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Glutamate homeostasis revisited - neuronal transport and metabolism

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Symposia
S01 History of Neurochemistry

S01-01
Canadian neurochemists and roles in ISN/ASN

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Canadians have made diverse contributions to neurochemistry, including notable scientific advances, service to ISN and Journal of Neurochemistry (JNC). The 17 Canadian members at ISN's foundation (1967) came from different areas of biochemistry, physiology and medicine. First ISN Chairman (1967-69) Roger Rossiter, Professor of Biochemistry, University of Western Ontario, was truly international being an Oxford trained Australian. Other ISN Presidents were Allan Boulton (1984-7) and Roger Butterworth (2007-9). Canadian representation on ISN Council has been limited, but consistent through the years, with Theodore Sourkes and Leonhard Wolfe important contributors in 1970s. Historically, prominent Canadian neurochemists contributed to 1st Meeting of Section of Neurochemistry of American Academy of Neurology (Boston, 1957), Juda Quastel was a member of Commission of Neurochemistry (1959) and Theodore Sourkes was a member of first elected ASN Council (1971). Allan Boulton was only non-USA President (1995-7) of ASN, which has held a single meeting in Canada (Vancouver, 1976). Vancouver was also site of the first ISN Meeting in Canada (1983) with Patrick and Edith McGeer both members of Local Organizing Committee. Marco Prado is Chairperson of Local Host Committee for 2019 Montreal ISN-ASN Meeting. Before ISN owned JNC (1970) Roger Rossiter, Alan Elliott and Juda Quastel, who were active in international neurochemical symposia in 1950s, served on its Editorial Board. Elliott and Quastel were co-authors on the landmark neurochemical text book with Irvine Page, "The Chemical Dynamics of Brain and Nerve" (1955). Some 15 Canadians have served on Editorial Board of JNC with Allan Boulton (1990-5) and Brian Collier (1996-2006) being Chief Editors. There have been notable Canadian contributions to growth of neurochemical knowledge across the basic neurochemistry of synaptic transmission (GABA, acetylcholine, catecholamines), lipid biochemistry, and understanding neuropathologies.

S01-02
Foundation of ASN and ISN and key USA names

A. Boullerne

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Neurochemistry emerged in USA after World War II, fueled by generous funding and new technologies– electron microscope, nuclear magnetic resonance, lipid extraction... Another driving force was neurochemical societies traced back to Francis Otto Schmitt 1949-1950 bimonthly neuroscience seminars at the Massachusetts Institute of Technology. The first international neurochemical forum was held in 1954 in Oxford, followed by national and international neurochemical conferences across the Atlantic and in Japan. Early organizer Russian-born Eugene Roberts at Washington University had discovered in 1950 gamma-Aminobutyric acid (GABA) in brain. In 1967, the International Society for Neurochemistry (ISN) was founded by four key players: Americans Jordi Folch-Pi and Heinrich Waelsch, and British Henry McIlwain and Derek Richter. ISN founder Alfred Pope at Harvard McLean Hospital did small sample analysis in 1952 that lead to anticholinesterase treatment in dementia. American Society for Neurochemistry (ASN) founded in 1969 by Folch-Pi, Donald Tower and Wallace Tourtellotte held its first annual meeting in spring 1970. Bernard Agranoff, pioneer of inositol signal transduction, had ASN sponsor the first Basic Neurochemistry textbook in 1972. Spanish-born Folch-Pi outstanding McLean Hospital research head founded complex lipid structural chemistry, and his charismatic personality contributed to formal recognition of ISN and ASN. Folch-Pi student Marjorie Lees purified in 1951 myelin protein Proteolipid and together reported in 1957 a now classic method for brain lipid extraction. In 1970 Julius Axelrod won Nobel prize for neurotransmitter re-uptake, and in 1971 Earl Sutherland won Nobel prize for cyclic AMP second messenger. In 1973, William Norton at Einstein College devised a sucrose gradient launching purified myelin molecular era, while Richard Quarles (NIH) discovered the first glycoprotein Myelin-Associated Glycoprotein. USA has proved a formidable force driving neurochemistry and hopefully will continue.

S01-03
Fine brains of Latin America: five decades of flourishing neurochemistry in the region

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Latin American neurochemistry was born in different countries between the 1950s and 1960s with different degrees of representation and participation. Right from the start, neurochemistry was very prominent in Venezuela, Argentina, Mexico, Uruguay, Chile and Brazil. As a matter of fact, due to the important development of neuroscience en Venezuela, ISN organized the first meeting in Latin America in La Guaira, Venezuela, in 1987, the second one in Buenos Aires, Argentina, in 2001, and the third one in Cancun, Mexico. In terms of leading researchers in the field, Venezuela gave us the outstanding work of Boris Drujan, Horacio Vanegas and Miguel Laufer. Argentina was the home of Eduardo De Robertis, Ranwell Caputto and Eduardo Soto. In turn, Uruguayan neurochemistry had a key figure in Clemente Estable, while Chilean neurochemistry gave us the fine work of JoaquínLuco Valenzuela. Finally, Mexico had prominent neuroscientists in Ricardo Tapia and Herminia Pasante. Brazil instead was more prone to biophysics and neurophysiology with important names like Miguel Covian and Carlos Chagas Filho, under whose direction at the Institute of Biophysics Rita Levi Montalcini conducted crucial experiments for

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the discovery of the neural growth factor after the Second World War. After this brief introduction, we will take a look at the development of neurochemistry in Latin America, all those scientists who make everyday efforts for the growth of neuroscience in each country, and how the neuroscience map has changed over the years.

S01-04

Julius axelrod: the second act was a smash

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Julius ("Julie") Axelrod was born in 1912 in the lower Manhattan, the son of Polish-Jewish immigrant parents. His father supported the family as a basket weaver. In 1933, Julie graduated from tuition-free City College of New York with a BS in Biology. Rejected from several medical schools, he took a position as a technician testing vitamin supplements at the Laboratory of Industrial Hygiene, where he remained for 11 years. During that time, he married, had 2 sons and completed a Masters in Chemistry. In 1946, Bernard Brodie hired him as a technician at the Goldwater Hospital. Julie joined Brodie at National Heart Institute in 1949 where he published nearly 30 papers on drug metabolism. Disenchanted with the lack of recognition, he enrolled in the PhD program in Pharmacology at George Washington University, completing it in a year. The National Institute of Mental Health appointed him the Chief of the Section on Pharmacology in 1957 at the age of 45. Over the next dozen years, he published over 20 reports in *Science* and *Nature* on the disposition of biogenic amines including defining the mechanism of action of antidepressant drugs. He received the Nobel Prize in Medicine in 1970. He hired his first post-doctoral fellow in 1962: Lincoln Potter, MD. Over the next 20 years, a score of distinguished scientists trained in his laboratory including Richard Wurtman, MD, Solomon Snyder, MD, Leslie Iversen, PhD, Jacques Glowinski, MD, Jacques de Champlain, MD, Ira Black, MD, Perry Molinoff, MD, Richard Weinshilboum, MD, Juan Saavedra, MD, Fred Wooten, MD, Michael Brownstein, MD, Roland Ciaranello, MD, Ronald Holz, MD, PhD, Joseph Coyle, MD, Steven Paul, MD, PhD, Manny Diberto, MD, Warren Strittmater, MD, and Fulton Crews, PhD.

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S02 Dysfunction at the presynapse

S02-01

Neurodevelopmental synaptopathies: presynaptic dysfunction in intellectual disability

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Neurodevelopmental disorders (including intellectual disability, autism spectrum disorder and movement disorders) affect 2-5% of children worldwide. The cause of neurological impairment in the vast majority of individuals with these brain disorders remains unknown, however advances in gene technology is now enabling the identification of novel substrates underlying neuronal dysfunction. This provides a new starting point for understanding the relationships between specific genetic mutations, brain function, neurodevelopment and cognition. Moreover, this provides a novel avenue for uncovering the molecular mechanisms underlying normal protein function. Importantly, mutations in proteins involved in neurotransmitter release and synaptic vesicle cycling have been identified in a range of neurodevelopmental disorders, including intellectual disability, epilepsy, and autism spectrum disorders. Alterations to the efficiency with which exocytosis or endocytosis occurs have adverse effects on neurotransmitter release, and therefore all coordinated neuronal activity. Newly identified mutations in key presynaptic proteins including synaptotagmin-1 and synaptophysin have been found in children with intellectual disability. We used model systems to examine the effect on these mutant proteins on presynaptic function, revealing mutation-specific effects on exocytosis, endocytosis and protein trafficking. These findings provide a framework for unravelling how disruption to synaptic vesicle dynamics and neurotransmitter release produces overlapping and distinct clinical phenotypes.

S02-02

Altered synaptic vesicle recycling in huntington's disease

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Neurodegenerative diseases affect millions of people worldwide and with society generally living longer, this means the number of affected individuals is increasing. This creates a need to understand the molecular mechanisms in order to develop new treatments. An emerging theme in neurodegenerative diseases, including Huntington's Disease (HD), is the premise that early presynaptic dysfunction plays a role towards later pathological outcomes. Huntington's disease is an inherited autosomal dominant disease whereby affected individuals have extended numbers of a CAG repeat in the *huntington* gene. When translated, this results in an expanded polyglutamine stretch in the huntingtin protein (htt) likely causing altered protein function. One of the early hallmarks of neurodegeneration in HD is synaptic atrophy in the striatum potentially caused by failure of efficient neurotransmission. Synaptic failure can be caused by an inability of the presynaptic nerve terminal to maintain neurotransmitter release, through either defects in exocytosis or in the subsequent endocytic processes required to retrieve the excess membrane and recycle synaptic vesicle proteins. We have uncovered activity-dependent signatures of presynaptic dysfunction in primary neuronal cultures from a knock-in mouse model of HD ($htt^{Q140/Q140}$). Furthermore, we have shown that this is due to loss of wt htt function and can be rescued with expression of wt htt in the $htt^{Q140/Q140}$ background. These results suggest that presynaptic dysfunction in HD may render neurons susceptible to repeated insults, culminating in synapse failure and degeneration. Understanding the molecular basis could lead to identification of new pathways for future therapeutic intervention.

S02-03

Molecular mechanisms underlying STXBP1/MUNC18-1 linked encephalopathies and rational rescue strategies

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Heterozygous de novo mutations in the neuronal protein STXBP1/Munc18-1 are linked to epilepsies, intellectual disability, movement disorders, and neurodegeneration. These devastating diseases have a poor prognosis and no known cure, due to lack of understanding of the underlying disease mechanism. To determine how mutations in Munc18-1 cause disease, we use newly generated *S. cerevisiae* strains, *C. elegans* models, and conditional Munc18-1 knockout mouse neurons expressing wild-type or mutant Munc18-1, as well as in vitro studies. We find that at least five disease-linked missense mutations of Munc18-1 result in destabilization and aggregation of the mutant protein. Aggregates of mutant Munc18-1 incorporate wild-type Munc18-1, depleting functional Munc18-1 levels beyond hemizygous levels. We demonstrate that the three chemical chaperones 4-phenylbutyrate, sorbitol, and trehalose reverse the deficits caused by mutations in Munc18-1 in vitro and in vivo in multiple models, offering a novel strategy for the treatment of varied encephalopathies.

S02-04

Function and dysfunction of the PD related LRRK2 protein at the presynaptic site

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Parkinson's disease (PD) is characterized by the progressive degeneration of dopaminergic neurons within the substantia nigra pars compacta and the formation of protein aggregates in surviving neurons. LRRK2 G2019S mutation is the major determinant of

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familial PD cases and leads to late-onset PD with pleomorphic pathology, including alpha-synuclein accumulation and deposition of protein inclusions. LRRK2 G2019S mouse model demonstrates an age-dependent motor and cognitive impairment. We observed the presence of aggregates containing N-ethylmaleimide sensitive factor (NSF) in basal ganglia specimen from G2019S carrier PD patients and in cellular and animal model expressing LRRK2 G2019S variant. We found that LRRK2 G2019S kinase activity affects NSF degradation and induces its accumulation in toxic aggregates. Noteworthy, induction of autophagy cleared NSF aggregation and rescued motor and cognitive impairment observed in aged hG2019S BAC mice. We suggest that LRRK2 G2019S pathological phosphorylation hampers substrates catabolism thus causing the formation of cytotoxic protein inclusions.

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S03 Complement: sculpting the developing and diseased brain

S03-01

Complement and blood-brain barrier integrity

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Emerging evidence highlights the critical role of the dysfunctional blood-brain barrier (BBB) in initiation and perpetuation of brain pathology in neuroinflammatory settings. Activation of the network of complement proteins in these settings results in the release of byproducts such as anaphylatoxins C3a and C5a, which forms a part of the clinical profile in diseases such as lupus. In the studies described here, *in vitro* 2D BBB model that closely emulates the BBB *in vivo*, constructed using human brain microvascular endothelial cells (HBMVEC) and astroglial cells, helped understand the role of complement in alteration of BBB integrity. Our data demonstrate that in lupus and other inflammatory settings, the proteins generated on complement activation along with other factors such as oxidative stress and calcium channel function compromise the endothelial cells. The endothelial layer has increased permeability monitored by changes in transendothelial electrical resistance. The cells are reprogrammed into a proinflammatory phenotype with altered tight junctions such as claudin-5 and zona occludens, cytoskeletal remodeling, as well as matrix function and viability resulting in a 'leaky' BBB. In addition, NFkB signaling is altered with transition from the cytoplasm into the nucleus. Bioenergetics via mitochondrial function is impaired by complement activation along with bioenergy-sensing signals by AMPK and SIRT1. Gaining insight into the complexity of complement mediated signaling in inflammatory and reparatory processes in endothelial cells will help identify effective therapeutic targets to combat inflammation in different settings and will bring the field one-step closer to understanding the translational potential of these targets.

S03-02

Complement C3A shapes the plasticity of the post-stroke brain

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Complement is part of the innate immune system that plays a major role in the initiation of inflammation and host defence against pathogenic bacteria. C3a is a 77 amino acid, 9 kDa peptide generated through the proteolytic activation of the central molecule of the complement system, the third complement component, C3. C3a exerts its functions through a G-protein coupled receptor, C3aR, that is expressed by many cell types including neurons and glia. This talk will discuss recent insights into the novel roles of C3a-C3aR signaling in the CNS with focus on synaptogenesis, axonal plasticity and regulation of reactive gliosis. I will also present findings from our laboratory pointing to C3aR as a target for therapies aiming at improving recovery after ischemic stroke and birth asphyxia.

S03-03

Deciphering key roles for complement peptide receptors in brain development

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Complement is an important immune system that has key pathogenic roles in the adult brain during neurodegeneration. However, emerging roles for complement in neurodevelopmental diseases have also been documented. Whilst significant attention has been focused on classical complement components, C1q and C4, and their roles in synaptic pruning, less is known about the roles of the 'anaphylatoxins' C3a and C5a, which are generated by all pathways of complement activation. Here we present data demonstrating essential physiological roles of C3a and C5a receptors during the neurogenic period of mammalian neurodevelopment. We utilized *in vivo* and *in vitro*models to modulate anaphylatoxin receptor (C3aR, C5aR1) function during critical periods of neurogenesis. Human development was modelled using human embryonic stem cells differentiated to form neural rosettes, or cultured as neurospheres. We show marked conservation in localization of C5aR1 and C3aR expressed on neural progenitor cells in both human and mice. Both receptors are expressed on the apical membrane of neural progenitors, and C5aR1 promotes their proliferation through activation of PKCz, a known mediator of polarity. *In vivo,* transient inhibition of C5aR1 through *in utero*injection of complement inhibitors to the embryonic ventricle resulted in a reduction of proliferating cells at the ventricular surface. In contrast, C3aR inhibition increased proliferation at this site, and *in vitro* experiments mimicked these findings. Remarkably, mice subjected to brief and transient pharmacological C5aR1 blockade during development demonstrated behavioral abnormalities and MRI-detected microstructural alterations in adulthood. Our current research is focused on identifying the source(s) of complement C3a and C5a in the embryo, and roles for other terminal complement proteins in embryonic neurogenesis. Together, these data demonstrate fundamental roles for complement anaphylatoxin receptors in the normal development of the embryonic mammalian brain.

S03-04

Complement: the culprit in neurodegeneration?

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Background: Complement is the body's host defense system against pathogens and is involved in microglia-mediated synaptic pruning during brain development. Complement is elevated in Alzheimer's disease (AD) brain and appears to contribute to the response to amyloid-β (Aβ) oligomers in early pre-plaque stages. Therefore, we asked whether complement plays a role in brain aging and/or later stages of AD pathogenesis.

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Methods: We crossed an amyloid model of AD, APPswe/PS1dE9 Tg mice, with complement *C3* germline knockout (*C3* KO) mice. Male APP/PS1;*C3* KO mice, wildtype (WT), APP/PS1 and *C3* KO mice were compared for cognitive flexibility using the Water T Maze (WTM) at 16 months of age. Aβ plaque load, gliosis, hippocampal synaptic changes and neuron number were evaluated. We also generated an inducible *C3* KO mouse model (*C3* fl/fl;UBC-Cre-ERT2) (*C3*iKO) in which tamoxifen treatment leads to global knockdown of *C3*.

