Early Secretory Pathway

Marijn Kuijpers, Ka Lou Yu, Eva Teuling, Dick Jaarsma, Casper Hoogenraad
Department of Neuroscience, Erasmus Medical Center, Rotterdam, The Netherlands

The vesicle-associated membrane protein (VAMP) associated protein B (VAPB) is an ubiquitously expressed integral membrane protein localized to the ER. A missense mutation (P56S) in de gene encoding for VAPB has been linked to motor neuron degeneration in amyotrophic lateral scleroses (ALS) affected patients. In the central nervous system VAPB is abundant in motor neurons and when mutated VAPB-P56S proteins accumulate in ER-like subcompartments. To understand the physiological and pathological functions of VAPB in causing neurodegenerative diseases, we searched for potential in vivo binding partners of VAPB protein. Via pull-down assays and mass spectrometry we identified interacting factor of YIP1 (YIF1A) as a VAPB binding partner and found that VAPB and YIF1A interact via their transmembrane regions. By immunofluorescent labeling of cultured hippocampal neurons we demonstrate that YIF1A is localized to the endoplasmic reticulum - Golgi intermediate compartments (ERGIC), which is involved in ER-to-Golgi trafficking. We show that VAPB knockdown affects the distribution of YIF1A localization in neurons. We also found that YIF1A is recruited to VAPB-P56S structures and thereby loses its ERGIC localization. These data suggest that YIF1A missorting and disruptions in the early secretory pathway might play an important role in VAPB associated motor neuron disease.

Presented by: **Kuijpers, Marijn**

Poster No 059
Green Session

Analysis of in vivo Glycine Transporter Function by Transgenic Approaches

Deepti Lall, Heinrich Betz, Volker Eulenburg

Glycine is one of the major inhibitory neurotransmitters in caudal regions of the central nervous system where it binds to strychnine sensitive glycine receptors. Additionally, glycine acts as a essential co-agonist on ionotropic glutamate receptors of the NMDA receptor subtype. To ensure neurotransmission to proceed with high spatial and temporal resolution, the extracellular glycine concentration has to be tightly regulated. This is achieved by two independent transport, the predominately glial expressed glycine transporter 1 (GlyT1) and the glycine transporter 2 (GlyT2), which is exclusively expressed by glycinergic neurons [1]. They facilitate the uptake of glycine from the extracellular space into the cytosol of the presynaptic terminal or sourrounding glial cells and belong to the large family of Na+/ Cl- dependent transport proteins that includes transporters for monoamines (seratonin, dopamine etc.) and gamma-aminobutyric acid (GABA). The detailed analysis of the in vivo GlyT1 function has been hampered by the fact that GlyT1 knockout mice show early postnatal lethality due to over-inhibition of motor neurons resulting from accumulation of glycine at glycinergic synapses. Therefore we propose to investigate the role GlyT1 in neurotransmission by the generation of an transgenic mouse line, allowing time and/or tissue specific overexpression of an epitope tagged GlyT1 using the Cre/loxP system. Biochemical, immunocytochemical as well as electrophysiological and behavorial analysis of mice with altered transporter expression might extend our understanding of the specific roles of the GlyT1 at inhibitory and excitatory synapses in the CNS.

Presented by: **Lall, Deepti**

Poster No 060
Blue Session

Sphingomyelin Influences Dendritic Spine Size through the Modulation of Actin Cytoskeleton

Maria Dolores Ledesma [1], *Ana Arroyo* [1], *Paola Camoletto* [2], *Laura Morando* [3], *Marco Sassoe-Pognetto* [3], *Mauricio Giustetto* [3]
[1] *Centro Biologia Molecular Severo Ochoa (Madrid, Spain)*
[2] *KU Leuven (Belgium)/ University of Turin (Italy)*
[3] *University of Turin (Italy)*

Dynamic changes in dendritic spine size and morphology underlie learning and memory processes. The actin cytoskeleton drives such changes. Although the protein machinery controlling spine actin dynamics has been characterized in detail, little is known about the influence of membrane lipids despite many lipidosis lead to cognitive impairment and spine anomalies. Niemann Pick disease type A (NPA) is a severe mental retardation syndrome caused by the lack of acid sphingomyelinase (ASM) activity. ASM converts sphingomyelin (SM) into ceramide. We here report a reduction in dendritic spine size and actin polymerization in the neurons of ASMko mice, which mimic the disease. We also provide with the underlying molecular mechanism. Hence, the aberrant accumulation of SM in ASMko dendritic spines and the addition of SM to wt neuronal cultures and synaptosomes leads to reduced levels, membrane attachment and activity of the small GTPase RhoA. This in turn could be explained by the low levels of mGluR receptors in ASMko synapses, which impair RhoA membrane binding and activation.

Altogether these results unveil a RhoA-mediated role for SM in dendritic spine actin polymerization through the modulation of mGluR membrane levels. These results could also explain, at least in part, the cognitive deficits of NPA patients.

Presented by: **Ledesma, Maria Dolores**

Poster No 061
Red Session

Regulation of Neurofibromin RasGAP Activity and Localization during Neuronal Differentiation

George Leondaritis [1], *Theodora Kalpachidou* [2], *Dimitra Mangoura* [2]
[1] *Department of Pharamcology, Medical School, University of Thessaly, Larissa, Greece*
[2] *Neurosciences, Biomedical Research Foundation Academy of Athens, Athens, Greece*

Ras and ERK1/2 signalling is pivotal to differentiation along the neuronal cell lineage. One crucial protein that may play a central role in this signalling pathway is the tumor suppressor RasGAP, neurofibromin (NF1). By studying the dynamics of PKC/Ras/ERK pathway signalling during differentiation of neuroblastoma cells upon treatment with a PKC agonist, we identified a phosphorylation event at the C-tail of NF1 on Ser2808 which correlated perfectly with acute and prolonged activation of Ras and ERK1/2. Importantly, Ser2808 phosphorylation resulted in a shift of NF1 localization from the nucleus to the cytosol and mutation to Ala caused reduced PKC-dependent phosphorylation and increased nuclear localization of the NF1-C-terminal domain. Thus, sustained Ser2808 phosphorylation may result in prolonged recruitment of nuclear NF1 to cytosolic membranous Ras complexes. In search for NF1 domains that may confer cytosolic, cytoskeletal or vesicular localization we focused on a Sec14-homology domain adjacent to GRD, the central RasGAP domain. Overexpressed Sec14 and GRD+Sec14 had a microtubule-associated and vesicular localization pattern reminiscent of the endogenous cytosolic pool of NF1 in differentiated neurons and COS-7 cells. Most importantly however, Sec14 induced an upregulation of RasGAP activity of GRD in vivo presumably by atypical direct interactions with Ras. A detailed kinetic analysis of NF1-GRD and NF1-GRD+Sec14 activities in EGF-stimulated cells suggested that Sec14 imposed a digital-like mode on the analog GAP activity of GRD. Thus, distinct NF1 domains regulated by distinct mechanisms may dictate the localization and activity of the protein during differentiation.

Presented by: **Leondaritis, George**

Poster No 062
Green Session

Development of the Corticospinal Tract: Integration into Spinal Circuitry Important for Coordinating Complex Motor Behaviors

Kathryn Lewallen, Ariel Levine, Sam Pfaff
Salk Institute

The corticospinal tract (CST) forms the longest axon trajectory in the mammalian central nervous system, extending processes from the motor cortex to functional targets throughout the length of the spinal cord. As a graduate student in the laboratory of Dr. Sam Pfaff at the Salk Institute, I am interested in understanding how corticospinal motor neurons integrate intrinsic and extrinsic cues to carry out specified functions, such as coordinating complex motor behaviors. Specifically, my thesis project involves the identification of cellular players and molecules that direct synaptic specificity during development. I am using novel genetic tools combined with single axon fiber resolution to study the CST during axonal growth, synaptic target selection, and activity-dependent maturation. My analysis includes a comprehensive descriptive analysis of the CST in normal development and in mutants that perturb CST formation and axon guidance. By understanding the mechanisms that direct corticospinal connectivity, we can gain additional insight into the pathways that can be recapitulated in a regenerative context.

Presented by: **Lewallen, Kathryn**

Poster No 063
Blue Session

Decreased GSKβ Activity by Downregulation of Tyr216 Phosphorylation Underlies the Gain of Regenerative Capacity Following Conditioning Lesion

Márcia Liz, Helena Pimentel, Daniela Silva, Ana Marques, Mónica Sousa
Nerve Regeneration Group, IBMC

CNS axons regenerate under certain conditions such as following a conditioning lesion (CL): the central branch of a dorsal root ganglia (DRG) neuron is capable of growing within the CNS inhibitory environment after its peripheral branch has been previously injured. We aimed at disclosing intrinsic mechanisms enabling CNS axonal regeneration using the CL as a model and used two proteomic approaches, iTRAQ and phosphoproteomic arrays. From the proteins identified in the DRG as differentially regulated following CL in comparison with spinal cord injury (SCI), the Glycogen synthase kinase 3β (GSK3β) pathway, involved in microtubule dynamics, emerged as a key player. GSK3β activity was downregulated following CL. Significantly, despite the focus on the inhibition of GSK3β through Ser9 phosphorylation, our data shows that a decreased phosphorylation of Tyr216, the activator of kinase activity, both in DRG and in the SCI site, is the regulatory event leading to gain of regenerative capacity following CL. Supporting this hypothesis, treatment of conditioned DRG neurons with lysophosphatidic acid, an inducer of Tyr216 phosphorylation, reduces neurite outgrowth. To further explore the relevance of GSK3β phosphorylation at Tyr216, we are analysing DRG neurons from animals with SCI overexpressing GSK3β Tyr216Phe, which should mimic the conditioning effect. Moreover, we are studying the mechanism responsible for the modulation of Tyr216 phosphorylation, namely the regulatory role of described GSK3β Tyr216 kinases and phosphatases in the gain of axonal regenerative capacity. Finally, to confirm the role of GSK3β in axonal growth in vivo, we are studying CNS regeneration in i) transgenic mice expressing constitutively active GSK3β (GSK3βS9A knockin mice), ii) heterozygous knockout mice presenting a partial decrease of GSK3β activity and iii) mice devoid of GSK3β activity in neurons (floxed GSK3β x Thy1-cre mice). Our in vitro and in vivo work will clarify the mechanism and relevance of GSK3β in promoting CNS axonal regeneration.

Presented by: **Liz, Márcia**

Poster No 064
Red Session

Role of KCC2 in Morphological Plasticity of Dendritic Spines

Olaya Llano, Anastasia Ludwig, Shetal Soni, Claudio Rivera
Neuroscience Center, Helsinki

Our recent findings show that the neuron-specific potassium chloride cotransporter KCC2, which is known to play a major role in the developmental maturation of GABAergic neurotransmission, also has a role in the development of dendritic spines that does not depend on the transporter's chloride extrusion activity. Here we continue our studies and demonstrate that KCC2 is involved in activity-induced modulation of spine morphology. Using a protocol for strong patterned synaptic activation we found that the distribution of KCC2 in dendrites and spines of dissociated hippocampal neurons is significantly affected. There is an activity-dependent redistribution of KCC2 to a specific class of spines that correlates with the synaptic marker PSD-95. These changes may be related to KCC2 interaction with proteins involved in the remodeling of the actin cytoskeleton. In particular we have found that the relative level of phospho-cofilin in KCC2 deficient neurons is significantly increased. A similar effect is also induced by overexpression of the dominant negative C-terminal domain of KCC2. In summary, our study indicates that KCC2 is involved in activity-dependent mechanisms regulating dendritic spine morphology.

Presented by: **Llano, Olaya**

Poster No 065
Green Session

Structural and Biochemical Characterization of CRMPs and their Complexes Involved in the Axonal Growth and Related Disease

Bernhard Lohkamp, Rajesh Ponnusamy
Department of Medical Biochemistry and Biophysics, Karolinska Institutet, 171 77 Stockholm, Sweden

Alzheimer's disease (AD) is a chronic and progressive neurodegenerative disorder. WHO has estimated that there are currently about 18 million people worldwide with AD, which is projected to nearly double by 2025. Therefore identification of new targets and understanding their intracellular mechanism is imperative.

CRMPs (Collapsin Response Mediator Proteins) are cytosolic phosphoproteins strongly expressed in the developing nervous system that induce growth cone collapse. CRMPs probably play a central role in normal and pathological events in the nervous system. CRMPs have been found to be regulated in many diversified contexts, both in the developing and the mature brain. Despite their high homology, CRMPs are probably differentially regulated at the post-translational level, which could increase the specificity. Phosphorylation of CRMP-2 is implicated in the formation of degenerating neuritis in AD. Recently, CRMP-2 and GSK-3β have been introduced as targets for the treatment of AD and nerve injury.

In contrast to the rapid progress in identification and characterization of axon guidance molecules and their receptors, much remains to be explored about the intracellular mechanism by which signals are transduced into the eventual response of the growth cone. In order to understand the role of CRMPs and their interaction partner in neurodegenerative diseases and development, several variants of CRMPs and the interaction partners were cloned, the proteins expressed, and purified. Biophysical and biochemical investigations of the individual CRMPs and their complexes will be presented, together with initial structural characterization. This study may help to clarify causes of and treatments aimed at reversing AD and nerve injury.

Presented by: **Lohkamp, Bernhard**

Poster No 066
Blue Session

Tracking Autophagosome Dynamics in Primary Neurons

Sandra Maday, Karen Wallace, Erika Holzbaur
University of Pennsylvania School of Medicine

Autophagy is a lysosomal degradation process that is particularly important in post-mitotic cells such as neurons. Inhibition of autophagy leads to neurodegeneration (Komatsu et al., 2006; Hara et al., 2006). To determine the dynamics of autophagosomes in neurons, we isolated dorsal root ganglion neurons from transgenic mice expressing the autophagosome marker GFP-LC3 and performed live-cell imaging. Autophagosomes displayed robust motility along neuronal processes, strongly biased toward the retrograde direction (82 ± 2 [± SEM] % of autophagosomes were retrograde). Autophagosomes moved at an average velocity of 0.45 ± 0.01 (± SEM) μm/sec, exhibited few reversals in direction, and paused ~10% of their journey. Disruption of dynein/dynactin function arrested autophagosome motility. These results suggest that autophagosomes are robustly transported along the neurite in a predominantly retrograde direction by the microtubule motor cytoplasmic dynein. This unidirectional transport of autophagosomes in the axon is distinct from the motility exhibited by other organelles such as lysosomes that predominantly move bidirectionally. To determine the maturation state of autophagosomes in the axon, we analyzed the composition of autophagosomes with regard to lysosomal markers. Our results support a model in which autophagosomes undergoing active transport along the neurite are acidified but have not yet fused with lysosomes. This model is distinct from the prevailing paradigm based on work in non-polarized cells. Our data suggest that neurons, due to their unique morphology, may have specialized pathways where the spatial and temporal dynamics of organelles may be linked to their function.

Presented by: **Maday, Sandra**

Poster No 067
Red Session

Tubulin Polyglutamylation: a Role in Neuronal Polarity and Transport

Maria Magiera, Judith Souphron, Diana Zala, Frederic Saudou, Carsten Janke
Institut Curie

Neurons are polarized cells and their cell body, dendrites and axon differ radically in terms of morphology, function and molecular composition. The specification of the axonal and dendritic identity occurs early in neuronal development and is maintained for decades. The mechanisms of neuron polarity establishment, maintenance and loss are not fully understood, but microtubules (MTs) seem to play an important role in these processes. Many MT-associated proteins (MAPs) and motors can be found exclusively in one of the cellular compartments, and strikingly, this distribution can be perturbed in degenerating neurons. Neuronal MTs are heavily polyglutamylated, and we have shown that deregulation of the levels of this posttranslational modification of tubulin leads to neurodegeneration. We are now interested by which mechanism tubulin polyglutamylation controls neuronal survival and we want to investigate whether it could influence the establishment and maintenance of neuronal polarity. Using primary cultures of mouse hippocampal neurons we modulate tubulin glutamylation and study the consequences of these changes on cell morphology, MAP distribution, motor traffic, axon branching, growth cone function and possible degenerative processes. First experiments have shown that tubulin is differentially modified in axons vs. dendrites and that changing tubulin glutamylation pattern in developing neurons can influence MAP localization. We are now establishing an assay allowing quantifying the impact of polyglutamylation levels on axonal transport parameters. In the next step we will investigate the role of MT polyglutamylation on MAP and motor binding and behaviour in cell-free in vitro systems.

Presented by: **Magiera, Maria**

Poster No 068
Green Session

Identifying Injury Signals and Regeneration Enhancers in the Conditioning Lesion Model

Fernando Mar [1], Marques Ana [1], Brites Pedro [1], António Barbosa [2], Vitor Costa [2], Mónica Sousa [1]
[1] IBMC - Nerve Regeneration group
[2] IBMC - cellular and applied microbiology

Following PNS lesion, injury is signaled by retrograde transport of positive injury signals, such as p-ERK, which lead to transcription of regeneration enhancers anterogradely transported to the injury site. These changes induced by a PNS lesion are thought to enable DRG neurons to regenerate central branch axons following a subsequent spinal cord injury (SCI) - conditioning lesion effect. However, the identity of the newly synthesized enhancers remains unclear.

To assess if the inability of central DRG axons to regenerate is at least partially due to lack of synthesis and transport of injury signals to the DRG, dorsal root crush was performed and transport of known injury signals was tested. Following dynein immunoprecipitation, an increase in p-ERK was detected in the injured dorsal root similarly to what is seen after sciatic nerve injury (SNI). This suggests that transport of injury signals following a lesion to the DRG central branch occurs. However it argues against the role of p-ERK in mounting a regenerative response since after a dorsal root crush, DRG neurons do not present increased neurite outgrowth as observed following SNI.

To identify regeneration enhancers synthesized in the DRG and anterogradely transported to the SCI site following a conditioning injury, these were radiolabeled, tracked and compared in animals following SCI or conditioning lesion. We found an increase in radiolabeled proteins transported to the spinal cord of animals with a conditioning injury. This increase was mainly due to higher synthesis of cytoskeleton-related and glycolitic proteins although the synthesis and transport of putative novel regeneration enhancers, including RhoGDI-1 and CRMP-5, was also detected.

Future work will concentrate on clarifying the role of described injury signals in the regenerative process and on determining the relevance for axonal growth of the putative novel regeneration enhancers identified.

