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Assembly and Disassembly of the Nervous System

A molecular journey along the life of the neuron

January 19-21, 2015
The David Lopatie conference centre
Weizmann Institute of Science, Rehovot, Israel

Abstract Book

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Program:

Monday, January 19th

09:00-09:15 Registration

09:15-09:30 Opening remarks

Session 1: Neurodevelopment

09:30-10:05 **Larry Zipursky**, UCLA
"Cellular recognition and the assembly of neural circuits"

10:05-10:40 **Franck Polleux**, Columbia University
"Cellular and molecular mechanisms underlying axon morphogenesis in the mammalian CNS"

10:40-10:55 Short talk: **Idan Alyagor**, WIS
"Deciphering the genetic program of developmental axon remodeling"

10:55-11:10 Short talk: **Michael Ratz,** Max Planck Institute for Biophysical Chemistry
"CRISPR-Cas9 knock-in cells for live-cell RESOLFT super-resolution microscopy"

11:10-11:40 Coffee break

11:40-12:15 **Gil Levkowitz**, WIS
"Assembly and functionality of oxytocin neuronal circuits using the zebrafish model"

12:15-12:50 **Lisa Goodrich**, Harvard
"Intrinsic and extrinsic regulation of neuronal morphogenesis in-situ"

12:50-13:05 Short talk: **Peter Engerer**, TUM
"Uncoupling of mitosis and differentiation allows for fast and synchronous CNS development in vivo"

13:05-13:20 Short talk: **Ramanathan Narayanan**, UMG
"Proteasome-mediated switching of BAF subunits controls cortical development"

13:20-14:50 Lunch + poster session

Session 2: Post developmental events

14:50-15:25 **Yehezkel Ben-Ari**, Inmed/Neurochlore
"Understanding brain development to treat disorders: the case of autism"

15:25-15:40 Short talk: **Natalia Marahori**, TUM
"The fate of synaptic mitochondria explored by optical pulse chase imaging"

15:40-15:55 Short talk: **Andrzej Bialowas**, SISSA
"Impaired spike-timing dependent plasticity at immature MF-CA3 synapses in the hippocampus of NL3 R451C knock-in mice, an animal model of Autism"

15:55-16:30 **Inna Slutsky**, TAU
"Interplay between population firing stability and single neuron dynamics in hippocampal networks"

16:30-17:00 Coffee break

17:00-17:35 **Noam Ziv**, Technion
"Synaptic remodeling and proteostasis: The river of constant change"

17:35-17:50 Short talk: **Venera Khuzakhmetova**, Kazan/Russian Academy of Science
"Septins are involved in the regulation of neurotransmitter secretion at mice neuromuscular junction"

17:50-18:05 Short talk: **Iryna Hlushchenko,** University of Helsinki
"Role of Gelsolin in Actin cytoskeleton regulation in dendritic spines"

18:05-18:40 **Nicholas C. Spitzer**, UCSD
"Cellular and molecular mechanisms of neurotransmitter switching in the CNS"

20:00 Social event

Tuesday, January 20th

Session 3: Degenerative mechanisms

09:00-09:35 **Elizabeth Fisher**, UCL
"Modeling Down syndrome - a human chromosomal disorder - in mice, with a focus on neurodegeneration"

09:35-09:50 Short talk: **Aleksandra Deczkowska**, WIS
"Aging-induced choroid plexus signature negatively affects neurogenesis and cognitive function"

09:50-10:05 Short talk: **Ketty Mishnaevsky**, Technion
"JNK pathway activation synchronizes neuronal death and glial phagocytosis in Drosophila"

10:05-10:40 **Frederic Saudou**, GIN
"Huntington's disease: huntingtin and the control of intracellular dynamics"

10:40-11:10 Coffee break

11:10-11:45 **Erika Holzbaur**, UPenn
"Autophagosome dynamics and neurodegeneration"

11:45-12:00 Short talk: **Keith Harris**, TAU
"Why are neurotransmitters neurotoxic? An evolutionary perspective"

12:00-12:35 **Bart De Strooper**, KU Leuven
"Gamma-and beta secretases: fascinating proteases at the membrane and crucial drug targets for Alzheimer disease"

12:35-12:50 Short talk: **Tatjana Kleele,** TUM
"Cellular mechanisms of neuromuscular synapse elimination"

12:50-13:50 Lunch

13:50-14:30 Round table session

Session 4: Regeneration of the adult nervous system

14:30-15:05	**Mike Fainzilber**, WIS "The Importins of axonal transport in neuronal growth and regeneration"
15:05-15:40	**Yishi Jin**, UCSD "Regulation of MT dynamics in axon regeneration"
15:40-15:55	Short talk: **Neta Kollet**, WIS "Sprouting assay of primary dissociated Drosophila mushroom body neurons is instrumental in assessing intrinsic axon growth potential"
15:55-16:10	Short talk: **Dragomir Milovanovic,** Max Planck Institute for Biophysical Chemistry "Length Matters: Hydrophobic mismatch sorts SNARE proteins into distinct membrane domains"
16:10-16:40	Coffee break
16:40-17:15	**Larry Benowitz**, Harvard "Optic Nerve Regeneration"
17:15-17:30	Short talk: **Katya Zelentsova**, HUJI "A Protein S-dependent mechanism for neural stem cell / progenitor proliferation and differentiation"
17:30-17:45	Short talk: **Kathrin Hemmer**, Luxembourg Centre for Systems Biomedicine "Induced neural stem cells achieve long-term survival and functional integration in the adult mouse brain"
17:45-18:20	**Martin Schwab**, ETH/UZH "Nerve fiber regeneration, new circuit formation and functional recovery after spinal cord and brain injury"
18:20-18:30	Closing remarks
18:30	Dinner in the lobby

Wednesday, January 21st

Tour to Jerusalem

Abstracts-
Invited speakers

Cellular Recognition and the Assembly of Neural Circuits

Larry Zipursky

Dept. of Biological Chemistry; HHMI; David Geffen School of Medicine, University of California, Los Angeles, USA

How precise patterns of synaptic connectivity emerge during development remains a central issue in neuroscience. The cellular diversity of the nervous system and the vast numbers and specificity of synapses put enormous demands on the cellular recognition mechanisms regulating this process. In the medulla region of the Drosophila visual system, for instance, each synaptic module repeated some 750 times comprises over 100 different neuronal cell types interlinked by tens of thousands of synaptic connections. How neurites of different neuronal cell types discriminate between one another during circuit assembly is the central goal of my laboratory. In the first part of my talk I will describe how molecular diversity at the Dscam1 locus regulates circuit assembly. Here each neuron acquires a unique cell surface identity by virtue of expressing in a probabilistic fashion a discrete set of Dscam1 isoforms. This provides neurons with a way to discriminate between self and non-self and plays a crucial in dendritic and axonal pattern, as well as in the assembly of multiple contact synapses. In the second part of the talk, I will describe an RNA-sequencing approach to identifying determinants of synaptic specificity. Here we analyzed expression of cell surface proteins in seven different neurons with discrete patterns of synaptic specificity in the medulla region of the fly visual. We demonstrate a complex cell-type specific pattern of expression in these neurons. Of particular interest was the complementary pattern of expression of one family of some 20 Ig superfamily proteins expressed in a cell type specific pattern in these neurons and the complementary pattern of expression of their binding partners within discrete layers where they make synaptic connections. We speculate that the cell type specific expression of these protein families plays an instructive role in regulating synaptic specificity.

Cellular and molecular mechanisms underlying axon morphogenesis in the mammalian CNS

Franck Polleux

Dept of Neuroscience, Mortimer B. Zuckerman Mind Brain Behavior Institute and Kavli Institute for Neural Sciences, Columbia University, New York, USA.

During axon development, mitochondria represent one of the largest cargo trafficked anterogradely and retrogradely along the axon. However, we and others recently found that in synaptically connected, adult cortical axons, over 95% of mitochondria are immobile both in vitro and in vivo. Mitochondria are often immobilized at presynaptic release sites in adult mammalian CNS axons. We also recently identified a signaling pathway centered around a kinase dyad (LKB1-NUAK1) required for proper terminal axon branching in vivo through its ability to regulate presynaptic mitochondrial capture (Courchet, Lewis et al. Cell 2013). Two main questions arose from this work: (1) what is the function(s) of presynaptic mitochondria underlying their critical role in axon morphogenesis? And (2) what are the cellular and molecular mechanisms anchoring mitochondria presynaptically? I will present preliminary results on both projects. Regarding the first question, we accumulated data demonstrating that in cortical axons, the serine/threonine kinase LKB1 plays an essential role in presynaptic Ca2+ homeostasis by promoting mitochondria-dependent Ca2+ clearance. We generated two genetically-encoded Ca2+ sensors targeted to the mitochondrial matrix ([Ca2+]m; mito-GCaMP5G) or to presynaptic boutons ([Ca2+]c; vGlut1-GCaMP5G). We found a significant decrease of [Ca2+]m uptake in LKB1-null axons which prevents normal [Ca2+]c clearance during neurotransmission. This deficit in mitochondria-dependent [Ca2+]c clearance is associated with decreased expression of the mitochondrial calcium uniporter (MCU). Using rescue experiments, we further demonstrate that in LKB1-deficient axons, the reduction of MCU-dependent [Ca2+]m uptake at glutamatergic synapses causes drastic changes in neurotransmitter release properties. Our results unravel a new signaling pathway linking mitochondria-dependent presynaptic Ca2+ clearance to neurotransmitter release properties. Regarding the second question, we are testing the potential role of organelle interface between mitochondria and endoplasmic reticulum (ER) as a candidate mechanism for presynaptic mitochondria anchoring along the axon.

Assembly and functionality of oxytocin neuronal circuits using the zebrafish model

Gil Levkowitz

Dept. of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel.

Oxytocin is an evolutionary old neuropeptide, which is found in species ranging from invertebrates to mammals. Oxytocin is synthesized in hypothalamic neurons whose axons project onto the pituitary and form the hypothalamo neurohypophyseal system (HNS). The HNS is neurovascular interface between axons and blood vessels, where oxytocin is secreted through fenestrated capillaries and enters the blood to controls parturition and lactation in mammals and aspects of reproductive processes in fish. Besides these well-known neuroendocrine (humoral) effects the activities of oxytocin in the central nervous system are important for the regulation of stress, social behaviors and appetite. Disruptions of the oxytocinergic system in humans have been implicated in Prader-Willi syndrome and autism. Our lab utilizes zebrafish as a vertebrate model organism to investigate the molecular and cellular processes underlying morphogenesis and function of oxytocinergic neurons. The zebrafish is an excellent model to study these processes as the fish's optically transparent embryo allows us to accurately trace oxytocinergic circuits. We previously showed that zebrafish have merely 20-25 oxytocin neurons, whereas mammals are known to have tens of thousands of neurons. This presents us with the exceptional possibility to analyse the connectivity, molecular composition and function of each neuron at a single-cell resolution. In the past few years we have generated unique transgenic lines that allow us to monitor and modulate the development and activity of zebrafish oxytocinegic neurons. Specifically, we have been engaged in the following projects: 1) mapping the complete set of axonal connection made by each oxytocinergic neuron. 2) studying the molecular cues that regulate morphogenesis of the neuro-vascular interface between oxytocinergic neurons and neurohypophyseal vasculature. 3) identifying new regulators of oxytocin production and generating tools to study neuropeptide transport and secretion. 4) studying the role of specific oxytocinergic circuits in animal behavior using a set of behavioral assays and physiological biomarkers that measure stress, feeding and social behavior in fish Taken together, the use of the zebrafish model in conjunction with unique genetic tools, fluorescent imaging and behavioral assays, places us in an excellent position to systematically study the embryonic development and functionality of oxytocin neuronal circuits.