Results: Lifelong *C3*-deficiency improved cognitive flexibility in APP/PS1 mice even though they had more Aβ plaque deposition at 16 months of age. Although the number of hippocampal glia did not change, fewer microglia were recruited to the plaques and plaque-associated microglia appeared to be less activated in the *C3*-deficient APP/PS1 mice. Hippocampal synapses and neuron numbers were rescued by *C3*-deficiency in APP/PS1 mice. Tamoxifen treatment of 9 mo-old male C3iKO mice to globally knockdown C3 expression led to reduced C3 protein levels in plasma, an increase in synaptic puncta, and improved LTP in hippocampal slices 3 months later. Current studies are underway to determine whether C3 lowering is protective in early stage neurodegenerative diseases.

Conclusions: Complement signaling appears to play a key role neuronal health and function in the aging brain and AD.

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S04 Glutamate at the crossroad

S04-01

The energetic cost of not resting

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While it is generally well accepted that increased glutamatergic activity results in increased metabolic activity, the metabolic impact of inhibitory (GABAergic) activity is less well understood. Inhibitory activity requires membrane hyperpolarization which is energetically expensive but it can also induce a global downturn in metabolic activity. This means that the balance between hyperpolarization and metabolic inhibition can determine whether or not there is a global increase or decrease in brain energy consumption [1].

The mechanism of excitatory stimulation is important, with some routes tolerated better than others [2,3]. The interplay of different cell types and compartments is highly important when interpreting what a metabolic profile represents: Normal metabolic activity? Overstimulation? Hyperexcitability? Metabolic exhaustion [4]?

Similarly, the mechanism of inhibitory input is also crucial. Delivered in just the right place, tiny amounts of GABA can have immensely strong inhibitory effects through activity at "master" switch GABAergic receptors [5].

How the system reacts depends on the baseline metabolic situation. Systems which are perturbed from the typical "resting" situation may react differently to input. This is an important consideration when designing interventions in clinical populations as baseline status may predict response [6].

1. Nasrallah F. et al. (2007) Journal of Cerebral Blood Flow and Metabolism **27**, 1510-1520

2. Rae C. et al. (2006) Journal of Cerebral Blood Flow and Metabolism **26**, 1005-1017.

3. Rae, C. et al (2005) Journal of Neurochemistry **92**, 405-416

4. Miller et al (2017) Sleep 40, zsx148

5. Nasrallah et al, (2011) Journal of Neuroscience Research **89**, 1935-1945

6. Rae et al (2013) International Journal of Neuropsychopharmacology **16**, 1695-1706

S04-02

Glutamate-glutamine cycling and oxidative metabolism in astrocytes from the perspective of magnetic resonance spectroscopy in vivo

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Astrocytes play an important role in glutamatergic neurotransmission, namely by clearing synaptic glutamate and converting it into glutamine that is transferred back to neurons. The rate of this glutamate-glutamine cycle is known to couple to that of glucose utilization and of neuronal metabolism. On the other hand, astrocytes are often considered to be glycolytic cells with meagre mitochondrial oxidative metabolism. Magnetic resonance spectroscopy has been used for ^{13}C tracing experiments *in vivo*, namely for detecting labelling incorporation from [^{13}C]glucose into brain amino acids. Such approach allows to determine rates of energy metabolism in neurons and astrocytes, and the glutamate-glutamine cycle. Recent work in the cerebral cortex of animal models suggests that variations of the glutamate-glutamine cycle rate upon cortical stimulation are coupled to the rates of mitochondrial metabolism in both neurons and astrocytes. Moreover, while the rate of resting energy metabolism is slower in astrocytes than neurons of the cortex *in vivo*, somatosensory stimulation induces oxidative metabolism increments of similar magnitude in the two cell types. This is in line with an active role of astrocyte bioenergetics in glutamatergic neurotransmission, which may be key in disorders characterised by dysfunction of excitatory synapses.

S04-03

Glutamate homeostasis revisited - neuronal transport and metabolism

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Glutamate homeostasis is mainly thought to be regulated within the frame of the glutamate -glutamine cycle. Vesicular glutamate release from the pre-synapse, uptake via high-affinity glutamate transporters in astrocytes, conversion of glutamate to glutamine and ultimately transfer of glutamine to the neuron and deamidation to glutamate. In addition, the importance of glutamate oxidation via glutamate dehydrogenase (GDH) has primarily been addressed mainly in astrocytes. However, it is now clear that presynaptic glutamate uptake via neuronal GLT1 (nGLT1) (McNair et al. 2019) is of functional importance and knock out of nGLT1 leads to disturbed amino acid and energy homeostasis and reduced oxidation of glutamate. In addition, glutamate oxidation via GDH (Hohnholt et al 2017) is essential in neurons for maintenance of the energetic machinery especially during increased energetic demand. We have preliminary data showing that enzymes, associated with glutamate metabolism is significantly affected in neurons derived from iPSCs of patients suffering from dementia. Interestingly, also the human isoform of GDH, i.e. GDH2, is affected, underlining the importance of employing a human model to study neurodegenerative pathologies. Overall, we hypothesize that GDH is important to sustain capabilities of neuronal mitochondria by maintaining a minimum amount of TCA cycle intermediates necessary during energetic demands induced by neuronal activation.

References:

Glutamate dehydrogenase is essential to sustain neuronal oxidative energy metabolism during stimulation. Hohnholt et al. J Cereb Blood Flow Metab. 2017. Deletion of Neuronal GLT-1 in Mice Reveals Its Role in Synaptic Glutamate Homeostasis and Mitochondrial Function. McNair et al. J Neurosci. 2019

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S04-04

Point-Counterpoint: glutamate production and oxidation

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The abstract has not been provided.

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S05 Reactive astrocytes in waste clearance and regeneration - context-dependent responses and treatment opportunities

S05-01

Reactive gliosis as a target - why and when

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The abstract has not been provided.

S05-02

Reactive gliosis and the consequences for cognition in stroke and alzheimer's disease

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Astrocytes are essential for the maintenance of CNS homeostasis and normal CNS function. In chronic neurodegenerative disorders (e.g. Alzheimer's disease[1]) and in acute neurological disorders (e.g. stroke[2]) astrocytes become activated and change both morphology and function. In response to astrocyte-derived signals, microglia remove synapses in a complement system-dependent manner. Two cellular hallmarks of reactive astrocytes are hypertrophy of their processes and upregulation of the part of the cytoskeleton known as intermediate filaments, which are composed of glial fibrillary acidic protein (GFAP), vimentin, nestin, and synemin. These intermediate filaments are highly dynamic structures that are involved in cell signalling, both in health and disease[3–5]. We have shown in a mouse model for Alzheimer's disease that reactive astrocytes have an altered expression of genes coding for extracellular matrix proteins, neuron-supporting genes, and immune response-related genes[6,7]. This implies a change in astrocyte-microglia and astrocyte-neuron interaction. We are now beginning to understand what the functional consequences are of reactive gliosis, how reactive gliosis can contribute to cognitive decline, and what the function is of intermediate filaments in these processes.

1 Osborn LM et al. Prog Neurobiol 2016; 144: 121–141.
2 van Dijk BJ et al. Biochim Biophys Acta - Mol Basis Dis 2016; 1862: 492–505.
3 Middeldorp J, Hol EM. Prog Neurobiol 2011; 93: 421–443.
4 Pekny M et al. Acta Neuropathol 2016; 131: 323–45.
5 Hol EM, Pekny M. Curr Opin Cell Biol 2015; 32: 121–130.
6 Kamphuis W et al. Glia 2015; 63: 1036–1056.
7 Orre M et al. NeurobiolAging 2014; 35: 2746–2760.

S05-03

Astrocytes and waste clearance in CNS – from physiology to intervention opportunities

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The abstract has not been provided.

S05-04

Astroglia define plasticity responses in the diseased brain

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The common and prevailing set of neurological thoughts considers neurones as the primary substrate of pathological progression. This " neurone-centric" concept, however, undergoes a rapid change. It has become universally acknowledged that the homeostasis of the nervous tissue is regulated by a complex fabric of neuroglial cells. Astroglia in particular represent a main element in the maintenance of homeostasis and providing defence to the brain. Consequently, dysfunction of astrocytes underlies many, if not all, neurological, neuropsychiatric and neurodegenerative disorders. Astrogliopathology comprises diametrically opposing morpho-functional changes in astrocytes, i.e. their hypertrophy along with reactivity or atrophy and astrodegeneration with asthenia. These complex plastic changes underlie pathophysiology of all neurological disorders including genetic (e.g. Alexander disease, which is a primary astrogliopathy), environmentally caused (e.g. heavy metal encephalopathies or hepatic encephalopathies), neurodevelopmental (e.g. different forms of autistic spectrum disorder), neuropsychiatric (including major depressive disorder, schizophrenia and addictive disorders) or neurodegenerative (e.g. amyotrophic lateral sclerosis, Alzheimer's and Huntington's diseases).

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S06 Interneuron Development and Interaction with other Cell Types in the Developing Brain

S06-01

Mechanisms controlling GABAergic interneuron plasticity in the adult brain

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Persistence of fear memories is important for survival, while the inability to effectively adapt to trauma is characteristic of post-traumatic stress disorder (PTSD), anxiety disorders and phobias. Fear memories in juvenile rodents are thought to be erased following extinction training, while extinction training only temporarily and weakly suppresses fear memories in adults. The development of GABAergic circuits, in particular of Parvalbumin-positive (PV) cells, a GABAergic interneuron subtype innervating hundreds of postsynaptic targets with multiple synapses clustered around the cell body and proximal dendrites, is one of the factors believed to restrict plasticity in the adult brain. Several recent studies, including our own work, suggest that controlled manipulation of cortical PV cell connectivity might help reinstate heightened plasticity in the adult brain. Our overarching goal is to investigate the molecular mechanisms controlling PV cell plasticity in the adult brain, since a better understanding of these mechanisms may help develop novel tools to securely foster brain plasticity to aid rehabilitation. Here, I will present findings showing that reducing histone deacetylase 2 expression in PV cells increases their plasticity and improve retention of fear extinction memories.

S06-02

Neuromodulatory control of inhibitory network arborization in the nascent neocortex

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Subcortical neuromodulatory systems project long-range axons to the cortex where they adjust processing modes of cortical circuits to environmental and behavioral states. Although their indispensable functions in the adult cortex have been extensively studied, the developmental role of neuromodulation in cortical circuit wiring remains poorly understood. Here we show that intracellular signaling driven by acetylcholine (ACh) derived from basal forebrain cholinergic neurons plays a key role in establishing local, dense inhibitory networks of neocortical chandelier cells (ChCs), which powerfully control spike generation of excitatory principal neurons (PNs) through innervation of their axon initial segments. Activation of nicotinic ACh receptors (nAChRs) promotes filopodia initiation that underlies axonal arborization. This ACh dependent filopodia initiation is mediated through downstream low voltage gated T-type calcium channels (T-type VGCCs) that shape transient calcium elevation in axonal varicosities. The blockade of ACh release from subcortical cholinergic neurons as well as genetic deletion of nAChRs and T-type VGCCs dramatically decreases the number of ChC axonal branches *in vivo*. These findings reveal a novel role for cholinergic neuromodulation in axonal arborization of developing ChCs and raise the possibility that the degree of inhibition at the spike initiation sites of PNs is shaped by the activity level of cholinergic neurons during development.

S06-03

Roles of long-lasting interactions between gabaergic interneurons and oligodendrocyte progenitors in the neocortex

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Most cortical interneurons and the first wave of oligodendrocyte precursors (firstOPCs) arise from the same embryonic origin: the medial ganglionic eminence (MGE) and the pre-optic area (POA). However, firstOPCs are supposed to have completely disappeared at posntatal day 10. Here we re-evaluated the death of firstOPCs and tested whether its common embryonic origin with interneurons contribute to the assembly of interneuron-OPC synaptic innervation at postnatal stages that is known to be transient and highly structured (Orduz et al., 2015, eLife). By using different transgenic mice, we followed the fate and functional properties of interneurons and firstOPCs from MGE and POA. First, we found that a small proportion of firstOPCs survives after the second postnatal week. Interestingly, these firstOPCs forms cell clusters with their lineage-related interneurons with which they display an unexpected high synaptic connectivity. Later in postnatal development, surviving firstOPCs differentiate into mature oligodendrocytes inside these cell clusters where they myelinate different types of neuronal fibers in vicinity of their lineage-related interneurons. These results show that a common embryonic origin favor a specific spatial organization and functional interaction between interneurons and surviving firstOPCs in the postnatal neocortex. To understand the significance of these clusters during cortical development, we genetically prevented the death of both MGE- and POA-derived interneurons and firstOPCs that had not normally survived during the first postnatal week. We found that the aberrant survival of interneurons and firstOPCs causes a strong decrease of interneuron-firstOPC connectivity and a general imbalance in the total oligodendroglia population. Therefore, the death/survival balance of interneurons and firstOPCs is crucial for the regulation of the interneuron-firstOPC connectivity and the entire population of oligodendroglia.

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S06-04

Morphological determinants of cortical gabaergic interneuron myelination

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Cortical GABAergic fast-spiking parvalbumin-positive (PV) interneurons are frequently myelinated with a proximally-biased topography and account for a substantial fraction of neocortical myelin. Conversely, somatostatin-positive (SOM) interneurons contribute only modestly to myelin content in the cerebral cortex. Previous studies have demonstrated that myelinating glia are sensitive to fiber caliber for initiating axonal wrapping, however the majority of studies have focused on the peripheral nervous system or have been performed in cell culture settings. Given the substantial differences in axonal morphology between local PV+ and SOM+ interneurons, we therefore sought to examine whether cortical interneuron myelination might be related to axonal morphology *in vivo*. We now demonstrate that segmental axonal myelination of cortical interneurons is strongly predicted by the joint combination of interbranch-point distance and local axon caliber in both mouse and human neocortex. We further explored the robustness of this model by either increasing PV+ interneuron size with cell-type specific deletion of *Tsc1* or reducing PV+ interneuron size by cell-type specific deletion of *Ube3a*. In both cases, although the frequency of myelinated segments was significantly altered, the joint combination of interbranch-point distance and local axon caliber remained highly predictive of myelin topography. Lastly, we considered regular-spiking SOM+ cells, which normally have relatively shorter interbranch distances and thinner axon diameters than PV+ cells, and are rarely myelinated. Enlargement of SOM+ cell size by cell type-specific deletion of *Tsc1* dramatically increased the frequency of myelinated axonal segments and with a topography accurately predicted by the model. Together, our results suggest that local axonal morphology is an important determinant underlying the topography of cortical GABAergic interneuron myelination.

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S07 RNA Control of Axonal Functions

S07-01

Subcellular localization of RNA-binding proteins for axon growth regulation

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Nucleolin is a multifunctional RNA-binding protein (RBP) found in the nucleus, cytoplasm and plasma membrane of the cell. Previously we have shown that nucleolin localizes to axons through interaction with the anterograde microtubule associated motor kinesin-1 (Kif5) and that the complex transports a number of key growth-regulating mRNAs including importin beta1 and mTOR. Perturbation of nucleolin-kinesin interactions leads to reduced levels of axonal nucleolin and its associated transcripts and enhances neuronal growth. Here we identify the kinesin-binding domain (KBD) in nucleolin, and show that the same domain mediates nucleolin localization to the cell cortex and plasma membrane. Heterozygous KBD-deletion mice reveal reduced axonal localization of nucleolin in dorsal root ganglion (DRG) neurons and enhanced axonal outgrowth. Homologous domains may exert similar functions in other RNA-binding proteins. The current study provides new mechanistic insights on subcellular localization of RBPs, and how changes in subcellular RBP localization regulate axon growth.

S07-02

Signaling mechanisms for regulation of protein synthesis in axons

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Axons of cultured neurons contain 1000's of different mRNAs, and proteins synthesized in adult PNS axons have been shown to support regeneration after injury. It is clear that axons can regulate which proteins are generated when, but how this translational regulation is achieved at a molecular level remains unknown. We recently showed that mRNAs are stored in stress granule-like structures in uninjured PNS axons (Sahoo et al. 2018). Over the first 6 hours following axotomy, the stress granule proteins TIA1 and G3BP1 show further increased aggregation in axons that then falls to below naïve levels thereafter. Aggregated G3BP1 binds to axonal mRNAs and attenuates their translation. Preventing G3BP1 aggregation increases axonal mRNA translation and accelerates axon growth in culture and *in vivo.* The fall in G3BP1 aggregation as axons start to mount a regenerative response is accompanied by phosphorylation of G3BP1 on Ser 149. Reineke et al. (2017) reported that Casein kinase 2a (CK2a) could phosphorylate G3BP1 in other cellular systems. We find that injury increases axonal CK2a levels as G3BP1 aggregation declines at 16 hours post axotomy. This CK2a upregulation is translation dependent and requires initial translation of mTOR mRNA in axons. Axonal translation of CK2a mRNA is inhibited by elevated axoplasmic Ca^{2+}; in contrast translation mRNAs needed for the initial injury response in axons is increased when axoplasmic Ca^{2+} is elevated. Together, these data indicate that axotomy is accompanied by sequential waves of local mRNA translation, where we suspect that newly synthesized proteins enable translation of subsequent mRNAs needed for regeneration.

S07-03

The secret life of 3'UTRS in developing axons

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Understanding how cells translate extracellular cues into specific patterns of gene expression is one of the major goals of modern neurobiology. Neurons are cells with a complex morphology, which maintain their cellular structure through the compartmentalized expression of proteins essential for growth and plasticity. Asymmetric localization of RNA is an evolutionarily conserved mechanism that allows spatial restriction of protein synthesis to specific cellular compartments. Incorrect processing and delivery of mRNA causes developmental defects and severe human neurological disorders. In neurons, mRNA transcripts are transported to both dendrites and axons where they are rapidly translated in response to stimuli.