Presented by: **Mar, Fernando**

Poster No 069
Blue Session

The Nogo-66 Receptor Restricts Both Anatomical and Functional Plasticity in Visual Cortex

Aaron McGee
Children's Hospital Los Angeles, Keck School of Medicine at the University of Southern California

The nogo-66 receptor (NgR1) inhibits plasticity in the central nervous system. In the developing visual system, NgR1 restricts experience-dependent plasticity to a defined 'critical period' (P20-P32 in mice). Monocular deprivation (MD) during the critical period, but not thereafter, shifts ocular dominance and decreases visual acuity. In NgR1 mutant mice, plasticity during the critical period is normal, but continues abnormally such that ocular dominance at P60 is subject to the same plasticity as at juvenile ages. We propose that an absence of inhibition from NgR1 and the myelin-associated ligand Nogo-A permits enhanced anatomical rearrangements that mediate continued cortical plasticity and failure to close the critical period. We are testing this hypothesis with chronic multi-photon in vivo imaging to measure the stability of synaptic structures, axonal boutons and dendritic spines, in NgR1 mutant mice relative to wild-type mice. In our preliminary experiments, only a small percentage of axons (~2%/week) and axonal boutons (3-5%/week) were dynamic. The length of axon extension and retraction in wild-type mice was similar before and after MD, yielding a ratio of extension/retraction length near one. In contrast, while NgR1 mutant mice displayed a similar ratio of extension/retraction prior to MD, this ratio increased several fold as the number and length of retractions decreased after MD concomitant with an increase in the stabilization of new axonal boutons. In similar experiments, we are examining the dynamics of dendritic spines. Updated findings from these two studies will be presented.

Presented by: **McGee, Aaron**

Poster No 070
Red Session

Mechanism of Draxin Signaling in Axonal Guidance

Rajeshwari Meli, Friedrich Propst
Max F. Perutz Laboratories-University of Vienna

We have recently identified a novel signal transduction pathway that links nitric oxide signaling to rearrangements of the growth cone cytoskeleton leading to growth cone collapse and axon retraction. This pathway is dependent on S-nitrosylation of the microtubule-associated protein MAP1B on a specific cysteine residue (Stroissnigg et al., Nature Cell Biol.Sep;9(9):1035-45; 2007). MAP1B can interact with both microtubules and F-actin and has been proposed to play a role in microtubule-actin coupling in growth cone steering. MAP1B controls directionality of growth cone migration and axonal branching. In embryonic development it is essential for the formation of the corpus callosum, a prominent fiber tract containing axons that link the left and right hemispheres of the cerebral cortex.

We have since obtained evidence that MAP1B is an essential component of a general mechanism involved in repulsive axon guidance cue signaling. For example, repulsive cues such as lysophosphatidic acid and semaphorin 3a depend on MAP1B for their effects on axon outgrowth and retraction. Moreover, draxin, a recently identified repulsive axon guidance protein essential for the formation of the corpus callosum, restricts axon growth of wild-type but not MAP1B deficient neurons. Participation at the EMBO workshop would put me in a superior position to unravel further molecular details of repulsive guidance cue signaling and the role of MAP1B in microtubule-actin coupling.

Presented by: **Meli, Rajeshwari**

Poster No 071
Green Session

Different requirements for Sad Kinases in Hippocampal and Cortical Neurons during the Establishment of Neuronal Polarity

Sindhu Menon [1], *Daniela Lutter* [1], *Myriam Müller*, *Andreas Püschel* [1]
[1] *Institut für Molekulare Zellbiologie, Westfälische Wilhelms-Universität Münster*

Neurons are highly polarized cells with a single axon and several dendrites. Several intrinsic and extrinsic factors have been shown to be involved in the establishment of neuronal polarity. One of these are the Ser/Thr kinases SadA and SadB. Loss of these kinases causes a severe polarity defect in neurons.

We performed a closer analysis of this phenotype, which revealed differential requirements for Sad kinase function in the hippocampus and cortex:

1) Cultured hippocampal neurons from SadA/B double knockout embryos show a phenotype where all the neurites are positive for both axonal and dendritic markers while cultured cortical neurons from these brains are unpolarized.
2) Analysis of brain sections from SadA/B double knockout embryos showed that hippocampal neurons extend axons as revealed by staining for the axonal marker neurofilament. By contrast, cortical neurons do not show any staining for neurofilament.
3) Hippocampal and cortical neurons also show differences in the requirement for SadB.

These differences could be explained by a differential requirement for Sad kinase targets in cortical and hippocampal neurons. In hippocampal neurons Sad kinases regulate polarity by targeting the cell cycle checkpoint kinase Wee1. Further analysis of Wee1 and other potential targets of Sad kinases in cortical neurons will reveal the role of different Sad kinase targets during brain development. In addition, we investigate the regulation of SadA by generating mutants of SadA and analyzing their ability to phosphorylate tau at Serine-262,which is one of the known substrates of Sad kinase. Taken together, these experiments will elucidate the mechanism underlying the cell-type specific differences in neuronal polarization.

Presented by: **Menon, Sindhu**

Poster No 072
Blue Session

Proteomics Reveals Novel Sets of Proteins Involved in Axonal Growth

Igarashi Michihiro, Nozumi Motohiro
Niigata Univ Sch Med Dent Sci

The growth cone plays crucial roles in neural wiring, synapse formation, and axonal regeneration. Continuous rearrangement of cytoskeletal elements and targeting of transported vesicles to the plasma membrane are essential to growth cone motility; and identification of proteins in the mammalian growth cone has the potential to advance our understanding of formation of neural circuit. However, the proteins directly involved in these processes and their specific functions are not well established, and to date, only one growth cone marker protein, GAP-43, has been generally accepted. We successfully used a proteomic approach to identify approximately1,000 proteins present in developing rat forebrain growth cones, including highly abundant, membrane- and actin-associated proteins. Almost one hundred of the proteins appear to be highly enriched in the growth cone, and for 17 proteins, the results of RNAi suggest a role in axon growth. Most of the proteins we identified have not previously been implicated in axon growth and thus, their identification presents a significant first step forward, providing candidate neuronal growth-associated proteins. To determine whether these 17 proteins are growth cone markers in other neuronal cell types, we examined their expression and function in PC12D cells. We found that all 17 nGAPs were highly concentrated in the growth cones of PC12D cells, and that knockdown of all of them by RNAi reduced neurite outgrowth, indicating that all of them may be general growth cone markers, operating axonal growth. We will also discuss the mechanisms of the proteins identified by our proteomic approach, involved in axonal growth and polarity.

References: 1) Nozumi M et al. PNAS 106: 17211-6 (’09); 2) Lu J et al. Neurosci Res, in press

Presented by: **Michihiro, Igarashi**

Poster No 073
Red Session

The Role of the Drosophila Formin dDAAM in Axon Growth and Filopodia Formation

József Mihály [1], *Tamás Matusek* [1], *Catarina Gonçalves-Pimentel* [2], *Natalia Sánchez-Soriano* [2], *Andreas Prokop* [2], *Rita Gombos* [1]
[1] *BRC HAS, Institute of Genetics, Szeged*
[2] *Faculty of Life Sciences, Wellcome Trust Centre for Cell-Matrix Research, Manchester*

In the developing nervous system growth cones have an essential role in guiding axons to their correct target sites. Directed growth cone motility in response to extracellular cues is produced by the coordinated regulation of peripheral F-actin and central microtubule networks. Key regulators of actin dynamics are the so called nucleation factors, such as the Arp2/3 complex and formins, which use different mechanisms to seed new actin filaments.

We have previously examined the function of the Drosophila formin dDAAM in the embryonic CNS, where this protein shows a strong accumulation in the developing neurites. Genetic analysis suggested that this protein plays a major role in the regulation of axonal growth by promoting filopodia formation in the growth cone. Subsequently, we revealed a dDAAM requirement in the adult brain as well by detecting axonal projection defects in the mushroom body. Additionally, we identified Enabled, Profilin, Rac and the Arp2/3 complex as potential partners that work together with dDAAM during axonal growth regulation. Currently, we are investigating the mechanism as to how dDAAM induced actin assembly might contribute to filopodia formation and how dDAAM cooperates with the other cytoskeletal regulators identified as interaction partners. Our poster will provide detailed information on these studies involving primary neuronal cultures, mushroom body neurons and mouse model systems.

Presented by: **Mihály, József**

Poster No 074
Green Session

Role of Neuronal Ca2+-Sensor Proteins in Golgi-to-cell-surface Membrane Ttraffic

Marina Mikhaylova [1], *Johannes Hradsky* [2], *Parameshwar Reddy* [1], *Eckart D Gundelfinger* [3], *Yogendra Sharma* [4], *Michael R Kreutz* [1]
[1] *PG Neurplasticity, Leibniz Institute for Neurobiology, Magdeburg Germany*
[2] *PG Neurplasticity, Leibniz Institute for Neurobiology, Magdeburg Germany and DZNE, Helmholz Association, Magdeburg, Germany*
[3] *Dept. of Neurochemistry and Molecular Biology, Leibniz Institute for Neurobiology, Magdeburg Germany*
[4] *CCMB, Hyderabad, India*

Recently we have identified a new subfamily of neuronal calcium sensor proteins named Calneuron-1 and -2. Calneurons are widely expressed in neuronal cells and show a tight association particularly with trans-Golgi network (TGN). The membrane localization is provided by a C-terminal transmembranal region and classifies Calneurons as tail-anchored proteins - a distinct class of membrane proteins that are characterized by a C-terminal membrane insertion sequence and a capacity for post-translational integration. For proper Golgi targeting also the EF-hands and a preferencial association with subset of membranal lipids are required. One of the functions of Calneurons at the Golgi membranes is calcium dependent regulation of TGN - to - plasma membrane (PM) trafficking. The regulated local synthesis of PI(4)P and PI(4,5)P2 is crucial for this process. Phosphatidylinositol 4-OH kinase IIIβ (PI-4Kβ) is involved in the regulated local synthesis of PI(4)P. Calneurons physically associate with PI-4Kß, inhibit the enzyme profoundly at resting and low calcium levels, and negatively interfere with TGN-to-PM trafficking. At high calcium levels this inhibition is released and PI-4Kβ is activated via a preferential association with neuronal calcium sensor-1 (NCS-1). In accord to its supposed function as a filter for subthreshold Golgi calcium transients, neuronal overexpression of Calneuron-1 enlarges the size of the TGN caused by a build-up of vesicle proteins and reduces the number of axonal Piccolo-Bassoon transport vesicles. The opposing roles of Calneurons and NCS-1 provide a molecular switch to decode local calcium transients at the Golgi and impose a calcium threshold for PI-4Kβ activity and vesicle trafficking.

Presented by: **Mikhaylova, Marina**

Poster No 075
Blue Session

Diffusion/Trapping of AMPA Receptors at Neurexin-Neuroligin Adhesions through PSD-95

Magali Mondin [1], *Eric Hosy* [1], *Martin Heine* [2], *Daniel Choquet* [1], *Olivier Thoumine* [1]
[1] *IINS CNRS UMR 5297*
[2] *Leibniz institute for neurobiology*

Neurexin/neuroligin adhesion plays a central role in synapse formation, but the mechanisms linking initial contact to the assembly of functional pre- and post-synaptic macromolecular complexes remain unclear. Here we investigated whether the recruitment of AMPA receptors at early neurexin/neuroligin contacts could occur through a diffusion/trap mechanism. We show by single nanoparticle tracking, that surface-diffusing AMPA receptors in primary hippocampal neurons stop reversibly at PSD-95 clusters triggered by neurexin/neuroligin adhesion. In addition, the accumulation of functional AMPA receptors at new neurexin/neuroligin contacts is prevented upon blocking AMPA receptor surface diffusion by antibody cross-link. Using neuroligin-1 mutants and inhibitory RNAs, we further show that the membrane mobility of AMPA receptors inversely correlates with neuroligin-1 level, and that this effect requires the interaction between neuroligin-1 and PSD-95. The triggering of new neurexin/neuroligin adhesions causes a depletion of PSD-95 from pre-existing synapses, accompanied by a drop in AMPA miniature EPSCs, suggesting a competitive mechanism. Finally, AMPA synaptic transmission is significantly reduced in CA1 hippocampal cells of slices from neuroligin-1 knock-out mice at early developmental stage. Overall, we propose that a pool of membrane-diffusing PSD-95 and AMPA receptors can be rapidly trapped at newly forming neurexin/neuroligin adhesions during post-synapse formation.

Presented by: **Mondin, Magali**

Poster No 076
Red Session

The End of 35-year Quest has Come by FRET Imaging - the Molecular Mechanism of dbcAMP-Induced Neurite Outgrowth

Takeshi Nakamura [1], *Akihiro Goto* [2], *Mikio Hoshino* [3], *Michiyuki Matsuda* [2]
[1] *Research Institute for Biological Sciences, Tokyo University of Science,*
[2] *Graduate School of Biostudies, Kyoto University*
[3] *National Institute of Neuroscience, NCNP*

The second messenger cAMP plays a pivotal role in neurite/axon growth and guidance, but its downstream pathways leading to the regulation of Rho GTPases, centrally implicated in neuronal morphogenesis, remain elusive. We examined spatiotemporal changes in Rac1 and Cdc42 activity and phosphatidylinositol 3,4,5-triphosphate (PIP3) concentration in dibutyryl cAMP (dbcAMP)-treated PC12D cells using Förster resonance energy transfer-based biosensors. During a 30-min incubation with dbcAMP, Rac1 activity gradually increased throughout the cells and remained at its maximal level. There was no change in PIP3 concentration. After a 5-h incubation with dbcAMP, Rac1 and Cdc42 were activated at the protruding tips of neurites without PIP3 accumulation. dbcAMP-induced Rac1 activation was principally mediated by protein kinase A (PKA) and STEF/Tiam2. STEF depletion drastically reduced dbcAMP-induced neurite outgrowth. PKA phosphorylates STEF at three residues (Thr749, Ser782, Ser1562); T749 phosphorylation was critical for dbcAMP-induced Rac1 activation and neurite extension. During dbcAMP-induced neurite outgrowth, PKA activation at the plasma membrane became localized to neurite tips; this may contribute to local Rac1 activation at the same neurite tips. Considering the critical role of Rac1 in neuronal morphogenesis, the PKA-STEF-Rac1 pathway may play a crucial role in cytoskeletal regulation during neurite/axon outgrowth and guidance which depend on cAMP signals. This work could provide a clue to how to regenerate injured axons. It is because the specific reinforcement at the appropriate point in signaling pathways in addition to cAMP administration is expected to synergistically promote regeneration.

Presented by: **Nakamura, Takeshi**

Poster No 077
Green Session

NMDA Receptor Regulates Migration of Newly Generated Neurons in the Adult Hippocampus via Disrupted-In-Schizophrenia 1 (DISC1)

Takashi Namba [1], *Shigeo Uchino* [2], *Kohsaka Shinichi* [2], *Kaibuchi Kozo* [1]
[1] *Department of Cell Pharmacology, Nagoya University Graduate School of Medicine*
[2] *Department of Neurochemistry, National Institute of Neuroscience*

In the mammalian brain, new neurons are continuously generated throughout life in the dentate gyrus (DG) of the hippocampus. Previous studies have established that newborn neurons migrate a short distance to be integrated into a pre-existing neuronal circuit in the hippocampus. How the migration of newborn neurons is governed by extracellular signals, however, is not fully understood. Here, we report that N-methyl-D-aspartate receptor (NMDA-R)-mediated signaling is essential for the proper migration and positioning of newborn neurons in the DG. An injection of the NMDA-R antagonists into adult male mice caused the aberrant positioning of newborn neurons, resulting in an overextension of their migration in the DG. Interestingly, we revealed that the administration of NMDA-R antagonists led to a decrease in the expression of Disrupted-In-Schizophrenia 1 (DISC1), a candidate susceptibility gene for major psychiatric disorders such as schizophrenia, which is also known as a critical regulator of neuronal migration in the DG. Furthermore, the overextended migration of newborn neurons induced by the NMDA-R antagonists was significantly rescued by the exogenous expression of DISC1. Collectively, these results suggest that the NMDA-R signaling pathway governs the migration of newborn neurons via the regulation of DISC1 expression in the DG.

Presented by: **Namba, Takashi**

Poster No 078
Blue Session

Cytoplasmic Linker Proteins Regulate Neuronal Polarization through Microtubule and Growth Cone Dynamics

Dorothee Neukirchen, Frank Bradke
Max Planck Institute for Neurobiology

Axon formation is a hallmark of initial neuronal polarization. This process is thought to be regulated by enhanced microtubule stability in the subsequent axon and changes in actin dynamics in the future axonal growth cone. Here, we show that the microtubule end-binding proteins Cytoplasmic Linker Protein (CLIP) 115 and CLIP 170 were enriched in the axonal growth cone and extended into the actin rich domain of the growth cone. CLIPs were necessary for axon formation and sufficient to induce an axon. The regulation of axonal microtubule stabilization by CLIPs enabled the protrusion of microtubules into the leading edge of the axonal growth cone. Moreover, CLIPs positively regulated growth cone dynamics and restrained actin arc formation, which was necessary for axon growth. In fact, in neurons without CLIP activity, axon formation was restored by actin destabilization or myosin II inhibition. Together, our data suggest that CLIPs enable neuronal polarization by controlling the stabilization of microtubules and growth cone dynamics.

Presented by: **Neukirchen, Dorothee**

Poster No 079
Red Session

A Genetically Encoded Dendritic Marker Sheds Light on Neuronal Connectivity in Drosophila and Reveals Conserved Features of Dendritic Development

Laura Nicolaï [1], Ariane Ramaekers [2], Tim Raemaekers [1], Andrzej Drozdzecki [3], Alex Mauss [4], Jiekun Yan [3], Matthias Landgraf [4], Wim Annaert [3], Bassem Hassan[5]
[1] *VIB/ KULeuven School of Medicine, 3000 Leuven, Belgium*
[2] *VIB*
[3] *VIB, Belgium*
[4] *University of Cambridge, UK*
[5] *VIB/ KULeuven School of medicine, 3000 Leuven, Belgium*

Drosophila melanogaster has emerged as a powerful model for neuronal circuit development and function. A major impediment has been the lack of a genetically encoded specific and phenotypically neutral marker of the somatodendritic compartment. We have developed such a marker, which we named DenMark (Dendritic Marker), and showed that it is a powerful tool for revealing novel aspects of the neuroanatomy of developing dendrites, identifying previously unknown dendritic arbors and elucidating neuronal connectivity.

One intriguing observation is the gradual restriction of DenMark to dendrites during development; in 1st instar larval mushroom bodies (MBs) the marker is homogenously distributed, by the 3rd instar stage DenMark is somatodendritically localized as in the adult. What intracellular mechanisms are responsible for this gradual restriction of DenMark to dendrites during development? Interestingly, DSCAM17.1::GFP also shows gradual restriction to the somatodendritic compartment suggesting that segregation of dendritic factors is acquired progressively during MB development and is a common feature of fly and mammalian neurons, supporting suggestions of a common origin of vertebrate and invertebrate dendrites. This also implies that a combination of specific targeting and selective degradation might account for dendrite-cargo specificity. The Rab protein family represents possible candidate molecules for such a mechanism. Rab GTPases are well known to regulate many steps of intracellular trafficking of cell surface proteins. We are performing a screen of all Rab GTPases to identify intracellular trafficking pathways responsible for DenMark specificity.