Intrinsic and extrinsic regulation of neuronal morphogenesis *in situ*

Lisa Goodrich

Dept. of Neurobiology, Harvard Medical School, Boston, USA.

Nervous system function depends on an extraordinary diversity of neurons that vary in size, shape, and patterns of connectivity. Efforts to understand how individual neurons acquire their unique morphologies have largely relied on in vitro assays, which have established an important role for intrinsic changes at the level of the cytoskeleton. Much less is known about how these events are regulated in response to extrinsic signals, such that neurons develop stereotyped morphologies that are oriented with the surrounding tissue. For example, the amacrine cells of the retina are situated in the inner nuclear layer (INL) and extend a single primary dendrite that arborizes within a discrete layer of neuropil called the inner plexiform layer (IPL). In order to learn how intrinsic and extrinsic programs of neuronal morphogenesis are coordinated in vivo, we have developed a time lapse imaging system that allows us to visualize individual amacrine cell progenitors in situ as they migrate and develop asymmetric morphologies. We find that developing amacrine cells are initially bipolar and extend leading processes into the nascent IPL while they are still migrating, followed by retraction of the trailing process once the cell body has settled at the bottom of the INL. In contrast, in mice mutant for the atypical cadherin Fat3, amacrine cells show less directed migration, with trailing processes that branch excessively and fail to retract, instead forming a second ectopic plexiform layer that interrupts the INL. Since Fat3 is a transmembrane protein that is localized to the leading process, we hypothesize that it acts as a receptor that polarizes the cytoskeleton in response to cues in the IPL. In support of this idea, the Fat3 intracellular domain binds to many known cytoskeletal proteins, including members of the Ena/VASP family of actin regulators. Moreover, when the distribution of endogenous Ena/VASP proteins is made more uniform, amacrine cells develop bipolar morphologies. These findings suggest that Fat3 integrates extrinsic and intrinsic cues to ensure that amacrine cells develop a single dendrite that is reliably oriented towards the IPL.

Understanding brain development to treat disorders: the case of autism

Yehezkel Ben-Ari

INMED, INSERM and Neurochlore, Marseille, France

We have recently shown that the diruetic NKCC1 chloride importer antagonist bumetanide reduces the severity of autism in children (1). We also reported that the same treatment enhanced visual communication in adolescents with autism (2). Furthermore, in the Valproate and fragile X mice animal models, we discovered (3) that the entire oxytocin mediated neuroprotective GABA excitatory/inhibitory developmental shift durign delivery is abolished (4). Maternal administration of the diuretic shortly before delivery restores in off springs inhibitory GABA and attenuates exacerbated oscillations in vivo and autistic behavioral features. Likewise, administration of an antagonist of oxytocin receptors to naive mothers before delivery produces autisitic features in off spring. Therefore, the polarity of GABA during delivery exerts a priming action on the manifestations of autism. These observations raise many fundamental issues that concern both the role of delivery as a possible critical period, the roles of activity dependent mechanisms in the buildup of neuronal ensembles –see the « check point » concept (5), whether and how they are deviated by genetic mutations and environmental insults –the « neuroarcheology » concept (6). They also suggest that innovative treatments might not come from a genocentric approach but rather one that relies on determination of developmental sequences and how they are deviated by insults. It is suggested that these deviations rather than the mutation per se are the cause of the clinical manifestations and that drugs acting on these immature currents that persist in adult brains are promising therapeutic targets.

1. Lemonnier et *al*. A randomised controlled trial of bumetanide in the treatment of autism in children. *Translational psychiatry* Dec 11; 2:e202.
2. Hadjikhani N, et *al*. Improving emotional face perception in autism with diuretic bumetanide: A proof-of-concept behavioral and functional brain imaging pilot study. *Autism*. 2013 Dec 16.
3. Tyzio R, et *al*. Maternal oxytocin triggers a transient inhibitory switch in GABA signaling in the fetal brain during delivery. *Science*. 2006 Dec 15; 314(5806):1788-92.
4. Tyzio R, et *al*. Oxytocin-Mediated GABA Inhibition During Delivery Attenuates Autism Pathogenesis in Rodent Offspring. *Science*. 2014 Feb 7; 343 (6171): 675-679.
5. Ben-Ari Y, Spitzer NC. Phenotypic checkpoints regulate neuronal development. *Trends Neurosci.* 2010 Nov;33 (11):485-92.
6. Ben-Ari Y. Neuro-archaeology: pre-symptomatic architecture and signature of neurological disorders. *Trends Neurosci*. 2008 Dec;31(12):626-3.

Interplay between population firing stability and single neuron dynamics in hippocampal networks

Inna Slutsky

Dept. of Physiology and Pharmacology, Sackler School of Medicine, Tel Aviv University, Israel

The ability of neuronal circuits to maintain the delicate balance between stability and flexibility in changing environments is critical for normal neuronal functioning. Despite a progress in understanding the homeostatic mechanisms that underlie stability of network firing properties, several key questions remain open. In my presentation I will address several key questions: What are the basic properties of neural networks that are subjected to homeostatic control? Are homeostatic control systems equally precise at the level of individual neurons and neuronal populations? What is the trigger of synaptic homeostatic mechanisms? How do compensatory changes in synaptic strength affect network's functions? What are the molecular mechanisms enabling stability of population firing properties at extended timescales? I will describe the basic relationships between ongoing spiking properties of individual neurons, population dynamics and synaptic adaptive mechanisms.

Synaptic Remodeling and Proteostasis: The River of Constant Change

Noam E. Ziv

Technion – Israel Institute of Technology, Haifa, Israel

Activity-dependent modifications to synaptic connections – synaptic plasticity – is widely believed to represent 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". Over recent years, molecular imaging studies have changed our notion of the synapse, from that of a “structure” to that of a dynamic molecular assembly at steady state. These studies, combined with proteomics and additional approaches, collectively indicate that synaptic molecular dynamics are dominated by the exchange and interchange of synaptic molecules, rather than protein synthesis and degradation, with the latter acting over longer time scales. Yet, regardless of their source and time scales, these continuous dynamics would seem to challenge the tenacity exhibited by individual synaptic sites. Indeed, recent studies from our lab and others indicate that the tenacity of individual synapses is inherently limited and that synaptic properties change spontaneously and extensively. We have also found, however, that these changes do seem to be governed by certain principles which become apparent when synapses are studied as individual entities on the one hand and populations on the other. This work, and the insights it has provided will be described.

Cellular and molecular mechanisms of neurotransmitter switching in the CNS

Nicholas C. Spitzer

Neurobiology Section and Kavli Institute for Brain and Mind
University of California, San Diego, USA

The brain changes in response to changes in the environment and experience, and these changes underlie processes such as learning and memory. Substantial evidence demonstrates that this brain plasticity results from changes in the strength and number of synapses – the connections that neurons make. But is there more to it than that? The neurotransmitters made by neurons, which they use to signal to one another, have long been thought to be fixed and unchanging, and to be part of neuronal identity. Transmitter switching – substituting one neurotransmitter for another - is a relatively newly recognized form of plasticity. It occurs both during development and in the mature brain, it regulates behavior, and it may provide a basis for treating neurological disorders. We have visualized transmitter switching in single neurons in the adult brain and begun to understand the signaling cascade by which transmitter switching is achieved in the embryonic nervous system.

Modeling Down syndrome - a human chromosomal disorder - in mice, with a focus on neurodegeneration.

Elizabeth M.C. Fisher

Dept. of Neurodegenerative Disease and Dept. of Experimental Epilepsy, UCL Institute of Neurology, London, UK

Approximately one in every 750 people worldwide is born with Down syndrome, and has an extra copy of chromosome 21 (and this number is not necessarily diminishing in countries with pre-natal diagnosis). Trisomy 21 gives rise to a complex set of features that includes cognitive impairment, a greatly increased risk of Alzheimer disease (AD) from mid-life onwards, and many variable features including heart malformations, increased susceptibility to infections and autoimmune disease. The severity of individual features is highly variable between different people with Down syndrome. Down syndrome is a gene dosage disorder and arises from having three copies of an entire chromosome, and so identifying individual genes that contribute to different features of the disorder is challenging. We have chosen to tackle the problem by creating a series of novel mouse models that are enabling us to identify important dosage sensitive genes for aspects of Down syndrome. We are particularly interested in Alzheimer disease in Down syndrome. An additional copy of the chromosome 21 encoded gene, APP, promotes the development of AD in the euploid population, in rare families with early onset AD. However, in Down syndrome, the effect of trisomy of the other ~300 chromosome 21 genes on disease is unclear. We have now shown that triplication of chromosome 21 genes other than APP makes a significant contribution to the development of AD. Trisomy of chromosome 21 significantly enhances Aβ deposition and associated synaptic transmission and cognitive deficits, and reduces survival. This is an exciting time for understanding Down syndrome as we are starting to understand the biological basis of the disorder and as new therapies are being proposed for aspects of the syndrome.

Huntington's disease: huntingtin and the control of intracellular dynamics

Frédéric Saudou[1,2,]

[1]Grenoble Institute of Neurosciences, Univ. Grenoble Alpes, [2]INSERM U836, Grenoble, France

Huntington's disease is caused by the abnormal polyglutamine expansion in the N-ter part of huntingtin (HTT), a large protein of 350kDa. Over the past years, we proposed that HTT acts a scaffold for the molecular motors and through this function, regulates the efficiency and directionality of vesicular transport along microtubules in neurons. This function is conserved in Drosophila. In particular, HTT controls the microtubule-based fast axonal transport (FAT) of neurotrophic factors such as BDNF. PolyQ expansion in HTT alters this function, leading to a decrease in neurotrophic support and death of striatal neurons. Interestingly, the defect in transport might not be restricted to axons but could also involve defects in the retrograde transport of TrkB in striatal dendrites.
In addition to the role of HTT in scaffolding the molecular motors both in cortical and striatal neurons, we found that HTT scaffolds GAPDH on vesicles and that vesicular GAPDH is necessary to propel vesicles in GAPDH deficient neurons. Here we will extend these findings and discuss how HTT by specifically localizing the glycolytic machinery on vesicles may supply constant energy for the transport of vesicles over long distances in axons. We will also discuss how this machinery is altered in disease situation. Finally, we will extend the function of HTT as a scaffold for dynamin1 for regulating intracellular dynamics in health and disease.

Autophagosome Dynamics and Neurodegeneration

Erika L.F. Holzbaur

Perelman School of Medicine, University of Pennsylvania, Philadelphia, USA

Maintenance of neuronal homeostasis is particularly dependent on efficient degradative pathways such as autophagy. We used live-cell imaging to investigate autophagosome dynamics in primary neurons expressing the autophagosome marker GFP-LC3. We find that constitutive autophagosome biogenesis is restricted to the distal end of the axon, and occurs via an ordered pathway of protein recruitment following stereotypical kinetics. Concomitant with autophagosome formation, we observed the engulfment of cargos including mitochondrial fragments. We followed up on these observations by analyzing the dynamics of mitophagy in HeLa cells, and found that the ubiquitin-binding protein optineurin is a required receptor, mediating the formation of LC3-positive autophagosomes around damaged mitochondria. An ALS-linked mutation in optineurin that blocks ubiquitin binding inhibits the degradation of depolarized mitochondria, suggesting that defects in mitophagy may contribute to the pathogenesis of motor neuron disease.