This talk will explore how transcripts localized in sympathetic neuron axons are transported, processed and translated in response to neurotrophins. Special emphasis will be given to the nature of the 3'UTRs of targeted axons and to the presence of unique elements that may determine their fate. I will also discuss our important findings indicating that the 3'UTR of localized transcripts undergo axonal cleavage and remodelling, thereby generating mRNA isoforms expressing a shorter 3'UTR, which are rapidly translated, and **a**xonally **c**leaved RNA fragments (acRNA) with yet unknown function.

S07-04

Axonal BDNF/TRKB signaling endosomes regulation of MTOR-dependent local translation and dendritic branching in somas

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Brain Derived Neurotrophic Factor (BDNF) is broadly express in different circuits of the central nervous system (CNS) and binds its

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receptors TrkB and p75 to trigger different signaling pathways regulating dendritic growth and synaptic plasticity. When binding to BDNF, TrkB and p75 are endocytosed to signaling endosomes, that are organelles transmitting trophic signals. Whether BDNF-TrkB-p75 signaling endosomes in axons are regulating long-distance signaling to cell bodies to modify neuronal morphology is unknown. Here, we studied the functional role of BDNF-TrkB-p75 signaling endosomes and BDNF signaling pathways in long-distance regulation of dendritic growth using compartmentalized cultures of rat and mouse cortical neurons derived from p75 knock out or TrkBF616A knock-in mice. By applying BDNF to distal axons we showed the capacity of axonal BDNF to increase dendrites, to activate the transcription factor CREB in the nucleus and the PI3K-mTOR pathway in cell bodies increasing somatodendritic protein synthesis. TrkB activation and not p75 was required for this effect. Locally in axons, increased activity of PLC-gamma and calcium was required for long-distance signaling; in addition to Rab5 (early endosomes regulator) and dynein activities. Our results suggest a compartmentalized activation of BDNF signaling pathways in axons and dendrites a role of BDNF-TrkB signaling endosomes in coordinating this process as well as wiring of circuits in the CNS.

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S08 The NMDA receptors: from synapse physiolgy to pathology

S08-01

The NMDA receptor co-agonist D-serine is essential for dopamine modulations of prefrontal neuronal activity and cognitive function

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Dopaminergic modulation of glutamatergic neurotransmission in the prefrontal cortex (PFC) plays an important role in the control of cognitive functions. Accordingly, disruption of frontocortical dopamine(DA)-glutamate cross-talk is a hallmark of several neuropsychiatric disorders, including schizophrenia. In addition, hypoactivity of NMDA receptors (NMDAR) due to reduced availability of their co-agonist D-serine is implicated in schizophrenia. Whether dopaminergic modulations of neuronal activity and cognitive functions involve D-serine is not known. Herein, we show that pharmacologically- and genetically-driven depletions of D-serine impair positive and negative modulations of glutamatergic transmission, neuronal excitability and plasticity by D_1 and D_3-receptor activation, respectively. Furthermore, we report that the selective blockade of the D_3-receptors increases global PFC activity and cognition in wild-type but not in null-mutant mice for serine racemase the enzyme that synthesizes D-serine. All these aberrant physiological and behavioral signatures found in the mutant mice were fully alleviated by chronic treatment of the mice with D-serine. Finally, we reveal that D_1R and D_3R activations coordinately regulate in opposite directions the extracellular levels of D-serine in the PFC and identify the cAMP/PKA pathway as a molecular hub through which DAergic receptors control the activity of the co-agonist at NMDARs. Collectively, our results reveal a key role for D-serine in the healthy neuromodulation by DA of PFC activity, findings highly relevant to the etiology and treatment of schizophrenia but also to disease where the dopamine-glutamate cross-talk is disrupted

S08-02

Glutamate is required for depression but not potentiation of long-term presynaptic function

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High-resolution fluorescence imaging of synaptic transmission, performed in living mammalian brain tissue, reveal data that offer a robust counterpoint to a widely held view that central synapse plasticity occurs exclusively at the post-synaptic locus. A meta-analysis of the plasticity literature offered a conceptually significant thread that has lead us to an experimental demonstration that glutamate functions to depress its own release at central synapses during Hebbian plasticity. The mechanistic basis of this form of plasticity has been explored using the optical quantal analysis technique to reveal that it is critically dependent upon glutamate's interaction with presynaptic NMDA receptors. This surprising result is likely to be of some importance, as it underscores the unique significance of presynaptic plasticity in synaptic transmission.

S08-03

NMDA receptor C-terminal domain signaling in health and disease

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The GluN2 subtype (2A vs. 2B) determines key biophysical properties of forebrain NMDA receptors. During development, GluN2A becomes incorporated into previously Glu2NB-dominated NMDARs, but both are highly expressed in the adult forebrain. In addition to controlling channel properties, GluN2A and GluN2B have large and highly divergent cytoplasmic C-terminal domains. Using genetically modified mice with targeted mutation of exchange of GluN2 C-terminal domains, we are investigating their role in development and disease. Key questions include their role in directing the switch in NMDA receptor subunit composition, and in pro-death signaling in acute and chronic neurological conditions.

References:

Martel et al (2012) Neuron; Hardingham & Do (2016) Nat. Rev. Neuro; McQueen et al (2017) ELife; McKay et al (2018) Cell Rep.

S08-04

Metabotropic NMDA receptor signaling underlies beta-amyloid induced synaptic dysfunction

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Until recently, NMDA receptor (NMDAR) functions have been attributed to its ability to conduct calcium ions. However, growing evidence demonstrates that the NMDAR can induce synaptic depression without ion-flux, suggesting that is has a metabotropic function. Our results show that glutamate binding or elevated amounts of beta-amyloid can trigger a conformational change in the NMDAR c-terminal domain. We have shown previously that this movement affects interactions between the NMDAR and signaling molecules, which results in synaptic depression. PSD-95, a major scaffolding protein at the synapse, binds directly to NMDARs and is significantly depleted in neurons exposed to beta-amyloid as well as in brain tissue of patients with Alzheimer's disease. In this talk, we will focus on recent experiments showing that increased PSD-95 can block metabotropic NMDAR signaling and thus prevent synaptic weakening induced by beta-amyloid. Our results show that large spines, containing increased amounts of endogenous PSD-95 have a similar NMDAR conformation as spines not exposed to beta-amyloid. Also, beta-amyloid overexpression specifically reduced PSD-95 content in small spines, leaving larger spines unaffected.

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Moreover, overexpressed PSD-95 does not potentiate synaptic transmission in tissue lacking the AMPA receptor subunit GluA1 while elevated PSD-95 still blocks beta-amyloid-induced synaptic depression in GluA1-lacking tissue. These results indicate that PSD-95 rescue of beta-amyloid induced depression is not due to synaptic potentiation but by a blockade of NMDAR metabotropic signaling. We are now testing a pharmacological approach to increase synaptic PSD-95 in vitro and in APP/PS1 model mice. Preliminary experiments suggest that this approach could lead to a possible new treatment against Alzheimer's.

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S09 Neuroprotection through Autophagy: the next milestones

S09-01

The molecular interplay between autophagy and proteasome in motoneuron diseases

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Several adult onset motoneuron diseases (MNDs) are linked to the presence of misfolded proteins which aberrantly behave in affected cells perturbing the normal cell functions. Amyotrophic lateral sclerosis (ALS) is a typical MNDs associated with misfolded proteins. We studies how these MNDs-proteins accumulate into aggregates during disease showing that they are typically poorly removed by, or may impair, the protein quality control (PQC) system. The PQC system is composed of chaperones and degradative pathways (proteasome and autophagy). We found that the potentiation of the chaperone-assisted selective autophagy (CASA) is sufficient to clear aggregated misfolded species. CASA relies of the CASA complex formed by the small heat shock protein B8 (HSPB8) its co-chaperone BAG3, the chaperone HSC70 and the E3-ubiquitin ligase CHIP. The CASA complex recognizes and ubiquitinated misfolded proteins for the insertion into autophagosomes. Notably, HSPB8 overexpression is sufficient to improve CASA complex activity and aggregate clearance, while is downregulation has the opposite effect. We found that the inhibition of CASA complex correlates with the activation of an alternative co-chaperone, BAG1, which sequesters HSC70/CHIP from the CASA complex, routing misfolded proteins to proteasome for degradation. Any alteration of this fine equilibrium results in misfolded protein accumulation. We thus postulated that when misfolded proteins are poorly transported to degradation by autophagy or stored in aggresome, the cells activate a compensatory mechanism based on BAG1 to target the HSC70-bound cargo to the proteasome in a active transport-independent manner.

S09-02

Selective autophagy: fighting neurodegeneration one protein at a time

A. M. Cuervo

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Cells count on surveillance systems to handle protein alterations and organelle damage. Malfunctioning of these systems occurs with age and is on the basis of different neurodegenerative conditions. Our studies have focused primarily on autophagy. We have found a double interplay whereby, different autophagic pathways contribute to clearance of pathogenic proteins but, conversely, these pathogenic proteins often became toxic for the autophagic system. Our current efforts are oriented to investigate the consequences of this toxicity on two selective forms of autophagy, chaperone-mediated autophagy (CMA) and in endosomal-microautophagy. We have developed conditional mouse models with modulatable CMA, where we have identified that decline on CMA activity contributes to neurodegeneration, increases proteotoxicity, accelerates the course of disease and facilitates propagation of the proteotoxic signature. We are currently utilizing genetic and chemical approaches to upregulate CMA in the neurodegenerative setting to determine the possible therapeutic value of such intervention.

S09-03

Autophagy in neurons: from development to degeneration

P. Boya

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Autophagy is an essential intracellular degradation pathway that recycles cell components, thereby generating new building blocks and energy to maintain cellular homeostasis. Autophagy plays an important part in the response to nutrient starvation and the recycling of damaged organelles, and serves as a key survival mechanism in conditions of stress. Our group has shown that autophagy is necessary for neuronal differentiation and for the removal of apoptotic cells during normal development of the nervous system. Furthermore, our findings suggest a metabolic role of autophagy, which may enable the production of ATP through the degradation of cellular components. We have recently demonstrated that mitophagy (the selective degradation of mitochondria) regulates metabolic reprogramming that is essential for neuronal differentiation. We are also exploring how autophagy defects are associated with age-related diseases such as glaucoma and Parkinson's disease and whether manipulation of this process could represent new therapeutic strategies for neurodegenerative conditions.

S09-04

Alterations in RAB-mediated membrane trafficking in neurological disease

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Membrane trafficking controls the levels and localization of proteins, and thus cellular function, and alterations in trafficking pathways contribute to human disease. Rab GTPases are key switches turning trafficking on and off. Our discovery that the differentially expressed in normal and neoplastic cells (DENN) domain functions enzymatically as a guanine-nucleotide exchange factor (GEF) to activate Rabs provided new understanding in the regulation of Rabs in membrane trafficking. There are 26 DENN domain (DENND) proteins in humans making them a critical new class of trafficking regulators. Here I will describe how alterations in DENN domain proteins and their Rab substrates contribute to neurological disease. Specifically I will discuss the role of DENND1 and its substrate Rab35 in the development of brain tumors and our recent discovery that mutations in DENND5A cause a severe neurodevelopmental disorder called epileptic encephalopathy.

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S10 Insights on organoid and 3D models to study brain diseases and development

S10-01

Modelling human brain developmental diseases using on-chip human brain organoids

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Human brain folding has been implicated in neurodevelopmental disorders such as lissencephaly. Here, we will describe using our on-chip human brain organoid platform to study the appearance of surface folds during the in vitro development and self-organization. Our micro-fabricated devices supports in situ imaging over a timescale of weeks. Lissencephalic (smooth brain) organoids display reduced convolutions, modified scaling and a reduced elastic modulus. Whereas we could also observe size reduction in microcephalic brain organoids. Our on-chip approach offers a means for studying the emergent properties of organoid development, with implications for the embryonic human brain.

S10-02

Using human cerebral organoids to probe the consequences of rare highly-penetrant mutations in major mental illness

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Schizophrenia and other major mental illnesses including classic neurodevelopmental disorders are highly heritable. Large-scale studies have shown that genomic variation, in the form of copy number variants (CNVs), accounts for a significant portion of risk. CNVs in the disrupted in schizophrenia 1 (DISC1)-interactor and nuclear distribution factor E-homolog 1 gene (*NDE1*) on chromosome 16q13.11, that lead to SCZ and neurodevelopmental disorders are proposed to result in abnormal neuronal precursor cell (NPC) proliferation and differentiation. Our group has tested this hypothesis by generating a platform of human iPSCs from patients with schizophrenia, and other neurodevelopmental disorders, who are known to have CNVs affecting *NDE1*. We have differentiated these iPSCs into NPCs *in vitro* and have undertaken comparative studies between mutant and control cell lines. In parallel we have also studied the effects of *NDE1* on developmental pathways in 'cerebral organoids'; a three-dimensional tissue culture of human iPSC that mimics early stages of human cortical development. Studying neurodevelopmental disorders in three-dimensional *in vitro* cultures can teach us fundamental aspects of the development of the human cortex, that are beyond reach in current animal model systems. Human brain imaging of affected carriers of the 16p13.11 microduplication showed reduced brain volume. IPSC-derived brain organoids from these patients were smaller and showed reduced neuronal progenitor cell proliferation. Transcriptomic and proteomic data shows deficits in key intracellular signaling pathways associated with proliferation which we have been able to rescue both genetically and pharmacologically. This study shows as a proof-of-principle that cerebral organoid technology holds much promise to probe the mechanistic underpinnings of neurodevelopmental and neuropsychiatric disorders.

S10-03

Genetic evolution of cerebral cortex size and folding

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One of the most prominent features of the human brain is the fabulous size and folding of the cerebral cortex, which emerge during development. Cortex size is determined by neurogenesis. We have found that direct neurogenesis from Radial Glia Cells (RGCs), with limited neuron production, dominates the avian, reptilian and mammalian paleocortex, whereas in the evolutionarily recent mammalian neocortex most neurogenesis is indirect via intermediate progenitors. Our experiments in mouse, chick and snake embryos, and human cerebral organoids, demonstrate that Slit/Robo signaling is necessary and sufficient to drive direct neurogenesis. Attenuating Robo signaling in snakes and birds promotes the formation of intermediate progenitors and indirect neurogenesis, as in mammals. Further expansion and folding of the mammalian neocortex depends on the abundance of basal RGCs found in a unique germinal layer, the Outer Subventricular Zone (OSVZ). We have found that during a brief developmental period, RGCs in the ventricular zone generate a burst of bRGCs that become founders of the OSVZ, after which they follow a lineage completely independent from other germinal zones. This brief period is confined by the dynamic temporal regulation of genes key for bRGC formation, determining the emergence of the OSVZ and folding of the cortex. Cortex folding occurs in highly stereotyped patterns, and we have identified unique transcriptional signatures along germinal zones of the human and ferret cortex, but not mouse, mapping the prospective location of folds and fissures. These include genes mutated in human cortical malformations, and may define cortical folding patterns. Our studies identify modulation in expression and activity levels of conserved signaling pathways as a primary mechanism driving the expansion, folding and increased complexity of the mammalian neocortex during evolution.

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S10-04

LGALS3BP modulates local gyrification in the human brain

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Basal radial glial cells (bRGs) are a neural progenitor type enriched in primates and humans and conto the expansion of neugenerated during cortical dein gyrencephalic species. Shortly after their generation, bRGs delaminate towards the outer subventricular zone, where they divide multitimes before terminal diffe The reguof bRGs generation is essential for the establishment of correct gyrification within the human cortex. Here, we study the role of LGALS3BP, a secreted prowhose RNA exis enriched in bRGs. By using cerebral organoids, human fetal tissues and mice, we show that manipulation of *LGALS3BP* regubRG generation. Ad individuals with unique *de novo* variants in *LGALS3BP* demonabnormal gyrification and thickness at multisites over the cerebral cortex. Single-cell-RNA-sequencing reveals the extracellular matrix involvement in the LGALS3BP mediated mecha We find that LGALS3BP is required for bRGs delamination and influences cortical deand gyrification in humans.

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S11 Neuron-Glia signaling in synaptic plasticity: Astrocytes Rule!

S11-01

Astrocytes as key drivers in NMDA receptor-depedent long term depression

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Astrocytes are increasingly being recognized as active players for neuronal synaptic communication, with multiple functions for central nervous system physiology. Nevertheless, astrocytes are typically considered as modulators of core mechanisms driven by the neuronal components, or are thought to provide metabolic fine-tuning for neuronal function (for instance by regulating neurovascular-neuroenergetic coupling). We will discuss new conclusive evidences that challenge this view, specifically for a form of synaptic plasticity involved in cognitive function: NMDA receptor-dependent long-term depression (LTD) in the hippocampus. Even though the molecular mechanisms for LTD are still being elucidated, the basic sequence of events that leads to synaptic depression appears to be well established (and widely accepted): prolonged, low-frequency release of glutamate from the presynaptic terminal, activation of postsynaptic NMDA receptors and engagement of specific signaling cascades that lead to the removal of AMPA receptors from the postsynaptic membrane. I will present a fundamental change of paradigm, in which the axis composed of presynaptic neuron-astrocyte-postsynaptic neuron defines an obligatory relay for information processing leading to synaptic plasticity.