Presented by: **Nicolaï, Laura**

Poster No 080
Green Session

Cux1 and Cux2 Regulate Dendritic Branching, Spine Morphology and Synapses of the Upper Layer Neurons of the Cortex

López Nieto

Summary
Dendrite branching and spine formation determines the function of morphologically distinct and specialized neuronal subclasses. However, little is known about the programs instructing specific branching patterns in vertebrate neurons and whether such programs influence dendritic spines and synapses. Using knockout and knockdown studies combined with morphological, molecular and electrophysiological analysis we show that the homeobox Cux1 and Cux2 are intrinsic and complementary regulators of dendrite branching, spine development and synapse formation in layer II-III neurons of the cerebral cortex. Cux genes control the number and maturation of dendritic spines partly through direct regulation of the expression of Xlr3b and Xlr4b, chromatin remodeling genes previously implicated in cognitive defects. Accordingly, abnormal dendrites and synapses in Cux2-/- mice correlate with reduced synaptic function and defects in working memory. These demonstrate critical roles of Cux in dendritogenesis and highlight novel subclass-specific mechanisms of synapse regulation that contribute to the establishment of cognitive circuits.

Presented by: **Nieto, López**

Poster No 081
Blue Session

Mek2 and Pin1 Regulate BNIP-H (Caytaxin) Ability to Promote Axonal and Dendritic Growth by Trafficking Glutaminase KGA on Kinesin Heavy Chain KIF5B

Catherine Qiurong Pan, Boon Chuan Low
National University of Singapore, Mechanobiology Institute

BNIP-H (or Caytaxin) is a brain-specific BNIP-2 family member implicated in Cayman cerebellar ataxia. Its overexpression potentiates neurite outgrowth in PC12 cells by acting as an adaptor to traffic glutaminase KGA on kinesin KIF5B towards neurite termini and regulates the production of glutamate. However, nothing is known about how formation and functions of such KGA/BNIP-H/KIF5B complex and their trafficking can be regulated and their physiologic outcomes perturbed. We recently showed that nerve growth factor stimulates interaction of BNIP-H with peptidyl-prolyl isomerase Pin1 in differentiating neurons. Here by co-immunoprecipitation studies, we further showed that constitutively active Mek2, but not the kinase-dead Mek2, strongly enhanced the interaction between BNIP-H with KGA as well as BNIP-H with KIF5B. However, the presence of Pin1 could disrupt the ability of BNIP-H to act as the adaptor that linked KGA to KIF5B. Consequently, co-expressing wildtype Pin1 (but not the catalytic mutant Pin1, H157A) with BNIP-H and KGA attenuates the ability of BNIP-H to potentiate PC12 cells differentiation. These results therefore reveal a novel duet role of Mek2 and Pin1 in activating and suppressing the functional ternary interaction of BNIP-H with KIF5B and KGA, and together they represent a novel feedforward and feedback determinant loop in regulating neuronal maturation and differentiation.

Presented by: **Pan, Catherine Qiurong**

Poster No 082
Red Session

Developmental Stabilization and Activity-Dependent Regulation of Gephyrin Scaffolds at Hippocampal GABAergic Postsynapses

Andreas Vlachos [1], Suneel Reddy-Alla [2], Theofilos Papadopoulos [2], Thomas Deller [1], Heinrich Betz [2]
[1] Goethe-University, Neuroscience Center, Institute of Clinical Neuroanatomy, Frankfurt a.M., Germany
[2] MPI for Brain Research, Department of Neurochemistry, Frankfurt a.M., Germany

Gephyrin is a scaffolding protein essential for synaptic clustering of inhibitory glycine and GABA(A) receptors. Here, we investigated the dynamics of gephyrin at individual synapses in organotypic entorhino-hippocampal slice cultures prepared from a newly generated mouse line, which expresses green fluorescent protein-tagged gephyrin under the control of the Thy1.2 promoter. Fluorescence recovery after photobleaching indicates that gephyrin clusters are stabilized at GABAergic postsynapses upon developmental maturation. This stabilization is accompanied by an increase in gephyrin scaffold size and inhibitory synaptic strength. Furthermore, by pharmacologically modulating GABA(A) receptor function we provide evidence for an activity-dependent regulation of gephyrin scaffold stability and size. We conclude that the maturation and plasticity of inhibitory synapses entail changes in the size of the synaptic gephyrin scaffold and its ability to undergo dynamic structural rearrangements. We are currently investigating the underlying molecular mechanisms controlling activity-dependent stabilization of gephyrin scaffolds at GABAergic Synapses.

Presented by: **Papadopoulos, Theofilos**

Poster No 083
Green Session

Tubulin Modifications that Control Microtubule Stabilizers vs Microtubule Depolymerizers

Leticia Peris, Didier Job, Annie Andrieux
Grenoble - Institut des Neurosciences Centre de recherche Inserm U 836-UJF-CEA-CHU Equipe 1: Physiopathologie du Cytosquelette Université Joseph Fourier - Faculté de Médecine Domaine de la Merci 38706 La Tronche Cedex

Tubulin is subject to a special cycle of tyrosination/detyrosination in which the C-terminal tyrosine of alpha-tubulin is cyclically removed by a carboxypeptidase and readded by a tubulin-tyrosine-ligase (TTL). This tyrosination cycle is conserved in evolution, yet its physiological importance is a matter of conjecture. TTL suppression in mice causes perinatal death due to severe brain disorganization. Spindle positioning in neuronal progenitors was perturbed with resulting imbalance of proliferation/differentiation events. In addition, mitotic spindle orientation was also altered in cultured TTL null cells grown in special restrictive micro patterns. In culture, TTL null neurons display morphogenetic anomalies including an accelerated and erratic time course of neurite outgrowth, premature axonal differentiation and increased axonal branching. We also observed an impaired microtubule disassembly in these cells. Analysis of microtubule dynamics in TTL null cells refleted a reduced catastrophe frequence and an increased time growing. We demonstrated that detyrosinated tubulin failed to interact with a family of microtubule stabilizers, CAP-Gly family proteins (CLIP-170, CLIP-115 and p150Glued) which links microtubule tips with the cell cortex. Additionally, we reported an inhibition of Kinesin-13 depolymerizing motor activity on detyrosinated microtubules.

We propose that the tyrosination cycle is required for the control of microtubule interactions with the cell cortex throughout the regulation of + tips proteins, and the regulation of microtubule dynamics by the activity of depolymerizing motors. In cells, the detyrosination of transiently stabilized microtubules may give rise to a different protein composition of +Tips complex and a persistent subpopulations of disassembly-resistant polymers to sustain subcellular cytoskeletal differentiation.

Presented by: **Peris, Leticia**

Poster No 84
Blue Session

Studying the Function, Regulation and Coordination of Kinesin-1 and Kinesin-3 in the Drosophila Nervous System

Isabel Peset-Martin, Lucy Williams, Matthias Landgraf, Isabel Palacios
University of Cambridge

Directed transport by microtubule-based motors is crucial to generate and maintain the polarity of the neuron, which is essential for neuronal activity. Kinesin-1 and Kinesin-3 are key factors in anterograde axonal transport and in synaptogenesis. This suggests that these motors transport to the axon terminus the elements required to sustain such a rapid morphogenesis and growth. However, what the cargoes responsible for nerve ending development are and how the motors are regulated is completely unknown. We are therefore studying Kinesin-1 and Kinesin-3 function using Drosophila melanogaster as a model system. In the germline Kinesin-1 is essential for localizing various cargoes that act as body axes determinants. By tracking these cargoes in oocytes that both lack endogenous motor and express mutated versions of Kinesin heavy chain (KHC) we have found that KHC has several cargo-specific domains that act independently or cooperatively (manuscript in preparation). These data introduce novel modes of motor attachment and possibly regulation of transport, since they show that various cargoes within the same cell do not share a common transport mechanism. We are currently performing similar studies in neurons with the goal of i) identifying Kinesin-1 and Kinesin-3 specific cargoes and associating them with the altered morphology of nerve endings observed in Kinesin-1 and Kinesin-3 mutant neurons; ii) characterizing the functional domains of the motors, with the goal of understanding how Kinesin-1 and Kinesin-3 achieve specificity of transport; iii) identifying and characterizing novel factors involved in Kinesin-1 and Kinesin-3 function and iv) studying how these motor proteins cooperate to regulate axonal transport.

Presented by: **Peset-Martin, Isabel**

Poster No 85
Red Session

β-Arrestin2 Mediates Spatial Control over Cofilin Activity in NMDA-Iinduced Dendritic Spine Remodeling in Hippocampal Neurons

Crystal Pontrello, Katie DeFea, Iryna Ethell
University of California, Riverside

Dendritic spines are the postsynaptic sites of most excitatory synapses in the brain, and F-actin dynamics underlie their structural plasticity. We have shown that the F-actin-severing protein cofilin, which is regulated by phosphorylation, can induce remodeling of mature spines in hippocampal neurons. Here we demonstrate that NMDA-mediated dendritic spine remodeling is dependent on cofilin activity and localization. NMDAR activation shifts the p-cofilin/cofilin equilibrium toward active cofilin through calcineurin- and PI3K-dependent signaling pathways that trigger cofilin dephosphorylation, and dominant-negative cofilinS3D prevents NMDA-induced dendritic spine remodeling in wt neurons.

NMDAR activation promotes the translocation of cofilin to dendritic spines and spine remodeling, events that require cofilin dephosphorylation and are blocked by overexpression of dominant-negative cofilinS3D. Cofilin clustering in spines is also dependent on β-Arrestins. Hippocampal neurons lacking β-Arrestin2 develop normal mature spines, but fail to remodel in response to NMDA. While NMDA or overexpression of constitutively-active cofilinS3A induces dendritic spine remodeling in wt neurons, this spine remodeling does not occur in β-Arrestin2-deficient neurons, and constitutively-active cofilinS3A fails to translocate to spines in response to NMDA in β-Arrestin2-deficient neurons. Impaired structural plasticity in response to NMDA may underlie spatial learning deficits that are also found in β-Arrestin2-deficient mice. Our studies demonstrate a novel function of β-Arrestin2 in NMDAR-mediated dendritic spine plasticity through spatial control over cofilin activity.

Presented by: **Pontrello, Crystal**

Poster No 086
Green Session

Visualization of Activity-Induced Local Translation Using TimeSTAMP

Fillip Port [1], *Michael Lin* [2], *Simon Bullock* [1]
[1] *MRC Laboratory of Molecular Biology, Department of Cell Biology, Hills Road, Cambridge, UK*
[2] *Department of Pediatrics and Programs in Gene Therapy and Molecular Imaging, Stanford University, Stanford, California, USA*

Local translation of selected mRNAs is of pivotal importance for axon guidance and synaptic plasticity. Spatial restriction of gene expression to individual growth cones and synapses enables these neuronal compartments to respond to external stimuli autonomous from the rest of the cell. How local translation in synapses is regulated in response to stimulation is a major focus of current research. Here we present a method to directly visualize newly synthesized proteins in neurons. TimeSTAMP is based on the drug-dependent preservation of epitope tags fused to the protein of interest and allows monitoring of local translation with great temporal and spatial precision. This system can be applied to any experimental system amenable to transgene expression. We will present the latest results we obtained using this method in neurons of the adult Drosophila brain. We also report on a new version of TimeSTAMP that is based on a split-fluorophore system that allows monitoring local translation in living cells.

Presented by: **Port, Fillip**

Poster No 087
Blue Session

Spectraplakins - Cytoskeletal Integrators with Key Roles in Neuronal Growth

Andreas Prokop, Juliana Alves-Silva, Robin Beaven, Natalia Sanchez-Soriano
Faculty of Life Sciences, The University of Manchester

The molecular mechanisms that coordinate the dynamics of actin and microtubule (MT) networks in developing, mature or regenerating neurons are poorly understood. To advance our understanding, spectraplakins provide a promising avenue of research. They are actin-MT linker molecules which act as key integrators of cytoskeletal regulation and play prominent roles in axonal/dendritic growth, neuronal migration and neurodegeneration. Their roles in axonal growth are highly conserved between mammalian ACF7 and its close Drosophila homologue Short stop (Shot). We now use Shot as the genetically more amenable paradigm to explore the underlying molecular mechanisms. To this end, we systematically apply combinatorial genetics of Drosophila, using readouts provided by neurons in the embryo and by our newly established embryo-derived primary neuron culture system. We find that Shot regulates filopodia formation in pathfinding, through its EF-hand motifs and interaction with eIF5c. Furthermore, Shot regulates MT networks in axon extension, through its F-actin-binding calponin-homology domains, its MT-stabilising Gas2 domain, and its Ctail which enhances association along MTs (via high arginine content) and mediates recruitment to MT plus ends (via EB1-binding motifs). Accordingly, we find that manipulations of F-actin influence Shot function, that MTs in shot mutant neurons are sensitive to nocodazole, and that loss of EB1 causes shot-like phenotypes and displays strong genetic interactions with shot. Systematic analyses of actin and MT regulators are now being carried out to explore the regulatory networks that link to Shot function during axonal growth. Supported by: BBSRC, Wellcome Trust

Presented by: **Prokop, Andreas**

Poster No 088
Red Session

Regulation of Neuronal Polarity by Rap1 GTPases

Daniela Lutter, Andreas Püschel
Institut für Molekulare Zellbiologie, Schlossplatz 5, Westfälische Wilhelms-Universität Münster, Germany

The establishment of neuronal polarity in hippocampal neurons is directed by a pathway that depends on the sequential activity of several GTPases. Rap1B performs a central function in this pathway and is necessary and sufficient to specify axonal identity. The analysis of conditional knockout mice for Rap1a and Rap1b shows that Rap1 GTPases are required for axon formation in the hippocampus not only in cultured neurons but also in vivo.

Rap1B accumulates in the growth cone of a single neurite in stage 2 neurons before they are polarized morphologically. This restriction of Rap1B localization is an essential step in neuronal polarization and is mediated by its destruction through the proteasome. We identified the E3 ubiquitin ligase Smurf2 as the enzyme that modifies inactive, GDP-bound Rap1B to initiate its degradation. The selective degradation of inactive Rap1B mediates its removal from minor neurites that contain a low level of active GTPase while the active Rap1B in the future axon is protected from proteolysis.

In addition to Smurf2, Rap1B is also regulated by Rheb and the mTOR pathway. Knockdown of Rheb by RNAi or inhibition of mTOR blocks the formation of axons while activation of mTOR induces supernumerary axons. One of the targets for mTOR is Rap1B. Activation of the mTOR pathway in neurons increases the amount of Rap1B and expression of Rheb induces supernumerary axons in an mTOR- and Rap1B-dependent manner. The mTOR-dependent translation of Rap1B may balance its degradation by the proteasome. While loss of Smurf2 alone induces multiple axons and suppression of Rheb results in the loss of axons, neurons establish normal neuronal polarity with a single axon when both pathways are blocked by knockdown of Smurf2 and Rheb.

Presented by: **Püschel, Andreas**

Poster No 089
Green Session

A Novel Stress-Regulated Protein that Acts on Actin Bundling, Synaptic Efficacy and Cognition

Mathias Schmidt, Jan Schülke, Claudia Liebl, Michael Stiess, Florian Holsboer, Mike Stewart, Frank Bradke, Matthias Eder, Marianne Müller, Theo Rein

Stress has been identified as a major causal factor for many mental disorders. However, our knowledge about the chain of molecular and cellular events translating stress experience into altered behavior is still rather scant. Here, we have characterized a novel stress-induced protein in mouse brain. It binds to actin, promotes bundling and stabilization of actin filaments, and impacts on actin-dependent neurite outgrowth. The endogenous protein localizes to distinct synapses, with preference for the presynaptic region. Hippocampal virus-mediated overexpression of the protein reduced spine density, diminished the probability of synaptic glutamate release and altered cognitive performance. We propose it as the first identified protein to link stress with actin dynamics, with important repercussions on synaptic function and cognition.

Presented by: **Rein, Theo**

Poster No 090
Blue Session

p140Cap and Citron-N: a "spiny" Cooperative Relationship

Daniele Repetto [1], *Noemi Morello* [2], *Eleonora Calcagno* [2], *Federico Bianchi* [1], *Maurizio Giustetto* [2], *Ferdinando Di Cunto* [1], *Emilia Turco* [1], *Paola Defilippi* [1], *Paola Di Stefano* [1], *Paola Camera* [1]
[1] *Molecular Biotechnology Center, Department of Genetic Biology and Biochemistry, University of Torino. V. Nizza 52. Torino 10126. Italy.*
[2] *National Institute of Neuroscience Italy and Department of Anatomy, Pharmacology and Forensic Medicine, University of Torino. Torino 10126. Italy.*

Dendritic spines are specialized protrusion with a pivotal role in memory and learning working as the major site of excitatory synaptic input. p140Cap is an adaptor protein able to directly bind and inhibit Src kinase and downstream signalling. We have already shown that p140Cap is highly expressed in brain, particularly in hippocampal neuron dendritic spines. Here we report that p140Cap deficient mice (p140Cap-/-) display an altered distribution of the spine density and a compromised dendritic spine morphology in hippocampal CA1 region. Interestingly p140Cap-/- cultured hippocampal neurons show a reduced number of mature spines and a mis-localized F-actin staining inside to the dendritic shaft. Purified p140Cap-/- synaptoneurosomes display an increased Src kinase and Rac1 activities and a reduced RhoA activation. Moreover, in sinaptosomes p140Cap and Src kinase co-immunoprecipitate with Citron-N (CIT-N), an adaptor protein responsible to recruit active RhoA into spines. Overexpression of CIT-N into rat hippocampal neurons silenced for p140Cap expression can rescue the immature filopodia phenotype due to p140Cap downregulation, whereas overexpression of CIT-N mutant unable to bind RhoA fail to rescue this phenotype. Taken together, our results suggest the importance of p140Cap, Src kinase and CIT-N cooperation in dendritic spine maintenance and synaptic plasticity.

Presented by: **Repetto, Daniele**

Poster No 091
Red Session

The Role of Perisynaptic Schwann Cells in Nerve Terminal Regeneration

Michela Rigoni [1], Erik Tedesco [1], Giorgio Carmignoto [2], Ornella Rossetto [1], Cesare Montecucco [1]
[1] Dept. of Biomedical Sciences, University of Padova, Italy
[2] Institute of Neuroscience-Consiglio Nazionale delle Ricerche (CNR), University of Padova, Padova, Italy

The neuromuscular junction (NMJ) is a tripartite system composed of a presynaptic element (the nerve terminal, NT), a postsynaptic element (the muscle) and the more recently identified perisynaptic Schwann cells (PSCs). PSCs are the glia of the NMJ and they are implicated (among other functions) in its recovery after damage. Indeed, PSCs dedifferentiate upon nerve terminal (NT) damage, remove NT debris and protrude prolongations to guide reinnervation. Some PSCs activation mediators have been identified (ATP, acetylcholine), but we hypothesized that also other mediators such as arachidonic acid and its derivates could lead to PSCs activation.

The nerve damage models employed till now (cut or crush model) provide a non controllable experimental system since they lead to Wallerian degeneration, a degenerative/inflammatory process that involves many cell types and inflammatory mediators. We therefore chosed another experimental model, the intramuscular injection of presynaptic neurotoxins (α-latrotoxin, from the black widow spider venom and SPANs, snake presynaptic PLAs neurotoxins) to investigate PSCs involvement in nerve terminal regeneration. These two classes of toxins cause a specific damage limited to the presynaptic element of the NMJ; their mechanism of action is different but Ca2+ overload plays a major role in NTs degeneration induced by both class of neurotoxins.