Gamma-and beta secretases: fascinating proteases at the membrane and crucial drug targets for Alzheimer disease

Bart De Strooper

VIB Center for the Biology of Disease; KU Leuven Center for Human Genetics, Leuven, Belgium

Over the last decade important progress has been made towards the understanding of the molecular pathobiology of Alzheimer's disease. Major breakthroughs were the identification of presenilin and its crucial role in the γ-Secretase processing of APP and Notch and of BACE1 and its role in Neuregulin processing. We will show in the current presentation that our knowledge on Secretase functions and regulation has dramatically progressed in the last years. We will explain how different γ-Secretase complexes have different biological roles, and that selectively targeting them could provide safer drugs. Also increasing insights into structure and function could lead to safer drugs, such as gamma-secretase modulators, but also drugs that specifically interfere with docking of specific substrates to the different γ-Secretases. Finally insight into the regulation of the γ-Secretase complex only starts to emerge, which also could open new opportunities for safer drugs. Inhibitors of BACE1 are going forward in the clinic. However, increasing insight into the biological function of this crucial protease e.g. in growth cone collapse and axonal outgrowth, calls for caution.

The Importins of Axonal Transport in Neuronal Growth and Regeneration

Mike Fainzilber

Dept. of Biological Chemistry, Weizmann Institute of Science, Rehovot, Israel

Retrograde axonal injury signaling stimulates regenerative responses by the cell body in lesioned peripheral neurons (1). We have previously shown essential roles for importins in dynein-dependent axonal injury signaling (1, 2). My presentation will cover recent (3) and unpublished data on new components of this mechanism, and how it might be connected to intrinsic length and size sensing in neurons (4), with implications for axonal growth and maintenance.

1. Rishal & Fainzilber, 2014: Axon-soma communication in neuronal injury. *Nature Reviews Neuroscience 15*, 32-42.
2. Perry *et al*., 2012: Subcellular knockout of importin β1 perturbs axonal retrograde signaling. *Neuron 75*, 294-305.
3. Rishal *et al*., 2012: A motor driven mechanism for cell length sensing. *Cell Reports 1*, 608-616.
4. Albus *et al*., 2013: Cell length sensing for neuronal growth control. *Trends Cell Biol. 23*, 305-310.

Regulation of MT dynamics in axon regeneration

Yishi Jin

University of California, San Diego, USA

Axon injury triggers a profound reorganization of the axonal cytoskeleton that is a prerequisite for effective axon regeneration. Using the C. elegans axon regeneration model, we have performed large-scale screening for conserved factors affecting axon regeneration. Our studies have discovered a critical role of the conserved DLK-1 kinase in injury response. We have also identified a novel axon regeneration inhibitor, EFA-6/Exchange-Factor-for-Arf6. The EFA-6/EFA6 protein family is conserved from yeast to mammals, and is defined by its Sec7 domain, which confers guanine exchange factor (GEF) activity for Arf6 GTPases. Unexpectedly, we find that the regrowth-inhibitory function of EFA-6 is independent of its GEF activity, and instead is mediated by its N-terminal domain. Our recent studies have shown that axon injury triggers EFA-6-dependent inhibition of microtubule dynamics, concomitant with a rapid relocalization of EFA-6 to the axonal MT cytoskeleton. We have identified conserved MT associated proteins that interact with the EFA-6 N-terminus. We propose a model by which EFA-6 acts as a bifunctional injury-responsive regulator of axonal MT dynamics.

Optic Nerve Regeneration

Larry Benowitz

Boston Children's Hospital; Dept. of Neurobiology, Harvard Medical School, Boston, USA

Like other pathways in the mature central nervous system (CNS) of mammals, the optic nerve cannot regenerate if injured, resulting in lifelong visual losses. In agreement with earlier studies, we found that induction of an inflammatory reaction in the eye leads to appreciable axon regeneration after optic nerve injury. We identified the prin-cipal mediator of this phenomenon as oncomodulin (Ocm), a small Ca2+-binding pro-tein that is secreted by activated neutrophils and macrophages and binds to a high-affinity receptor on RGCs in a cAMP-dependent manner. When combined with a cAMP analog and pten deletion, inflammation-induced Ocm enables some RGCs to regener-ate axons from the eye to the brain. These axons form synapses in appropriate target areas (e.g., dLGN, SCN) and restore some visual responses. Nonetheless, even under optimal conditions, almost 2/3 of RGCs continue to die after nerve injury and most of the surviving RGCs fail to regenerate axons. These observations point to the existence of additional suppressors of cell survival and axon regeneration. Using autometallog-raphy or the fluorescent Zn2+ sensor ZinPyr1 (courtesy Stephen Lippard, MIT), we ob-served a sharp rise in ionic zinc (Zn2+) in the inner plexiform layer (IPL) of the retina within an hour of optic nerve injury. The IPL contains synaptic inputs from amacrine cells and bipolar cells onto the dendrites of RGCs. Levels of Zn2+ within RGCs them-selves increased more slowly. Co-localization studies showed that the initial accumula-tion of Zn2+ occurs primarily in the terminals of amacrine cells that express ZnT3, the protein that transports Zn2+ into synaptic vesicles. Chelating Zn2+ using either TPEN or ZX1 (S. Lippard) eliminated the Zn2+ signal in the IPL and led to enduring survival of many RGCs as well as extensive axon regeneration. Combining Zn2+ chelation with other pro-regenerative treatments enabled some RGCs to regenerate axons the full length of the optic nerve in just 2 weeks. Because the elevation of Zn2+ precedes al-most all other known changes after nerve injury, and because the extent of regeneration obtained by chelating Zn2+ is as great as that of any single treatment reported to date, these studies point to Zn2+ as a major suppressor of axon regeneration in the op-tic nerve and perhaps in other parts of the CNS as well.

Nerve fiber regeneration, new circuit formation and functional recovery after spinal cord and brain injury

Martin E. Schwab

Brain Research Institute, University of Zurich and ETH Zurich, Switzerland

Large spinal cord or brain injuries lead to life-long structural and functional deficits. In contrast, small lesions of the CNS often have a good prognosis with extensive functional recovery; the underlying mechanisms are not well understood, however. Major changes in the neuronal wiring including formation of new circuits and maps were found after spinal cord lesions in adult rats. Spinal cord hemisections induced sprouting of spared reticulo-spinal fibers across the midline of the lower cord, as well as regenerative sprouting of lesioned fibers and formation of a detour pathway via propriospinal neurons around the lesion site.Electrical stimulation of this system induced locomotion in animals with 80-90% destroyed spinal cords. - In a stroke model, destruction of the forelimb motor cortex led to sprouting of hindlimb corticospinal fibers into the cervical spinal cord and the formation of a new, functional forelimb projection from former hindlimb neurons. However, in all these cases extent and length of fiber growth was limited to about 0.2 - 2 mm.

Specific neurite growth inhibitory factors restrict plastic and regenerative neurite growth in the adult CNS. The membrane protein Nogo-A is currently the most potent known neurite growth inhibitor. Nogo-A activates a RhoA cascade via two multisubunit receptor complexes. Function blocking antibodies against Nogo-A have been applied to rats and macaque monkeys with spinal cord injuries as well as animals with very large stroke lesions of the sensory-motor cortex. In the spinal cord, injured fibers showed enhanced regenerative sprouting as well as long-distance regeneration with formation of large terminal arbors. Spared fiber tracts showed enhanced compensatory sprouting, often covering relatively long distances. In animals with cortical strokes, fibers from the intact corticobulbar or corticospinal system crossed the midline, supplying innervation to the denervated brain stem or spinal cord under the influence of anti- Nogo-A antibodies. Behavioral tests for locomotion, grid and beam walk, swimming, as well as skilled forelimb reaching showed marked improvements of functional recovery in the Nogo-A antibody treated injured animals which could be further enhanced by intense rehabilitative training. Pharmacogenetically silencing of the midline crossing corticospinal fibers in the stroke animals abolished the regained skilled forelimb movements. - Antibodies against human Nogo-A are currently used in clinical trials for spinal cord injury, MS and ALS.

Short talks abstract

Deciphering the genetic program of developmental axon remodeling

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Developmental neuronal remodeling is essential for sculpting the mature nervous systems of vertebrates and invertebrates during development. Neuronal remodeling often involves pruning of exuberant neuronal connections and regrowth to new targets as a mechanism to refine neural circuits during development. The stereotypical remodeling of the *Drosophila* mushroom body (MB) γ neurons offers a unique opportunity to study both axon pruning and axon regrowth. Mounting evidence from our lab and others suggest that both axon pruning and developmental axon regrowth are regulated, at least partially, by distinct transcription factors. Thus, the goal of our study is to uncover the genetic program underlying neuronal remodeling of MB γ neurons during development. To mark and isolate small populations of distinct MB neurons, we tested several GAL4 lines from the FlyLight GAL4 collection, and identified one insertion, which enabled us to selectively label MB γ neurons and not any other neuron type. We purified the labeled cell populations using Fluorescence activated cell sorting (FACS) and utilized state of the art techniques in next generation sequencing in order to obtain high quality gene expression profiles from 1000 isolated γ neurons. Preliminary sequencing data indeed highlighted many genes known to participate in remodeling with a developmental transcriptional expression pattern that fits the time points at which the remodeling occurs. These include the proteasome system genes (Uba1, Rpn6, and Mov34) and ecdysone targets (Sox14, Cullin1, and Hdc). These data suggest that our profiling is a robust method to uncover the transcriptional programs of axon pruning and developmental regrowth. We are currently improving our developmental data by obtaining more developmentally relevant time points as well as isolating mutant neurons to identify targets of specific transcription factors. Following up this genomic data with genetic screens should increase our mechanistic understanding of neuronal remodeling. Finally, our database should also serve the entire MB community for studying other developmental processes.

CRISPR-Cas9 knock-in cells for live-cell RESOLFT super-resolution microscopy

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The majority of live-cell microscopy studies rely on cells transiently overexpressing a host protein fused to a green fluorescent protein (GFP)-like protein. However, there is substantial evidence that transiently or constitutively overexpressed proteins may cause a multitude of artifacts including mislocalizations, protein aggregation, violated balanced gene dosage, and others. These overexpression induced problems are presumably even more articulate in studies using diffraction-unlimited super-resolution microscopy, or nanoscopy, which facilitates the visualization of protein localizations and dynamics on a scale inaccessible by conventional light microscopy. Still, so far all live-cell super-resolution microscopy studies of mammalian cells using FPs relied on overexpressed proteins. Recent advances in genome engineering technologies, such as the CRISPR-Cas9 system, permit site-specific endogenous tagging of proteins from their chromosomal loci. Genomic labeling might eliminate overexpression concerns and would allow direct quantification of the abundance, localization and dynamics of proteins under native expression conditions. Therefore we generated various CRISPR-Cas9 knockin neuronal cells and show that live-cell super-resolution microscopy (RESOLFT) can be used for imaging protein dynamics at endogenous expression levels. In particular, RESOLFT microscopy reliably produced super-resolved images of different model structures with optical resolutions of ~ 30-40 nm while allowing multiple recordings to visualize sub-cellular dynamics on different timescales in the genome edited cells. A particular future interest will be the use of quantitative live-cell super-resolution RESOLFT imaging of genome-edited neuronal cells for investigating synaptic vesicle dynamics.