S11-02

Signaling of CB1 receptors in astrocytes

G. Marsicano

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Type 1 cannabinoid receptors (CB1) are expressed at very low levels in astrocytes as compared to other brain cell types. However, they play key roles in specific responses to cannabinoid drugs and in the fine physiological regulation of behavior. In this presentation, I will go through our studies on the functions exerted by astroglial CB1 receptors in the brain, ranging from the control of synaptic plasticity, the regulation of learning and memory and the participation in key bioenergetic processes.

S11-03

The involvement of astrocytes in cognitive processing

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Astrocytes interact with neurons at the cellular level through modulation of synaptic formation, maturation, and function, but the impact of such interaction in circuit activity that results in behavior remains unclear. Here, we studied the mouse models with impaired exocytosis in astrocytes to dissect the role of astrocyte-derived signaling in cortico-hippocampal circuits, with implications for cognitive processing. We found that the blockade of gliotransmitter release in astrocytes triggers a critical desynchronization of neural theta oscillations between the dorsal hippocampus and prefrontal cortex. Moreover, we found a strong cognitive impairment in tasks depending on this network. In this talk, I will discuss also further evidence suggesting the involvement of astrocyte-released signals in mechanisms of long-distance network modulation, with direct implications to cognitive function.

S11-04

Astrocyte signalling control of spike timing-dependent plasticity

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Critical periods of synaptic plasticity facilitate the reordering and refining of neural connections during development, allowing the definitive synaptic circuits responsible for correct adult physiology to be established. Presynaptic spike timing-dependent long-term depression (t-LTD) exists in the hippocampus, which depends on the activation of NMDARs and that probably fulfills a role in synaptic refinement. This t-LTD is present until the 3rd postnatal week in mice, disappearing in the 4th week of postnatal development. We were interested in the mechanisms underlying this maturation related loss of t-LTD and we found that at CA3-CA1 synapses, presynaptic NMDA receptors (preNMDARs) are tonically active between P13 and P21, mediating an increase in glutamate release during this critical period of plasticity. Conversely, at the end of this critical period (P22-P30) and coinciding with the loss of t-LTD, these preNMDARs are no longer tonically active. Using immunogold electron microscopy, we demonstrated the existence of preNMDARs at Schaffer collateral synaptic boutons, where a decrease in the number of preNMDARs during development coincides with the loss of both tonic preNMDAR activation and t-LTD. Interestingly, this t-LTD can be completely recovered by antagonizing adenosine type 1 receptors (A_1R), which also recovers the tonic activation of preNMDARs at P22-P30. By contrast, the

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induction of t-LTD was prevented at P13-P21 by an agonist of A_1R, as was tonic preNMDAR activation. Furthermore, we found that the adenosine that mediated the loss of t-LTD during the fourth week of development is supplied by astrocytes. These results provide direct evidence for the mechanism that closes the window of plasticity associated with t-LTD, revealing novel events probably involved in synaptic remodeling during development.

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S12 ASN Folch-Pi Award Symposium - Lipidomics as a tool to study metabolic and environmental contributors to Alzheimer's Disease

S12-01

Lipidomic characterization of pro-repair lipid pathways in Alzheimer's Disease

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Background: Alzheimer's Disease (AD) is a progressive brain disorder characterized by persistent inflammation and neuronal damage. Specialized lipid mediators known to resolve neuroinflammation and repair damaged neurons have been reported to be reduced in post-mortem brains of AD patients. However, the underlying cause of this reduction is not known.

Hypothesis and objective: The present study tested the hypothesis that pathways regulating pro-repair lipid mediator metabolism are altered in post-mortem brains of AD patients.

Methods: Post-mortem pre-frontal cortex from pathologically confirmed AD subjects (n=21) and unaffected controls (n=20) was subjected to lipidomic analysis with ultra-high pressure liquid chromatography coupled to tandem mass-spectrometry following separation of brain esterified and unesterified lipid pools with solid phase extraction.

Results: Compared to controls, concentrations of several pro-repair lipid mediators esterified to neutral lipids were significantly reduced by ~50% in AD patients ($P<0.05$). No significant changes were observed in free or phospholipid-bound pro-repair lipid mediators.

Conclusion: This study provides novel evidence of reduced esterified pro-repair lipid mediators in post-mortem AD pre-frontal cortex. Targeting pro-repair lipid mediator turnover within neutral lipids may stimulate neuronal repair and resolution of inflammation in AD.

S12-02

Chronic exposure to real-time traffic related air pollution increases neuroinflammation and plaque burden in TGF344-ad rats

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Epidemiological studies have linked traffic-related air pollution (TRAP) to increased risk of Alzheimer's disease (AD). However, this association has yet to be confirmed in a preclinical model. Moreover, the mechanism(s) by which TRAP influences AD are unclear. To address these issues, we exposed male and female TgF344-AD rats and congenic controls to real-time TRAP or filtered air over the course of 15 months, using a mobile exposure facility that samples air from a highway tunnel in the Bay Area of California. Rats were exposed to TRAP or FA from postnatal day 28 to 15 months of age. At 3, 6, 10, and 15 months of age, brain samples were collected, and analyzed for plaque burden, bioactive lipids, microgliosis, astrogliosis, and cytokine protein levels. Chronic TRAP exposure increased plaque burden in AD transgenic rats at 6 months. In addition, we found that TRAP exposure increased pro-inflammatory cytokines as early as 3 months of age, and modulated levels of both pro- and anti-inflammatory cytokines at later time points. Finally, both microgliosis and astrogliosis were increased by TRAP exposure. These data suggest that TRAP may exacerbate AD-relevant phenotypes, and that these results may be mediated through neuroinflammation. Supported by the NIEHS (grants R21 ES025570, P30 ES023513 and T32 ES007059) and NIA (grant P30AG010129).

S12-03

Determining blood and brain bioavailability of omega-3 fatty acids in APOE4 carriers

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Approximately 20% of Canadians carry at least one epsilon 4 allele of apolipoprotein E (*E4)* allele, which almost doubles their risk of late-onset Alzheimer's disease (AD). AD risk is closely linked to changes in lipid metabolism and there are evidences that docosahexaenoic acid (DHA) may exert a preventive effect in AD and that higher DHA levels in the blood is associated with better cognition. The suggested mechanism of this link is that DHA is more available to be taken up by the brain to fulfill brain DHA turnover. However, this mechanism relies on efficient DHA transport across the blood brain barrier (BBB) and there are two conditions to achieve that:

1) Plasma DHA is packaged in the right compartments and is available to the brain for its uptake.

2) There are enough brain transporters at the BBB interface, such as *MFSD2A* to increase DHA concentrations within the central nervous system.

However, the apoE4 protein has lower affinity for the LDL receptor and VLDL remains for a longer period in their blood compared to non-carriers. This means that, in *E4* carriers, DHA is probably packed more in triglycerides than to another compartment, hence promoting its β-oxidation instead of its incorporation in membrane and tissues. Metabolic defects in fatty acid packaging in the blood may accentuate how much vulnerable *E4* carriers are to brain DHA deficiency during aging. There is no cure to AD and hence, a reduction of even 10% in the prevalence of AD, by providing innovative supplements, would markedly improve the quality of life of affected Canadians, especially the 3.6 million *E4* carriers aged between 30-60 years old.

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S12-04

Serum soluble epoxide hydrolase derived oxylipins as cognitive biomarkers related to cerebral small vessel disease and mixed alzhe

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Background: Cerebral small vessel disease is commonly comorbid with late-onset Alzheimer's disease (AD). The soluble epoxide hydrolase (sEH) enzyme inactivates anti-inflammatory and vasoactive cytochrome p450 derived polyunsaturated fatty acid epoxides by converting them into cytotoxic dihydroxy oxylipin species. Here we investigate serum concentrations of the sEH-derived dihydroxy species and their parent epoxides as correlates of cerebral small vessel disease and the related cognitive deficits in patients with and without AD.

Methods: The present study included participants with AD and cognitively healthy elderly controls, each separated into groups with either extensive or minimal small vessel disease as determined by visual rating of white matter hyperintensities on multimodal 3.0 T MRI. The oxylipins were extracted from serum using solid phase extraction, then quantified with a targeted ultrahigh pressure liquid chromatography mass spectrometry (UPLC-MS/MS) lipidomics platform. A unit-weighted composite Z-score of speed, attention and executive function was derived from age, gender and education corrected norms from the Digit Symbol Substitution Test, Trial-Making Test Part B, Stroop Color-Word Interference Test, and FAS Verbal Fluency Test. Memory was assessed using the California Verbal Memory Test, 2nd Edition. Multivariate analyses of covariance, and linear regression models, were used to investigate the association between the oxylipins and white matter hyperintensities and cognitive performance, respectively.

Results: Preliminary data included 30 participants with AD and 54 participants without AD. The serum concentration of 12,13-dihydroxyoctadecamonoenoic acid (12,13-DiHOME; an sEH derived linoleic acid oxylipin) relative to its epoxide sEH substrate 12,13-epoxyoctadecenoic acid (12,13-EpOME) was higher among patients with extensive small vessel disease. The 12,13-DiHOME/12,13-EpOME ratio was negatively associated with a composite score of executive function, processing speed, and attention in all participants with extensive small vessel disease, including subgroups with AD and without AD. The ratio was not related to memory performance. Replication is now underway in a larger sample.

Conclusions: Oxylipins derived from sEH activity were associated with a cognitive profile consistent with vascular cognitive impairment. A putative linoleic acid-derived biomarker appears to be relevant to cognition in elderly people with extensive cerebral small vessel disease, both with and without dementia due to concomitant Alzheimer's disease.

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S13 Emerging biomarker concepts in neurodegenerative diseases

S13-01

The value of a-synuclein as a diagnostic or prognostic biomarker

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In Neurodegenerative Disorders there is a wide field of recent and ongoing search for useful biomarkers for early and differential diagnosis, disease monitoring or subtype characterization. Cerebrospinal fluid (CSF) is often used as a source for biomarker development in different neurological disorders because it reflects changes in central-nervous system homeostasis. The presentation gives an overview about different biomarker approaches, mainly focusing on CSF analyses. Current state and future perspectives regarding classical protein markers, but also different "omics" techniques are described. In conclusion, technical advancements in the field already yielded promising results, but further multicenter trials with well-defined cohorts, standardized protocols and integrated data analysis of different modalities are needed before successful translation into routine clinical application.

S13-02

From biomarkers to clinical studies

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Most neurodegenerative disorders, including the hereditary forms, are slowly progressive. The functional decline is measured with validated clinical scales that reflect the impairment of the patients. The slow progression of the functional impairment, the day-to-day variation of patients performing the scales, as well as the interpretation by the observers require large patient numbers and long trial durations in order to reveal an effect of disease-modifying interventions. Molecular biomarkers, which reflect the pathophysiology of a disorder, address the question of whether an interventional treatment is on target, and may also help address the effectiveness and the effect size of a given intervention. In neurodegenerative diseases it has also been shown that magnetic resonance imaging (MRI) is sufficiently sensible to measure focal atrophy before patients become symptomatic, and to measure volume loss more sensibly than clinical scales can detect functional deterioration[1].

I will use Friedreich ataxia as an example for the use of biomarkers. Friedreich ataxia is autosomal-recessively inherited, the most prevalent genetic form of ataxia with a typical onset before the age of 25 years. It is caused by a loss of Frataxin and subsequent loss of mitochondrial dysfunction in several neuronal and non-neuronal tissues. Epigenetic modifications, particularly HDAC inhibition by nicotinamide, leads to an increase in Frataxin. Based on natural history data[2,3]it was calculated that a clinical trial needs to include 225 patients and a treatment period of two years to prove efficacy of the therapeutic intervention. Volumetric measurements of the spinal cord may help substantiate the protective effect.

References:

1. Dogan I, ..Schulz JB, Reetz K. Structural characteristics of the central nervous system in Friedreich ataxia: an in vivo spinal cord and brain MRI study. J Neurol Neurosurg Psychiatry. 2019 May;90(5):615-617. https://doi.org/10.1136/jnnp-2018-318422.
2. Reetz K, .., Schulz JB; EFACTS Study Group. Progression characteristics of the European Friedreich's Ataxia Consortium for Translational Studies (EFACTS): a 2 year cohort study. Lancet Neurol. 2016 Dec;15(13):1346-1354. https://doi.org/10.1016/s1474-4422(16)30287-3.
3. Reetz K, . . . Schulz JB. Biological and clinical characteristics of the European Friedreich's Ataxia Consortium for Translational Studies (EFACTS) cohort: a cross-sectional analysis of baseline data. Lancet Neurol. 2015 Feb;14(2):174-82. https://doi.org/10.1016/s1474-4422(14)70321-7.

S13-03

Alpha-synuclein as a potential biomarker and therapeutic target for parkinson's disease

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Parkinson's disease is the most common neurodegenerative movement disorder. Yet diagnostic biomarkers, which are measures that detect the presence of Parkinson's disease or identify individuals with a subtype of Parkinson's disease, remain elusive as none are yet available or approved for clinical use. Multiple lines of evidence support a role of the protein alpha-synuclein in the pathophysiology of Parkinson's disease. Hence an important focus for the development of disease-modifying therapies is on targeting alpha-synuclein. In parallel, major ongoing efforts to identify diagnostic biomarkers are aimed at measuring alpha-synuclein in peripheral tissues and biofluids. This talk will review the evidence for alpha-synuclein as a potential therapeutic target for Parkinson's disease, examine the necessity of diagnostic biomarkers for clinical trials testing disease-modifying therapies, and discuss the possible pitfalls of a single biomarker approach for Parkinson's disease.

S13-04

Exploration and validation of fluid biomarkers in manifest and prodromal Parkinson's disease

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The causative treatment of α-synuclein (aSyn) aggregation disorders [i.e. Parkinson's disease (PD), multiple system atrophy

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and dementia with Lewy Bodies] is hampered by the lack of disease-specific biomarkers to signal the risk of developing the disease (trait), to indicate its manifestation (state), the speed of its progression and response to therapy (rate), and to predict its clinical course (fate).

Several cross-sectional and longitudinal single- and multicenter cohorts of PD have been established, including the single center DeNoPa-[1] and multicenter Parkinson Progression Marker Initiative (PPMI)[2]. These cohorts enable biomarker research based on clinical phenotyping, imaging, blood/cerebrospinal fluid (CSF) biomarkers for biochemical analyses. DeNoPa also includes microbiome studies. To study prodromal aSyn aggregation disorders people with isolated REM sleep behaviour disorders are also recruited to determine the evolution of biomarkers.

Several biomarkers have shown to be interesting for diagnostic purposes of aSyn aggregation disorders, like CSF aSyn, that due to the overlap of single values and the lack of longitudinal change has limited clinical utility. Other biomarker (like neurofilament light chain; NfL) and other technologies (like PMCA or RT-QuIC) show promising results. CSF NfL discriminated PD from other (atypical) Parkinson's syndromes, but also metabolomic and microbiome analyses reveal interesting results as biomarker, but also to understand the pathophysiology of the disease.

Due to the clinical heterogeneity of aSyn disorders, a multimodal panel of different biomarkers is needed for clinical practice and as outcome measure. Newer biomarker will have to be identified and explored, including inflammatory response and including several cross-omics approaches.

1. Mollenhauer B, Zimmermann J, Sixel-Doring F, et al. Monitoring of 30 marker candidates in early Parkinson disease as progression markers. *Neurology* 2016; **87**(2): 168-77.

2. Mollenhauer B, Caspell-Garcia CJ, Coffey CS, et al. Longitudinal CSF biomarkers in early Parkinson's disease, prodromal PD and healthy controls. *Movement Disorders in press* 2019.

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S14 Glial phagocytic clearance in health and disease

S14-01

Clearing the corpses: are microglia eating enough in the diseased brain?

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Microglia are the brain professional phagocytes, equipped with sensors to detect the apoptotic cell debris generated during development, in adult neurogenic niches as well as during ageing and neurodegenerative diseases. We will discuss recent lab findings showing that phagocytosis goes beyond debris removal, and in fact, it activates a coordinated transcriptional and metabolic program in microglia that impacts on their function. We will also discuss discus that microglial phagocytosis is very efficient in physiological conditions and that when challenged with an increasing number of apoptotic cells, microglia plastically adapt their behaviour and boost their phagocytic output proportionally using different cellular strategies. In contrast, we will show that microglial phagocytosis is impaired under pathological conditions such as epilepsy and stroke, and discuss different cellular and molecular mechanisms underlying this impairment. In summary, we propose that harnessing microglial phagocytosis may serve to control tissue damage and inflammation as a novel strategy to accelerate brain recovery.

S14-02

Neuroprotective properties of the innate immune cells

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We recently found that the progressive cognitive decline and decrease in expression of numerous synaptic markers and neurotrophins in the brain of mouse models of Alzheimer's disease (AD) correlated with major changes in the proportions of peripheral blood monocyte subsets when compared with age-matched controls. Indeed, there is a defect in the production of circulating M1 monocytes in APP/PS1 mice, whereas the population of M2 monocytes remains normal in this mouse model of AD. Injecting M-CSF to transgenic mice that spontaneously develop AD on a weekly basis prior to the appearance of learning and memory deficits prevented cognitive loss. The treatment also restored the population of M1 monocytes in the circulation and greatly decreased Aβ levels. In addition, M-CSF treatment resulted in the stabilization of the cognitive decline state in transgenic mice that already had Aβ pathology. These results are quite encouraging as they suggest that stimulating circulating monocytes may have a great therapeutic potential for AD. It is therefore likely that stimulating monocytic cells may be a new therapeutic avenue for treating brain diseases, such as AD. In this presentation, we will show new data regarding the potent effects of new molecules to stimulate innate immune cells as a preventive and curative treatment for brain diseases. We will also show the central role of the neurovascular unit in diseases of the CNS and how it can be targeted for novel therapeutic strategies.