First of all we monitored the kinetic of the degeneration/regeneration process by comparing the progressive loss and recovery of the staining of the presynaptic marker VAChT (that identifies the presynaptic element of the NMJ) with a functional assay (the DAS assay). Once identified this "time window ", we followed PSCs activation by staining with a set of markers whose intensity is reported to change following cell activation: S100, nestin, GFAP.

Preliminary results indicate that, following α-latrotoxin poisoning, Schwann cells are activated (as indicated by a decrease in S100 staining), but no prolongations were observed (these processes should guide the reinnervation of the terminal), maybe due to the fast recovery kinetic of the terminal after toxin treatment.

We are currently setting up another model, an ex-vivo model of soleus muscle from mouse, that is more controllable than the in vivo one and keeps unaltered the tripartite organization of the NMJs; by this system we are trying to follow via calcium imaging the activation of PSCs following administration of a puff of α-latrotoxin.

Presented by: **Rigoni, Michela**

Poster No 092
Green Session

Frequency-Based Motor-Driven Mechanisms in Axon Length Sensing

Ida Rishal [1], Na'aman Kam [1], Vera Shinder [1], Elizabeth Fisher [2], Giampietro Schiavo [3], Mike Fainzilber [1]
[1] Dept. of Biological Chemistry, Weizmann Institute of Science, 76100 Rehovot, Israel
[2], Institute of Neurology, University College London, London WC1N 3BG, U.K.
[3] Molecular Neuropathobiology Laboratory, Cancer Research UK London Research Institute, Lincoln's Inn Fields Laboratories, London WC2A 3PX, U.K

Size homeostasis is a fundamental characteristic of eukaryotic cells, but how cells might assess their own size remains largely unknown. In neurons, the size-sensing problem can be approximated to a one-dimensional challenge, specifically how can neurons assess the lengths of their own neurites? Here we propose that neurons use microtubule motor dependent signaling to this end. Computer simulations demonstrate that bidirectional motor dependent signaling can give rise to frequency-encoded readout of neurite lengths, similar to chirp signals used in radar or sonar technology. A counter-intuitive prediction arising from the model is that decreasing the amount of retrograde or anterograde signal carrier should result in longer axons. Indeed, RNAi-mediated partial knockdown of cytoplasmic dynein heavy chain 1 or of certain kinesin heavy chain isoforms, caused increases in process length in adult sensory neurons in culture. The same phenomenon was also observed in cultured sensory neurons from a mutant mouse with reduced levels of axonal dynein. Thus, frequency-based motor-driven mechanisms may ensure robustness in length sensing in neurons.

Presented by: **Rishal, Ida**

Poster No 093
Blue Session

Effects of Microtubule Stabilization on Axon Regeneration in Clinically Relevant Approaches

Jörg Ruschel [1], Andres Hurtado [2], Farida Hellal [1], Vance Lemmon [3], John Bixby [3], Frank Bradke [1]
[1] Max-Planck-Institute of Neurobiology,Martinsried,Germany
[2] Hugo Moser Research Institute at Kennedy-Krieger, Johns-Hopkins University, Baltimore, USA
[3] Miami Project to cure paralysis, Miller school of medicine, University of Miami

Microtubule stabilization promotes regeneration of injured spinal cord axons by increasing intrinsic neuronal growth capacity and removing extrinsic inhibitory factors.

Here we aimed to demonstrate the benefit of the microtubule stabilizing drug Taxol for spinal cord regeneration and functional recovery in a clinically relevant injury model. After contusion injury, Taxol treatment decreases fibrotic scarring and stimulates axonal regeneration resulting in a significant improvement of fine-tuned locomotion. In addition, we tested a new class of microtubule stabilizing agents, the Epothilones which are, in contrast to Taxol, crossing the Blood Brain Barrier and without pro-inflammatory side effects. In vitro, Epothilone-B increases axonal growth competence enabling cultured neurons to overcome growth inhibitory substrates. Moreover, in vivo live-imaging studies revealed that Epothilone-B treatment reduces dystrophic-endbulb formation of cut dorsal column axons. Additionally, Epothilone-B inhibits fibrotic scarring and increases axonal regeneration after spinal cord hemisection in rats.

Taken together these results emphasize the clinical relevance of microtubule stabilization to stimulate axonal regeneration in the injured central nervous

Presented by: **Ruschel, Jörg**

Poster No 094
Red Session

Cell-Contact Induced Eph Receptor Trans-Endocytosis Activates an SFK Dependent Phagocytic Pathway

Maria Sakkou, Rüdiger Klein
Max Planck Institute of Neurobiology, Department of Molecular Neurobiology, Martinsried, Germany

Cells have self-organizing features that control their behavior in complex tissues. Ephrins are membrane tethered guidance cues that bind to Eph receptor tyrosine kinases (RTKs). During development the Eph/ephrin signaling system controls a large variety of cellular responses including contact-mediated attraction or repulsion, adhesion or de-adhesion and migration. Moreover, unlike other RTKs, Eph/ephrin signaling dynamics are regulated by a large number of components like proteolytic cleavage, trans-endocytosis, bi-directional signaling, higher order clustering and cis-interactions of Ephs and ephrins. Their relative contribution though during brain development is not yet known. Ongoing work indicates that bidirectional endocytosis is a critical regulator of growth cone collapse and in cell culture is required to convert an otherwise adhesive force provided by the interaction of the receptors and ligands to a repulsive signal. My aims are to study the role of endocytosis in Eph/ephrin signaling, to characterize the endocytic pathways that are activated by Ephs and ephrins and to investigate their relative functional role. In a candidate approach we undertook we found that although Src family kinases (SFKs) are phosphorylated and activated downstream of both Eph receptor and ephrin ligand they are required for ephrin "reverse", but not Eph "forward" trans- endocytosis. Furthermore, we show that Src requirement is exclusive for cell-cell interaction but not for soluble ligand stimulation and that trafficking of Eph/ephrin complexes require Rab5 activation, actin cytoskeleton polymerization and PI3K activation. To further assess the functional role of endocytosis we blocked SFK activation in primary neurons and observed that de-attachment between ephrinB growth cones and EphB2 cells was significantly slower. Taken together these data indicate that Eph/ephrin complexes are internalized via a phagocytic –like mechanism and that downstream of ephrin SFKs activate a novel pathway independent of the phosphotyrosine and PDZ dependent signaling networks that is required for receptor internalization and cell de-attachment.

Presented by: **Sakkou, Maria**

Poster No 095
Green Session

Linking Synaptic Endocytosis to Retrograde Axonal Transport: the Role of the Coxsackievirus and Adenovirus Receptor in Neuronal Trafficking of Adenoviruses

Sara Salinas [1], *Charleine Zussy* [1], *Daniel Henaff* [1], *Neus Bayo-Puxan* [1], *Giampietro Schiavo* [2], *Eric Kremer* [1]
[1] *Institut de Génétique Moléculaire de Montpellier-CNRS*
[2] *Molecular NeuroPathobiology Laboratory, Cancer Research UK, London Research Institute, London, United Kingdom,*

The coxsackievirus and adenovirus receptor (CAR) is an adhesion molecule and a key component of tight junctions in epithelia. Its role as adenovirus receptor was mainly described as a docking factor in the first step of membrane binding with no subsequent role in the internalisation process. This molecule is also largely expressed in the nervous system but its function in neurons remains uncharacterised. Axonal transport is responsible for the movement of cargoes between nerve termini and cell bodies of neurons and can be diverted by pathogens to reach the central nervous system (CNS). Here, we characterised the axonal traffic of an adenovirus (CAV-2) that preferentially infects neurons. In addition to its relevance to disorders associated with adenovirus infections of the CNS, the potential use of adenoviral vectors to treat brain pathologies makes the understanding of their neuronal trafficking crucial. We show that CAV-2 displays bidirectional motility in axons of motor neurons and, in contrast to adenovirus trafficking in epithelial cells, its transport occurs in Rab7+ pH neutral vesicles. CAV-2 binding is CAR-dependent and, unexpectedly, CAR is found associated with axonal vesicles containing CAV-2 and a variety of cargoes such as tetanus toxin and neurotrophin receptors. These observations suggest that some axonal Rab7 organelles provide a protective environment for endogenous molecules and pathogens to be transported over long-distances. Finally, sub-cellular localisation studies showed that a portion of CAR is localised to the pre-synaptic part of central synapses and neuromuscular junctions, raising a potential role of this adhesion molecule during synaptogenesis or for synaptic stability

Presented by: **Salinas, Sara**

Poster No 096
Blue Session

Regulation of the Ubiquitin-Proteasome System by BDNF at Hippocampal Synapses

Ana Rita Santos, Miranda Mele, Blanka Kellermayer, Diogo Comprido, Bruno J Manadas, Carlos B Duarte
Center for Neuroscience and Cell Biology, Department of Life Sciences, University of Coimbra, Coimbra, Portugal

The neurotrophin brain-derived neurotrophic factor (BDNF) plays important roles in synaptic plasticity, neuronal development, and in cell survival. Evidences from a proteomics study performed in our laboratory suggest that BDNF exerts a fine control of the proteome of hippocampal neurons by modulating the intricate balance of protein synthesis and degradation. In this work we investigated the effect of BDNF on the activity of the Ubiquitin-Proteasome System (UPS) in hippocampal neurons. We found that BDNF regulates several components of the UPS, as shown by gene and protein expression studies. Additionally, BDNF down-regulated the proteasome activity in both cultured hippocampal neurons and in synaptoneurosomes isolated from the hippocampus of adult rats. This subcellular fraction (synaptoneurosomes) contains the pre- and post-synaptic regions. The protein levels of Uch-L1, a deubiquitinating enzyme, are also regulated by BDNF in cultured hippocampal neurons, and experiments addressing its specific activity showed that the neurotrophin specifically activates Uch-L1 at the synapse. The maintenance of a free ubiquitin pool is determinant for the normal neuronal function, and is determined by the activity of both the proteasome and deubiquitinating enzymes. We next examined the effect of BDNF on the levels of ubiquitin using synaptoneurosomes. Incubation with BDNF for 15min reduced the free ubiquitin levels, but this effect was not observed for longer stimulation periods with the neurotrophin. These changes in the activity of the UPS and the consequent alterations in the amount of free ubiquitin may modulate the ubiquitination state of some synaptic proteins. In order to identify potential targets of the UPS at the synapse we are conducting a proteomic analysis using fusion proteins that recognize poly-ubiquitinated proteins. Additional experiments will be performed to address the role of BDNF on the same UPS targets. The coordination of two opposing limbs (protein translation and degradation) may explain the multiple roles of BDNF in the central nervous system.

Presented by: **Santos, Ana Rita**

Poster No 097
Red Session

+TIPs are Regulated by MAP1B in Neuronal Cells

Elena Tortosa [1], Niels Galjart [2], Jesus Avila [1], Laura Sayas [1]
[1] Centro de Biología Molecular "Severo Ochoa", CSIC-UAM, Madrid, Spain
[2] Department of Cell Biology and Genetics, Erasmus MC, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands.

Classical microtubule associated proteins (MAPs) and microtubule-plus-end tracking proteins (+TIPs) regulate microtubule dynamics during neuronal morphogenesis. However, a crosstalk between MAPs and +TIPs in neuronal cells has not been described yet. Here, we show that +TIPs are regulated by MAP1B, the first structural MAP expressed in neurons. Using ectopic expression and protein depletion approaches, we find that MAP1B levels influence the binding of +TIPs to microtubule-plus ends in N1E-115 neuroblastoma cells. In MAP1B stably knocked-down cells, EB1 (and EB3)-positive comets were longer than in control cells and EB1 binds along stretches on the MT lattice. By time-lapse confocal microscopy and tracking of GFP-tagged +TIPs, we show that average microtubule growth speed and length are increased in depleted cells. Different +TIPs, like EB3 and CAP-Gly proteins (CLIP-115, CLIP-170 and p150Glued) coimmunoprecipitated with MAP1B. In correlation with a delay in axon outgrowth, primary hippocampal neurons from MAP1B deficient mice present an increased binding of different +TIPs to microtubule-distal-ends, more prominent in enlarged growth cones. Our data point to a new function of MAP1B as regulator of +TIPs during neuronal differentiation; MAP1B might facilitate axon extension by preventing and excessive accumulation of +TIPs at microtubule-plus ends in growth cones.

Presented by: **Sayas, Laura**

Poster No 098
Green Session

Eph Receptor Clustering is a Signaling-Integrator to Elicit Cellular Responses

Andreas Schaupp [1], *Ola Sabet* [2], *Irina Dudanova* [1], *Philippe Bastiaens* [2], *Rüdiger Klein* [1]
[1] *Max-Planck Institute of Neurobiology*
[2] *Max-Planck Institute of Molecular Physiology*

Eph receptor tyrosine kinases (RTKs) and their corresponding membrane-bound ephrin ligands are essential regulators of cell movement and positioning required for establishing and stabilizing patterns of cellular organization. They have been described as 'graded molecular tags' that monitor the abundance or density of their binding partner on opposing cells and relay this information to elicit correspondingly graded cellular responses. In contrast to most RTKs that bind soluble ligands, Eph receptor dimerization is insufficient to induce intracellular signaling; rather higher order Eph/Ephrin complexes are required to transduce signals. To what extend Eph ectodomain interactions with ephrins (in trans) and other Ephs (in cis) regulate cluster formation is a matter of debate. Moreover, correlating the sizes of Eph clusters with functional readouts have been difficult when using soluble ephrins as inducers, because ephrin-Eph cluster size distributions remained poorly defined. Here we used chemical dimerizers to generate defined cluster size distributions of Ephs independent of ephrin contact. Dimerizer-induced Eph clustering was sufficient to activate Eph signaling and the degree of Eph clustering positively correlated with cellular responses such as Eph autophosphorylation and cell contraction. Unexpectedly, large Eph clusters produced stronger cellular responses than smaller Eph oligomers suggesting that the degree of clustering is sensed by the cell to produce graded cellular responses. Intracellular dimerization of Ephs increased the sensitivity towards ephrin-induced extracellular clustering, suggesting that Eph clustering is the central integrator to elicit appropriate cellular Eph-dependent signaling responses.

Presented by: **Schaupp, Andreas**

Poster No 099
Blue Session

Deciphering the Mechanisms Underlying the Refinement of Synaptic Axonal Branches

Marlen Schlieder [1], *Marion Langen* [1], *Robin Hiesinger* [2], *Bassem Hassan* [1]
[1] *VIB/KULeuven*
[2] *UT Southwestern Medical Center*

Early phases of nervous system development are characterized by excessive axon outgrowth and exuberant branch formation. To refine synaptic connections and establish mature neuronal networks, redundant or inappropriate synaptic axon branches are pruned via mechanisms like degeneration, retraction and axosome shedding. Whether the majority of these mechanisms are genetically programmed or dependent on synaptic activity, or both remains controversial.

The Dorsal Cluster Neurons (DCN) are a valuable model to investigate axon branch pruning in the central nervous system of Drosophila melanogaster. The DCN extend their axons towards the lobula and medulla of the optic lobe, whereby medulla innervating axons establish a highly stereotyped pattern of synaptic axon branches. We found that this pattern is achieved by regulated axon pruning after an excessive axon growth phase during pupal development. We have developed high-resolution 4D live imaging of pupal brain culture which allowed us for the first time to observe branch growth and pruning in Drosophila in real time.

To identify the mechanisms involved in pruning we investigated alteration of neuronal activity. We found that neuronal inactivation of DCNs with various available genetic tools fails to alter the normal branch pattern, suggesting that the absence of spontaneous electrical activity does not modify pruning processes.

Genetic analysis of the pruning event reveals that the process is developmentally regulated by EGF-receptor signaling. Phenotypes of hypomorphic, null and dominant negative receptor mutations as well as receptor knock-down results support these conclusions. Further experiments are underway to determine the precise signaling cascade and the source of the genetically determined, activity independent, pruning signal.

Presented by: **Schlieder, Marlen**

Poster No 100
Red Session

Expression of Pax6 in Retinal Ganglion Cells Mediates SFRP1 Axonal Response

Alvaro Sebastian-Serrano [1], *Africa Sandonis* [2], *Pilar Esteve* [2], *Paola Bovolenta* [2], *Marta Nieto* [1]
[1] *CNB/CSIC*
[2] *CBMSO/CSIC*

The selective response of axons to the guidance cues encountered along their paths allows the formation of topographic maps during development. Mechanisms that specify neuronal subclasses are coupled to those that specify their axonal response. In the eye, subpopulations of retinal ganglion cells (RGCs) stereotypically project to different areas of the primary visual centers according to the relative position of their cell bodies in the neuroretina. However, there is a limited knowledge of the molecules and signals patterning these maps. The molecular identity of RGCs is partly defined by the expression of the homedomain transcription factor Pax6. The expression of Pax6 in RGCs is graded, with higher levels in the ventral and temporal distal cells, and lowers in the proximal domains. Despite this suggestive pattern of expression, the functions of Pax6 in the postmitotic RGCs are poorly explored. Here we show that knock down of Pax6 in mouse retinal explants electroporated ex vivo abolishes RGCs axonal growth induced by SFRP1. Loss of Pax6 did not change the response of RGCs axons to Shh function neither block normal differentiation, as assessed by the expression of Islet2 or Brn3 identity markers. Axonal growth was restored when knock down was performed in the presence of silent mutant form of Pax6 resistant to shRNA discarding off-target effects. We also show that while most postmitotic cortical neurons do not express Pax6 or show axonal stimulation after SFRP1 treatment, ectopic overexpression of Pax6 rendered these cells competent to respond to exogenous SFRP1. Hence, we concluded that expression of Pax6 in postmitotic neuronal populations of retinal and cortical origin is necessary and sufficient to confer neurons with response to SFRP1. These results situate Pax6 and SFRP1 as pair regulators of axonal connectivity in the retina and revealed new functions of Pax6 in the postmitotic populations.

Presented by: **Sebastian-Serrano, Alvaro**

Poster No 101
Green Session

An Extracellular Steric Seeding Mechanism for Eph-Ephrin Signaling Platform Assembly

Elena Seiradake [1], *Karl Harlos, Geoff Sutton, A. Radu Aricescu, E. Yvonne Jones*
1 University of Oxford

Erythropoetin-producing hepatoma (Eph) receptors comprise the largest family of receptor protein tyrosine kinases. They are cell surface signalling receptors that mediate cell-to-cell communication and thereby control vital processes, especially during the development and repair of the nervous system. Binding to the ligand (ephrin) triggers Eph activation and the formation of extended Eph/ephrin signalling clusters at the cell surface. To understand the architecture of these assemblies, and to find out how cluster formation is triggered, we solved crystal structures of the complete extracellular part of human EphA2 comprising five domains (eEphA2), alone and in complex with the receptor-binding domain of ephrinA5 (ephrinA5RBD). Unliganded eEphA2 forms linear arrays of staggered parallel receptors involving two patches of residues conserved across A-class Ephs. eEphA2-ephrinA5RBD forms a more elaborate assembly, whose interfaces include the same conserved regions on eEphA2, but re-arranged to accommodate ephrinA5RBD. Cell surface expression of mutant EphA2s demonstrated that these interfaces are critical for localization at cell-cell contacts and activation-dependent degradation. Our results suggest a 'nucleation' mechanism whereby a limited number of ligand-receptor interactions seed an arrangement of receptors which can propagate into extended signaling arrays.