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

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

Proteasome-mediated switching of BAF subunits controls cortical development

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Composed of multiple neuronal subtypes and diverse glial cells, the cerebral cortex underwent a pronounced expansion during evolution that is assumed to underlie the high intellectual capability of primates. Research in the recent years indicated that the immense neuronal diversity in the mammalian cortex, including lower layer (early-born) and upper layer (late-born) neurons, is generated by sequential expression of specific genes during early and late corticogenesis, respectively. However, it remains elusive how such precise spatio-temporal gene expression programs are initiated and maintained. The contribution of epigenetic mechanisms, including DNA/histone modifications and chromatin remodelling is an emerging topic in development. There are increasing number of evidences that point towards the significance of BAF complexes in organ development, including knowledge from human developmental disorders and transgenic mice. BAF (Brg1/Brm Associated Factors) complex is a multi-subunit chromatin remodelling complex that alters the position of nucleosomes thereby regulating gene expression. Although, it is clear that specific BAF subunits selectively interacts with transcription factors to regulate gene expression programs, the logic underlying the composition of the BAF complex remains largely unknown. This is particularly a relevant question, considering the antagonistic functions featured by two of the core subunits of this complex, namely BAF155 and BAF170. Our previous study indicated that the switch from early to late mouse corticogenesis involves the exchange of a BAF170 subunit for a BAF155 subunit within the BAF complex in late cortical progenitors, resulting in the elimination of the REST-repressor complex on Pax6 target gene promoters and inducing euchromatin state that enhances accessibility of Pax6 to its target promoters. The ultimate biological outcome of this BAF subunit exchange is an increase in the expression of IP specific genes and premature expression of late cortical progenitor genes, endowing indirect neurogenesis. To better understand this essential switch in BAF complex composition during neural development, we investigated proteasome-mediated degradation of BAF155 and BAF170. Our preliminary data indicated that overexpression of Trim21, an E3 ubiquitin ligase leads to differential regulation of protein levels of BAF subunits in vitro. With earlier studies suggesting that the transcript levels of most BAF subunits remains largely unaltered during neural differentiation, our data suggests a novel proteasome-mediated mechanism that regulates BAF complex composition during corticogenesis.

The fate of synaptic mitochondria explored by optical pulse chase imaging

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Mitochondria are responsible for producing the majority of cellular ATP in eukaryotic cells, but are also key players in other cellular processes such as apoptotic cell death or calcium buffering. Therefore proper function and distribution of mitochondria is crucial for cellular viability. Given the large size and complex morphology of a neuron, a sophisticated axonal transport machinery has evolved to supply a neuron's periphery with mitochondria. Not surprisingly, disturbed mitochondrial dynamics often contribute to neurodegenerative diseases, such as Parkinson's disease or amyotrophic lateral sclerosis. Various models have been developed to study mitochondria in living cells, which have greatly contributed to gain further insight into the dynamic behavior of mitochondria. However, many questions regarding the life cycle and behavior of individual mitochondria in fully mature neurons in situ still remain unanswered. To address this, we have developed a new imaging technique based on transgenic mice that express photo-activateable proteins in neuronal mitochondria that allow "pulse-chase" experiments and studying the behavior of individual organelles. With this approach, we will test different hypotheses about the supply and turnover of mitochondria in presynaptic terminals. For example, we will measure the residence time of an anterogradely delivered synaptic mitochondrion and ask what determines its retention vs. its retrograde return. Moreover, we hope to gain deeper insight into the role of mitochondrial degradation, fission and fusion in synapses. Preliminary data point to a combination of previously described models, where a subset of newly arriving mitochondria in the synapse becomes anchored in the presynaptic terminal, while another subset is recycled back to the axon.

Impaired spike-timing dependent plasticity at immature MF-CA3 synapses in the hippocampus of NL3 R451C knock-in mice, an animal model of Autism

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Autism Spectrum Disorders (ASDs) comprise a heterogeneous group of neurodevelopmental disorders, characterized by impaired social interactions, communications deficits, stereotyped and repetitive behaviors. In rare cases, ASDs have been linked to single mutations in genes encoding for neuroligins (NLs), postsynaptic adhesion molecules which interact with presynaptic proteins neurexins to ensure the crosstalk between post and presynaptic specializations. The R451C mutation of the NL3 gene (NL3 R451C knock-in) resulted in impaired social behaviors in mice, symptoms reminiscent of those observed in ASD patients. Major alterations in both GABAergic and glutamatergic transmission were observed in NL3 R451C knock-in mice depending on the brain region and the developmental stage. In a previous study (*Pizzarelli and Cherubini, Front Cell Neurosci. 2013*) we found that CA3 principal cells in hippocampal slices from P0-P6 old animals, exhibit an increased GABA release from immature GABAergic interneurons. This may impact on spike precision and synaptic plasticity processes. Here, we used whole cell recordings to test the hypothesis that spike-timing-dependent plasticity (STDP) is altered at immature MF-CA3 synapses of NL3 R451C knock-in mice. The experiments were performed in presence of glutamatergic blockers to isolate MF-GABA mediated postsynaptic currents (MF-GPSCs). Positive pairing (in current-clamp) of presynaptic activity with postsynaptic spiking (10 spikes at 0.1 Hz, presynaptic stimulation 20 ms before postsynaptic spike) induced on the average a reduction in the amplitude of MF-GPSCs in NL3 R451C knock-in vs WT mice (GPSCs; 15-20 min post pairing, the normalized MF-GPSCs were 236 ± 45 % and 93 ± 18 % in WT and in NL3 knockin, respectively). These data show impaired synaptic potentiation after positive pairing at immature MF-CA3 synapses in NL3 R451C knock-in mice. Thus, early GABAergic dysfunctions affecting synaptic plasticity processes can play a critical role in the pathogenesis of ASDs.

Septins are involved in the regulation of neurotransmitter secretion at mice neuromuscular junction

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Exocytosis is facilitated by the formation of a complex between vesicular and plasma membrane SNARE proteins. Interaction of SNARE proteins with septins, GTP-binding cytoskeletal proteins that form hetero-oligomeric complexes with each other and other proteins, suggested the involvement of septins in exocytosis, but the mechanism is unclear. We used the inhibitor of septin organization, forchlorfenuron (FCF), which specifically impairs assembly and disassembly of septin hetero-oligomers without affecting actin or tubulin polymerization. To study the involvement of septins in exocytosis of neuronal synaptic vesicles, we measured neurotransmitter release in mouse phrenic nerve–diaphragm preparations using electrophysiological methods. The preparations were superfused with the following low-Ca, high-Mg^{2+} Ringer solution. The synaptic delay of evoked endplate currents (EPCs) was measured. The EPCs after 1000 nerve stimuli were collected to build the synaptic delay histograms. To quantify the components of the neurotransmitter release, the EPC signals within the time range of ≤3 ms were counted to quantify the synchronous release, and signals in the range of 3 ms to 50 ms were counted to quantify the delayed asynchronous release. The exposure to FCF decreased both spontaneous and stimulated neurotransmitter release. The frequency of spontaneous miniature endplate currents (mEPCs) was decreased in the presence of FCF (20 μM) as like as quantal quantity of synchronous and delayed asynchronous phases of evoked secretion. In addition, FCF altered the kinetics of secretion by altering the duration of a synaptic delay, which is the time interval between the arrival of an action potential at the nerve terminal and the initiation of the evoked EPCs. The results indicate that normal assembly and disassembly of septin oligomers is important for both spontaneous and evoked secretion of neurotransmitters.

Role of Gelsolin in Actin Cytoskeleton Regulation in Dendritic Spines

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The majority of the excitatory postsynaptic sites in the brain reside on dendritic spines - the small protrusions in neuronal dendrites. During development and experience-dependent circuit refinement dendritic spines undergo continuous refurbishment. In this ongoing project we aim to elucidate the molecular mechanisms of actin cytoskeleton remodeling that transduce synapse activation into structural and functional changes in the spine. The actin cytoskeleton is strictly regulated by actin binding proteins. Among those the calcium (Ca2+) - activated protein gelsolin is of particular interest as it can play a role in translating Ca2+ entry into the cell to actin filament disassembly. Gelsolin is expressed ubiquitously in mammalian tissues including the nervous system, and has also been reported to generally be localized to actin-rich structures. Gelsolin knockout mice show some abnormalities in neurons, including impairment in activity-dependent stabilization of actin. This result is surprising in the light of the established role of gelsolin as an actin-severing factor. Thus, it is important to clarify the role of gelsolin in neurons and especially in dendritic spines. We first check how gelsolin is distributed in neurons and how its over-expression influences spine morphology and dynamics. These studies revealed that gelsolin accumulates to dendritic spines. Over-expression of gelsolin in days-in-vitro (DIV) 14 hippocampal neurons resulted in a reduced number of filopodia and thin spines. The analysis of spine dynamics in living neurons revealed that neurons overexpressing gelsolin have a more stable head size compared to control. These results are in line with earlier results of impaired LTP stabilization and indicates that the role of gelsolin differs between neurons and fibroblasts. In the future, we plan to investigate how gelsolin is regulated by synapse activation, Ca2+ entry and pH level changes and how it influences actin dynamics in dendritic spines.

Aging-induced choroid plexus signature negatively affects neurogenesis and cognitive function

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Multiple lines of evidence indicate that non-tissue-autonomous factors modulate brain senescence; however, the sources and roles of signals that shape brain's function in the aged body remain enigmatic. Using multi-organ genome-wide analysis of aged mice, we found that the choroid plexus (CP), an interface between the circulation and the cerebrospinal fluid, shows a type I interferon (IFN-I) dependent gene expression profile (often associated with anti-viral response), whereas IFNγ-dependent signaling in this compartment was downregulated. Uncoupling the aging process of the non-CNS tissues from that of the brain, in a surgical parabiosis model in which old mice shared vasculature with young mice, we further found that the IFNγ signature was induced by the aged systemic milieu, while IFN-I response was triggered by brain-derived signals found in the cerebrospinal fluid of old animals. Finally, adult mice deficient in IFNγ signaling showed premature cognitive decline, while blocking IFN-I signaling within aged CNS partially restored cognitive function and hippocampal neurogenesis, and re-established IFNγ-dependent choroid plexus activity. Our data identify a chronic aging-induced IFN-I response at the CP as a negative regulator of the CP and brain function, and thus suggest a novel target for therapeutic anti-aging intervention.

JNK pathway activation synchronizes neuronal death and glial phagocytosis in Drosophila

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The role of glia in neurodegeneration remains controversial. Vertebrate phagocytic microglia accumulate in the lesions of a variety of neurodegenerative disorders, such as Alzheimer's disease (AD), Parkinson's disease, and multiple sclerosis, and may play both toxic and protective functions for neuronal survival. Multiple factors secreted from activated glia cause neurotoxicity making hard to understand whether glial phagocytosis is a consequence or a reason for neuronal loss. Drosophila does not have inflammation and adaptive immunity, which enables studying the role of glial phagocytosis in neurodegeneration with no influence of inflammation. In this work we show that glia overexpressing the phagocytic receptor SIMU cause neuronal loss in the adult brain. We reveal that the reduction in neuronal number is not a result of apoptosis and provide evidence that SIMU phagocytic ability is required for the neuronal loss. Moreover, SIMU specific overexpression in the adult Drosophila glia is accompanied by motor dysfunction and shorter life span of the affected flies. Our study proposes a novel mechanism of neuronal loss, which might affect neurodevelopment as well as be considered as a cause of neurodegenerative disorders where the brain pathology originates from abnormal phagocytic function of glial cells.

Why are neurotransmitters neurotoxic? An evolutionary perspective

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In the CNS, minor changes in the concentration of neurotransmitters such as glutamate or dopamine can lead to neurodegenerative diseases. We present an evolutionary perspective on the function of neurotransmitter toxicity in the CNS. We hypothesize that neurotransmitters are selected because of their toxicity, which serves as a test of neuron quality and facilitates the selection of neuronal pathways. This perspective may offer additional explanations for the reduction of neurotransmitter concentration in the CNS with age, and an additional role of the blood-brain barrier. It may also suggest a connection between the specific toxicity of the neurotransmitters released in a specific region of the CNS, and is role as a chemical that is optimal for testing the quality of cells in that region.