The Fonds de la Recherche du Québec – Santé (FRQS), Canadian Institutes in Health Research (CIHR) and the Multiple Sclerosis Scientific Research Foundation of Canada support this research.

S14-03

Brain remodeling by phagocytic astrocytes in the penumbra region after stroke

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The brain consists of neurons and much higher number of glial cells, astrocytes, oligodendrocytes and microglia. They communicate each other, by which they control brain functions. The brain is highly vulnerable to several insults such as ischemia, but has a self-protective and self-repairing mechanism against these, for which glial cells have central roles. Astrocytes, the most abundant glial cells in the brain, sense changes in brain environments, and cause phenotypical changes dramatically, leading to unexpected regulation of brain functions. Adult astrocytes are quiescent but become rather reactive in response to various brain insults such as brain ischemia. However, the functions of reactive astrocytes are poorly understood. Here we show that astrocytes become reactive and function as a main player of phagocytosis after transient ischemic injury in a limited spatiotemporal pattern. After transient brain ischemia, phagocytic astrocytes were observed within ischemic penumbra region in the later stage of ischemia. On the contrary, phagocytic microglia, a well-known as professional phagocytes in the brain, were mainly observed within ischemic core region in the early stage of ischemia. Phagocytic astrocytes upregulated ABCA1 and its pathway molecules, MEGF10 and GULP1, which were required for their phagocytosis. In addition, upregulation of ABCA1 was sufficient for the phagocytosis. Together, these findings suggest that astrocytes should be transformed into phagocytic phenotype via increasing ABCA1 and its related molecules. Judging from the spatiotemporal pattern of the phagocytic astrocytes, they have distinct roles from microglia, and would contribute to remodeling of the penumbra networks.

S14-04

The role of astrocytes in accumulation and spreading of pathogenic proteins

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Alzheimer's disease (AD) and Parkinson's disease (PD) are neurodegenerative diseases, affecting a large proportion of the elderly population. Knowledge about the cellular mechanisms

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behind the propagation of these diseases in the brain is limited. Decades of research have focused on neuronal abnormalities in AD and PD, but recently more attention has been given to glial cells. The aim of our research is to clarify the involvement of astrocytes in the progression of AD and PD and to investigate their therapeutic potential. Our results demonstrate that astrocytes engulf large amounts of aggregated amyloid beta (Aβ) and alpha-synuclein (α-SYN) that is stored, rather than degraded by the cells. The accumulation of Aβ/α-SYN in astrocytes results in lysosomal defects and spreading of neurotoxic protein aggregates via tunneling nanotubes and extracellular vesicles. Our hypothesis is that astrocytes try to be "helpful" by ingesting the pathogenic proteins, but are overwhelmed by the challenge and instead promote disease spreading and neuronal cell death. Being the most numerous glial cell type in the brain, astrocytes constitute a compelling treatment target. Interestingly, our recent data demonstrates that antibody treatment effectively prevent accumulation of toxic Aβ/α-SYN aggregates in the astrocytes.

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S15 Pathophysiological mechanisms producing early onset epilepsies with severe comorbid neurodevelopmental disorders

S15-01

Epilepsy-associated intellectual disability triggered by abnormal interactions of ion channels with signaling pathways

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The *KCNT1* gene encodes a sodium-activated potassium channel termed Slack (also KNa1.1 and Slo2.1). Slack is expressed predominantly in neurons, where it regulates excitability and patterns of firing in response to synaptic stimulation. Mutations in *KCNT1* lead to several types of early onset epilepsies, as well as to autism. Many of these mutations are located in the large cytoplasmic C-terminus of the protein and in, all cases, the mutations are associated with very severe intellectual disability. Expression of the KCNT1 mutants in heterologous cells or in human IPS-derived neurons reveals that the disease-causing Slack channels are fully functional but have a greater open probability than wild type channels. This gain-of-function results in a hyperexcitable phenotype. To address the impact of these mutations on cellular function, we have analyzed the interactions of the cytoplasmic C-terminus with other cellular proteins. We have found that the channels bind to Phactr1 (Phosphatase and Actin Regulatory Protein-1), the RNA-binding protein FMRP (Fragile X Mental Retardation Protein), and to CYFIP1 (Cytoplasmic FMRP-Interacting Protein-1). The two latter proteins are known regulators of RNA-translation. Using a fluorescent reporter for mRNA translation we have found that stimulation of Slack channels increases translation rate. This channel-dependent translation is markedly potentiated by loss of FMRP or by a disease-causing Slack mutant. Our results suggest activity-dependent mRNA translation is directly linked to channel activation and that this is impaired by such channel mutations. This deficit may underlie the intellectual disability that results from enhanced channel activity.

S15-02

Pathophysiological mechanism of PHACTR1 mutations causing west syndrome, an infantile epilepsy with intellectual disability

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Trio-based whole exome sequencing identified two *de novo* heterozygous missense mutations [c.1449T>C/p.(Leu500Pro) and c.1436A>T/p.(Asn479Ile)] in *PHACTR1*, encoding a molecule critical for the regulation of protein phosphatase 1 (PP1) and the actin cytoskeleton, in unrelated Japanese individuals with West syndrome (infantile spasms with intellectual disability (ID)). We then examined the role of *Phactr1* in the development of mouse cerebral cortex and the pathophysiological significance of these two mutations and others [c.1561C>T/p.(Arg521Cys) and c.1553T>A/p.(Ile518Asn)], which had been reported in undiagnosed ID patients. Immunoprecipitation analyses revealed that actin-binding activity of Phactr1 was impaired by the p.Leu500Pro, p.Asn479Ile and p.Ile518Asn mutations while the p.Arg521Cys mutation exhibited impaired binding to PP1. Acute knockdown of mouse *Phactr1* using *in utero* electroporation caused defects in cortical neuron migration during corticogenesis, which were rescued by an RNAi-resistant Phactr1 but not by the four mutants. Experiments using knockdown combined with expression mutants, aimed to mimic the effects of the heterozygous mutations under conditions of haploinsufficiency, suggested a dominant negative effect of the mutant allele. As for dendritic development *in vivo*, only the p.Arg521Cys mutant was determined to have dominant negative effects, because the three other mutants appeared to be degradated with these experimental conditions. Electrophysiological analyses revealed abnormal synaptic properties in *Phactr1*-deficient excitatory cortical neurons. Our data show that the *PHACTR1* mutations may cause morphological and functional defects in cortical neurons during brain development, which is likely to be related to the pathophysiology of West syndrome and other neurodevelopmental disorders.

S15-03

Mechanisms of sensory circuit hyperexcitability in mouse models of autism

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Autism Spectrum Disorders (ASD) in humans is characterized by sensory processing and sensitivity problems. In Fragile X Syndrome (FXS), a monogenic cause of ASD and intellectual disability, individuals display behavioral sensory hypersensitivity that is correlated with hyperexcitable cortical activity, both at rest, and in response to sensory stimuli such as sound, as measured with electroencephalogram (EEG). Using the mouse model of FXS, *Fmr1* knockout (KO), we have identified hyperexcitable cortical circuit oscillations in acute slices of either auditory or somatosensory cortex. Such hyperexcitable cortical circuit oscillations are mediated by overactive metabotropic glutamate receptor 5 (mGluR5), as a result of disrupted interactions with the synaptic scaffolding protein Homer. I will present data on the synaptic mechanisms of hyperexcitable cortical oscillations in *Fmr1* KO mice, as well as new data from a different mouse ASD model, where we observe mGluR5-dependent hyperexcitable cortical oscillations, but these effects are sex-dependent. These later results may provide insight into sex-dependent alterations in ASD-relevant behaviors and circuit dysfunction.

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S15-04

Molecular mechanisms underlying brain wiring and asd-like behaviours

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Individuals with the 15q11.2 microdeletions, which include the *CYFIP1* gene, can present with a diverse array of symptoms such as neurobehavioral disturbances, epilepsy and psychiatric problems. The core behavioral features and the underlying molecular mechanisms of this genetic condition, however, remain unclear. In brain, CYFIP1 regulates synapse structure and plasticity by orchestrating two processes: actin remodeling and protein synthesis. We now show that in mice *CYFIP1* haploinsufficiency causes deficits in functional brain connectivity and behaviour. I will discuss the importance of our findings for other neurodevelopmental disorders.

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S16 Signaling Mechanisms in Cortical Development

S16-01

A non canonical role for proneural genes in maintaining the neural stem cell pool

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The origins of adult neural stem cells (NSCs) has been elusive until recently when it was shown that slow-dividing embryonic NSCs are set aside to populate the adult NSC niche. To prospectively identify embryonic NSCs marked for retention, we stratified the neocortical NSC pool into four populations based on proneural gene expression (negative, Neurog2$^+$, Ascl1$^+$, double$^+$). Neurog2/Ascl1 double$^+$ NSCs cycle the slowest, accumulating in S-phase due to the elevated expression of negative cell cycle regulators. Double$^+$ NSCs are also uncommitted and are maintained in this state into the postnatal period by *Neurog2-Ascl1* cross-repression. We have thus identified a novel mechanism for embryonic NSC retention involving proneural gene cross-repression and multilineage priming.

S16-02

WNT signaling regulates key telencephalic midline structures

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The hippocampus is induced by Wnt signaling from a telencephalic midline organizer, the cortical hem. Though the hem expresses and secretes Wnt ligands, it does not itself display indicators of canonical Wnt signaling, suggesting that it may not respond to the ligands it secretes. The cortical hem produces at least two mature cell types, the Cajal-Retzius neurons and the non-neuronal choroid plexus epithelium. We examined the cortical hem and its lineage in the context of either loss of function or constitutive activation of β-catenin, a key component of canonical Wnt signaling. Both perturbations cause distinct cell-autonomous and non-cell-autonomous disruptions of midline patterning. Together, our results indicate that the ability to experience and process canonical Wnt signaling in the hem lineage is a carefully controlled phenomenon that is critical for normal development of the brain.

S16-03

Intermediate progenitors in cerebral cortex development

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Intermediate progenitors (IPs) are a type of transit-amplifying cell in developing cortex, marked by expression of transcription factor Tbr2. IPs produce the majority of cortical pyramidal-projection neurons, and are themselves derived from neural stem cell (NSC)-like radial glial progenitors (RGPs) that express transcription factor Sox9. Lineage tracing shows that RGPs produce large columnar clones of neurons, while IPs produce small clones of neurons (average size ~2 cells), often limited to one layer. IP progeny frequently undergo asymmetric daughter cell apoptosis. In IPs, Tbr2 regulates hundreds of downstream target genes to drive the transition from progenitor to cortical neuron identity, and regulate diverse biological processes, such as the IP cytoskeleton, cell-cell signaling, and regional and laminar identity of daughter neurons. Comprehensive analysis of gene regulation by Tbr2 reveals context-specific functions of Tbr2 in cortical development.

S16-04

Mechanisms generating cell-type diversity in cerebral cortex

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The concerted production of the correct number and diversity of neurons and glia is essential for intricate neural circuit assembly. In the developing cerebral cortex, radial glia progenitors (RGPs) are responsible for producing all neocortical neurons and certain glia lineages. We recently performed a quantitative clonal analysis by exploiting the unprecedented resolution of the genetic MADM (Mosaic Analysis with Double Markers) technology and discovered a high degree of non-stochasticity and thus deterministic mode of RGP behavior. However, the cellular and molecular mechanisms controlling the precise pre-programmed RGP lineage progression through proliferation, neurogenesis and gliogenesis remain unknown. To this end we use quantitative MADM-based experimental paradigms at single RGP resolution to define the cell-autonomous functions of candidate genes and signaling pathways controlling RGP-mediated cortical neuron and glia genesis and postnatal stem cell behavior. Ultimately, our results shall translate into a deeper understanding of brain function and why human brain development is so sensitive to disruption of particular signaling pathways in pathological neurodevelopmental and psychiatric disorders.

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S17 The needs of a synapse: How dendritic and axonal organelles serve synaptic function

S17-01

Positioning of secretory organelles in dendrites: focus on f-actin

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Organelle positioning within neurites is required for proper neuronal function. In dendrites with their complex cytoskeletal organization, transport of organelles is guided by local specializations of the microtubule and actin cytoskeleton, and by coordinated activity of different motor proteins. Here, we focus on the actin cytoskeleton in the dendritic shaft and describe dense structures consisting of longitudinal and branched actin filaments. These actin patches are devoid of microtubules and are frequently located at the base of spines, or form an actin mesh around excitatory shaft synapses. Using lysosomes as an example, we demonstrate that the presence of actin patches has a strong impact on dendritic organelle transport, as lysosomes frequently stall at these locations. We provide mechanistic insights on this pausing behavior, demonstrating that actin patches form a physical barrier for kinesin-driven cargo. In addition, we identify myosin Va as an active tether which mediates long-term stalling. This correlation between the presence of actin meshes and halting of organelles could be a generalized principle by which synapses control organelle trafficking.

S17-02

Retrograde trafficking and local signaling of trkB-containing amphisomes at presynaptic terminals

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Amphisomes derive from fusion of autophagosomes with late endosomes and biogenesis of both organelles occurs predominantly at axon terminals. Non-degradative roles of autophagy have been barely described. Here we show that in neurons BDNF/TrkB traffick in amphisomes and signal at presynaptic boutons during retrograde transport to the soma. Local signaling and inward transport is orchestrated by the Rap GTPase-activating (RapGAP) protein SIPA1L2, which connects TrkB amphisomes to a dynein motor. The association with autophagosomes regulates the RapGAP activity of SIPA1L2, and thereby the retrograde trafficking and local signaling of TrkB. Following induction of presynaptic plasticity amphisomes dissociate from dynein at boutons, and this enables local signaling and promotes transmitter release. Accordingly, *sipa1 l2* knockout mice show impaired BDNF-dependent presynaptic plasticity. Collectively, the data suggest that TrkB-signaling endosomes are in fact amphisomes that during retrograde transport have local signaling capacity in the context of presynaptic plasticity.

S17-03

Unconventional protein trafficking in neurons

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Regulated synthesis and movement of proteins between cellular organelles is central to diverse forms of biological adaptation and plasticity. In neurons, the repertoire of channel, receptor and adhesion proteins displayed on the cell surface directly impacts cellular development, morphology, excitability and synapse function. The immensity of the neuronal surface membrane and its division into distinct functional domains presents a challenging landscape over which proteins must navigate to reach their appropriate functional domains. I will present recent data from my lab dissecting the trafficking itinerary of nascent integral membrane proteins as they travel from their sites of synthesis inside the cell to their sites of function at the cell surface. I will introduce a new approach we have developed for selectively initiating protein trafficking from different subcellular locations, which will help unravel when, where and how proteins traffic to and from different neuronal compartments.

S17-04

Unveiling unconventional golgi-related organelles in peripheral axons and their role in regeneration

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New models for the regulation of the axonal proteome have emerged not only based on local synthesis but also on trafficking of proteins. Axons contain mRNAs for membrane proteins, ribosomes and endoplasmic reticulum elements that support the presence of a local biosynthetic machinery. The increasing number of studies reporting axonal traffic of transmembrane proteins and the presence of some Golgi apparatus (GA) markers suggests the presence of this organelle in axons. Nevertheless, the canonical stacked membranous structure of GA has not been detected in axons. We hypothesized that a simplified but efficient GA-like structure is present in axons that allows its maintenance and assist in the restoration functionality after injury.

The presence and distribution of Golgi components was evaluated in axons from mice sciatic nerve. We locally disrupted the ER to Golgi trafficking exposing a distal portion of sciatic nerve to the ArfGEFs inhibitors Golgicide-A (GCA) and Brefeldin-A (BFA). We evaluated the axonal regeneration in embryonic DRG cultures using a microfluidic chamber that allows the separation of axons and cell

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bodies in different compartments. Disruption of ER to Golgi trafficking in isolated axons was achieved with GCA and BFA.

The Golgi markers TGN38, Golgi satellite (pGolt), Galactosyltransferase (Gal-T) and Mannosidase-II (Mns-II) were detected in nodal and internodal regions of myelinated axons. pGolt displayed a high co-distribution (~70%) with Gal-T and Lamp. GCA and BFA reduce the particles of pGolt, Gal-T and Mns-II in isolated axons from sciatic nerve.

Our data suggest that Golgi components are present in peripheral axons. These components may constitute a mixed organelle conformed by Lamp, pGolt and Gal-T markers that might be part of an unconventional Golgi apparatus adapted to axonal requirements.