Presented by: **Seiradake, Elena**

Poster No 102
Blue Session

Regulation of Odorant Receptor Genes

Benjamin Shykind
Weill Cornell Medical College in Qatar

Olfaction is an essential sense in animals, conferring the ability to search out food and mates and to detect and avoid predators. While smell is considered to be aesthetic in humans, olfactory disorders may presage neurological diseases including Parkinson's, Alzheimer's, and schizophrenia. The odorant receptors (ORs), numbering more than 1,000 in the genome, endow an animal with the ability to smell. Critical to the development and function of the olfactory system is the regulation of the ORs, with each sensory neuron selecting just one OR for expression, at random, from only one allele. In one model of singular OR choice a kinetic mechanism assures that only one OR allele may be activated in a given time window. This model posits that OR loci are transcriptionaly non-permissive. To examine this possibility we have undertaken in vivo genetic experiments in mice to examine the transcriptional permissiveness of an endogenous OR locus during the development of the olfactory neuroepithelium. We placed the tetracycline-dependent transactivator responsive promoter, at the start site of transcription of the OR P2 gene by homologous gene targeting. Using this modified P2 allele we functionally “interrogate” the OR locus in vivo by attempting to activate it with the tetracycline-dependent transactivator (tTa). This strategy also lets us take advantage of the conditional activation of the tTa system to probe temporal changes in OR chromatin by staged administration of doxycycline.

Presented by: **Shykind, Benjamin**

Poster No 103
Red Session

Extracellular Histones: A Novel Inhibitor of Axonal Regeneration in the CNS?

Mustafa Siddiq, Marie Filbin
Hunter College, New York

Axons in the injured adult CNS do not regenerate, in part due to inhibition by myelin debris. Outside the CNS, released histones are detected in response to inflammation and contributes to cellular damage. In the CNS, up-regulation of a cytoplasmic isoform of Histone H1 was reported in neurons and astrocytes in a mouse model of prion and Alzheimer's disease. Examination of conditioned media from naïve astrocytic cultures revealed secretion of Histones H2A and H4. This suggests that histones are released extracellularly in the CNS. Here, we show that primary cortical and hippocampal neurons isolated from P1-2 rat pups extended long neurites when grown on permissive monolayers of Chinese Hamster Ovary (CHO) cells; however, when we simultaneously added exogenous histones (a mix of all isoforms from calf thymus) to the co-cultures, we observe significantly shorter neurites (up to 70% shorter) for both cortical and hippocampal neurons. This histone-induced inhibiton of neurite outgrowth is reversed with the simultaneous addition of cAMP. Using microfluidic chambers, a technique which isolates the cell bodies from the neurites, we plated cortical neurons on PLL and after one week we observed long neurites growing across the micro-grooves of the chamber. In contrast, when histones were applied to either the cell bodies or neurite-containing part of the chambers, we observed that neurites were unable to grow a significant distance past the micro-grooves. Once again, the application of cAMP to the cell bodies resulted in improved neurite outgrowth of the cortical neurons when compared to treatment with histones only. Our data suggest that extracellular histones could be a potent inhibitor of axonal regeneration following CNS injury and that the addition of cAMP can overcome this inhibition. We also have preliminary data showing that extracellular histones are up-regulated in the CSF of mice with lesions to the dorsal column when compared to the CSF from mice with laminectomy alone, which suggests that histones may inhibit regeneration in vivo as well.

Presented by: **Siddiq, Mustafa**

Poster No 104
Green Session

A Mechanistic Model Underlying Inhibitory Synapse Assembly

Tolga Soykan [1], Alexandros Poulopoulos [1], Theofilos Papadopoulos [1], Heinrich Betz [2], Nils Brose [1], Frederique Varoqueaux [1]
[1] Max Planck Institute of Experimental Medicine
[2] Max Planck Institute for Brain Research

Synaptic inhibition is vital to brain function. GABAergic and glycinergic synapses, mediating fast inhibition in the CNS, balance the activity of neural circuits. At the inhibitory postsynapse, GABA and glycine receptors accumulate around a scaffold composed of the protein Gephyrin, which is recruited to sites apposed to GABA or glycine releasing terminals through interaction with Neuroligin 2, a member of the Neuroligin family of synaptic adhesion molecules that function as powerful synapse organizers. Another Gephyrin-binding molecule, Collybistin, regulates this recruitment process, functioning as a switch that is activated at sites of Neuroligin 2 accumulation, leading to the tethering of the Gephyrin scaffold to the postsynaptic membrane and allowing the subsequent clustering of receptors at sites of transmitter release. Despite the well-documented studies about the critical role of Collybistin in the development of synaptic inhibition, the mechanistic events involving Collybistin that underlie the formation of the inhibitory postsynapse have not yet been determined. We performed a structure-function analysis on Collybistin followed by protein-lipid overlay assays, which led to the identification of two crucial molecular mechanisms: a conformational switch in Collybistin induced by the interaction with NL2 and subsequent membrane targeting of Gephyrin-Collybistin complex via the protein-lipid interactions. These data allows formulation of a coherent molecular model of the assembly of inhibitory synapses and covers a gap in our understanding of the development of synaptic inhibition in the mammalian CNS.

Presented by: **Soykan, Tolga**

Poster No 105
Blue Session

Otx2 Homeoprotein Ttransfer and Signaling in Visual Cortical Plasticity

Julien Spatazza [1], Marine Beurdeley [1], Hing-Cheong Lee [2], Sayaka Sugiyama [2], Clémence Bernard [1], Ariel Di Nardo [1], Takao Hensch [2], Alain Prochiantz [1]
[1] CIRB - Collège de France - Paris, France
[2] Harvard Medical School - Boston, USA

Homeoprotein (HP) transcription factors play fundamental and various roles in development. Aside from their classical cell autonomous activity, HP also undergo intercellular transfer. In the latter context, we have previously shown that postnatal Otx2 HP internalization by parvalbumin (PV)-positive GABAergic interneurons initiates their maturation and leads to the opening of the critical period for ocular dominance in the binocular visual cortex.

The specific internalization of Otx2 by PV-cells led us to study whether glycosaminoglycans (GAGs) present at the surface of PV-cells (Perineuronal nets, PNNs) play a role in Otx2 capture. We find that PNN hydrolysis by Chondroitinase ABC, which reopens ocular dominance plasticity in adulthood, reduces the amount of endogenous Otx2 in PV-cells. A short domain of Otx2 necessary for GAG recognition on PV-cells was further identified. This domain has a high affinity for chondroitin sulfates, antagonizes Otx2 transfer in vivo and reactivates ocular dominance plasticity in the adult leading to changes in visual acuity beyond the critical period.

Accumulation of cortical Otx2 inside PV-cells is driven by visual experience. Accordingly, Otx2 can be transported along the thalamocortical pathway, supporting the possibility that Otx2 in cortical PV-cells originates in the eye. However this eye to cortex transport does not exclude the existence of alternative sources of Otx2. Among them the choroid plexus is an interesting candidate given that it is in the vicinity of the cortex and massively expresses this HP. Our recent results strongly suggest that Otx2 from the choroid plexus may play a role in maintaining the postnatal PV-cell phenotype in the adult visual cortex.

Presented by: **Spatazza, Julien**

Poster No 106
Red Session

Wnt Signalling Regulates Actin Dynamics during Axonal Remodelling

Eleanna Stamatakou, Monica Hoyos-Flight, Patricia Salinas
Research Department of Cell and Developmental Biology, University College London

Wnt secreted proteins play a key role in the formation of neuronal circuits by regulating axon guidance, axonal remodelling, dendritic development and synaptogenesis. In the spinal cord, we found that Wnt3 expressed by motor neurons promotes the remodelling of NT-3 responsive dorsal root ganglia neurons. Remodelling is manifested by enlargement of growth cones and axonal branching. We have previously demonstrated that axon remodelling is associated with change in the organization of microtubules. However, Wnts also induce profound changes in the actin cytoskeleton. Here we examined in detail the effect of Wnt on the actin organization and dynamics during axonal remodelling. Wnt3/Wnt3a induces F-actin accumulation in the growth cone within 15mins. Time-lapse imaging shows that Wnt increases the formation of lamellipodia. Using pharmacological inhibitors of actin dynamics we found that upon application of Wnt3a, F-actin is becoming more susceptible to Cytochalasin D and Latruculin B. Recovery experiments after actin depolymerization showed that Wnt3a also increases actin polymerisation. Taken together these data strongly suggest that Wnt3a regulates actin dynamics during axonal remodelling. Our preliminary results indicate that Wnt3a regulates the actin cytoskeleton through inhibition of Gsk3. We are currently examining in detail the molecular mechanism by which Wnt3a regulates actin during axon remodelling.

This work is funded by the BBSRC and MRC.

Presented by: **Stamatakou, Eleanna**

Poster No 107
Green Session

Axon Extension Occurs Independently of Centrosomal Microtubule Nucleation

Michael Stiess [1], *Nicola Maghelli* [2], *Lukas Kapitein* [3], *Susana Gomis-Rüth* [1], *Michaela Wilsch-Bräuninger* [2], *Casper Hoogenraad* [3], *Iva Tolic-Norrelykke* [2], *Frank Bradke* [1]
[1] *Max Planck Institute of Neurobiology, Martinsried*
[2] *Max Planck Institute of Molecular Cell Biology and Genetics, Dresden*
[3] *Erasmus Medical Center, Department of Neuroscience, Rotterdam*

The centrosome is the classical site of microtubule nucleation and is thought to be essential for axon growth and neuronal differentiation; processes that require microtubule assembly. Here, we found that the centrosome loses its function as a microtubule organizing center (MTOC) during neuronal development. Axons still extended and regenerated through acentrosomal microtubule nucleation and axons continued to grow after laser ablation of the centrosome in early neuronal development. Thus, decentralized microtubule assembly enables axon extension and regeneration and, after axon initiation, acentrosomal microtubule nucleation arranges the cytoskeleton, which is the source of the sophisticated morphology of neurons.

Presented by: **Stiess, Michael**

Poster No 108
Blue Session

Cyclin-Dependent Kinase 5 Regulates Calcium Influx through N-type Calcium Channels

Susan Su, Jennifer Pan, Benjamin Samuels, Khaing Win, Susan Zhang, David Yue, Li-Huei Tsai

Cyclin-dependent kinase 5 (Cdk5) is a proline-directed serine/threonine kinase expressed in postmitotic neurons of the central nervous system and requires p35, its binding partner, for activity. Besides its well-known role in establishing the cytoarchitecture of the developing brain and its implication in a number of neurodegenerative diseases such as Alzheimer's disease, amyotrophic lateral sclerosis (ALS), Neiman Pick's Type C disease, and ischemic brain injury, recent evidence suggest that Cdk5 plays a key role in modulating synaptic functions. A growing list of characterized synaptic substrates include amphiphysin, dynamin, ephexin1, Munc-18, synapsin, and PSD-95, proteins that are involved in various functions such as vesicle trafficking, synapse formation and function or synaptic transmission. We previously showed that Cdk5 regulates synaptogenesis through phosphorylation of the presynaptic scaffolding molecule CASK. Upon phosphorylation by Cdk5, CASK dissociates from liprin-α, localizes to the synaptic compartment and associates with several presynaptic scaffolding proteins such as Mint1, Veli, and the α1B pore-forming subunit of the N-type voltage-gated calcium channels (CaV2.2), where it promotes calcium influx through CaV2.2 at presynaptic terminals. We now demonstrate that Cdk5 interacts with and phosphorylates CaV2.2. Furthermore, from whole-cell patch clamp recordings in heterologous cells we observe that Cdk5/p35 causes a two-fold increase in calcium influx through CaV2.2. Our results can provide novel insights into how Cdk5 modulates CaV2.2 to regulate synaptic function. Understanding Cdk5 signaling could lead to the development of new therapeutics targeting Cdk5 or CaV2.2 for neuropathic pain or neurodegenerative conditions.

Presented by: **Su, Susan**

Poster No 109
Red Session

Expression of the Natriuretic Peptide Receptor 2 (Npr2) and its Role in Axonal Branching

Gohar Ter-Avetisyan, Agne Stonkute, Renè Jüttner, Hannes Schmidt, Fritz G. Rathjen
Max-Delbrück-Centrum für Molekulare Medizin (MDC)

Axonal branching is a key mechanism to form complex circuits of the mature nervous system. Branching allows neurons to innervate multiple targets and is therefore essential for integration and distribution of information. The forms of axonal branching include growth cone splitting and interstitial branching. Signalling mechanisms conducting axonal branching during the development of nervous system are still poorly understood.

Recently we revealed that a cGMP-signalling pathway including the secreted peptide CNP, its receptor Npr2 and the cGMP-dependent protein kinase I (cGKI) controls sensory axon bifurcation within the spinal cord: the binding of CNP by the particulate guanylyl cyclase Npr2 activates the intracellular guanylyl cyclase domain of Npr2, which results in the generation of cGMP from GTP. In the absence of one of these components sensory axons are unable to bifurcate at the entry zone of the spinal cord.

The second messenger cGMP binds to cGKI which elicits the phosphorylation of substrates. Therefore, using phosphorylation-motif specific antibodies we search for phosphorylation targets of cGKI in dorsal root ganglions (DRGs).

To investigate the role of Npr2-dependent axonal branching in other neural systems than sensory axons and to define the expression pattern of Npr2 we generated mouse models (Npr2-LacZ and Npr2-CreERT2), which allow studies on the expression pattern of Npr2 and on axonal branching of single Npr2-expressing axons in the developing mouse nervous system.

Presented by: **Ter-Avetisyan, Gohar**

Poster No 110
Green Session

Rac1 Function in Cortical Interneuron Development

Simona Tivodar [1], Marina Vidaki [1], Katerina Doulgeraki [1], Victor Tybulevicz [2], Nicoletta Kessaris [3], Vassilis Pachnis [4], Domna Karagogeos [1]
[1] 1: Department of Basic Science, Faculty of Medicine, University of Crete, Heraklion, Greece, 2: Institute of Molecular Biology & Biotechnology (IMBB, FORTH), Heraklion, Greece.
[2] Division of Immune Cell Biology, Medical Research Council, National Institute for Medical Research, London, UK.
[3] Wolfson Institute for Biomedical Research and Department of Cell & Developmental Biology, University College London, UK
[4] Division of Molecular Neurobiology, Medical Research Council, National Institute for Medical Research, London, UK.

Cortical GABAergic interneurons are characterized by extraordinary neurochemical and functional diversity. Although recent studies have uncovered some of the molecular components underlying interneuron development, including the cellular and molecular mechanisms guiding their migration to the cortex, the intracellular components involved are still unknown. Rac1, a member of the Rac subfamily of Rho GTPases, has been implicated in various cellular processes such as cell cycle dynamics, axonogenesis and migration. In this study we have addressed the specific role of Rac1 in interneuron progenitors originating in the medial ganglionic eminence, via Cre/loxP technology. We show that ablation of Rac1 from mitotic progenitors, results in a delayed cell cycle exit, which in turn leads to a later onset of migration towards the cortex. As a consequence, only half of GABAergic interneurons are found in the postnatal cortex. Ablation of Rac1 from postmitotic progenitors does not result in similar defects, thus underlying a novel, cell autonomous and stage-specific requirement for Rac1 activity, within proliferating progenitors of cortical interneurons. Rac1 is necessary for their transition from G1 to S phase, at least in part by regulating CyclinD levels and Retinoblastoma protein phosphorylation. In addition, MGE cells grown in vitro, show cytoskeletal alterations such as a significant reduction in lamellipodia formation in the absence of Rac1 protein.

Presented by: **Tivodar, Simona**

Poster No 111
Blue Session

MAP1B is required for Dendritic Spine Development and Synaptic Maturation

Elena Tortosa [1], *Carolina Montenegro-Venegas* [2], *Marion Benoist* [1], *Steffen Härtel* [3], *Christian Gonzalez-Billault* [4], *Jose Esteban* [1], *Jesus Avila* [1]
[1] *Centro de Biologia Molecular "Severo Ochoa"*
[2] *Universidad de Chile and Institute for Cell Dynamics and Biotechnology (ICDB)*
[3] *Universidad de Chile,Programa de Anatomía y Biología del Desarrollo (ICBM),Facultad de Medicina*
[4] *Universidad Chile and Institute for Cell Dynamics and Biotechnology (ICDB)*

MAP1B is a microtubule associated protein that is expressed prominently during early stages of neuronal development and it has been implicated in axonal growth and guidance. However, MAP1B is still expressed in adult brain areas with high synaptic plasticity. Here we show that MAP1B is present in dendritic spines, and neurons obtained from MAP1B-deficient mice show a decrease in density of mature dendritic spines and an increase of immature filopodia-like protrusions. Although these neurons show normal passive membrane properties and action potential firing, their synaptic currents mediated by AMPA receptors have a significant diminution. Moreover, using post-synaptic densities from adult MAP1B +/- mice, we show a significant decrease in Rac1 activity and an increase in RhoA activity. These MAP1B +/- fractions present a decrease in phosphorylated cofilin. Taken together, these results define a new and important function of MAP1B in dendritic spine formation and maturation through the regulation of actin cytoskeleton. This eventually could contribute to the regulation of the synaptic activity and plasticity in the adult brain.

Presented by: **Tortosa, Elena**

Poster No 112
Red Session

Functions of Neuronal Nogo-A for Neurite Growth in the Developing Nervous System

Flóra Vajda, Marija M. Petrinovic, Carri S. Duncan, Martin E. Schwab
Brain Research Institute - ETH/UZH Zürich

Nogo-A has been initially characterized as a myelin membrane protein in the central nervous system that inhibits axonal regeneration after injury. However, during development Nogo-A is expressed predominantly in neuronal cells, especially in those that project axons to distal brain or spinal cord regions. In particular neuronal populations (such as in the hippocampus, deep cerebellar nuclei and dorsal root ganglia) this expression persists in the adulthood as well. Possible neuronal functions of Nogo-A are the subject of emerging interest, especially those fulfilled during development, when the expression level of the protein suggests crucial roles in neurite outgrowth.

Our studies have recently demonstrated developmental functions of neuronal Nogo-A. Neutralization of neuronal Nogo-A by a function- blocking antibody or its genetic ablation led to increased fasciculation and decreased branching of cultured dorsal root ganglion neurons. These observations suggest that Nogo-A acts as a negative regulator of axon-axon adhesion and as a facilitator of neurite branching. Localization of Nogo-A to the axon-shaft might render non-myelinated neurite surfaces repulsive to each other, however, Nogo-A is also present in the axonal growth cones where the relation of the protein to the growth machinery needs to be further investigated. Novel underlying mechanisms are evolving on how neuronal Nogo-A could influence neurite growth, acting either intracellularly or on the neural cell surface.