Cellular mechanisms of neuromuscular synapse elimination

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Synapse elimination is a central event in establishing neuronal networks. During this process, redundant synaptic connections are removed to ultimately result in a precisely wired neural circuit. This process involves local dismantling of axon branches, a process that likely reoccurs in neurodegenerative conditions. The mammalian neuromuscular junction and its transition from multiple to single axonal innervation is an excellent model system for studying synapse elimination. During embryonic development motor neurons built connections with a large number of muscle cells and as a result, each muscle fiber receives multiple inputs at a of individual axonal branches from motor units leaves each muscle fiber with only one innervating axon. The elimination of axonal inputs is driven by an activity-dependent competition between co-innervating inputs, during which one input's synaptic territory shrinks and is taken over by the competitor. The cellular and molecular mechanisms, which are involved in such axonal withdrawal, however, are mostly unknown. In this project we aim to analyse the mechanisms that underlie axon branch dismantling by monitoring axonal transport and cytoskeletal stability in axon branches during different stages of synapse elimination. Time-lapse imaging in neuromuscular explant preparations showed that while organelle delivery correlates with synaptic territory in the winning (i.e. growing input), the dismantling axon branch, once it shrinks below a specific threshold of synaptic occupancy (<40%), showed a relative lack of anterograde organelle delivery. At more advanced stages of dismantling, transport ceases altogether, as we could demonstrate for mitochondria and peroxisomes. Corresponding to this, an increase in microtubule dynamics is seen in branches with less than 40% of synaptic occupancy that correlates with altered posttranslational modifications of microtubules. Pharmacological stabilization of microtubules is sufficient to delay synapse elimination significantly, underscoring the causative role of cytoskeleton destabilization during axon branch dismantling. Currently we are using genetic manipulations to define the molecular mechanisms that mediate microtubular destabilization in single branches. Altogether, this study aims to elucidate how axonal remodelling can occur in a compartmentalized fashion within healthy developing neurons to allow establishment of a mature connectivity pattern.

Sprouting assay of primary dissociated Drosophila mushroom body neurons is instrumental in assessing intrinsic axon growth potential

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Axons in the mammalian central nervous system undergo little or no regeneration following injury. In contrast, axons in the peripheral nervous system as well as young neurons have increased capacity to regenerate and resume functionality after nerve injury. What underlies the intrinsic growth capacity of neurons during development or following injury remains mostly unknown. During metamorphosis, the Drosophila brain undergoes stereotypical neuronal remodeling, which includes pruning and regrowth of mushroom body (MB) γ neurons to form adult specific connections. We have recently found that the nuclear receptor UNF (Nr2E3) is required for developmental axon regrowth but not initial outgrowth of MB axons. While several lines of evidence suggest that developmental regrowth and regeneration following injury share molecular mechanisms, we set out to better understand the relationship between these processes. To better compare between different types of axon growth, we have established a spouting assay in culture of dissociated primary neurons. We used the MARCM technique to both mark and manipulate MB neurons, and compared the sprouting abilities of dissociated WT and mutant neurons originating from flies at different developmental stages. As early as one day following dissociation, neurons grew neurites in culture, and these neurites became longer over time. The sprouting ability of WT MB neurons depended on the developmental stage the neurons were derived from: Neurons derived from third-instar (L3) larvae or the pupal stages had increased capacity to sprout, whereas neurons derived from adult flies sprouted very short neurites. In addition, we found that, as expected, the TOR pathway plays a role in sprouting: TOR-/- neurites derived from L3-larvae were shorter than WT, whereas PTEN-/- neurites derived from adult flies were longer than WT. Likewise, we found that DLK (also known as wallenda in the fly), previously shown to be required for regeneration in vertebrates and invertebrates, was also required for sprouting of dissociated neurons. Interestingly, however, DLK was not required for developmental axon regrowth, suggesting that these are distinct axon growth programs. In contrast, we found that UNF, which is required for developmental regrowth, was not required for sprouting. These results suggest that some genes are required for both developmental regrowth and sprouting (TOR pathway), others are required only for sprouting (DLK) or developmental regrowth (UNF) suggesting that these processes are governed by distinct transcriptional programs, and this sprouting following dissociation assay is a complimentary analysis of intrinsic axon growth potential.

Length Matters: Hydrophobic Mismatch Sorts SNARE Proteins into Distinct Membrane Domains

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The clustering of proteins and lipids in distinct microdomains is emerging as an important principle for the spatial patterning of biological membranes. Such domain formation can be the result of hydrophobic and ionic interactions with membrane lipids as well as of specific protein-protein interactions. In this meeting, I will present true multidisciplinary approach to tackle membrane patterning that includes biochemical assays (in vitro membrane reconstitutions, analysis of cell membranes), cutting-edge biophysical analysis (twocolor- STED nanoscopy, FCS, FRET, imaging ellipsometry) and molecular dynamics simulations. Using plasma membrane-resident SNARE proteins as model, we now show that cholesterol induced hydrophobic mismatch between the transmembrane domains and the membrane lipids not only suffices to induce clustering of proteins, but can also lead to the segregation of structurally closely homologous membrane proteins in distinct membrane domains. Domain formation is further fine-tuned by interactions with polyanionic phosphoinositides and proteins. Our findings demonstrate that the structural organization of membranes is governed by a hierarchy of interactions with hydrophobic mismatch emerging as one of the fundamental physical principles. Segregating SNARE proteins into distinct clusters at the plasma membrane has three key functional implications: (i) clusters act as the local hot spots for the vesicle recruitment, (ii) the local enrichment provides sufficient number of proteins necessary for the fast, evoked synaptic release, (iii) closely homologous SNARE proteins such as synataxin 1 and 4 are segregated in non-overlapping membrane domains which is essential for their distinct roles in regulated (sytnaxin 1) and constitutive (syntaxin 4) exocytosis.

A Protein S-dependent mechanism for neural stem cell / progenitor proliferation and differentiation

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Neurogenesis is the process through which proliferation and maturation of neural stem cells (NSCs) results in the continuous generation of new, functional neurons and glia throughout life. Therefore, NSCs are a continuous pool for generating new neurons, which may replace damaged or diseased ones, making them a promising therapeutic tool. We conditionally deleted Pros1 expression in the nervous system (Pros1 cKO) by crossing the Pros1fl/fl mouse line to the Nestin-Cre driver, and tested the effects of Pros1 deletion on proliferation, survival and differentiation of NSCs. Proliferating NSCs in adult control and Pros1 cKO littermates were labeled by bromodeoxyuridine (BrdU) incorporation. Stem cell self- renewal, proliferation and long-term survival, migration and incorporation of newborn neurons into the hippocampal granular zone, were evaluated. PROS1 expression in NSCs was characterized by immunohistochemistry and real-time qPCR. Finally, the effect on NSC signaling pathways was evaluated. We report the expression of PROS1 in NSC of the hippocampal subgranular zone, in young as well as mature neurons, and in astrocytes. Deletion of Pros1 increased NSC proliferation and self-renewal both in-vivo and in neurospheres derived from either embryonic or adult mice. These prolific NSCs survive to adulthood and are found as mature neurons which incorporate into the hippocampal Dentate Gyrus. Significantly less young (DCX+) and mature (NeuN+) neurons were generated from Pros1 cKO NSCs, suggesting an instructive role for PROS1 in neuronal fate determination. Loss of NSC quiescence following Pros1 deletion was mediated through Notch signaling. Pros1 is expressed in mouse adult and embryonic NSCs. Neural-specific deletion of Pros1 expression results in increased NSC proliferation and increased stem cell self-renewal both in vivo and in vitro, suggesting Pros1 regulates NSC proliferation. Pros1 deletion affected the fate of dividing NSCs progeny, reducing the number of newborn neurons. Together, our results suggest Pros1 is a new and important regulator of adult neurogenesis.

Induced Neural Stem Cells Achieve Long-Term Survival and Functional Integration in the Adult Mouse Brain

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Recently, we have shown that mouse fibroblasts were directly converted into expandable multipotent neural stem cells (i.e., induced neural stem cells; iNSCs) without passing through a pluripotent stage. iNSCs show the defining properties of primary neural stem cells; therefore, these cells offer an attractive alternative to induced pluripotent stem cells (iPSCs) technology with regard to regenerative therapeutic applications. In order to be relevant for cell replacement therapies long-term survival, differentiation and integration of iNSCs and their progeny is of crucial importance. To address this issue we transplanted GFP labeled iNSCs into the cortex and the hilus of the dentate gyrus and conducted their analysis six month after transplantation. In order to minimize immune rejection we used NOD.SCID mice. This study is the first to perform an in vivo long-term analysis of transplanted iNSCs in the adult mouse brain. iNSCs showed sound in vivo long term survival rates without graft overgrowths. The cells displayed a neural multilineage potential with a clear bias toward GFAP- positive astrocytes. The cells also showed a permanent downregulation of progenitor and cell cycle markers, indicating that iNSCs are not predisposed to tumor formation. Furthermore, the formation of synaptic connections as well as neuronal and glial electrophysiological properties revealed that differentiated iNSCs migrated, functionally integrated, and interacted with the existing neuronal circuitry. We conclude that iNSC long-term transplantation is a safe procedure; moreover, it might represent an interesting and novel tool for future personalized cell replacement therapies.

Poster Abstracts

Cerebroventricular nitric oxide represses choroid plexus NFκB activation for leukocyte trafficking

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Chronic neuroinflammation is evident in brain aging and neurodegenerative disorders, and is often associated with excessive nitric oxide (NO) production within the central nervous system (CNS). Under these conditions, increased NO levels are observed the choroid plexus (CP), an epithelial layer that forms the blood-cerebrospinal fluid-barrier (B-CSF-B) and serves as a gateway for physiological immunosurveillance of the CNS and beneficial leukocyte recruitment following injury. Here, we hypothesized that excessive cerebral NO levels may interfere with CP function for leukocyte trafficking to the CNS. We found that induction of leukocyte trafficking determinants by the CP, as well as subsequent myeloid and T cell migration to the CSF, are dependent on the CP epithelial NFB/p65 signaling pathway, which was experimentally suppressed by NO. We further found in the 5XFAD transgenic mouse model of Alzheimer's disease, that elevated NO levels at the CP suppress local epithelial NFB/p65 signaling, an effect that was reversed following administration of an NO scavenger. Together, our findings identify cerebral NO as a negative regulator of CP gateway activity for immune cell trafficking to the CNS.

Rapamycin suppresses development of Alzheimer-like pathology in senescence-accelerated OXYS rats

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Alzheimer's disease (AD) is the most common neurodegenerative disorder that causes dementia against atrophic changes in the brain. Experimental studies aiming to understand the mechanisms of pathogenesis and progression as well as promising new targets for AD-directed therapeutics are complicated by the limitations of animal models. In recent years, our group found strong evidence promising for the study of the mechanisms of brain aging and neurodegenerative processes similar to those seen in AD in the senescence-accelerated OXYS rats. The manifestation of the behavioral alterations, learning and memory deficits are develops after 4th week of age, i.e. simultaneously with first signs of neurodegeneration detected by MRI and light microscopy. With age, neurodegenerative changes in the brain of OXYS rats amplified. We have shown that this is happening against the background of overproduction of amyloid precursor protein, accumulation of β-amyloid protein, and hyperphosphorylation of tau in the hippocampus and cortex. OXYS rats have been used successfully to assess the effectiveness of new treatments. For instance we showed that rapamycin treatment (0.1 or 0.5 mg/kg from the age of 1.5 to 3.5 months) decreased anxiety and improved locomotor and exploratory behavior in OXYS rats. In untreated OXYS rats, MRI revealed an increase of the area of hippocampus, substantial hydrocephalus and 2-fold increased area of the lateral ventricles. Rapamycin treatment prevented these abnormalities, erasing the difference between OXYS and Wister rats (used as control). All untreated OXYS rats showed signs of neurodegeneration, manifested by loci of demyelination. Rapamycin decreased the percentage of animals with demyelination and the number of loci. Levels of Tau and phospho-Tau were increased in OXYS rats (compared with Wistar). Rapamycin significantly decreased Tau and inhibited its phosphorylation in the hippocampus of OXYS and Wistar rats. Importantly, rapamycin treatment caused an increase in levels of S6 and phospo-S6 in the frontal cortex, indicating that some downstream events were compensatory preserved, explaining the lack of toxicity. We conclude that rapamycin in low chronic doses can suppress brain aging.