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S18 Repeating themes of tandem repeat toxicity in neurological disorders

S18-01

Molecular mediators and environmental modulators of pathogenesis in huntington's disease

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We have been investigating how various environmental manipulations selectively alter gene expression, cellular plasticity and associated cognitive processes and behaviours. Huntington's disease (HD) is one of over 50 tandem repeat disorders and involves a triad of psychiatric, cognitive and motor symptoms. In a transgenic mouse model of HD we have shown that expansion of the tandem repeat encoding a polyglutamine tract of the mutant huntingtin protein leads to a spatiotemporally specific cascade of molecular, cellular and behavioural abnormalities. We have also demonstrated that environmental enrichment can delay onset of the affective (depression-like), cognitive and motor endophenotypes. Environmental enrichment and physical exercise induce changes in gene expression, which exhibit temporal specificity and regional selectivity, and also act as cognitive enhancers. These findings have been extended to include stress manipulations in HD mice, and environmental manipulations in other preclinical models. Most recently, we have discovered that gut microbiota are altered at an early stage of pathogenesis. We are pursuing this first evidence of gut dysbiosis in HD, with respect to pathogeneic mechanisms and novel therapeutic targets. These approaches may also facilitate the development of 'enviromimetics', novel therapeutics which mimic or enhance the beneficial effects of cognitive stimulation and physical activity. We are further exploring the impact of specific environmental and pharmacological interventions, including environmental enrichment, exercise and stress, and the relevance of these discoveries in mice to clinical HD. Our findings have implications for the development of novel therapeutic approaches to delay onset and slow progression of HD.

S18-02

The role of oligodendroglia in Huntington disease

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White matter abnormalities and oligodendroglial changes are common features of neurodegenerative disorders, although their aetiology is poorly defined. A long-held assumption is that the white matter atrophy observed in neurodegenerative disorders is simply a secondary outcome of the progressive neuronal loss that manifests with advancing disease. This assumption has been difficult to examine on the molecular and microstructural levels directly in pre-symptomatic individuals prior to onset of neuronal loss, owing to the invasiveness of the techniques involved. In this talk, I will present our recent studies investigating the aetiology and consequences of oligodendroglial dysfunction in animal models of Huntington disease, a trinucleotide repeat disorder and the most common genetic cause of dementia. I will further discuss the implications of a better understanding of white matter pathology for the development of therapies for neurodegenerative diseases.

S18-03

Targeting ran proteins improves phenotypes in *C9orf72* BAC ALS/FTD mice

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Microsatellite expansion mutations cause more than 48 neurologic diseases. In 2011, we discovered that in the absence of an AUG or near cognate initiation codon, expanded CAG and CUG repeats can express homopolymeric proteins from all three reading frames. We and others have demonstrated that RAN translation occurs in a growing number of repeat expansion disorders including: spinocerebellar ataxia type 8 (SCA8), *C9orf72* amyotrophic lateral sclerosis / frontotemporal dementia (ALS/FTD), Huntington's disease (HD) and myotonic dystrophy (DM). An emerging theme is that coding and non-coding expansion mutations are bidirectionally expressed, producing two mutant RNAs and up to six mutant proteins. We now show that RAN translation can be regulated both *in vitro* and *in vivo* through the PKR/eIF2α phosphorylation pathway. In cells, steady state levels of several types of RAN proteins are increased by PKR overexpression and decreased by inhibiting PKR. In *C9orf72* BAC transgenic ALS/FTD mice, inhibiting PKR through AAV expression of the dominant negative PKR-K296R protein decreases RAN protein pathology *in vivo* and improves behavioral phenotypes. These data are consistent with a model in which chronic activation of the PKR pathway by repeat expansion RNAs favor RAN translation and that blocking this pathway in mice reduces RAN protein accumulation and mitigates disease. These data suggest that targeting the PKR pathway may be a fruitful therapeutic approach to treat *C9orf72* ALS/FTD and for other repeat expansion diseases. In a separate study we show that targeting RAN proteins with human antibodies improves behavior, decreases neurodegeneration and increases survival in *C9orf72* ALS/FTD BAC transgenic mice. These data demonstrate RAN proteins play a central role in *C9orf72* ALS/FTD and describe novel approaches for the treatment of C9 and other RAN-protein diseases.

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S18-04

Non-AUG initiated translation of nucleotide repeats in fragile x-associated disorders

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Fragile X-associated tremor/ataxia syndrome (FXTAS) is an age-related inherited neurodegenerative disorder affecting > 1 in 4000 people. FXTAS results from a CGG nucleotide repeat expansion in the 5' untranslated region (UTR) of *FMR1*. Expanded CGG-repeats allow aberrant translation of cryptic homopolymeric proteins through a repeat-associated non-AUG initiated translation mechanism (CGG RAN translation). The most abundant CGG RAN generated protein, FMRpolyG, accumulates in ubiquitin-positive neuronal inclusions in *Drosophila*, CGG repeat-expressing mice and FXTAS patients. RAN translation is necessary for CGG repeats to elicit toxicity in *Drosophila*, neurons and mice. This lecture will cover recently published and unpublished work exploring the mechanisms by which RAN translation occurs at CGG repeats and other nucleotide repeat expansions, with a special emphasis on the roles of cellular stress pathways in this process. In addition, I will present data related to a potential native function for CGG repeats and RAN translation in the regulation of the fragile X gene, and provide evidence that either directly or indirectly targeting RAN translation initiation suppresses disease relevant phenotypes and enhances survival in disease model systems.

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S19 Cellular and molecular mechanisms of glial development

S19-01

Role of transcriptional and epigenetic regulation in glial cells

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Glial represent major actors in neural differentiation/physiology and the absence or defects in this cell population leads to major human pathologies as diverse as cancer, neurodegeneration and autoimmune disorders. It is therefore of paramount importance to understand the biology of glial cells. We have identified and characterized the molecular pathway leading to the differentiation of glia in Drosophila. These cells arise from multipotent precursors that can be assimilated to the vertebrate neural stem cells. More recently, we have started the analysis of the glial chromatin landscape and have identified a cell-specific epigenetic signature that allows glial cell function. This constitutes the first evidence of a single signature controlling specific biological processes in a differentiated cell type and highlights the importance of chromatin modifications in the function of the nervous system.

S19-02

Glia-ECM interactions control peripheral nerve integrity and function

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Glial cells are critical the protection and function of the nervous system and disruption of glial function leads nervous system defects. The *Drosophila* nervous system is encased in a highly conserved layer of glial cells, the perineurial glia, which are in turn covered by a specialized extracellular matrix (ECM). The function of perineurial glia and their interaction with the ECM is just beginning to be elucidated and we are investigating the mechanisms and functional importance of this interaction in the peripheral nervous system. We found that integrins and laminins are key to glial sheath development and maintenance. Loss of integrins and focal adhesions disrupts the glial wrap of peripheral nerves, disrupts animal locomotion and is lethal. We have identified a new partner for integrin mediated glia-ECM interactions, the transmembrane Ig domain protein, Basigin/CD147/EMMPRIN. Loss of this highly conserved protein from the perineurial glia leads to an unexpected phenotype of compression of the glia and ECM in the peripheral nerves. Perineurial glial compression results in breakage of the glial cytoskeleton. Disruption of the peripheral nerves leads to reduced locomotion and death. We found Basigin is expressed in close proximity to integrin and functions with integrins in the perineurial glia. Reduction of integrins or the integrin-binding protein Talin can rescue the nerve compression phenotypes. Our results indicate that Basigin regulates the integrin-based focal adhesion complexes in order to uphold the structure of the glia-extracellular matrix sheath.

S19-03

Motor exit point (MEP) glia: novel myelinating glia that bridge CNS and PNS myelin

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Traditionally, the CNS and PNS have been considered two halves of a single organ, connected only by motor and sensory axons. However, identification of a CNS-derived peripheral glial population called perineurial glia challenged this view and led us to hypothesize that development of spinal motor nerves may involve other CNS-derived glial populations. To investigate ithis hypothesis, we used *in vivo*, time-lapse imaging, single cell ablation/fate-mapping and genetic perturbation in zebrafish, and discovered that spinal motor nerve root axons are populated by a second, CNS-derived glial population that is distinct from perineurial glia and neural crest-derived Schwann cells, which we call motor exit point (MEP) glia. Once in the periphery, these cells divide and produce glia that myelinate spinal motor root axons. Recently, we have focused on thoroughly characterizing this novel glial population by investigating its development, maintenance and function. Developmentally, we discovered that these cells share a common ventral spinal cord precursor with oligodendrocyte progenitor cells (OPC), the cells that ultimately differentiate into oligodendrocytes and ensheath CNS axons in a fatty membrane known as myelin. However, unlike OPCs, MEP glia migrate out of the CNS, associate with and myelinate axons in the PNS and function to restrict OPCs to the spinal cord. Therefore, for all intents and purposes, they are peripheral glia. However, this simple designation does not capture the complexity of this cell population. Therefore, using genetics and in vivo imaging, we are investigating whether MEP glia are more like oligodendrocytes or Schwann cells, or alternatively, if they are a hybrid cell population with characteristics of both lineages and determining the potential of these cells to replace both central and peripheral myelinating glia upon demyelination.

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S19-04

Transcriptional control in myelinating glia: from extrinsic signals to intrinsic factors and networks

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Development of myelinating glia is under control of a complex gene regulatory network with transcription factors at its core and chromatin modifying complexes and regulatory RNAs as additional components. By changing functional interactions within the network, extracellular signals drive lineage progression, eventually culminating in terminal differentiation and myelination. In my presentation, several examples will be given for important functional interactions within the regulatory network of oligodendroglial cells and how their alterations influence developmental myelination in the central nervous system of mammals. This will include the calcium-dependent activation of Nfat proteins and the induction of Myrf expression at the time of terminal differentiation as important modulatory events with impact on the transcriptional activity of Sox10 and as triggers of the myelination event.

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S20 SUMOylation in Health and Disease: From synaptic function to neurodegeneration

S20-01

Extranuclear protein sumoylation in neurons

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SUMOylation acts as a biochemical switch that regulates a wide and diverse array of cellular processes. The dynamic balance between SUMO conjugation, mediated by a restricted set of SUMOylation enzymes, and deSUMOylation mediated by SUMO proteases controls substrate protein function, and is essential for cell survival. While predominantly studied as a nuclear protein modification, it is now clear that SUMOylation of proteins outside the nucleus play direct roles in controlling synaptic transmission, neuronal excitability, mitochondrial dynamics and adaptive responses to cell stress. Furthermore, alterations in protein SUMOylation are observed in a wide range of neurological and neurodegenerative diseases, and several extranuclear disease-associated proteins have been shown to be directly SUMOylated. Nonetheless, how SUMOylation of specific substrates is orchestrated to control diverse cellular pathways is a major unresolved question. Here I will discuss our recent mechanistic findings on how SUMOylation and deSUMOylation of specific synaptic and mitochondrial proteins are central to neuronal function and viability.

S20-02

Sumoylation of alpha-synuclein in the pathogenesis of parkinson's disease

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Parkinson's disease (PD) is characterized by the neuronal accumulation of α-synuclein and death of dopaminergic neurons in the substantia nigra. At post-mortem examination, neurons from PD patients show the presence of inclusions, known as Lewy bodies, which are primarily composed of aggregated/fibrillated α-synuclein. Although the aggregation of α-synuclein is likely involved in the pathogenesis of the disease, the mechanisms responsible for its accumulation and aggregation in PD have remained elusive. We have previously shown that monoubiquitination by SIAH ubiquitin-ligase promotes the proteasomal degradation of α-synuclein. This monoubiquitination is dynamic, and when not properly degraded by the proteasome, monoubiquitinated α-synuclein promptly aggregates in cells. More recently, we found that the degradation and aggregation of α-synuclein are further controlled by another post-translational modification, SUMOylation. We identified PIAS2 as an endogenous SUMO-ligase for α-synuclein. SUMOylation by PIAS2 decreases α-synuclein monoubiquitination, leading to decreased α-synuclein proteasomal degradation and triggering α-synuclein accumulation. SUMOylation by PIAS2 also directly promotes the aggregation of α-synuclein *in vitro* and in cells. In addition, α-synuclein disease mutants are more readily SUMOylated, suggesting that increased SUMOylation may play a role in the aggregation of α-synuclein in patients with familial PD. Supporting a more widespread role of SUMOylation in PD, the levels of SUMOylated α-synuclein and PIAS2 are increased in sporadic PD brains. Therefore, we raise the possibility that SUMOylation may play a role in the accumulation and aggregation of α-synuclein in the disease. Targeted inhibition of α-synuclein SUMOylation may help prevent the build-up of pathological α-synuclein in PD.

S20-03

The role of FOXP1/2 sumoylation in neurodevelopment

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Post-translational modifications play an important role in directing the function or expression of proteins. Sumoylation has been shown to regulate protein function in numerous ways including subcellular localization, transcriptional regulation, and stability. In the central nervous system, sumoylation of proteins has been identified in regulating ion channel activity, synaptic formation and function, mRNA transport in axons, and mitochondrial function. However, few studies have demonstrated a role for sumoylation in directing mammalian organismal behavior. In our work, we have examined the role of sumoylation of two related transcription factors, FOXP1 and FOXP2, in the development of the neocortex and cerebellum, respectively. Both of these transcription factors have strong genetic links to human brain disorders: variants in FOXP2 are associated with speech and language disorders and variants in FOXP1 are among the most common de novo autism spectrum disorder variants. We have found that sumoylation of FOXP2 is necessary for Purkinje cell development and cerebellar-related behaviors including ultrasonic vocalizations. Sumoylation of FOXP1 is necessary for proper cortical lamination development and may underlie deficits in socially-relevant behaviors. Ongoing work in the lab is devoted to understanding the cell-type specific role of each of these transcription factors within the developing brain, and results from single-cell RNA-sequencing in a number of conditional knockout lines will be presented.

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S20-04

Sumoylation impact on synaptic function and alzheimer disease pathology

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Small ubiquitin-like modifiers (SUMOs) can affect a number of cellular pathways. Recent evidence has shown SUMOylation contributions to neuronal function and may be important factors in the amyloid and tau pathology in Alzheimer disease (AD) and related disorders. We demonstrated in a transgenic mouse model that over-expression of SUMO1 results in an impairment of synaptic development leading to cognitive deficient. In contrast, comparable SUMO2 transgenic animals display normal development and no changes in learning and memory. There has been a debate on the effects of SUMO1 and SUMO2 on amyloid pathology and, to resolve this issue, we have recently investigated the impact of SUMOylation on processing of the amyloid precursor protein (APP) leading to the production and deposition of the amyloid-β (Aβ) peptide. Using the SUMO1 transgenics, an *in vivo* model was developed by the generation of double transgenic mice over-expressing human SUMO1 and a mutant APP. The SUMO1-APP mice displayed normal APP processing but exhibited increased insoluble Aβ and plaque density accompanied by increased synaptic loss, more pronounced synaptic and cognitive deficits. These findings suggest a potential impairment in Aβ clearance as opposed to increased amyloid production. In contrast, SUMO2-APP double transgenic mice were less affected by amyloid deposition suggesting a more beneficial response of SUMO2 to the AD-related stress conditions. Our findings indicate a more detrimental impact of SUMO1 on amyloid and tau pathology and possible protective effects associated with higher levels of expression for SUMO2.

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S21 Regulation of Neuronal Development and Plasticity by Palmitoylating Enzymes

S21-01

Post-translational palmitoylation and its regulation of synaptic plasticity

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Palmitoylation is the most common post-translational lipid modification in the brain. It involves the addition of the fatty acid, palmitate, onto substrate proteins and is exceedingly important for protein trafficking and cell signaling. Enzymes that mediate palmitoylation consist of a family of 23 proteins zDHHC enzymes. Approximately 41% of *all* identified synaptic proteins are substrates for palmitoylation, and the differential palmitoylation of synaptic substrates has been reported in response to synaptic activity suggesting a role for palmitoylation in the regulation of synapse plasticity. Using proteomic analysis, we have identified a list of synaptic proteins that are differentially palmitoylated in the hippocampus of mice that have undergone fear conditioning, as well as in hippocampal cultures following chemical LTP. We have also identified zDHHC enzymes that are differentially expressed and modified in response to synaptic activity, to provide a more mechanistic understanding of how zDHHC enzymes regulate synapse plasticity.

S21-02

Control of neuronal excitability by palmitoylation-dependent ion channel clustering at the axon initial segment

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Precise control of neuronal excitability is essential for normal behaviour and cognition, while aberrant excitability is a hallmark of many neurological diseases. One key factor that controls the threshold of excitability is the clustering of voltage-gated ion channels at the Axon Initial Segment (AIS), but how such clustering is regulated is not fully understood. Ion channel clustering at other subcellular locations is often controlled by modification of Membrane-Associated Guanylate Kinase (MAGUK) family 'scaffold' proteins with the lipid palmitate, a process called palmitoylation. Using unbiased screening we identified PSD-93, the only MAGUK family member that localizes to the AIS, as a direct interactor and substrate of a palmitoyl acyltransferase (PAT), ZDHHC14. Using lentiviral-mediated shRNA knockdown we assessed how loss of *Zdhhc14* affects clustering of PSD-93 and AIS-localized potassium channels to which PSD-93 binds. Results of these studies provide new insights into the regulation of ion channel clustering at the AIS, and have broad implications for our understanding of physiological regulation of excitability and its dysfunction in conditions such as epilepsy.