Presented by: **Vajda, Flóra**

Poster No 113
Green Session

In vivo Pull Down using a Synapsin-GFP-Neogenin Transgenic Mouse Identifies Dock7 as a Novel Neogenin Interacting Protein Involved in RGMa Induced Axon Repulsion

Dianne van den Heuvel, Anita Hellemons, Jeroen Pasterkamp
Rudolf Magnus Institute of Neuroscience; UMC Utrecht; The Netherlands

Neogenin is a transmembrane receptor that regulates several key cellular processes in the developing brain, including axon guidance, neuronal differentiation, morphogenesis and apoptosis. In the process of neuronal network formation binding of Repulsive Guidance Molecule A (RGMa) to Neogenin elicits growth cone collapse and axon repulsion.

To further our understanding of the role and mechanism of Neogenin signalling in the developing nervous system, we generated Synapsin-GFP-Neogenin transgenic mice that express a GFP-Neogenin fusion construct under control of the neuron-specific Synapsin I promoter. An in vivo anti-GFP pull down of GFP-Neogenin from transgenic brain lysates followed by mass spectrometry identified Dock7 as a novel interactor of Neogenin. Dock7 is a Rac GTPase-activating protein and an important regulator of neuronal polarity with high expression levels in the developing embryonic mouse brain.

In order to study the Neogenin-Dock7 interaction in more detail immunohistochemistry was used to study protein colocalization both in mouse embryonic brain slices and cultured primary cortex neurons.

n addition, co-immunoprecipitation experiments were performed using Neogenin and Dock7 deletion mutants to identify the protein domains involved in the interaction. On a functional level, we were able to show that knockdown of Dock7 in mouse embryonic cortical neurons strongly reduces the axon repellent effect of RGMa.

Presented by: **van den Heuvel, Dianne**

Poster No 114
Blue Session

The bZIP Transcription Factors CREB, C/EBP and NFIL3 Coordinately Regulate Axon Regeneration

Loek van der Kallen [1], Harold MacGillavry [1], Joost Verhaagen [2], August Smit [1], Ronald van Kesteren [1]
[1] Center for Neurogenomics and Cognitive Research, Neuroscience Campus Amsterdam, VU University, Amsterdam, the Netherlands
[2] Department of Neuroregeneration, Netherlands Institute for Neuroscience, Amsterdam, The Netherlands

Regeneration of injured axons depends on the coordinated expression of regeneration-associated genes (RAGs). Insight into the transcriptional machinery that is required for appropriate RAG expression might provide new therapeutic strategies to promote axonal regeneration. We screened many transcription factors for axon growth-promoting properties, and identified NFIL3 as a potent repressor of neurite outgrowth. We further showed that NFIL3 binds to CREB binding sites. CREB and NFIL3 have opposite effects on transcription; CREB induces gene expression, whereas NFIL3 represses CREB-induced genes. To confirm this so-called inconsistent regulatory feed-forward loop, we next examined NFIL3 and CREB binding to target genes using chromatin immunoprecipitation combined with microarray technology (ChIP-chip). Although only a small subset of NFIL3 target genes also bound CREB, NFIL3 target genes showed a significant enrichment of C/EBP binding motifs. C/EBP binding to NFIL3 sites was confirmed by ChIP analysis, and knockdown of C/EBPα, C/EBPβ and C/EBPδ, but not C/ EBPγ, significantly reduced neurite outgrowth in vitro. Together, our findings indicate that CREB, C/EBPs and NFIL3 together regulate regenerative axon growth by fine-tuning RAG expression.

Presented by: **van der Kallen, Loek**

Poster No 115
Red Session

Neuronal microRNA Expression Profiling Identifies Novel microRNAs with Axon Growth Promoting Effects

S. van Erp, A.H.A.A. Derijck, E.Y. van Battum, R.J. Pasterkamp
Rudolf Magnus Institute, Univerity Medical Centre Utrecht

The role of microRNAs in the development of neuronal connectivity is still poorly understood. The presence of miRNAs and their targets in axons may enable local regulation of protein synthesis and thereby contribute to axon growth and guidance.

To investigate a potential link between miRNA expression and axon guidance, we determined miRNA expression profiles in primary cortical neurons exposed to the axon guidance protein Semaphorin7A (Sema7A). LNA-based microarray technology was used to assess miRNA expression in control and treated neuron cultures. Although Sema7A had no robust effect on miRNA expression, several miRNAs displayed very high levels of expression in cortical neurons. These candidates were further analyzed by in situ hybridization to determine their spatiotemporal expression during neural development. Based on this expression pattern analysis, cortical neurons and cerebellar granule cells were selected for further functional studies. Subsequent manipulation of miRNA expression in dissociated primary cultures revealed effects of select miRNAs on axon and dendrite growth. Our study has identified novel miRNAs with potent effects on axon outgrowth. Future work will focus on examining the role of these miRNAs during the development of the cortex and cerebellum and on the identification of the mRNA targets involved.

Presented by: **van Erp, S.**

Poster No 116
Green Session

Homeostatic Synaptic Scaling Contributes to the Structural Reorganization of Neurons after Denervation

Andreas Vlachos [1], *Denise Becker* [1], *Carlos Bas Orth* [1], *Moritz Helias* [2], *Peter Jedlicka* [3], *Maike Neuwirth* [3], *Raphael Winkels* [3], *Jochen Roeper* [4], *Markus Diesmann* [2], *Gaby Schneider* [5]

[1] *Institute for Clinicla Neuroanatomy, Neuroscience Center, Goethe-University Frankfurt, Germany*

[2] *RIKEN Brain Science Institute, Wako City, Japan*

[3] *nstitute for Clinicla Neuroanatomy, Neuroscience Center, Goethe-University Frankfurt, Germany*

[4] *Institute of Neurophysiology, Neuroscience Center, Goethe-University Frankfurt, Germany*

[5] *Department of Computer Science and Mathematics, Goethe-University Frankfurt, Germany*

Denervation-induced spine reorganization was studied following entorhinal deafferentation of hippocampal granule cells in organotypic slice cultures of Thy1-GFP mice. The highly laminar organization of entorhinal afferents to the dentate gyrus made it possible to selectively denervate distal dendritic segments of granule cells without deafferenting their proximal dendritic segments. Thus, we could study how spines located on denervated and non-denervated dendritic segments of the same neuron react to the loss of innervation. Time-lapse imaging revealed alterations in spine loss and in the stability of newly formed spines but not in spine formation rate in the denervated layer. Patch-clamp analysis revealed homeostatic scaling of excitatory synapses within the same layer, demonstrating that denervated neurons locally adapt their synapses to maintain their afferent drive. The layer-specific functional and structural adaptations observed after denervation required the layer-specific upregulation of tumor necrosis factor alpha (TNFa). Since induction of homeostatic scaling in non-denervated control cultures also resulted in a destabilization of newly formed spines, we propose that TNFa-mediated destabilization of spines could be a general mechanism by which neuronal networks homeostatically adapt spine and thus excitatory synapse numbers to the level of network activity.

Presented by: **Vlachos, Andreas**

Poster No 117
Blue Session

Functional Analysis of Vertebrate Orthologues of Synapse-Defective-1 (Syd-1)

Corinna Wentzel, Julia E. Sommer, Peter Scheiffele
Biozentrum, Universität Basel

Synapses are central processing units for neuronal communication. Therefore, identification of the mechanisms regulating the assembly of pre- and postsynaptic structures is crucial for understanding information flow in the brain. Genetic screens in C. elegans and D. melanogaster have let to the characterisation of two critical cytoplasmic molecules, Synapse-defective-1 and -2 (Syd-1, Syd-2), that are essential for active zone assembly (Zhen and Jin, 1999; Hallam et al., 2002; Owald et al., 2010). Syd-1 is required for the concentration of Syd-2 at the presynapse where it interacts with ELKS-1/Bruchpilot. Here, we identified two candidate mammalian orthologues for syd-1: msyd-1a and msyd-1b. Sequence comparison between C. elegans Syd-1 and mSyd-1A and mSyd-1B shows an overall amino acid sequence similarity of 40 %. As the invertebrate Syd-1 proteins, mSyd-1A contains C2 and rhoGAP domains, however, the N-terminal PDZ domain of invertebrate Syd-1 proteins is not conserved in mSyd-1.

Using a FRET-based assay we discovered that mSyd-1A shows GAP activity towards RhoA. This activity is inhibited by the N-terminal domain of the protein and relieved by targeting mSyd-1A to the plasma membrane with an N-terminal lipid anchor. Using biochemical and Yeast-2-Hybrid assays, we identified a collection of mSyd-1A interacting proteins that associate with the N-terminal domain and might serve as endogenous activators of mSyd-1A function. Amongst these binding partners is the Syd-2 orthologue Liprin-α2.

Thus, we identified mSyd-1A as a mouse orthologue of Syd-1. mSyd-1A interacts with active zone proteins and might play a role in the regulation of the presynaptic actin cytoskeleton via local regulation of rho-GTPase activity.

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Presented by: **Wentzel, Corinna**

Poster No 118
Red Session

The Microtubule Binding Protein SCG10 Regulates Axon Extension

Lena Will [1], *Ying-Hua Li* [2], *Percy Bondallaz* [3], *Gabriele Grenningloh* [3], *Andreas, W. Püschel* [1]
[1] *Institut für Molekulare Zellbiologie/ Westfälische Wilhelms-Universität Münster*
[2] *Institut für Molekulare Zellbiologie/ Westfälische Wilhelms-Universität Münster*
[3] *Center for Psychiatric Neuroscience/ University of Lausanne*

Small GTPases play an important role in the establishment of neuronal polarity. The Rho GTPase Rnd1 affects actin dynamics antagonistically to Rho and has been implicated in the regulation of neurite outgrowth, dendrite development and axon guidance. Here we show that Rnd1 is not only important for actin regulation, but is involved in microtubule dynamics as well. Rnd1 interacts with Superior Cervical Ganglion10 (SCG10), a neuron specific microtubule regulator protein. This interaction requires a central domain of SCG10 comprising about 40 amino acids located within the N-terminal half of a putative α-helical domain and is independent of phosphorylation at the four identified phosphorylation sites that regulate SCG10 activity. Rnd1 enhances the microtubule destabilizing activity of SCG10 in vitro. Both proteins colocalize in the axon, but not in the minor neurites of hippocampal neurons. Knockdown of Rnd1 or SCG10 by RNAi suppresses axon extension, indicating a critical role for both proteins during neuronal differentiation. Overexpression of Rnd1 in neurons induces the formation of multiple axons. The effect of Rnd1 on axon extension depends on SCG10. These results indicate that SCG10 acts as an effector downstream of Rnd1 to promote the formation of axons. The direct interaction of SCG10 with microtubules together with its membrane-localization mediated by the N-terminus makes it an interesting candidate for the regulation of the cell morphology by coordinating microtubule dynamics and vesicular transport. Currently we are analyzing SCG10 knockout mice to elucidate its role in neuronal differentiation in vivo.

Presented by: **Will, Lena**

Poster No 119
Green Session

The Role of Schwann Cell Notch Signalling in Peripheral Nerve Regeneration

Daniel Wilton [1], Jie Shen [2], Dies Meijer [3], Laura Feltri [4], Lawrence Wrabetz [4], Rhona Mirsky [5], Kristjan Jessen [5]
[1] *University College London*
[2] *Centre For Neurologic diseases, Brigham and Women's hospital, Harvard Med. Sch, Boston, MA*
[3] *Dept of cell biology and genetics, Erasmus MC, Rotterdam, Netherlands*
[4] *DIBIT, San Rafaele Scientific Inst., Milan, Italy*
[5] *University College London, London, UK*

Peripheral nerves, unlike their CNS counterparts, possess the ability to regenerate following injury and Schwann cell plasticity plays a fundamental role in this process. As a result of nerve injury and associated Wallerian degeneration, Schwann cells demyelinate and dedifferentiate to an immature phenotype broadly similar to that seen in perinatal nerves. This enables them to provide trophic support to neurons and a permissive surface for regrowing axons. One of the signals driving demyelination in Schwann cells is Notch (Woodhoo et al., Nature Neuroscience 12, 839 - 847 (2009)). Notch is down-regulated at the onset of myelination and suppressed by the pro-myelin transcription factor Krox-20. Following nerve injury Notch signalling is activated and in its absence demyelination is delayed; conversely enforced Notch signalling accelerates the breakdown of myelin.

Here we examined the role of the Schwann cell Notch signalling pathway in peripheral nerve regeneration. Unexpectedly, we found that inhibition of Notch signalling prior to crush injury actually increased the rate of subsequent axonal regeneration and remyelination. This was accompanied by a more rapid return of motor function as denoted by gait analysis. We show that one of the mechanisms underlying the negative impact of Notch signalling on regeneration is likely to be a direct suppression of genes known to be beneficial to regeneration through a reduced level of c-Jun. These findings demonstrate that Notch signalling in Schwann cells plays a role in peripheral nerve regeneration and its inhibition is able to alter the neuronal response to injury and promote a more rapid return of function.

Presented by: **Wilton, Daniel**

Poster No 120
Blue Session

Calmyrin1 Binds to SCG10 Protein (stathmin2) to Modulate Neurite Outgrowth

Katarzyna Debowska, Adam Sobczak, Magdalena Blazejczyk, Michael R. Kreutz, Jacek Kuznicki, Urszula Wojda

Calmyrin1 (CaMy1) is an EF-hand Ca2+-binding protein expressed in several cell types, including brain neurons. Using a yeast two-hybrid screen of a human fetal brain cDNA library, we identified SCG10 protein (stathmin2) as a CaMy1 partner. SCG10 is a microtubule-destabilizing factor involved in neuronal growth during brain development. We found increased mRNA and protein levels of CaMy1 during neuronal development, which paralleled the changes in SCG10 levels. In developing primary rat hippocampal neurons in culture, CaMy1 and SCG10 colocalized in cell soma, neurites, and growth cones. Pull-down, coimmunoprecipitation, and proximity ligation assays demonstrated that the interaction between CaMy1 and SCG10 is direct and Ca2+-dependent in vivo and requires the C-terminal domain of CaMy1 (residues 99-192) and the N-terminal domain of SCG10 (residues 1-35). CaMy1 did not interact with stathmin1, a protein that is homologous with SCG10 but lacks the N-terminal domain characteristic of SCG10. CaMy1 interfered with SCG10 inhibitory activity in a microtubule polymerization assay. Moreover, CaMy1 overexpression inhibited SCG10-mediated neurite outgrowth in nerve growth factor (NGF)-stimulated PC12 cells. This CaMy1 activity did not occur when an N-terminally truncated SCG10 mutant unable to interact with CaMy1 was expressed. Altogether, these data suggest that CaMy1 via SCG10 couples Ca2+ signals with the dynamics of microtubules during neuronal outgrowth in the developing brain.

Presented by: **Wojda, Urszula**

Poster No 121
Red Session

Rassf1 and Rassf5 are required for Neuronal Polarity

Rui Yang [1], *Eryan Kong* [2], *Andreas W. Püschel* [2]
[1] *Institut für Molekulare Zellbiologie, Westfälische Wilhelms-Universität Münster, Germany and International Graduate Program Cell Dynamics and Disease (CEDAD)*
[2] *Institut für Molekulare Zellbiologie, Westfälische Wilhelms-Universität Münster*

The polarized morphology of a neuron with a single axon and several dendrites is essential for its function in the directional flow of information in the central nervous system. Cultures of dissociated hippocampal neurons have proven invaluable to dissect the signaling pathways that direct the establishment of neuronal polarity. The early development of cultured neurons can be subdivided into 5 stages. Initially, neurons form several indistinguishable neurites, which all have the potential to become the axon (stage 1-2). In late stage 2, one of the neurites is specified as the axon and grows rapidly (stage 3) GTPases of the Ras family are central components of the signaling pathway that initiates axon extension. The Rassf (Ras-association domain family) proteins are a family of Ras effectors that are characterized by the shared Ras association domain and function as tumor suppressors. Through this domain, they interact with Ras GTPases, molecular switches that regulate a diverse range of cellular functions. In our study, we used cultured E18 rat hippocampal neurons to study the function of Rassf1 and Rassf5 in neuronal polarity. Neurons formed multiple axons when the expression of Rassf1 or Rassf5 was suppressed by RNAi. To address the question how Rassf1 and Rassf5 affect axon extension, we investigated the role of Ndr1 and Ndr2. Rassf1 interacts with MST (mammalian Sterile20-like 1) kinases, which in turn can activate the NDR (nuclear Dbf-2 related) kinases. The NDR kinases belong to the serine/threonine AGC (protein kinase A(PKA)/PKG/PKC-like) class of protein kinases that control important cellular processes, such as changes in cell morphology, mitotis, cytokinesis, and apoptosis. In neurons, NDR kinases have been implicated in mediating neurite tiling and regulating neurite growth. After knockdown of both NDR1 and NDR2, neurons formed multiple axons. Expression of NDR2 can rescue the knockdown phenotype of Rassf5, confirming that Rassf5 acts through NDR kinases. These experiments reveal a novel pathway that regulates neuronal polarity.

Presented by: **Yang, Rui**

Poster No 122
Green Session

NMDA Receptor Activation Suppresses Microtubule Dynamics in Neurons

Kah Wai Yau [1], Lukas C. Kapitein [1], Susana Montenegro Gouveia [2], Wouter A. van der Zwan [1], Phebe S. Wulf [1], Jeroen Demmers [3], Jacek Jaworski [4], Anna Akhmanova [2], Casper C. Hoogenraad [1]
[1] Department of Neuroscience, Erasmus Medical Center Rotterdam, The Netherlands
[2] Department of Cell Biology, Erasmus Medical Center Rotterdam, The Netherlands
[3] Proteomics Center, Erasmus Medical Center Rotterdam, The Netherlands
[4] International Institute of Molecular and Cell Biology, Warsaw, Poland

Dynamic microtubules are important to maintain neuronal morphology and function, but whether neuronal activity affects the organization of dynamic microtubules is unknown. Here we show that a protocol to induce NMDA-dependent long-term depression (LTD) rapidly attenuates microtubule dynamics in primary hippocampal neurons, removing the microtubule binding protein EB3 from the growing microtubule plus-ends in dendrites. This effect requires the entry of calcium and is mediated by activation of NR2B-containing NMDA type glutamate receptor. The rapid NMDA effect is followed by a second, more prolonged response, during which EB3 accumulates along MAP2-positive microtubule bundles in the dendritic shaft. MAP2 is both required and sufficient for this activity-dependent redistribution of EB3. Importantly, NMDA receptor activation suppresses microtubule entry in dendritic spines, while overexpression of EB3-GFP prevents NMDA-induced spine shrinkage. Therefore, short-lasting and long-lasting changes in dendritic microtubule dynamics are important determinants for NMDA-induced LTD.