The JNK pathway and the adhesion molecule Fasciclin 2 converge to regulate developmental axon pruning

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Neuronal remodeling is essential for the proper development of vertebrate and invertebrate nervous systems. Remodeling often involves pruning of exuberant neuronal connections in a process that shares molecular and mechanistic characteristics with axon degeneration during injury and disease and following injury. We use the stereotypical neuronal remodeling of the Drosophila mushroom body (MB) γ neurons to study the mechanism of axon pruning. Using a mosaic forward genetic screen we identified basket (bsk), the Drosophila JNK, as cell autonomously required for normal pruning of MB γ neurons axons. Remarkably, dendrite pruning occurs normally in bsk-/-MB clones. Genetic experiments suggest that Bsk does not function via its 'classical' targets such as c-Jun (Jra) and Fos (kay). Furthermore, both Bsk and phospho-Bsk are enriched within MB axons throughout development suggesting that Bsk functions within the axons to regulate pruning by phosphorylating a cytoplasmic target. A serendipitous finding led us to uncover that the pruning defect in bsk-/-clones is mediated by the cell adhesion molecule, Fasciclin 2 (FasII), the N-CAM ortholog. We found that FasII distribution is altered within MB γ neurons expressing a dominant negative form of Bsk (BskDN), such that it is concentrated towards the distal axon tips. Remarkably, MB γ neurons doubly mutant for bsk and fasII prune normally suggesting that FasII mislocalization is critical for the bsk pruning defect. Moreover, we found that overexpressing FasII is sufficient to inhibit pruning of MB neurons and this is significantly augmented by a mild perturbation of bsk, indicating that there is a strong genetic interaction between these proteins. Taken together, this study reveal a novel interaction between the JNK pathway and the adhesion molecule FasII in regulating developmental exon pruning.

Live imaging of neuronal remodeling in cultured Drosophila brains

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Following initial wiring of neural circuits, they often undergo neuronal remodeling in a process that is fundamental for the establishment of accurate neural circuits in both vertebrate and invertebrate brain. Neuronal remodeling often involves the elimination of specific axons and dendrites followed by regrowth to form adult specific connections. Currently, the molecular and cellular mechanisms that regulate developmental neuronal remodeling have not been fully delineated. Understanding these mechanisms should provide us with a broad understanding of axon fragmentation and elimination during development, disease and after injury.

Drosophila melanogaster provides a unique model to study developmental neuronal remodeling. During metamorphosis, stereotypic neuronal remodeling occurs on a massive scale thereby sculpting the adult fly nervous system. These processes include axon pruning of mushroom body (MB) γ neurons followed by regrowth of the adult specific axons to new targets. Even though this process was described more then a decade ago, our understanding of the cellular and molecular mechanisms has been limited. One major impediment to fully investigate the cellular mechanisms of neuronal remodeling has been that up until now it was not possible to live-image neuronal remodeling in Drosophila because it occurs during metamorphosis, when the brain is enwrapped by light scattering fat bodies as well as the pupal case.

In order to follow the dynamic process of neuronal remodeling we established a whole brain culturing system that allows robust live imaging of axon pruning and regrowth during development. This method permits proper ex-vivo development of the brains for about 20 hours. The use of live imaging will allow us to visualize this intricate process in unprecedented temporal resolution while answering basic questions regarding the cellular mechanisms involved.

Change of calcium transient under influence of cholinergic agents at the frog neuromuscular junction

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At the vertebrate neuromuscular junction acetylcholine causes not only the generation of post-synaptic potential but also the modulation of release intensity of subsequent portions of mediator via activation of presynaptic nicotinic and muscarinic autoreceptors. It was shown previously that carbachol, non-hydrolysable analogue of acetylcholine, modulates synaptic transmission by changing the quantity and secretion kinetics of mediator. These effects may be associated with changes in calcium entry into the nerve ending. In this work we studied effects of exogenous and endogenous acetylcholine as well as its mimetics on the relative change in the Ca2+ level (Ca2+-transient) in response to nerve stimulation in axon terminal branches in isolated frog nerve-muscle preparations (m. cutaneus pectoris). Ca2+-transient was evaluated using fluorescent dye Oregon Green Bapta 1. Dye was loaded through the stump of the nerve and optical records of Ca2+-transient were performed using photometric set-up. Non-selective cholinomimetic carbachol (10 mkM) reduced Ca2+-transient by 10%. Application of exogenous acetylcholine (10 mM) as well as acetylcholinesterase inhibition by 1 mkM neostigmine aimed to increase amount of endogenous acetylcholine in synaptic cleft caused similar reduction of Ca2+-transient (by 9 % and 13%, respectively). Blockade of neither nicotinic receptors by d-tubocurarine (10 mkM) nor muscarinic receptors by atropine (1 mkM) prevented the action of carbachol on Ca2+-transient. Effect of carbachol was abolished only by combined application of d-tubocurarine and atropine. Pharmacological methods of research have shown that cholinergic nicotinic and muscarinic (m2-subtype) receptors are involved in the realization of inhibitory action of endogenous acetylcholine on calcium content in the nerve ending. It can be concluded that acetylcholine may be involved in the negative feedback regulation of calcium entry. Action of cholinergic agents on the Ca2+-transient is associated with activation of nicotinic and muscarinic (m2-subtype) autoreceptors.

Molecular mechanisms of neurotrophic receptors spatiotemporal dynamics along neuron cell membrane

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Ligand activation of neurotropic receptors mediates dynamic re-organization along the cell membrane, leading to both local and long distance signaling events. However, the interconnection between their organization, activation and signaling is little understood. Here using live TIRF imaging, we explore the early steps of membranal re-organization of the TrkB receptor in response to its ligand, BDNF. First we measured the bulk mobility of TrkB receptor along the membrane of primary motor neuron cultures after BDNF by FRAP. We demonstrate a distinct reduction in the ratio of mobile to immobile without a change in the diffusion rates. Next, by utilizing single particle tracking analysis assay, we estimated motility profile of TrkB receptor along the cell membrane. While most of these maintained their diffusive mobility, a small subset of the receptor shifts into stationary mode, and another into directed transport mobility, as appreciated by distribution analysis of particle trajectories. By co-labeling receptor and microtubules, we demonstrate that these directed transported receptors move on cortical microtubule tracks. Thus, BDNF mediates formation of two activated TrkB populations along the membrane, a confine population in distinct microdomains that may activate local signaling, and a smaller mobile population that may undergo endocytosis and direct transport along microtubule, that responsible for the long distance signaling.

Studying sleep in the model organism *C. elegans*

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Sleep is essential for animal life and found throughout the entire animal kingdom. It is characterized by the absence of voluntary movement within a specific body posture, an increased arousal threshold, homeostatic regulations and altered neuronal activity. Even though sleep is well defined, the function of sleep and its molecular control remains a mystery. The most widely accepted and experimentally supported explanation of sleep is its critical importance for memory processing and synaptic rewiring. Sleep is found in mammals, but is also observed in many other organisms that have a nervous system. In the nematode C. elegans, a developmental sleep-like state that fulfills these behavioral criteria has been identified. Therefore, across distantly related species, sleep shows a critical role for nervous system development, learning and synaptic homeostasis. As sleep has a monophyletic origin and uses conserved neuronal routs and molecular mechanisms in vertebrates and invertebrates, C. elegans is a promising model to study sleep. The nervous system of C. elegans is consistent and has 302 neurons. Each neuron has a known cellular linage and synaptic connectivity. Additionally, C. elegans has a short generation time of 3 days, which allows the use of powerful genetics. Since C. elegans is transparent, many imaging strategies are possible. For behavioral evaluation of sleep, we image the animals in microfluidics devices. This technique allows in vivo imaging without mounting the animals. This increases the viability during image acquisition and enables to record the behavior of the worms, which is fundamental in studying sleep. Moreover, this technique allows functional Ca2+ imaging of neurons and even confocal microscopy in the mobile animal. It also allows us to apply a variety of methods while imaging. Optogenetic methods can be applied for single neuron activation or inhibition in vivo with simultaneous imaging of the behavior. Further, functions of single neurons in neuronal circuitries can be studied by single neuron laser ablation. In addition, RNAi experiments can be conducted to identify the function of genes in sleep. By using this automated technique, the lab identified a sleep-active and -promoting neuron, called RIS, and several more genes and cells which regulate sleep or are regulated during sleep. In summary, studying the neuronal activity underlying sleep in C. elegans is of high general interest, because sleep in C. elegans and vertebrates has common molecular mechanisms, similar neuronal routes and a monophyletic origin. Thus, future findings will also advance the sleep research in vertebrate models.

Changes in the neural network of reading following stroke: A preliminary study exploring similarities and differences between reading networks identified via task-based fMRI and resting-state fMRI

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Stroke can lead to significant disruption of normal neural function. How does the human brain recover cognitive function after such an event? In order to understand how lost functionality is recovered after stroke, it is useful to identify and track neurophysiological changes related to recovery. Numerous studies have looked at neural changes in individuals subsequent to stroke by having stroke participants perform a cognitive task during functional Magnetic Resonance Imaging (fMRI). However, many stroke participants are unable to perform tasks in the scanner due to hemiparesis or cognitive deficits that prevent them from following task instructions. Recently, the use of Resting-State fMRI (RS-fMRI) has allowed researchers to study these individuals, by using a resting paradigm during which participants lie in the scanner and are not required to perform any task. Critically, the correspondence between RS-fMRI and task-based fMRI has not been well established in many cognitive domains, including the domain of reading. In this study we examined the hypothesis that the RS reading network can serve as a good estimate of the neuro-functional network of reading. To do so, we explored the correspondence between a reading network that we identified in RS-fMRI and the reading network we observed during a reading task in neurologically intact participants. Results show that there is a strong correspondence between task-based and RS-fMRI reading networks, specifically in an area of the fusiform gyrus often referred to as the Visual Word Form Area (VWFA, Cohen & Dehaene, 2004), in the Inferior Frontal Gyrus (IFG), and in the right-hemisphere homologue of the VWFA (rVWFA). Using these results we are now able to evaluate the RS reading networks of individuals who have suffered a stroke. Specifically, we can determine how those networks change as a result of the stroke and how they change in response to rehabilitation. This research provides the foundations of a promising approach to understanding the neuroplastic capacities and limitations of the brain's response to neural injury and recovery of function.