S21-03

Activity-dependent palmitoylation regulates SynDIG1 function in excitatory synapse development and plasticity

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A key neural mechanism of learning and memory is thought to be activity-dependent changes in synaptic AMPA receptors (AMPAR) levels. Hebbian plasticity such as long-term potentiation and long-term depression is an associative, positive feedback mechanism to allow individual synapses to strengthen or weaken depending on increased or decreased activity, respectively. Homeostatic plasticity is a negative feedback mechanism that maintains the strength of synapses relative to each other and is referred to as 'synaptic scaling.' Together, Hebbian and homeostatic plasticity are necessary to allow for input-specific changes in synaptic strength while maintaining a dynamic yet stable framework of neuronal excitability. Palmitoylation is a reversible post-translational modification that regulates membrane association, trafficking, and protein–protein interactions. Activity-dependent palmitoylation regulates localization and function of many synaptic proteins including AMPARs and PSD-95 in an activity-dependent manner. Previously, we showed that overexpression or knock-down (KD) of the AMPAR-interacting transmembrane protein SynDIG1 (SD1, Synapse Differentiation Induced Gene 1) in dissociated rat hippocampal neurons increases or decreases, respectively, AMPA-R synapse size and number by ~50% with immunocytochemistry and electrophysiology (Kalashnikova et al., 2010). The magnitude of this effect matches that of the transmembrane AMPAR associated regulatory proteins (TARPs) and PSD-95, suggesting that SD1 is a critical regulator of AMPAR synaptic strength. Intriguingly, SD1 localization at excitatory synapses increases dramatically in response to network silencing by tetrodotoxin, a manipulation established to induce upscaling of synaptic AMPARs. Furthermore, KD of SD1 in hippocampal neurons prevents AMPAR-mediated homeostatic upscaling of synaptic strength, a form of non-Hebbian plasticity, in response to activity silencing. We propose that SD1 localization at synapses and regulation of synaptic strength is mediated by activity-dependent palmitoylation.

S21-04

Determining the role of palmitoylation in subcellular localization of ankyrins

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Ankyrins are a family of scaffolding proteins that recruit ion channels, transporters, and cell adhesion molecules to specialized membrane domains, critical for cell polarity and cell function. Two

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members of the ankyrin family, ankyrin-G (encoded by *ANK3*) and ankyrin-B (encoded by *ANK2*), exhibit distinct subcellular localization and unique functions in neurons, despite high homology. In neurons, ankyrin-G recruits voltage-gated sodium channels, the cell adhesion molecule neurofascin, as well as the cytoskeletal protein βIV spectrin at the axon initial segment, a critical membrane domain responsible for initiation of the action potential. In contrast, ankyrin-B is localized throughout the neuronal plasma membrane, with the exception of the AIS, where it plays roles in maintenance of the distal axon cytoskeleton, control of axonal projections, and participation of axonal transport. To date, the mechanisms underlying the distinct localization of ankyrin-G and ankyrin-B in neurons remain unclear. Previous studies showed that *S*-palmitoylation of ankyrin-G is required for its specific localization at the epithelial cell lateral membrane, as well as its ability to build this membrane. We show here the first evidence that all neuronal isoforms of ankyrin-G and ankyrin-B are *S*-palmitoylated in mouse cortex, providing precedence for studying palmitoylation as a regulator of ankyrin localization and function in neurons. Furthermore, we show that ankyrin-B and ankyrin-G palmitoylation is mediated by a partially distinct set of zDHHC PATs in HEK293T cells, suggesting that differential ankyrin recognition by zDHHC PATs may govern their distinct localization in neurons.

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S22 Is Multiple Sclerosis a Primary Cytodegenerative Disease?

S22-01

Primary neurodegeneration in multiple sclerosis

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Demyelination is the pathological hallmark of multiple sclerosis (MS). Neurodegeneration, however, is the major cause of irreversible neurological disability in MS and occurs as a consequence of demyelination. Axons are transected in acute white-matter (WM) lesions, and cortical and deep gray-matter (GM) demyelination cause neuronal and axonal loss. GM atrophy is one of the best MRI predictors of neurological disability in MS patients and can occur independently of brain WM lesions. Based upon these MRI observations, it has been proposed that demyelination and neurodegeneration can be independent events in MS. We recently described a novel subtype of MS (myelocortical MS; MCMS) characterized by demyelination of the spinal cord and cerebral cortex, but not of cerebral WM. MCMS patients were severely disabled at time of death and comprised ~12% of our autopsy cohort. Clinical histories of MCMS patients were indistinguishable from typical MS (TMS) patients with cerebral WM demyelination, which provided a platform to compare cortical neuronal density in MCMS, TMS, and aged-matched control brains. Compared to control cortices, neuronal densities were lower in MCMS cortices than in TMS. These studies provide pathological evidence that cerebral WM demyelination and cortical neuronal degeneration can be independent events in MCMS. MRI of MCMS brains detected cerebral WM regions that contained T2 hyperintensities, T1 hypointensities, and altered magnetization transfer ratios (MTR). Pathological analyses of these regions detected swollen myelinated axons. We propose that increased water content in swollen myelinated axons is partially responsible for regions with T1 hypointensities and reduced MTR. These studies all support the concept that brain WM demyelination and neurodegeneration can be independent events in MS. If a primary neurodegenerative process exists in individuals with MS, then associated molecular changes could initiate a secondary immune-mediated demyelination. This work was funded by the NIH.

S22-02

Inflammatory demyelination and the loss of oligodendroglial support of axonal energy metabolism

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Loss of CNS myelin in multiple sclerosis (MS) and its animal models (EAE) is invariably associated with axonal dysfunction and degeneration, but the underlying mechanisms are not well understood. Myelin sheaths isolate axonal compartments from the extracellular milieu and thus from rapid access to glucose. We hypothesize that the structural integrity of myelin, including the system of nm-wide cytosolic channel that connect the oligodendroglial soma with the periaxonal innermost layer of myelin, are required for efficient metabolic support of axons by lactate. Here, non-synaptic activation of oligodendroglial NMDA receptors by high frequency spiking, a proxy of energy demands, enhances glucose import and lactate supply. Moreover, myelin itself is an energy reserve and oligodendroglial lipid metabolism contributes to the axonal energy balance. Is myelin under acute immune attack most detrimental to axon function and survival compared to completely demyelinated axons, because the latter are less likely to 'starve'? This hypothesis can be tested in *Mbp neo*mice, a novel mouse mutant with mosaic dysmyelination of the spinal cord. These mice appear significantly more 'resistant' to the effects of MOG-EAE despite the large fraction of unmyelinated spinal cord axons.

S22-03

Neurodegeneration, grey matter pathology, and an aberrant AXO-myelinic synapse: lessons from histopathology and post mortem MRI

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Multiple sclerosis (MS) is classically considered to be an autoimmune, inflammatory demyelinating disease of the CNS. However, it has been questioned whether inflammation and/or autoimmunity are really at the root of the disease, and it has been proposed that MS might, in fact, be a primary degenerative disorder (Stys, 2012). This lecture will review several studies that contributed to this discussion from the viewpoint of human histopathology and (post mortem) MRI. Firstly, over the past twenty years it has become abundantly clear that gray matter damage in this 'prototypical WM disease' is widespread (Geurts 2008, 2012). Interestingly, it seems that demyelinated gray matter lesions are hardly, if at all, driven by inflammation. Instead, vast quantities of myelin products seem to gather extracellularly around blood vessels and in the meninges (Kooi 2009), presumably to be transported away to the cervical lymph nodes (Fabriek 2005). Chronic white matter damage, too, was shown to be largely non-inflammatory (Seewann et al, 2009) except for a microglial component reminiscent of that in primary neurodegenerative diseases such as Alzheimer's. Granted, these might be 'late stage effects', when neuroinflammation and neurodegeneration have largely become separate pathophysiological processes. However, even in the earliest phases of the disease, gray matter degeneration, especially thalamic atrophy, is already pertinent (Schoonheim 2012). And ongoing research suggests that, at the microscopic level, communication between MS axons and their surrounding myelin is fundamentally disrupted in several different ways, even before an inflammatory response is apparent. Whether these pathological processes build up to a sufficiently convincing model for 'inside-out' cytodegeneration in MS is now up for debate. However, they already seem to provide a tantalizing challenge to the existing dogma of primary autoimmunity.

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S22-04

Multiple sclerosis as a protein misfolding disorder

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Data in recent years support the notion that MS is a convolution of degeneration and auto-immunity, together conspiring to produce the broad spectrum of relapsing-remitting and progressive clinical phenotypes. However, after decades of intensive investigation, the primary trigger of the disease is unknown. Data will be presented supporting the hypothesis that MS is a protein misfolding disorder, similar to other traditional neurodegenerative diseases. Intracerebral inoculation of transgenic mice with human MS brain homogenate induces a myelinopathy together with an astro- and microgliosis similar to the non-lesional white matter pathology found in human MS. Subtle behavioral disturbances (spatial learning deficits on water maze testing) accompany the histological abnormalities. Passaging (inoculation of naïve mice with brain homogenate of MS-inoculated mice) also continued to transmit pathology. Control human brain homogenate (e.g. chronic encephalitis, Alzheimer's, Lewy body disease) did not reproduce the transmitted pathology. We hypothesized that prion protein may play a role: immunodepletion of PrP and intracerebral inoculation significantly reduced the transmitted pathology. Together these data suggest that a transmissible misfolded protein might play a role, and the prion protein could be directly or indirectly involved in the generation of the subtle MS-like pathology in recipient mice. This mechanism might lead to myelin disruption, subsequent axonal compromise, and release of antigenic debris that secondarily promote auto-immune inflammation in the human.

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S23 RNA modification in the brain and behaviour

S23-01

RNA modifications and memory

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In this talk, I will discuss our recent work characterized experience-dependent subcellular compartmentalization of m6A modified RNA and it's role in adaptive behavior. Specifically, we are testing the hypothesis that m6A is necessary for RNA localization and that there is a distinct pool of m6A modified RNA at the synapse that influences fear exitinction memory. I will also touch on other RNA modifications that appear to occur in a learning- or activity-dependent manner.

S23-02

RNA modifications and translational regulation at neuronal synapses

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Single-cell analyses have revealed that compared to other cell types in the brain, neurons not only contain higher RNA content, but also higher RNA species diversity, suggesting complex translational regulatory mechanisms that neurons actively engage to achieve their function. Our lab has focused on a cellular mechanism that physically uncouple transcription and translation through mRNA trafficking and local translation at neuronal synapses. We have found that the synaptically pre-deposited mRNA species can respond to stimuli that induce long-term synaptic plasticity and undergo synapse-, transcript-, and stimulus-specific translation. In search for the regulatory components accounting for such molecular specificity, we have found that methylation at adenosine RNA residues (m6A) can functionally mark the localized RNA species and positively regulate their translation. Reducing proteins that recognize and bind to m6A in neurons causes neuronal deficits in spinogenesis, activity-dependent spine maturation, synaptic transmission, learning, and memory retention. Furthermore, we show that RNA methylation-mediated translational mechanisms may play important roles in regulating dynamic microtubule networks that underlie building and remodeling of neuronal circuits.

S23-03

Neuronal allocation to an engram underlying memory

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Understanding how the brain uses information is a fundamental goal of neuroscience. Several human disorders (ranging from autism spectrum disorder to Alzheimer's disease) may stem from disrupted information processing. Therefore, this basic knowledge is not only critical for understanding normal brain function, but also vital for the development of new treatment strategies for these disorders. Memory may be defined as the retention over time of internal representations gained through experience, and the capacity to reconstruct these representations at later times. Long-lasting physical brain changes ('engrams') are thought to encode these internal representations. The concept of a physical memory trace likely originated in ancient Greece, although it wasn't until 1904 that Richard Semon first coined the term 'engram'. Despite its long history, finding a specific engram has been challenging, likely because an engram is encoded at multiple levels (epigenetic, synaptic, cell assembly). Here, I will discuss our previous studies examining how specific neurons are recruited or allocated to an engram, and our recent work examining how neuronal membership in an engram may change over time or with new experience.

S23-04

Epitranscriptomic regulation of protein synthesis, learning and memory by N6-methyladenosine (m6A)

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N^6-methyl(m^6A) is the most abundant mammalian inmRNA modification. It proreguRNA splicing, trans locali transand degradation, and may play a central role in the spatial and temporal control of protein synthesis. The emergence of m^6A research is largely facilitated by the disof its key effector pro m^6A "writers" (methyl e.g. METTL14) install m^6A, "erasers" (demethyl remove m^6A, and "readers" (e.g. YTHDF1, 2 and 3) recogand bind to m^6A to determine the fate of the modified RNA. Different m^6A readers may mediate different downfunctional consequences of m6A modification of mRNA. Recent data sugthat m^6A deficiency impairs both neurement and adult central nervous system func and impairs learning and memory. We have found that conditional deletion of Mettl14 in striatonigral neu striatopallidal neuor dopamine neurons impaired reinforcement learning and motor learning, and altered reto cocaine. Constitutive deletion of Ythdf1 impaired prosynthesis, altered synaptic trans and impaired many different forms of learning and memory. Because m^6A modifies thousands of transand proimpacts many neufunc it's been a challenge to systematically inhow the dynamic reguof the m^6A pathway may control speprotein synthesis with good spatial and temporal resolution to affect synaptic plasticity, learning and memory. We are using geapproto manipulate the effector pro What the targets of these effector prons are, how the above manipulations may affect transglobally or transof spemRNAs, synaptic proturnover, synaptic trans plasticity, denspine morphology and beare being systematically examined.

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S24 Biological and Therapeutic Roles of Lipids in Neurodegeneration

S24-01

Role of isoprenoids in autophagy and prion-like spread of abeta

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The development of disease-modifying therapies for Alzheimer's disease (AD) is hampered by the incomplete understanding of early pathogenic mechanisms that lead to disease. Brain accumulation of beta amyloid (Aβ) drives AD pathogenesis. Recent findings indicate that cell to cell transmission of Aβ by a 'prion-like' spread mechanism contributes to AD progression.

We discovered that $A\beta_{42}$ inhibits synthesis of cholesterol and isoprenoids (FPP and GGPP) by impairing maturation of SREBP2. This inhibition results in reduction of protein prenylation in neurons exposed to $A\beta_{42}$. We also demonstrated that protein prenylation is reduced in brain cortex of TgCRND8 mice.

A cellular process that relies heavily on prenylated proteins such as Rabs, is autophagy. Extensive autophagic-lysosomal pathology in AD brain plays a role in disease pathogenesis, although the underlying mechanisms are not well understood. Previous reports demonstrated that reversing autophagy dysfunction by genetic manipulation improves pathophysiology and rescues memory performance in TgCRND8 mice. Using the tandem reporter mcherry-GFP-LC3 we found that $A\beta_{42}$-induced inhibition of protein prenylation causes a blockade of autophagic flux in cultured cells and in vivo. Recovery of protein prenylation with GGPP normalizes autophagic flux in both paradigms. Moreover, Rab7 localization to autophagosomes, which is required for autophagy progression, is reduced in $A\beta_{42}$-treated neurons and GGPP corrects Rab7 prenylation and its subcellular localization.

When autophagy is compromised, cells may resource to protein secretion to alleviate stress. Aβ is released in extracellular vesicles (EVs). Using imaging flow cytometry and nanoparticle tracking analysis we showed that Aβ-induced autophagy blockade increases EVs secretion favoring cell-to-cell spreading of Aβ.

Our studies identify the reduction of protein prenylation as a key mechanism of autophagy dysfunction and prion-like spread in AD models and provide novel autophagy-related targets with disease-modifying value.

S24-02

Gangliosides in Huntington's disease and beyond

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Huntington's disease (HD) is a neurodegenerative disorder caused by the expansion of a CAG trinucleotide repeat in the first exon of the *HTT* gene. The resulting mutant huntingtin (mHTT) protein acquires toxic conformations and aggregates within the cells, leading to neuronal dysfunction and death. We have shown that levels of ganglioside GM1, a glycosphingolipid highly enriched in the brain, are decreased in HD models. Administration of exogenous GM1 reduces levels of soluble and aggregated mutant huntingtin in HD mouse brains, slows down neurodegeneration and corrects motor as well as cognitive and psychiatric-like dysfunctions in HD mice. The underlying mechanisms and potential implications for other protein misfolding diseases will be discussed.

S24-03

TREM2 regulates microglial cholesterol metabolism upon chronic phagocytic challenge

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Loss of function variants of TREM2, an immune receptor expressed in microglia, increase Alzheimer's disease (AD) risk. REM2 was shown to recognize lipids and mediate myelin phagocytosis, but its role in microglial lipid metabolism is unknown. Combining chronic demyelination paradigms and cell sorting techniques with RNA sequencing and lipidomics, we found that wildtype microglia acquire a disease-associated microglia (DAM) transcriptional state, while TREM2-deficient microglia remain largely homeostatic, which leads to neuronal damage. TREM2-deficient microglia maintain phagocytic activity of myelin debris, but are incompetent at clearing myelin lipids, including cholesterol, resulting in marked intracellular accumulation of cholesteryl esters. Defects in cholesterol metabolism were replicated in aged wildtype microglia and in cultured TREM2-deficient macrophages upon myelin challenge, where they required ACAT1 activity. TREM2 therefore mediates a transcriptional program required to process cholesterol overload during chronic phagocytic activity, which ultimately prevents neuronal damage. These results provide a potential mechanism for pathogenic lipid accumulation in AD.

S24-04

Effects of Niemann-Pick type C1-deficiency on synaptic function and brain energy metabolism

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Cholesterol is an essential component of all animal membranes, and influences membrane fluidity, permeability, membrane protein function, and fission and fusion processes. The brain is the most cholesterol-rich organ in the human body. While most brain cholesterol is in myelin and metabolically nearly inert, non-myelin cholesterol is actively turned over at rates comparable to peripheral tissues. Changes in brain cholesterol homeostasis are linked to synaptic dysfunction and neurodegeneration. Niemann-Pick Type C (NPC) disease, caused in most cases by loss of the late endosomal NPC1 protein, is characterized by cholesterol accumulation in the endocytic pathway, redistribution of cholesterol and a range of cellular dysfunctions. Here, I will discuss the effects of NPC1 deficiency on synaptic function and energy metabolism.