Presented by: **Yau, Kah Wai**

Poster No 123
Blue Session

Drosophila Melanogaster is an in vivo Platform for the Study of Huntingtin-Dependent Vesicular Trafficking and htt Phosphorylation Modifiers in a Huntington's Disease Context

Diana Zala, Yannick Elipot, Saudou Frédéric
Institut Curie / CNRS UMR 3306 / INSERM U1005 Orsay, FRANCE

Huntington's Disease (HD) is a genetic neurodegenerative disorder caused by a polyglutamine expansion (polyQ) in the huntingtin protein (htt). Htt is a processivity factor for vesicles containing brain-derived neurotrophic factor (BDNF). Htt-polyQ reduces intracellular transport and induces neuronal death. We have previously demonstrated that phosphorylation of htt at S421 by IGF-1/Akt restores vesicular transport and is neuroprotective.Our aim was to set up an in vivo HD model allowing the analyses of htt phosphorylation on survival and on vesicular transport. In rat primary neuronal culture we have replaced endogenous htt with Drosophila htt. We observed that fly htt localizes to rat vesicles and rescues for the loss of endogenous htt in vesicular transport. To confirm this in vivo we used Drosophila melanogaster L3 larvae expressing synaptic vesicles tagged with syt-GFP in motoneurons and recorded vesicular transport in control and htt KO. We could show that the transport function of huntingtin is conserved as KO larvae have a slower vesicular transport. Finally, we have analyzed the survival of flies expressing htt-polyQ, htt-polyQ constitutively phosphorylated at S421 (S421D), and flies co-expressing Htt-polyQ and Akt. We showed that promoting htt phosphorylation increases life span. We will use survival analyses and vesicular transport as a readout for genes and compounds modulating htt phosphorylation.

Presented by: **Zala, Diana**

Poster No 124
Red Session

Motor Neuron Differentiation and Topographic Mapping Depends on β- and γ-Catenin Activity

Niccolo Zampieri, Elena Demireva, Lawrence Shapiro, Thomas Jessell
Columbia University

Spinal reflex circuits contribute to the control of movement, and their assembly depends on the formation of specific synaptic connections between proprioceptive sensory neurons and motor neurons. Within the spinal cord, the positioning of motor neuron cell bodies and the elaboration of motor neuron dendrites are thought to be key steps in the establishment of sensory-motor reflex circuitry. Studies in chick embryos have provided evidence that classical cadherins participate in the patterning of motor neuron cell bodies, but the diversity of cadherins, and the lack of genetic approaches, has hindered analysis of the developmental role of cadherins in sensory-motor circuit assembly. Since cadherins mediate their activities through catenin proteins, we have combined mouse genetic and cellular assays to explore the relative contributions of β- and γ-catenin to the differentiation of spinal motor neurons, examining sequential phases of cell body positioning, dendritic development, axonal growth, and sensory-motor connectivity. Our findings reveal that β- and γ-catenin function in a redundant manner in spinal motor neurons, and that these two catenins are necessary for classical cadherin activity. Joint inactivation of β- and γ-catenin results in profound defects in motor neuron positioning, as well as in dendritic arborization. In contrast, the elimination of β- and γ-catenin function has no obvious impact on the growth and patterning of motor axons in the developing limb. More generally, our data show that the differentiation and topographic mapping of motor neurons relies on separable somato-dendritic and axonal programs, with the involvement of β- and γ-catenin signaling restricted to somato-dendritic differentiation.

Presented by: **Zampieri, Niccolo**

Poster No 125
Green Session

Localization and function of gamma-RIMs

Katrin Michel, Elena Alvarez-Baron, Tobias Mittelstaedt, Casper Hoogenraad, Albert Becker, Susanne Schoch
Institute of Neuropathology and Department of Epileptology, University Bonn, Germany

RIMs (Rab interacting molecules) are active zone enriched multidomain proteins. In vertebrates, the RIM family is composed by 7 proteins (RIM1α, 1β, 2α, 2β, 2γ, 3γ and 4γ) encoded by four different genes (RIM1-4). α-RIMs, contain the full set of domains (N-terminal zinc-finger; central PDZ-domain; and two C-terminal C2-domains). RIM2β lacks the whole zinc finger domain whereas in RIM1β only the N-terminal Rab-3-binding sequence is absent. In contrast, the γ-isoforms are composed of a short isoform-specific N-terminal sequence and a C-terminal C2B domain. In contrast to α/β-RIMs that have been shown to play a key regulatory role in synaptic vesicle exocytosis and presynaptic forms of short- and long-term plasticity the physiological role of the γ-isoforms is still unclear. Here, we show that RIM3γ and RIM4γ are coexpressed with the full-length RIM variants, but exhibit differences in their expression patterns and subcellular localization. We found their subcellular localization to be regulated by specific targeting sequences and protein interactions. Downregulation of either, RIM3γ or RIM4γ, resulted in alterations of neuronal morphology in vitro and in vivo and this phenotype could be rescued by overexpression of resistant variants of the protein. Taken together, our results suggest that γ-RIMs mediate synaptic and non-synaptic functions.

Presented by: **Michel, Katrin**

Poster No 126
Blue Session

PARTICIPANT LIST

Dr. Christian Alfano
INSERM U636, Nice, France
alfano@unice.fr

Prof. Claudia Bagni
Vesalius Research Center VIB,
Leuven, Belgium
claudia.bagni@med.kuleuven.be

Dr. Ales Balik
MRC Laboratory of Molecular Biology,
Cambridge, UK
abalik@mrc-lmb.cam.ac.uk

Ms. Kira Balueva
Max-Delbrück-Centrum für Molekulare Medizin (MDC), Berlin, Germany
kbalueva@mdc-berlin.de

Ms. Ellen Barker
University of Bristol, School of Medical Sciences, Bristol, UK
e.barker@bris.ac.uk

Dr. Anthony Paul Barnes
Oregon Health Sciences U, Portland Oregon, USA
barnesan@ohsu.edu

Ms. Justine Barry
Columbia University, New York, USA
jb2524@columbia.edu

Mr. Konstantinos Barsakis
University of Crete, Heraklion, Greece
bio1275@edu.biology.uoc.gr

Dr. Med. George Bastakis
IMBB-FORTH & University of Crete,
Heraklion, Greece
bastakis@imbb.forth.gr

Dr. Stephane Baudouin
Biozentrum of the University of Basel, Switzerland
stephane.baudouin@unibas.ch

Dr. Catherina G. Becker
University of Edinburgh, CNR, UK
catherina.becker@ed.ac.uk

Ms. Kinga Bercsenyi
London Research Institute, London, UK
kinga.bercsenyi@cancer.org.uk

Ms. Clémence Bernard
Collège de France, Paris, France
clemence.bernard@college-de-france.fr

Dr. Florence Besse
Institute of Developmental Biology and Cancer (IBDC), UMR6543, Nice, France
besse@unice.fr

Mr. Pallab Bhattacharya
Banaras Hindu University, School of Biomedical Engineering, Varanasi, India
pallab.bhu@gmail.com

Dr. Mariano Bisbal
Université Joseph Fourier,
Grenoble, France
mariano.bisbal@ujf-grenoble.fr

Prof. John Bixby
University of Miami, LPLC, Miami, USA
jbixby@miami.edu

Dr. Kieran Alexander Boyle
University College London, London, UK
k.boyle@ucl.ac.uk

Dr. Frank Bradke
Max Planck Institute of Neurobiology,
Martinsried, Germany
fbradke@neuro.mpg.de

Prof. Michael Brand
Dresden University, BIOTEC and CRTD, Dresden, Germany
michael.brand@biotec.tu-dresden.de

Dr. Christine Renee Cain
Hunter College, New York, USA
cain@genectr.hunter.cuny.edu

Dr. Laura Cancedda
Italian Institute of Technology, Genova, Italy
laura.cancedda@iit.it

Prof. Marco Canossa
Italian Institute of Technology, Genova, Italy
marco.canossa@iit.it

Mr. Thomas Chater
University of Bristol, MRC Centre of Synaptic Plasticity, Bristol, UK
antec@bris.ac.uk

Mr. Anaël Chazeau
University of Bordeaux, IINS, UMR CNRS 5297, Bordeaux, France
anael_chazeau@hotmail.com

Ms. Joanna Chiang
Westfälische Wilhelms-Universität, Münster, Germany
j_chia02@uni-muenster.de

Dr. Laurie Emmanuelle Chicha
F. Hoffmann-La Roche Ltd, Basel, Switzerland
laurie.chicha@roche.com

Dr. Daniel Choquet
University of Bordeaux, IINS, UMR CNRS 5297, Bordeaux, France
dchoquet@u-bordeaux2.fr

Dr. Eleanor Therese Coffey
Turku Centre for Biotechnology, Finland
ecoffey@btk.fi

Dr. Catherine Collins
University of Michigan, Ann Arbor, Michigan, USA
collinca@umich.edu

Dr. Julien Courchet
The Scripps Research Institute, Dorris Neuroscience Center, La Jolla, USA
courchet@scripps.edu

Mrs. Françoise Coussen-Choquet
University of Bordeaux, IINS, UMR CNRS 5297, Institut F. Magendie, Bordeaux, France
fcoussen@u-bordeaux2.fr

Mrs. Jaeda Coutinho-Budd
University of North Carolina at Chapel Hill, , USA
aplecloud@yahoo.com

Dr. Ann Marie Craig
University of British Columbia, Brain Research Centre, Vancouver, Canada
amcraig@interchange.ubc.ca

Dr. Beatriz Cubelos
Universidad Autonoma Madrid, Spain
bcubelos@cnb.csic.es

Dr. Katalin Czondor
University of Bordeaux, IINS, UMR CNRS 5297, Bordeaux, France
katalin.czondor@u-bordeaux2.fr

Dr. Patrizia D'Adamo
DTI at San Raffaele Scientific Institute, Milan, Italy
p.dadamo@hsr.it

Prof. Graeme W. Davis
University of California, San Francisco, Department of Biochemistry and Biophysics, USA
graeme.davis@ucsf.edu

Dr. Pietro De Camilli
Yale University, BCMM 236, New Haven, USA
pietro.decamilli@yale.edu

Dr. Joris de Wit
University of California San Diego, La Jolla, USA
jdewit@ucsd.edu

Dr. Katrin Deinhardt
NYU Medical Center, Skirball Institute, New York, USA
katrin.deinhardt@med.nyu.edu

Prof. Ferdinando Di Cunto
University of Torino, Molecular Biotecnology Center, Torino, Italy
ferdinando.dicunto@unito.it

Ms. Elizabeth Alisha Di Lullo
CIRB -Collège de France, Paris, France
elizabeth.dilullo@college-de-france.fr

Mr. Travis Lee Dickendesher
University of Michigan, Neuroscience Program, Ann Arbor, Michigan, USA
travlee@umich.edu

Prof. Carlos Dotti
Katholieke Universiteit Leuven, Belgium
carlos.dotti@med.kuleuven.be

Dr. Karin Dumstrei
The EMBO Journal, Heidelberg, Germany
karin.dumstrei@embo.org

Mrs. Liudmila V Efremova
University of Konstanz, Konstanz, Germany
liudmila.efremova@uni-konstanz.de

Mr. Paschalis Efstathopoulos
University of Crete, Heraklion, Greece
Pefstatho@gmail.com

Prof. Britta Johanna Eickholt
King's College London, MRC, London, UK
Britta.J.Eickholt@kcl.ac.uk

Dr. Jose A. Esteban
Centro de Biología Molecular CSIC, Madrid, Spain
jaesteban@cbm.uam.es

Ms. Marta Esteves da Silva
Erasmus MC, Rotterdam, Netherlands
m.dasilva@erasmusmc.nl

Prof. James Fawcett
University of Cambridge, Centre for Brain Repair, Cambridge, UK
jf108@cam.ac.uk

Dr. Monica Fernandez-Monreal
Universidad Autonoma Madrid, CBMSO/CSIC, Madrid, Spain
mfmonreal@cbm.uam.es

Ms. Joana Ferreira
University of Coimbra, Center for Neuroscience and Cell Biology, Coimbra, Portugal
joana.s.ferreira@gmail.com

Prof. Marie Filbin
Hunter College, Biology Dept., New York, USA
filbin@genectr.hunter.cuny.edu

Dr. Kevin C Flynn
Max Planck Institute of Neurobiology, Martinsried, Germany
flynn@neuro.mpg.de

Mrs. Catarina Ferraz Franco
Instituto de Tecnologia Química e Biológica,
Oeiras, Portugal
cfranco@itqb.unl.pt

Dr. Kristian Franze
University of Cambridge, Cavendish Laboratory, Cambridge, UK
kf284@cam.ac.uk

Ms. Meng-meng Fu
University of Pennsylvania, School of Medicine, Philadelphia, PA, USA
mfu@mail.med.upenn.edu

Dr. Annette Gaertner
KULeuven - Center for Human Genetics, Laboratory of Neuronal Differentiation
Leuven, Belgium
Annette.Gaertner@cme.vib-kuleuven.be

Mr. Mikael Garcia
University of Bordeaux, IINS, UMR CNRS 5297, Bordeaux, France
mikael.garcia84@gmail.com

Dr. Frank Gertler
MIT Koch Institute for Cancer Research, Cambridge, MA, USA
fgertler@mit.edu

Dr. Oleg O. Glebov
University of Bristol, MRC Centre for Synaptic Plasticity, School Biochemistry, Bristol, UK
anxog@bris.ac.uk

Dr. Yukiko Goda
University College London, MRC Cell Biology Unit, London, UK
y.goda@ucl.ac.uk

Dr. Inmaculada Maria Gonzalez
University of Bristol, MRC Centre of Synaptic Pplasticity, School of Medical Sciences, Bristol, UK
anmig@bristol.ac.uk

Prof. Magdalena Götz
Helmholz Zentrum München, Institute for Stem Cell Research, Neuherberg, Germany
magdalena.goetz@helmholtz-muenchen.de

Dr. Seema Grewal
Development, Company of Biologists, Cambridge, UK
s.grewal@biologists.com

Prof. Eckart D. Gundelfinger
Leibniz Institute for Neurobiology (LIN), Magdeburg, Germany
gundelfinger@ifn-magdeburg.de

Mrs. Anna Magdalena Guzik-Kornacka
University of Zurich, Brain Research Institute Zurich, Switzerland
guzik@hifo.uzh.ch

Mrs. Anja Hafner
University of Ljubljana, Department of Pharmaceutical Biology, Ljubljana, Slovenia
anja.hafner@ffa.uni-lj.si

Dr. Christine Hassler
Max Planck Institute of Neurobiology, Martinsried, Germany
hassler@neuro.mpg.de

Prof. Zhigang He
Harvard Medical School, Children's Hospital, Boston, USA
zhigang.he@childrens.harvard.edu

Mr. Matthias Heidenreich
Max-Delbrück-Centrum für Molekulare Medizin (MDC), Berlin, Germany
matthias.heidenreich@mdc-berlin.de

Dr. Farida Hellal
Max Planck Institute of Neurobiology, Martinsried, Germany
hellal@neuro.mpg.de

Prof. Jeremy Martin Henley
University of Bristol, MRC Centre for Synaptic Plasticity, School Biochemistry, Bristol, UK
j.m.henley@bris.ac.uk

Ms. Muna Hilal
University of Bordeaux, IINS, UMR CNRS 5297, Bordeaux, France
muna.hilal@inserm.fr

Ms. Keri Lyn Hildick
University of Bristol, MRC Centre for Synaptic Plasticity, Bristol, UK
keri.hildick@bristol.ac.uk

Prof. Christine Holt
University of Cambridge, Cambridge, UK
ceh@mole.bio.cam.ac.uk

Prof. Casper Hoogenraad
UMC Utrecht, Utrecht, Netherlands
c.hoogenraad@erasmusmc.nl

Ms. Hanna Hornberg
University of Cambridge, Department of Physiology, Cambridge, UK
hkh27@cam.ac.uk

Dr. Audrey Howell
Stanford University, Stanford, CA, USA
audrey.s.howell@gmail.com

Prof. Michihiro Igarashi
Niigata University, Niigata, Japan
tarokaja@med.niigata-u.ac.jp

Ms. Rachel Jackson
King's College London, Guy's Hospital Campus, London, UK
rachel.jackson@kcl.ac.uk

Prof. Yuh-Nung Jan
University of California, San Francisco, California, USA
yuhnung.Jan@ucsf.edu

Dr. Carsten Janke
Centre Universitaire, Institut Curie, Orsay, France
Carsten.Janke@curie.fr

Mr. Ignacio Jausoro
INIMEC-CONICET, Córdoba, Argentina
ijausoro@immf.uncor.edu

Dr. Yishi Jin
University of California, San Diego, Howard Hughes Medical Institute, La Jolla, USA
yijin@ucsd.edu

Prof. Kozo Kaibuchi
Nagoya University, Department of Cell Pharmacology, Nagoya, Japan
kaibuchi@med.nagoya-u.ac.jp

Dr. Adrianna Kalous
University of Cambridge, Department of Physiology, Cambridge, UK
ak666@cam.ac.uk

Dr. Lukas Kapitein
Utrecht University, Utrecht, Netherlands
l.kapitein@erasmusmc.nl

Dr. Helmut Kessels
The Netherlands Institute for Neuroscience, Amsterdam, Netherlands
h.kessels@nin.knaw.nl

Dr. Michael M Kessels
Friedrich Schiller University of Jena, Institute for Biochemistry I, Jena, Germany
michael.kessels@mti.uni-jena.de

Dr. Josef Kittler
University College of London, London, UK
j.kittler@ucl.ac.uk

Prof. Ruediger Klein
Max Planck Institute of Neurobiology, Martinsried, Germany
rklein@neuro.mpg.de

Dr. Dennis Koch
Friedrich Schiller Univ. of Jena, Institute for Biochemistry I, Jena, Germany
dennis.koch@mti.uni-jena.de

Mr. Nikos Kourtis
IMBB-FORTH, Heraklion, Greece
nkourtis@gmail.com

Dr. Joachim Kremerskothen
University Hospital Muenster, Muenster, Germany
kremers@uni-muenster.de

Dr. Robert Kruger
Cell, Cambridge, MA, USA
RKruger@cell.com

Ms. Marijn Kuijpers
Erasmus MC, Rotterdam, Netherlands
m.kuijpers.1@erasmusmc.nl

Ms. Deepti Lall
Emil Fisher Zentrum, Institute for Biochemie, Erlangen, Germany
deeptilall@gmail.com

Dr. Maria Dolores Ledesma
Universidad Autonoma Madrid, Centro de Biología Molecular "Severo Ochoa", Madrid, Spain
dledesma@cbm.uam.es

Dr. George Leondaritis
University of Thessalia, Department of Pharmacology, Larissa, Greece
leondaritis@bioacademy.gr

Dr. Christophe Leterrier
INSERM U641,
Marseille CEDEX 15, France
christophe.leterrier@univmed.fr

Dr. Joel Levine
Stony Brook University, Stony Brook New York, USA
Joel.Levine@sunysb.edu

Ms. Kathryn Lewallen
Salk Institute, Salk Institute,
La Jolla, USA
klewallen@salk.edu

Mrs. Márcia Liz
Instituto de Biologia Molecular e Celular- IBMC, Porto, Portugal
maliz@ibmc.up.pt

Ms. Olaya Llano
Neuroscience Center Helsinki, Helsinki, Finland
olaya.llano@helsinki.fi

Dr. Bernhard Lohkamp
Karolinska Institutet, Stockholm, Sweden
Bernhard.Lohkamp@ki.se