Sema6A-plexin-A4 pathway regulates mammary gland sensory innervation

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During development of the peripheral nervous system specific neuronal connections must be established to ensure proper function. Sensory neurons of the dorsal root ganglia (DRG) initially extend their axons to the targets by responding to attractive and repulsive cues expressed in the extracellular environment. At the target, limited amounts of neurotrophic factors control their innervation through regulation of axonal growth, neuronal cell death, and pruning. The mammary gland serves as a model for target innervation of BDNF-dependent sensory neurons. In search for cues that control the innervation process in combination with BDNF we have found a specific expression of the membrane bound repulsive molecule Sema6A in the gland epithelial cells. Importantly, ablation of Sema6A or its receptor, Plexin-A4, causes developmental hyper-innervation of the gland by sensory axons. In agreement, Sema6A collapse the axonal growth cones of the BDNF responsive neurons in a Plexin-A4 dependent manner. Overall our studies suggest that mammary gland innervation is restricted by expression of Sema6A in the gland that signals through Plexin-A4 receptor in the sensory axons.

Sensory experience affects hippocampal plasticity during development

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A fundamental feature of neural circuits is the capacity for plasticity in response to experience or learning. Neocortical circuits display well-defined 'critical periods', characterized by superior plasticity to sensory input in early life. While information from different sensory modalities converges onto the hippocampus, little is known on whether sensory experience influences hippocampal plasticity during postnatal development. Here, we explored the impact of whisker deprivation, applied during the critical period of barrel cortex plasticity, on functioning of CA3-CA1 hippocampal synapses. Bilateral whisker trimming promoted frequency-dependent short-term synaptic facilitation due to reduction in synapse release probability. These presynaptic modifications paralleled with an increase in the AMPA- and NMDA-mediated postsynaptic currents and in the fraction of NR2B-containing NMDARs. Despite these deprivation-induced synaptic modifications, input-output relationship in the CA3-CA1 connections was homeostatically preserved. Our findings suggest that sensory input drives adaptive reconfiguration of hippocampal circuits, limiting hippocampal plasticity during development.

Clickable Unnatural Amino Acids as Tags Secondary Ion Mass Spectrometry Imaging

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The isotopic composition of different materials can be visualized by secondary ion mass spectrometry (SIMS). A mainstream approach in biology is to investigate metabolism and protein turnover using amino acids labeled with stable isotopes (15N, 13C) at a lateral resolution of about 100 nm. This method offers a general protein labeling but in order to identify specific subcellular structures, such as organelles and individual proteins, it is necessary to correlate secondary ion mass spectrometry with fluorescence imaging. The aim of this study is to prove that a method based on specific protein labeling can be used for SIMS measurements making optional the need for correlation with fluorescence microscopy. For this, we have combined SIMS with confocal imaging to visualize proteins specifically labeled via genetic incorporation of the unnatural amino acid propargyl-L-lysine (PRK). This unnatural amino acid can be subjected post-fixation to a click reaction with a novel 19F-labeled fluorophore (SK155). The latter acts as a dual marker for both SIMS and fluorescence imaging. Using this approach, we were able to precisely visualize the proteins of interest labeled with SK155 in both SIMS and confocal microscopy, with minimum background and very good correlation between the 19F and fluorescence signals. In addition, this technique allowed us to analyze specific protein turnover in the context of general cellular metabolism. This proof-of-principle study shows that SK155 can be used as a probe in SIMS measurements for investigating any protein of interest without the constraint of correlating the results with fluorescence imaging. If fluorescence correlation is desired, then this method also allows for more cellular compartments to be labeled by immunostaining.

Chronic exposure to TGFβ1 regulates myeloid-cell inflammatory response in an IRF7 dependent manner

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Following acute injury in the central nervous system (CNS), there is an immediate and crucial phase of microglial activation; however, these cells fail to acquire an inflammation-resolving phenotype (M2-like phenotype), unlike the recruited monocyte-derived macrophages (mo-MΦ), which display distinct activities in the lesion site, and have a pivotal role in the repair process by resolving the microglial-induced inflammation. Here we demonstrate a novel microenvironment-derived molecular mechanism that regulates the distinct fate of resident myeloid-derived cells under physiological and pathological conditions. We found that long exposure to Transforming Growth Factor-β1 (TGFβ1), a constitutively expressed anti-inflammatory cytokine in the CNS milieu, causes changes in gene circuitry that render myeloid-cells refractory to switch from pro- to anti- inflammatory phenotype under inflammatory conditions. Using genome-wide expression analysis and chromatin immunoprecipitation followed by next generation sequencing, we show that the capacity to undergo phenotype-switch is controlled by the transcription factor Interferon regulatory factor-7 (IRF7) that is down-regulated by the TGFβ1 pathway. We found that following spinal cord injury (SCI), microglial failure to acquire resolving phenotype is correlated with reduced expression levels of IRF7, relative to mo-MΦ. While RNAi-mediated perturbation of IRF7 in macrophages inhibited the phenotype switch, the induction of IRF7 using IFNβ1, reversed the impairment to undergo phenotype switch induced by TGFβ1 imprint in vitro, and reduced the expression levels of microglia-derived pro-inflammatory cytokines in vivo following SCI. These results highlight the major effect of tissue-specific environmental factors on the phenotype of resident myeloid cells, not only during homeostasis, but also in their subsequent functional response to pathology

Behavioral Profile of *Nymphea lotus* Flowers in Animal Model of Depression

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Background and Objectives: Nymphaea lotus Linn, popularly called Water lily has been used traditionally for the treatment of anxiety, depression and as a nerve tonic. No clinical or animal experimental data demonstrate that N. lotus acts by biochemical mechanisms similar to a serotonin reuptake inhibitor (yohimbine). Thus we compare in this study, its efficacy to a clinically standard used antidepressant drugs. Materials and methods: The effect of aqueous extract of N. lotus was compared with Yohimbine, using an animal model of depression: the unpredictable chronic mild stress paradigm. Forced swim test (FST), Sucrose preference test and locomotor activity tests were performed at the end of treatment. Plant aqueous extract and Yohimbine were administered to rats orally. Results: Our findings indicated that 14-day administration of aqueous extract of N. lotus as well as Yohimbine administration produced antidepressant-like effects in the forced swim (FST) and sucrose preference tests. In the FST, N. lotus extract (75 and 200 mg/kg) caused a dose dependent reduction in immobility time in rats with maximal effect at 200 mg/kg. Yohimbine (2 mg/kg) produced similar reduction in the immobility time in rats. In the locomotor activity test (OFP), N. lotus increased the locomotors counts of rats similar to the standard antidepressant. Conclusion: N. lotus has antidepressant properties similar to Yohimbine. The antidepressant profile of N. lotus is closely related to Yohimbine, which is a alpha 2-adrenoreceptors inhibitors class of antidepressants.

Glutamatergic neuron-targeted loss of LGI1 epilepsy gene results in seizures

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Leucin-rich, glioma inactivated 1 (LGI1) is a secreted protein linked to human seizures of both genetic and autoimmune etiology. Mutations in LGI1 gene are responsible for autosomal dominant temporal lobe epilepsy with auditory features (ADEAF), while LGI1 autoantibodies are involved in limbic encephalitis, an acquired epileptic disorder associated with cognitive impairment. We and others previously reported that Lgi1-deficient mice have early-onset spontaneous seizures leading to premature death at 2-3 weeks of age. Yet, where and when Lgi1 deficiency causes epilepsy remains unknown. To address these questions, we generated Lgi1 conditional knockout (cKO) mice using a set of universal Cre-driver mouse lines. Selective deletion of Lgi1 was achieved in glutamatergic pyramidal neurons during embryonic (Emx1-Lgi1cKO) or late postnatal (CaMKIIα-Lgi1cKO) developmental stages, or in GABAergic parvalbumin interneurons (PV-Lgi1cKO). Emx1-Lgi1cKO mice displayed early-onset and lethal seizures, whereas CaMKIIα-Lgi1cKO mice presented late-onset occasional seizures associated with variable reduced lifespan. In contrast, neither spontaneous seizures nor increased seizure susceptibility to convulsant were observed when Lgi1 was deleted in parvalbumin interneurons. Together, these data showed that Lgi1 depletion restricted to pyramidal cells is sufficient to generate seizures, while seizure thresholds were unchanged following depletion in GABAergic parvalbumin interneurons. We suggest that Lgi1 secreted from excitatory neurons, but not parvalbumin inhibitory neurons, makes a major contribution to the pathogenesis of Lgi1-related epilepsies. Our data further indicates Lgi1 is required from embryogenesis to adulthood to achieve proper circuit functioning.

Transport and Localization of RNA-induced Silencing Machinery in Axons

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In order to survive and maintain normal function neurons depend on a dynamic system of spatial specificity and fidelity of signaling pathways that can respond to both internal and external changes over space and time. Local protein synthesis in neuronal axons far from the cell body plays an important role in this essential spatiotemporal signaling process. The molecular basis for this post-transcriptional regulation however, is still poorly understood. As microRNAs are key players in gene regulation and play a vital role in motor neuron survival and synapse maintenance, we tested the possibility that proteins involved in the biogenesis and silencing function of microRNA, namely Dicer and Argonaute, transport from the cell body to axons, and locally control synthesis. First, we identified the expression of microRNAs, Dicer and Argonaute in cultured embryonic motor neuron axons in vitro, as well as at the adult neuromuscular junction in vivo. Then, using live cell imaging techniques we track the transport of the microRNA machinery in the axon. Future work will focus on the characterization of transport and localization of the silencing machinery, and their contribution to neurodegeneration.

Chronic exposure to CNS microenvironment impairs microglia switch from pro- to anti- inflammatory phenotype

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Following central nervous system (CNS) injury, resident microglia often fail to resolve local inflammation, and recruitment of monocyte-derived macrophages (mo-MΦ) is required for repair due to their ability to acquire an inflammation-resolving (M2) phenotype. Microglia and mo-MΦ differ in origin and maintenance throughout adulthood; while mo-MΦ are freshly renewed in the bone-marrow from hematopoietic stem cells, microglia are long lived and maintained in the specialized CNS microenvironment. In the present study we address the hypothesis that this unique microenvironment is imprinting microglia incompetence to resolve inflammation under pathological conditions. We found that chronic exposure to TGFβ1, an abundant cytokine in the CNS microenvironment, impaired the ability microglia to switch phenotype from inflammatory to inflammation-resolving, anti-inflammatory (M2), phenotype. Using genome-wide expression analysis and chromatin immunoprecipitation followed by next generation sequencing, we show that the capacity to undergo pro- to anti- inflammatory phenotype switch is controlled by the transcription factor Interferon regulatory factor-7 (IRF7) that is down-regulated by the TGFβ1 pathway. In vivo induction of IRF7 expression in microglia following spinal cord injury reduced their pro-inflammatory activity. These results emphasize that the CNS microenvironment might be a double-edged sword, enabling important functions under normal physiological conditions, but imprinting microglia incompetence under severe pathological conditions.

The relative parts of specific activity histories and spontaneous processes in synaptic remodeling in ex-vivo networks of cortical neurons

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Activity-induced modification of synaptic connections (synaptic plasticity) is widely believed to represent a fundamental mechanism for modifying neuronal network function. It is also commonly, often implicitly, presumed that synapses do not change spontaneously or in manners unrelated to their activation histories. Recent studies indicate, however, that synapses also exhibit significant spontaneous remodeling even in the absence of electrical activity, that is, in the absence of specific instructive forces. An important question thus arises: What are relative parts of specific activity histories versus spontaneous processes in the remodeling synapses undergo? To address this question we have developed a system based on primary cultures of rat cortical neurons growing on multi-electrode array substrates, fluorescent reporters of synaptic size, and automated microscopy, and used this system to follow individual synapses belonging to pairs of connected neurons over periods of several days, while concomitantly recording activity in the same networks. Our preliminary results indicate that the part of specific activation histories shared by particular synaptically connected neurons in dictating the remodeling of individual synapses is surprisingly small in comparison to other processes that might be attributed to global activity histories or spontaneous, activity-independent remodeling processes.