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S25 Molecular dynamics of the inhibitory post synapse and the tuning of synaptic inhibition

S25-01

Membrane dynamics at the inhibitory synapse and the regulation of inhibitory transmission

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Synaptic inhibition plays a critical role in regulating the balance of excitation and inhibition in the brain and thus information processing. The strength of inhibition is determined to a large extent by the number of $GABA_A$receptors ($GABA_A$Rs) at synaptic sites, which can be controlled by receptor stabilisation in the synaptic membrane. I will talk about our ongoing work to better understand the machinery important for targeting and stabilization of $GABA_A$Rs at synapses and the role played by key inhibitory synaptic components including Neuroligin 2 and the $GABA_AR$ receptor accessory protein LHFPL4/Garlh. I will also focus on the mechanisms regulating the trafficking dynamics of Neuroligin 2 at the synapse and how this can regulate inhibitory synapse strength. Our elucidation of the mechanisms important for controlling the membrane dynamics of inhibitory synaptic components opens up new avenues for understanding the regulation of inhibitory transmission in the brain.

S25-02

Nanoscale organization of the inhibitory synapse

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Inhibitory synapses mediate the majority of synaptic inhibition in the brain, thereby controlling neuronal excitability, firing and plasticity. Although essential for neuronal function, the central question of how these synapses are organized at the subsynaptic level remains unanswered. Here, we utilize 3D super-resolution microscopy to image key components of the inhibitory postsynaptic domain and presynaptic terminal, revealing that inhibitory synapses are organized into nanoscale subsynaptic domains (SSDs) of the gephyrin scaffold, $GABA_A$Rs and the active zone protein, Rab3-interacting molecule (RIM). Gephyrin SSDs cluster $GABA_AR$ SSDs, demonstrating nanoscale architectural interdependence between scaffold and receptor. $GABA_AR$ SSDs strongly associate with active zone RIM SSDs, indicating an important role for $GABA_AR$ nanoscale organization near sites of GABA release. Finally, we find that in response to elevated activity, synapse growth is mediated by an increase in the number of postsynaptic SSDs, suggesting a modular mechanism for increasing inhibitory synaptic strength.

S25-03

Proteo-connectomics to discover novel synaptic proteomes and mechanisms of inhibition in vivo

S. Soderling

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This talk will present work on identifying novel proteins at synapses using *in vivo* proximity biotinylation and high resolution quantitative mass spectrometry. I will first present data on the diverse proteomes of synapses within multiple neuron types *in vivo.* This will be followed by data on the synaptic and behavioral analysis of knockout mice for a newly discovered GABAergic postsynaptic protein.

S25-04

Tuning of synaptic inhibition by the second messenger cl-

S. Levi

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Upon activation by GABA, $GABA_A$Rs open a selective chloride/bicarbonate conductance. The direction of chloride (Cl^-) flux through the channel depends on transmembrane Cl^- gradients. Therefore, Cl^- homeostasis critically determines the polarity and efficacy of GABAergic transmission in the brain. Pharmaco-resistant epilepsies are often associated with altered Cl^- homeostasis. It is therefore crucial to discover novel mechanisms regulating neuronal Cl^- homeostasis that may help develop new and efficient treatment for these forms of epilepsy and other diseases associated with impaired inhibition, such as neuropathies and some neuropsychiatric disorders.

The increase in $[Cl^-]_i$ and subsequent depolarized shift in the reversal potential of $GABA_AR$-mediated currents (E_{GABA}) observed in the epileptic brain are most often attributed to reduced surface expression/function of the neuronal K^+-Cl^- cotransporter KCC2, responsible for Cl^- export. Furthermore, up-regulation of the Na^+-K^+-Cl^- cotransporter NKCC1, which imports chloride into neurons, also increases $[Cl^-]_i$ and alters E_{GABA}. Although the mechanisms regulating KCC2 in the pathology have been extensively explored revealing altered membrane trafficking, those controlling NKCC1 remain largely unknown.

We recently demonstrated the contribution of a novel signaling pathway in the regulation of KCC2. We showed that KCC2 is regulated by GABAergic signaling through Cl^--dependent phosphorylation of KCC2 Threonine residues T906/1007. Cl^- acts as a secondary messenger in this regulation by tuning the activity of the Cl^- sensitive With No lysine (K) serine-threonine kinase WNK1 and its downstream effectors Ste20 Proline Asparagine Rich Kinase (SPAK) and Oxydative Stress Response kinase 1 (OSR1). Interestingly, WNK kinases not only promote KCC2 T906/T1007 but also NKCC1 T203/T207/T212 phosphorylation. This results in dual modulation of Cl^- transport by inhibiting KCC2 and by activating NKCC1; both regulations leading to elevation in intracellular Cl^- level. Therefore, inhibiting the neuronal WNK/SPAK/OSR1-dependent KCC2 and NKCC1 Threonine phosphorylation may normalize the membrane expression/function of the transporters and reduce $[Cl^-]_i$. The WNK/SPAK/OSR1 signaling pathway may thus

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represent a promising therapeutic target for preventing the emergence of acquired epilepsies.

We aim to characterize the WNK/SPAK/OSR1 pathway in central neurons and to determine whether genetic or pharmacological inhibition of this cascade has beneficial effects for epilepsy. Our project will help uncover novel and promising therapeutic strategies for several forms of acquired epilepsy, and other pathologies in which inhibition is altered, such as neuropathic pain and psychiatric disorders.

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S26 Emerging pathways in amyotrophic lateral sclerosis

S26-01

Coordinated disassembly and reassembly of the nuclear pore complex in C9orf72 and sporadic ALS

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An intronic GGGGCC hexanucleotide repeat expansion in the C9orf72 gene is the most common cause of Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD). Nucleocytoplasmic transport is tightly controlled by the nuclear pore complex and has recently emerged as a prominent pathomechanism underlying multiple neurodegenerative diseases including C9orf72 ALS/FTD. Using super resolution structured illumination microscopy, we evaluated the distribution of individual nucleoporins in nuclei isolated from control and C9orf72 iPSC derived motor neurons and postmortem human motor cortex to identify a subset of nucleoporins lost from the nuclear pore complex in an age dependent manner. A combination of overexpression and knock down experiments reveals that POM121, an integral scaffolding nucleoporin, coordinates the disassembly and reassembly of the nuclear pore complex in post-mitotic neurons impacting nucleocytoplasmic transport and cellular toxicity. Together, these data suggest that POM121 is an integral nucleoporin in the maintenance of the nuclear pore complex in post-mitotic neurons and loss of POM121 from the nuclear pore complex in C9orf72 ALS/FTD initiates a pathological cascade affecting nuclear pore complex integrity and function.

S26-02

Axonal transport defects in motor neurons derived from ALS patients

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The dissociation of the motor axon from the muscle, resulting in denervated neuromuscular junctions, leads to muscle atrophy and paralysis during amyotrophic lateral sclerosis (ALS). The retraction of the axon and ultimately the selective death of the motor neuron is the hallmark of the disease. The mechanism leading to this 'dying back' phenomenon is not known in approximately 90% of patients, while in the remaining patients a number of different genes are mutated. SOD1, TARDBP, FUS and C9orf72 are the most important ones. We generated and characterized induced pluripotent stem cells (iPSCs) from ALS patients with mutations in TARDBP, FUS and hexanucleotide repeats in C9orf72, as well as from healthy controls. Patient-derived motor neurons showed a number of typical characteristics for each of the mutations, including a higher fraction of insoluble TDP-43 in the lines from patients with mutated TARDBP, cytoplasmic mislocalisation of FUS in the lines from mutant FUS patients and the production of dipeptide repeat proteins (DPRs) in the C9orf72 patient lines. In addition, we observed hypoexcitability, as well as progressive axonal transport defects in all the ALS lines. These axonal transport defects could be rescued by genetic correction using CRISPR/Cas9 of the FUS mutation in the patient-derived iPSCs. Moreover, these defects could be reproduced by expressing mutant FUS in human embryonic stem cells (hESCs) confirming that these pathological changes were mutant FUS dependent. Pharmacological inhibition, as well as genetic silencing of histone deacetylase 6 (HDAC6), increased α-tubulin acetylation and restored the axonal transport defects in patient-derived motor neurons. In conclusion, we observed axonal transport defects in human-derived motor neurons from patients with different genetic causes that we could correct by using selective HDAC6 inhibitors.

S26-03

Pathogenic significance of aberrant glia phenotypes in amyotrophic lateral sclerosis

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Amyotrophic Lateral Sclerosis (ALS) is characterized by degeneration of upper and lower motor neurons accompanied by the proliferation of reactive microglia in affected regions. Previous reports have shown the occurrence of aberrant glial phenotypes associated to spinal motor neurons. However, the origin and pathogenic significance of glial diversity in ALS remain unknown. By using cell cultures and immunohistochemistry we have characterized abnormal microglia cell phenotypes interacting with motor neurons in the spinal cord of SOD1G93A rat spinal cords and autopsied tissues from sporadic ALS subjects. We will present evidence of two distinct and yet-unknown phenotypes of microglia identified by the expression of senescent and microglia progenitor markers. Both subsets of microglia cells accumulate adjacent to degenerating spinal motor neurons, representing intriguing cell targets for approaching ALS pathogenesis and therapeutic.

S26-04

Translational control of immune response in ALS

J. Kriz

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Microglia are the principal immune cells of the brain. Once activated, in injured and/or diseased brain, microglia can acquire a wide repertoire of the context-dependent immune profiles. However, at present, the molecular mechanisms involved in the control of microglia polarization profiles remain elusive. By using an in vivo model-system for analysis of the dynamic translational state of microglial ribosomes with mRNAs as input and newly synthesized peptides as an output, recently created in our laboratory (*Boutej et al., Cell Rep 2017*),we found a marked dissociation of microglia mRNA and protein molecular signatures following an acute innate immune challenge. The results revealed that highly up-regulated and

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ribosome-associated mRNAs were not translated resulting in creation of two distinct microglia molecular signatures: i) a highly specialized pro-inflammatory mRNA and ii) immunomodulatory/homeostatic protein signature. The most striking divergence was observed in the key immune NF-κB network where we found that the cluster of highly up-regulated LPS-induced and polysome-associated mRNAs such as Saa3, Lcn2 ccl3, ccl5 (from 15-30 fold increase at mRNA level) were indeed not translated. As mechanism, we discovered a selective 3'UTR-mediated translational suppression of highly expressed mRNAs. Moreover, we identified a novel and previously unknown role for RNA binding protein SRSF3 as a master suppressor/regulator of innate immune genes translation. The complex analysis of mRNA/protein networks in ALS affected and chronically activated microglia suggests existence of SRSF3 mediated, ribosome-based mechanism/check point involved in the control of highly regulated mRNAs *in vivo*.

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S27 ASN Haber Award Symposium - mTOR signaling in the CNS

S27-01

Differential impact of mTOR signaling in oligodendrocytes during myelination in spinal cord and brain

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The differentiation of oligodendrocyte precursor cells (OPCs) into mature, myelinating oligodendrocytes in the central nervous system involves numerous intracellular signaling cascades, including the mammalian target of rapamycin (mTOR) pathway. mTOR exists in two complexes: Raptor-containing mTOR complex 1 (mTORC1) and Rictor-containing mTOR complex 2 (mTORC2), and our studies deleting mTOR or the separate complexes in oligodendrocytes suggest that the signaling requirements for OPC differentiation and myelination may be region-specific. Our earlier studies demonstrated that deletion of mTOR or mTORC1 (Raptor) in 2',3'-cyclic-nucleotide 3'-phosphodiesterase (CNP)-Cre mice reduced myelination in spinal cord, with little impact in brain. By contrast, deletion of mTORC2 (Rictor) in CNP-Cre mice had little impact in either CNS region.The current study focuses on the role of mTORC2 in OPC differentiation by deleting Rictor in platelet-derived growth factor receptor alpha (PDGFRα)-Cre mice, where recombination is specific to OPCs. By contrast to the earlier studies, conditional deletion of Rictor in OPCs had a dramatic impact on OPC differentiation and myelination, but in these animals the impact was in brain, not spinal cord or optic nerve. Consistent with this phenotype, downstream mTORC2 signaling was impacted more in brain than spinal cord in PDGFRα-Cre x Rictor fl/fl mice. Interestingly, side-by-side analysis of control brain and spinal cord lysates revealed unexpected differences in signaling pathway usage in these CNS regions. These studies supported by NIH NS080223

S27-02

mTOR and telomerase-new partners in the brain?

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Telomerase is a special reverse transcriptase that in its canonical function maintains telomeres in dividing cells using a template on its inherent RNA component. Additionally, the protein part TERT (Telomerase Reverse Transcriptase) has various non-canonical functions. For example, it can localize to mitochondria under increased stress and protect cells *in vitro* from oxidative stress, DNA damage and apoptosis. Recently it has been demonstrated that TERT protein persists in adult neurons in the brain and data emerge suggesting that it might have a protective function in these post-mitotic cells as well. We have recently demonstrated that TERT protein accumulated specifically in brain mitochondria from of mice that have undergone short-term dietary restriction (DR) and rapamycin treatment. This increased mitochondrial localization correlated to lower levels of oxidative stress in brain mitochondria. Decreased mTOR signalling is a known mediator for the beneficial effects of DR. Feeding mice with rapamycin for 4 months increased brain mitochondrial TERT and reduced ROS release from brain mitochondria while telomerase activity was not changed. Importantly, the beneficial effects of rapamycin on mitochondrial function were absent in brains and fibroblasts from first generation TERT -/- mice, and when TERT shuttling was inhibited by the Src kinase inhibitor bosutinib. In summary, our data suggests that the mTOR signalling pathway impinges on the mitochondrial localisation of TERT protein, which might in turn contribute to the protection of the brain by DR or rapamycin against age-associated mitochondrial ROS increase and cognitive decline. Thus, we have discovered that the mTOR pathway might be involved in the TERT localization to mitochondria and its beneficial effects in brain mitochondria in vivo.

S27-03

Aberrant mTOR signaling contributes to development of alzheimer-like dementia

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The mTOR pathway represents a key growth and survival pathway involved in several diseases. Numerous studies linked the alterations of mTOR signaling to age-dependent cognitive decline, pathogenesis of Alzheimer disease (AD) and AD-like dementia in Down syndrome (DS). DS is the most frequent chromosomal abnormality that causes intellectual disability. The neuropathology of AD in DS is complex and involves impaired mitochondrial function, defects in neurogenesis, increased oxidative stress, altered proteostasis and autophagy. Recent studies from our laboratory employing specimens from DS individuals and DS mouse models showed that aberrant mTOR signalling is an early degenerating event in the brain that contributes to acceleration of Aβ and tau deposition and to the development of AD-like cognitive decline. Our results showed the hyperactivation of PI3K/AKT/mTOR axis in the brains of subjects with DS, with or without AD pathology, in comparison to healthy controls, as well as in a Tg mouse model of the disease. These data were associated with decreased autophagy, inhibition of IRS1 and GSK3β activity. Moreover, our results suggest that aberrant activation of PI3K/Akt/mTOR axis acts in parallel to RCAN1 in phosphorylating tau, in DS and DS/AD. These findings represent a strong rationale to test therapeutic strategies aimed to restore mTOR signaling and among drug candidates, we tested the effects of intranasal rapamycin treatment to slow the progression of AD in DS. We demonstrated that rapamycin, administered for 3 months by intranasal route, led to improved cognition in DS mice with no effects at peripheral organs. The favorable outcomes of rapamycin treatment seem to rely on its ability to rescue molecular pathways associated with aberrant mTOR

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phosphorylation, including metabolism of APP and Tau, activation of AMPK and reduction of oxidative stress.

S27-04

Antidepressant effect of ketamine via the mTOR pathway and eEIF4E-dependent mRNA translation

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mTOR controls many cellular functions, including mRNA translation through phosphorylation and inactivation of the eukaryotic initiation factor 4E (eIF4E) binding proteins (4E-BPs), which supress translation by binding to the eIF4E. The latter is the mRNA cap-binding protein that facilitates ribosome binding. The fast-acting antidepressant drug ketamine activates the mammalian target of rapamycin (mTOR) signaling pathway, which is essential for the antidepressant effect of ketamine. We sought to determine whether 4E-BPs play a role in the antidepressant effect of ketamine, and whether this pathway is activated in excitatory or inhibitory neurons. Ketamine did not affect the immobility in the force-swim test (FST) of *Eif4ebp1*$^{-/-}$ or *Eif4ebp2*$^{-/-}$ mice, but, as expected, it reduced immobility in wildtype mice. Moreover, the effect of ketamine on NSF (reduced latency to feed in a new environment) was not observed in *Eif4ebp2*$^{-/-}$ and *Eif4ebp1*$^{-/-}$ mice. Mice lacking either *Eif4ebp1* or *Eif4ebp2* in Camk2a+ cells, were resistant to the antidepressant effects of ketamine, but responded normally to an acute injection fluoxetine. Conditional KO mice in Gad2 + cells were also resistant to ketamine. Furthermore, *Eif4ebp2-/-* mice in Gad2 + cells displayed reduced immobility in the FST without any antidepressant treatment, suggesting a more preponderant role for 4E-BP2 in Gad2 + neurons in response to ketamine. These results demonstrate that activation of eIF4E-dependent translation initiation is required in both excitatory and inhibitory neurons for the antidepressant effect of ketamine.

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