Dr. Le Ma
University of Southern California, Zilkha Neurogenetic Institute, Los Angeles, USA
le.ma@usc.edu

Dr. Sandra Maday
University of Pennsylvania, School of Medicine, Philadelphia, USA
smaday@mail.med.upenn.edu

Ms. Maria M Magiera
Centre Universitaire, Institut Curie, Orsay, France
maria.magiera@curie.fr

Mr. Fernando Mar
IBMC, Porto, Portugal
fmar@ibmc.up.pt

Dr. Aaron W McGee
University of Southern California, Los Angeles, USA
amcgee@usc.edu

Mrs. Rajeshwari Siddesh Meli
University of Vienna, Max F. Perutz Laboratories, Wien, Austria
rajeshwari.meli@univie.ac.at

Mrs. Sindhu Menon
Westfälische Wilhelms-Universität, Institut für Molekulare Zellbiologie, Münster, Germany
s4saranya@gmail.com

Mrs. Llane Leslie Meyn
MPI Neurobiology, Planegg, Germany
meyn@neuro.mpg.de

Mrs. Katrin Michel
University Bonn, Institute of Neuropathology, Bonn, Germany
michel.katrin@yahoo.com

Dr. Jozsef Mihaly
Hungarian Academy of Sciences, Biological Research Center, Szeged, Hungary
mihaly@brc.hu

Dr. Marina Mikhaylova
Leibniz Institute for Neurobiology, Magdeburg, Germany
marina.mikhaylova@ifn-magdeburg.de

Dr. Magali Mondin
University of Bordeaux, IINS, UMR CNRS 5297, Bordeaux, France
magali.mondin@etud.u-bordeaux2.fr

Prof. Takeshi Nakamura
Tokyo University, Research Institute for Biological Sciences, Noda, Chiba, Japan
tnakamr@rs.noda.tus.ac.jp

Dr. Takashi Namba
Nagoya University, Department of Cell Pharmacology, Nagoya, Japan
nambatk@med.nagoya-u.ac.jp

Dr. Dorothee Neukirchen
Max Planck Institute for Neurobiology, Martinsried/Munich, Germany
neukirchen@neuro.mpg.de

Ms. Laura Nicolaï
Vesalius Research Center VIB, Leuven, Belgium
laura.nicolai@med.kuleuven.be

Dr. Marta Nieto
CNB, CSIC, CNB, CSIC, Madrid, Spain
mnlopez@cnb.csic.es

Dr. Catherine Qiurong Pan
National University of Singapore,
Mechanobiology Institute, Singapore,
dbspqc@nus.edu.sg

Dr. Sampson Akinleye Oludare Panneah-Ajiteru
Regional Center for Oral Health Research Initiative, Jos, Nigeria
rcortijosministryofhealth@gmail.com

Dr. Theofilos Papadopoulos
Max-Planck Institute of Experimental Medicine, Göttingen, Germany
papadopoulos@em.mpg.de

Mr. Iosif Pediaditakis
University of Crete, School of Medicine, Heraklion, Greece
jpediaditakis@med.uoc.gr

Dr. Leticia Peris
Institut of Neurosciences Grenoble U836 UJF-CEA-CHU, Grenoble, France
leticia.peris@ujf-grenoble.fr

Dr. Isabel Peset-Martin
University of Cambridge, Department of Zoology, Cambridge, UK
ip282@cam.ac.uk

Prof. Franck Polleux
The Scripps Research Institute,
Dorris Neuroscience Center,
La Jolla, USA
polleux@scripps.edu

Dr. Crystal Pontrello
University of California,
Riverside, USA
cpont001@ucr.edu

Dr. Fillip Port
MRC Laboratory of Molecular Biology,
Cambridge, UK
fport@mrc-lmb.cam.ac.uk

Dr. Andreas Prokop
The University of Manchester, UK
andreas.prokop@manchester.ac.uk

Dr. Andreas W Püschel
Westfälische Wilhelms-Universität,
Institut für Molekulare Zellbiologie,
Münster, Germany
apuschel@uni-muenster.de

Mr. Owen Randlett
University of Cambridge, Harris Lab,
Cambridge, UK
or235@cam.ac.uk

Dr. Theo Rein
Max Planck Institute of Psychiatry,
Munich, Germany
theorein@mpipsykl.mpg.de

Dr. Daniele Repetto
Molecular Biotechnology Center Torino,
Torino, Italy
daniele.repetto@unito.it

Dr. Michela Rigoni
University of Padova, Dept. of Biomedical Sciencesa, Padova, Italy
rigonim@bio.unipd.it

Dr. Ida Rishal
Weizmann Institute of Science,
Rehovot, Israel
ida.rishal@weizmann.ac.il

Mr. Jörg Ruschel
Max Planck Institute of Neurobiology,
Martinsried, Germany
ruschel@neuro.mpg.de

Mr. Abhishek Shrikant Sahasrabudhe
Indian Institute of Science Education and Research (IISER), Sai Trinity Garware Circle, Pune, India
a.sahasrabudhe@iiserpune.ac.in

Dr. Maria Sakkou
Max Planck Institute of Neurobiology, Department of Molecular Neurobiology, Martinsried, Germany
sakkou@neuro.mpg.de

Dr. Sara Salinas
Institute of Molecular Genetics of Montpellier, Montpellier, France
sara.salinas@igmm.cnrs.fr

Ms. Ana Rita Santos
University of Coimbra, Center for Neuroscience and Cell Biology, Coimbra, Portugal
rita.a.santos@gmail.com

Dr. Frederic Saudou
Centre Universitaire, Institut Curie, Orsay, France
frederic.saudou@curie.fr

Dr. C. Laura Sayas
Universidad Autonoma Madrid, Centro de Biología Molecular "Severo Ochoa", Madrid, Spain
lsayas@cbm.uam.es

Mr. Andreas Schaupp
Max Planck Institute of Neurobiology, Martinsried, Germany
schaupp@neuro.mpg.de

Mr. Peter Scheiffele
Biozentrum of the University of Basel, Basel, Switzerland
peter.scheiffele@unibas.ch

Ms. Marlen Schlieder
Vesalius Research Center VIB, Leuven, Belgium
Marlen.Schlieder@med.kuleuven.be

Prof. Dietmar Schmucker
Vesalius Research Center VIB, Leuven, Belgium
dietmar.schmucker@vib-kuleuven.be

Dr. Erin Schuman
Max Planck Institute for Brain Research, Frankfurt, Germany
schumane@brain.mpg.de

Prof. Martin Ernst Schwab
University of Zurich, Brain Research Institute Zurich, Switzerland
schwab@hifo.uzh.ch

Prof. Thomas L Schwarz
Harvard Medical School, Children's Hospital, Boston, MA, USA
thomas.schwarz@childrens.harvard.edu

Mr. Alvaro Sebastian
The National Centre for Biotechnology (CNB) /CSIC, Spain
asebastian@cnb.csic.es

Dr. Elena Seiradake
University of Oxford, Wellcome Trust Centre for Human Genetics, Oxford, UK
elena@strubi.ox.ac.uk

Prof. Benjamin Shykind
Weill Cornell Medical College in Qatar, Education City, Doha, Qatar
bes2027@qatar-med.cornell.edu

Dr. Mustafa M Siddiq
Hunter College,
New York, USA
siddiq@genectr.hunter.cuny.edu

Prof. Stephan Sigrist
Freie Universität Berlin,
Berlin, Germany
stephan.sigrist@fu-berlin.de

Mr. Tolga Soykan
Max Planck Institute of Experimental Medicine, Göttingen, Germany
soykan@em.mpg.de

Mr. Julien Spatazza
CIRB -Collège de France, Paris, France
julien.spatazza@college-de-france.fr

Ms. Eleanna Stamatakou
University College of London,
London, UK
e.stamatakou@ucl.ac.uk

Dr. Michael Stiess
Max Planck Institute of Neurobiology,
Martinsried, Germany
stiess@neuro.mpg.de

Ms. Susan Su
MIT, Cambridge, USA
scsu@mit.edu

Prof. Nektarios Tavernarakis
IMBB-FORTH, Heraklion,
Crete, Greece
tavernarakis@imbb.forth.gr

Dr. Gaia Tavosanis
Max Planck Institute of Neurobiology,
Martinsried, Germany
gaia@neuro.mpg.de

Ms. Gohar Ter-Avetisyan
Max-Delbrück-Centrum für Molekulare Medizin (MDC), Berlin, Germany
gohar.teravetisyan@mdc-berlin.de

Dr. Florencia Tevy
Institute for Research in Biomedicine (IRB), Barcelona, Spain
florencia.tevy@irbbarcelona.org

Dr. Simona Sandra Tivodar
IMBB-FORTH, Heraklion,
Crete, Greece
tivodar@imbb.forth.gr

Dr. Elena Tortosa
Universidad Autonoma Madrid, Centro de Biología Molecular "Severo Ochoa",
Madrid, Spain
etortosa@cbm.uam.es

Dr. Hanako Tsushima
Italian Institute of Technology,
Genova, Italy
Hanako.Tsushima@iit.it

Prof. Emilia Turco
University of Torino, Molecular Biotecnology Center, Torino, Italy
emilia.turco@unito.it

Ms. Flora Vajda
University of Zurich, Brain Research Institute, Zurich, Switzerland
vajda@hifo.uzh.ch

Ms. Dianne van den Heuvel
UMC Utrecht, Rudolf Magnus Institute for Neuroscience, Utrecht, Netherlands
d.m.a.vandenheuvel@umcutrecht.nl

Mr. Loek van der Kallen
Vrije Universiteit Amsterdam,
Amsterdam, Netherlands
loek.van.der.kallen@cncr.vu.nl

Ms. Susan van Erp
UMC Utrecht, Rudolf Magnus Institute for Neuroscience, Utrecht, Netherlands
s.vanerp-2@umcutrecht.nl

Dr. Med. Andreas Vlachos
Goethe-University Frankfurt, Clinical euroanatomy, Neuroscience Center, Frankfurt am Main, Germany
a.vlachos@med.uni-frankfurt.de

Ms. Corinna Wentzel
Biozentrum of the University of Basel, Basel, Switzerland
corinna.wentzel@unibas.ch

Mrs. Lena Will
Westfälische Wilhelms-Universität, Münster, Germany
lenawillpost@uni-muenster.de

Dr. Daniel Kenneth Wilton
University College London, London, UK
daniel.wilton@ucl.ac.uk

Dr. Urszula Wojda
International Institute of Molecular and Cell Biology, Warsaw, Poland
ulawojda@iimcb.gov.pl

Dr. Andrea Kuan Cie Wong
University of Padova, Padova, Italy
andreakuancie.wong@gmail.com

Ms. Rui Yang
Westfälische Wilhelms-Universität, Institut für Molekulare Zellbiologie, Münster, Germany
ryang_01@uni-muenster.de

Mr. Kah Wai Yau
Erasmus MC, Rotterdam, Netherlands
k.yau@erasmusmc.nl

Mrs. Diana Zala
Centre Universitaire, Institut Curie, Orsay, France
diana.zala@curie.fr

Dr. Niccolo Zampieri
Columbia University, New York, USA
nz2141@columbia.edu

Ms. Justyna Jolanta Zdrojewska
Turku Centre for Biotechnology, Turku, Finland
justyna.zdrojewska@btk.fi

Prof. Noam E. Ziv
Technion Faculty of Medicine, Fishbach Building (Electrical Engineering), Haifa, Israel
noamz@netvision.net.il

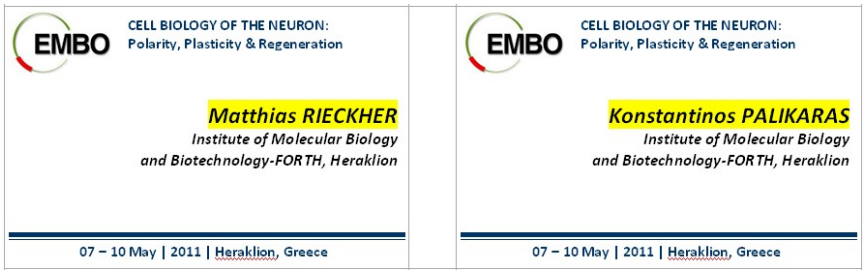
Dr. Hongyan Zou
Mount Sinai School of Medicine, Dept. Neuroscience, New York, USA
hongyan.zou@mountsinai.org

Workshop Secretary
Mrs. Georgia Houlaki
Local Organization
Abstract Book Editiing
IMBB – FORTH, Heraklion
gh@imbb.forth.gr

USEFUL INFORMATION

If you are a speaker:

There is a data projector connected to a PC (Windows XP – Microsoft Office XP); **Mr. Matthias Rieckher** and **Mr. Konstantinos Palikaras** are in charge at your disposal for any technical assistance. You should not forget to contact them during the break *prior to* your presentation's session and hand over to them your presentation data (CD, memory stick, notebook /laptop).



- Allocated time for oral presentations is **20** minutes with 10 minutes for discussion. For the short talks, which are selected from the abstracts, presentation time is 10 minutes with 5 minutes discussion.

If you are presenting a poster:

Poster Room: No 34 "Games Room" at the hotel plan – see page 221)

Poster boards are ready for presentation mounting. You are not allowed to use push-pins or any other mount material which could damage boards. Therefore, you should ask for proper mounting material (blue tack or scotch tape) at the secretariat.

Posters should be up for display by Saturday May 7th at 14.00. All posters will remain mounted for the whole duration of the meeting.

There are 3 Poster sessions: Saturday May 7, 21.00-23.00 (Red Session), Sunday May 8, 16.00-18.00 (Green Session) and Monday May 9, 14.00-16.15 (Blue Session) There is a poster list where you can check the poster session you present in and your poster number/board. You will also be notified for your poster session during registration.

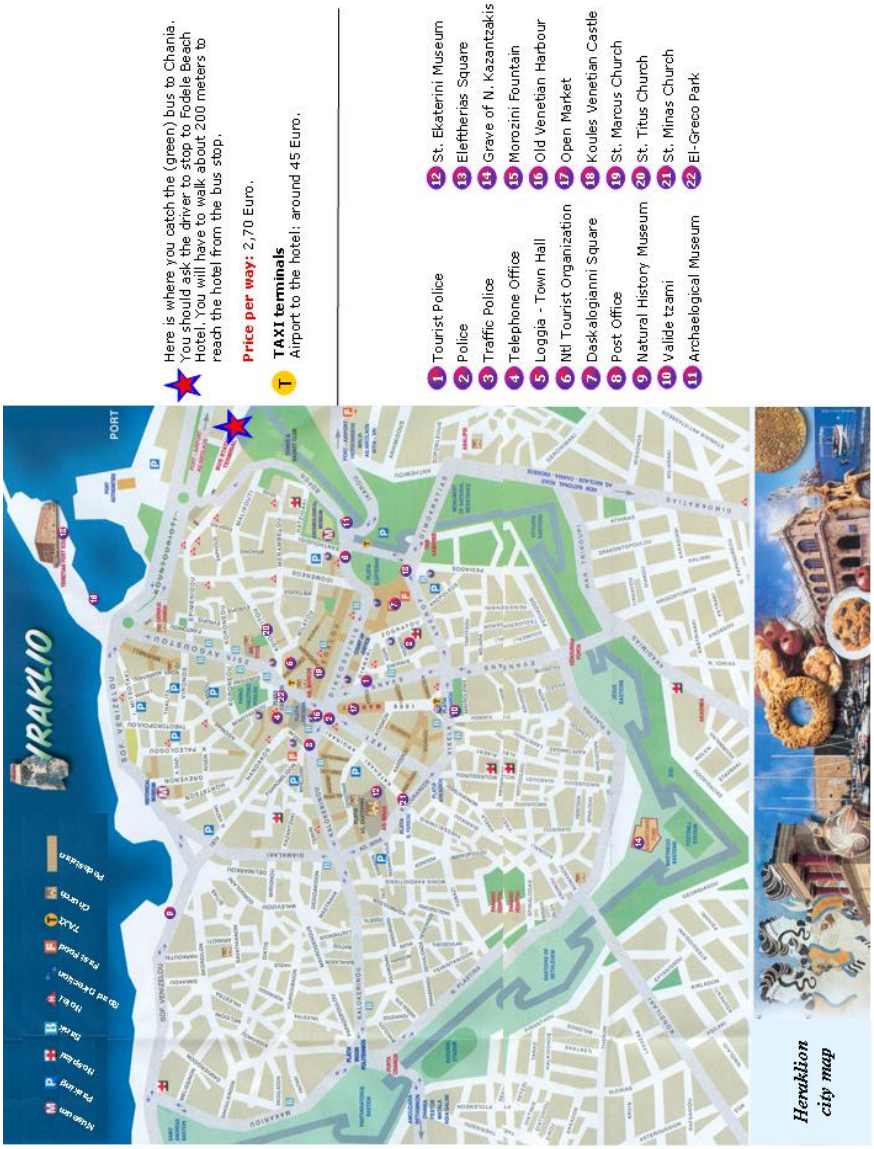
❖ Frequently Asked Questions

Will I have Internet access during the conference?

There is an "internet corner" just outside the conference hall with 3 desktop computers connected to the hotel LAN. For wireless internet access inside the conference room and the rest of the conference venue, you will need to buy an access card from the Reception of the hotel. We have a special deal for the participants: 4 days for 15 Euros.

What if I want to keep my room after 12 noon on checkout day?

12.00 is the regular check-out time. If you wish to keep your room past 12.00, you should notify the reception of the hotel in advance to check for availability. In case of extended day stay, you should arrange the additional payment directly with the hotel. Bear in mind that after 18.00 a full overnight stay charge is applicable.



Heraklion city map


1 MAIN ENTRANCE
2 MAIN BUILDING ENTRANCE
3 RECEPTION
4 MAIN BAR
5 MAIN RESTAURANT
6 INTERNET CORNER
7 A LA CARTE RESTAURANT "AMBROSIA"
8 SNACK BAR
9 BEACH BAR
10 DISCO
11 MINI CLUB
12 MEDICAL CENTER
13 FITNESS ROOM (GYM)
14 SAUNA
15. MAIN SWIMMING POOL
16 HEATED SWIMMING POOL
17 PLAZZA
18 STAR LIGHT BAR
19 SWIMMING POOL
20 MINI GOLF
21 TENNIS COURTS
22 BASKET BALL COURT
23 MINI MARKET
24 HAIRDRESSING SALON
25 RENT A CAR
26 BEACH
27 SEA SPORTS
28 AMPHITHEATRE
29 LUGGAGE ROOM
30 PARKING
31 ARCHERY
32 BEACH SOCCER
33 BEACH VOLLEY
34 GAMES ROOM
35 CONFERENCE ROOM
36 DIVING CENTER
37 ATM
38 WATERPARK
39 SNACK BAR "WINDMILL"
40 BOCCIA
41 A LA CARTE RESTAURANT
FODELE BEACH HOLIDAYS RESORT
P.O.BOX 1354
GR - 71500 HERAKLION
CRETE - GREECE
Tel. +302810521251/5
+302810521351/4
Fax. +302810521249
www.fodelebeach.gr
e-mail: fodele@fodelebeach.gr
FODELE BEACH
HOTEL PLAN

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