Rabies Virus Hijacks and Accelerates the p75NTR Retrograde Axonal Transport Machinery

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Rabies virus (RABV) is a neurotropic virus that depends on long distance axonal
transport along microtubules, in order to reach the central nervous system (CNS). The strategy RABV uses to hijack the cellular transport machinery is still not clear. It is thought that RABV interacts with membrane receptors in order to internalizeand exploit the endosomal trafficking pathway, yet this has never been demonstrated directly. The p75 Nerve Growth Factor (NGF) receptor (p75NTR) binds RABV Glycoprotein (RABV-G) with high affinity. However, as p75NTR is not essential for RABV infection, the specific role of this interaction remains in question. Here we used live cell imaging to track RABV entry at nerve terminals and studied its retrograde transport along the axon with and without the p75NTR receptor. First, we found that NGF, an endogenous p75NTR ligand, and RABV, are localized in corresponding domains along nerve tips. Furthermore, RABV and NGF were internalized at similar time frames, suggesting comparable entry machineries. Next, we demonstrated that RABV could internalize together with p75NTR. Characterizing RABV retrograde movement along the axon, we showed the virus is transported in acidic compartments, mostly with p75NTR. Interestingly, RABV is transported faster than NGF, suggesting that RABV not only hijacks the transport machinery but can also manipulate it. Intriguingly, co-transport of RABV and NGF identified two modes of transport, slow and fast, that may represent a differential control of the trafficking machinery by RABV. Finally, we determined that p75NTR-dependent transport of RABV is faster and more directed than p75NTR-independent RABV transport. This fast route to the neuronal cell body is characterized by both an increase in instantaneous velocities and fewer, shorter stops en route. Hence, RABV may employ p75NTR-dependent transport as a fast mechanism to facilitate movement to the CNS.

The Life Cycle of the Synaptic Vesicle

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Ageing is a phenomenon that describes the gradual functional deterioration that occurs in most complex lifeforms and has traditionally been studied mainly at the organism level. However, the cause of major ageing-related processes is often to be found at the cellular and organelle level. Particularly the ageing of the nervous system, more specifically of synaptic connections, is the basis for many degenerative effects linked to ageing in humans. However, the life cycle of synaptic vesicles, the principal organelles of synaptic transmission, is largely unknown, as is the number of times they can undergo release and recycling before undergoing degradation. Synaptic vesicles can be broadly divided into two functional categories: recycling pool vesicles which undergo fusion with the cell membrane to release neurotransmitter in response to a stimulus, and reserve pool vesicles which are largely inert and do not respond to stimulation. It has been unclear until now what distinguishes synaptic vesicles in these two pools, how transitions between these two pools are regulated, and in which sequence synaptic vesicles pass through these pools during their lifetime. We describe here the complete life cycle of the synaptic vesicle, from biogenesis via participation in neurotransmitter release to retirement and degradation. We found that the average synaptic vesicle recycles ~300 times at the beginning of its lifetime, then becomes inactivated via accumulation of molecular contaminations from the cell membrane during this recycling phase, which leads to functional deterioration of the organelle. However, it remains at the synaptic bouton for several days, serving as a buffer for soluble proteins involved in release and recycling of the next generation of vesicles, before eventually undergoing degradation in the cell body. This is the first complete description of the life cycle of a synaptic vesicle, including an explanation for functional pool transitions and providing a basis for understanding organelle ageing. We demonstrate that synaptic communication is critically dependent on constant replacement of ageing synaptic vesicles with freshly produced ones and provide a hypothesis of how the cell deals with functionally deteriorating organelles.

Newly Synthesized Neuropeptides with CNS activity in mice

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The modern drug design creates medicaments on the basis of well-known active peptides with improved pharmacokinetic properties. Object of present study are two newly synthesized neuropeptides with short chains (Pajpanova et *al*, 2013) - analogues of Tyr- MIF – 1 with code P1 and of Nociceptine with code P2. Their molecular design, similar to some neurotransmitters in the central nervous system (CNS) suggests their possible CNS activity. Our previous data demonstrated their low oral and intraperitoneal toxicity, biological activity and effective doses. Purpose: To study the CNS activity of both new neuropeptides in experimental mice. On male Albino mice we studied the changes in the cognitive functions of animals after 3, 7 and 14 days pretreatment with both compounds (5 mg/kg intraperitoneally) using classic cognitive methods: step through test (for learning and memory), Rot-a-rod test (for muscular coordination) and Hole board test (for exploratory activity). Their potential analgesic effect was evaluated by chemical irritation (Acetic acid test) and their central nervous activity via interaction with hexobarbital (HB- 100 mg/kg i.p). Molecular modeling of both new compounds was used for predicting their potential neuronal receptors . Statistic was performed with Student – Fisher test. On the 3rd day after treatment it wasn't found significant effect of compounds on cognitive functions of animals, but on the 7th day only the analogue of Tyr- MIF –1 (peptide P1) has significant improving effect on the memory (by 60 %) and decreased also the exploratory activity of treated animals. The analogue of Nociceptine- P2 demonstrated significant dose-dependent analgesic effect. On the 14th day both compounds improved neuro-muscular coordination of animals. In single doses two compounds shorten significantly duration of hexobarbital narcosis (P1 by 40% and P2 by 50%) via unknown mechanism, probably related to functional antagonism between the neuropeptides and hexobarbital on CNS level. Molecular docking gave some suggestions about compound- receptor interactions. Newly synthesized neuropeptides are biological active substances with effect on CNS. The analogue of Tyr- MIF –1 improves cognitive function of animals and the analogue of Nociceptine has significant dose-dependent analgesic effect.

Regulation of amyloid-beta 40/42 ratio by spiking patterns *in vivo* and *in vitro*

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Strong genetic evidence suggests that familiar Alzheimer's Disease (FAD) is linked to mutations in amyloid precursor protein (APP) and presenilin (PS), the catalytic subunit of the γ-secretase complex, leading to attenuation in theratio between cerebral Aβ40 and Aβ42 (Aβ40/42). However, FAD mutations account for only 1-2% of AD patients, leaving experience-dependent mechanisms leading to synaptic dysfunction an enigma. Recent work in our laboratory demonstrates that stimulation patterns regulate Aβ40/42 dynamics in acute hippocampal slices (Dolev et *al*, 2014).In particular, spike bursts boost the Aβ40/42 mainly via an increase in Aβ40production. In the present study, we wish to determine the relationship between stimulation pattern in the medial perforant path (mPP), the most vulnerable connections in the early AD stage, the Aβ40/42, and synaptic transmission in anesthetized and behaving FAD transgenic mouse model. Ourresults in anesthetized mice indicate that one hour of the mPP stimulation bybursts induces an increase in the levels of soluble Aβ40, augmenting theAβ40/42 ratio in a soluble fraction that is known to correlate with the severity of AD. Conversely, insoluble Aβ levels and the Aβ40/42 ratio were not affected by burst stimulation in the mPP. To determine the molecular mechanism underlying regulation of the Aβ40/42ratio by spike bursts, we utilize FRET) methodologies to monitor burst-induced conformational change within the presenilin-1 (PS1). Our results show that deletion of APP protein abolishes the effect of bursts on PS1 conformational change, indicating a crucial role of APP substrate in the sensibility of PS1 conformation to neuronal activity pattern. We continue to identify the APP molecular domains that mediate pattern-dependent change in the PS1 conformation. At the next step, we plan to start chronic stimulations in the mPP of behaving FAD model mice and examine how itaffects neuronal functioning and the disease progression. The proposed research may offer novel therapeutic and conceptual insights to prevent Aβ40/42 decline in AD.

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Transcriptional Profiling of Hypocretin/Orexin (HCRT) neurons, reveals new players in the regulatory network

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The hypothalamus is a central regulator that interacting with the sleep\wake centers, endocrine system, the amygdale and the circadian clock regulates the more basic physiological functions of the body. In particular, the HCRT (hypocretin/orexin) is one of the centers forming the hypothalamus that coordinates the regulation of those functions. To gain a further insight into the molecular underpinnings that distinguish this neurons from other centers of the brain, we have utilized the transgenic line *hcrt:EGFP* as a tool to isolate HCRT neurons for transcriptome profiling. The HCRT is composed by only 16-20 neurons that were sorted by FACS; mRNA was then purified, amplified and subjected to RNA-seq. A thorough bionformatic analysis of the data obtained led to the identification of new putative key players in the HCRT regulatory network. Exquisite expression in HCRT or adjacent cells was validated for tens of candidate genes using *in-situ* hybridization followed by immunofluorescent staining. Hybridization results also described novel hypothalamic populations probably involved in the control of the physiological functions. A further HCRT genome-wide analysis of transcription factors revealed genes that may be engaged in the control of the genes expressed by HCRT neurons and the hypothalamic populations described in this work.
The HCRT transcriptome data set presented here provides a platform for a thorough exploration of the genes active in the regulation of basic physiological processes such as metabolism, sleep-wake cycles and endocrine functions. Thus, a molecular function analysis of the genes, the transcription factors and the long non-coding RNA molecules found in this research will facilitate understanding the complex interaction between the HCRTs and additional nuclei of the hypothalamus and the brain in general. This will lead to a better understanding of how the genetic program controlling HCRT functions unfolds and is converted into regulatory modules that drive the different HCRT tasks. Our results point to a variety of molecules that have yet to be studied and offer a panoramic view of the transcriptome of the HCRT system.

Axonal Transcription Factors Are Retrograde Injury Signals in Lesioned Sensory Axons

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Sciatic nerve lesion induces retrograde transport of injury signals that initiate regeneration responses in the cell body. Earlier work has shown that nuclear import factors from the importins family are involved in injury signal transport in axons, but the identities of these importin-dependent retrogradely transported molecules are still largely unknown. Transcription factors require importin binding to access the nucleus for their primary functions in cells, and therefore seemed good candidates for importin-dependent retrograde injury signaling in axons. A combination of axonal phosphoproteomics and cell body transcriptome analyses suggested a number of transcription factor families as candidate axonal signaling molecules. This presentation will focus on recent data supporting roles for signal transducer and activator of transcription-3 (STAT3) and peroxisomal proliferator activated receptor γ (PPARγ) as retrograde injury signals in sensory axons.

Identifying Potential Pathways and Target Proteins for Head Injury Therapeutics

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A wide range of neuropathologies are associated with Traumatic Brain Injury (TBI), yet there is a lack of understanding of the disease mechanisms underlying TBI and no effective therapeutic strategies. To date, there are no U.S. Food and Drug Administration (FDA)-approved biomarkers for the diagnosis or prognosis of TBI, and the molecular mechanisms of TBI response remain poorly understood. This lack of understanding reflects the complex, multifactorial nature of secondary cellular responses to TBI, which are believed to involve a network of interweaving molecular pathways that mediate cellular response. Our preliminary data suggest that integrins, transmembrane proteins that couple the intracellular cytoskeleton with the extracellular matrix (ECM), are associated with pathological activation of mechanotransduction signaling events in axons after a blow to the head. In this work, we mimic axonal injury in vitro and identify the main biological pathways affected by consecutive injuries to identify therapeutic opportunities. Furthermore, we target key proteins that were preliminarily identified in our lab as potential biomarkers and therapeutic targets (e.g. Src kinase, Rho). The implications of this research may have wide-ranging impact on the diagnosis and treatment of TBI, particularly the identification of potential biomarkers of injury, drug targets for therapeutic intervention, and the development of new regulations on the design of head protection gear.