

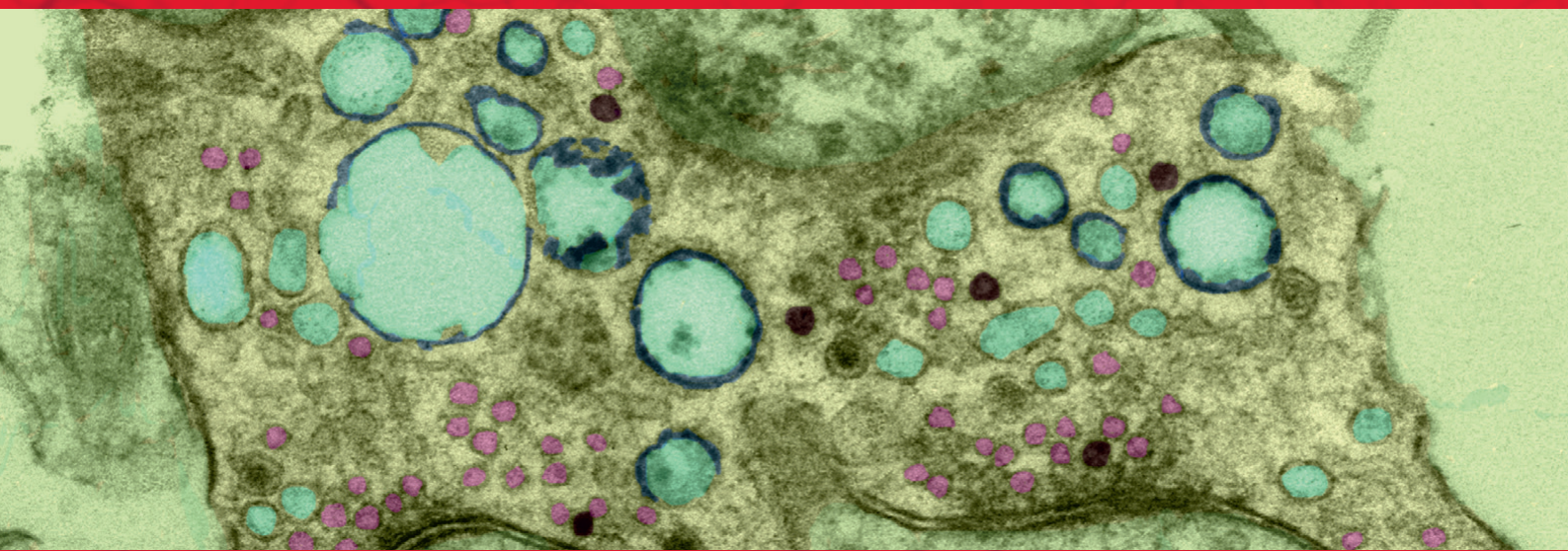
JNC

The Official Journal of the International
Society for Neurochemistry



Journal of Neurochemistry

VOLUME 150 | SUPPLEMENT 1 | JULY 2019



ISN-ASN 2019 Meeting
Montreal, Canada
4th–8th August 2019

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Fax: +49-241-80-82582
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Aims & Scope

Journal of Neurochemistry focuses on molecular, cellular and biochemical aspects of the nervous system, the pathogenesis of neurological disorders and the development of disease specific biomarkers. It is devoted to the prompt publication of original findings of the highest scientific priority and value that provide novel mechanistic insights, represent a clear advance over previous studies and have the potential to generate exciting future research.

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Front cover

Image content: The false colour image shows two presynaptic nerve terminals after a train of neuronal activity which triggered neurotransmitter release followed by both clathrin-mediated endocytosis (CME) and activity-dependent bulk endocytosis (ADBE). In addition to existing synaptic vesicles (pink) and endosomes (light blue) new organelles generated by both CME (purple synaptic vesicles) and by ADBE (dark blue endosomes) are displayed. Image modified from original version in Clayton et al (2010) *Nature Neuroscience* 13: 845–851.

Read the full article '*Dynamin 1 phosphorylation by GSK3 controls activity-dependent bulk endocytosis of synaptic vesicles*' by Emma L Clayton, Nancy Sue, Karen J Smillie, Timothy O'Leary, Nicolai Bache, Giselle Cheung, Adam R Cole, David J Wyllie, Calum Sutherland, Phillip J Robinson & Michael A Cousin (*Nature Neuroscience* volume 13, pages 845–851 (2010)) on doi: 10.1038/nn.2571

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Plenary Lectures

PL01 Plenary Lecture 1 – Professor Lynn Raymond

PL01

Synaptic dysfunction and altered plasticity in prodromal Huntington disease

L. Raymond

University of British Columbia, Psychiatry, Vancouver, Canada

Huntington disease (HD) is an inherited neurodegenerative disorder caused by expansion of a CAG triplet repeat in the *Huntingtin* gene. Neurodegeneration predominantly affects striatum and cortex. The clinical diagnosis is based on a characteristic movement disorder, but cognitive changes – especially impaired mental flexibility and skilled motor learning – often precede the motor diagnosis by 5–10 years. Studies in transgenic and knock-in mouse models of HD have shown alterations in cortical-striatal

synaptic transmission that precede an overt motor phenotype, and suggest aberrant cortical excitatory drive onto striatal neurons, as well as striatal cell autonomous mechanisms, underlie the early loss of striatal projection neurons. Here, work from our lab demonstrating a role for altered NMDA receptor trafficking and signaling in striatal neuronal vulnerability will be described. In addition, our recent studies reveal changes in cortical-striatal and cortical-cortical glutamatergic synaptic plasticity that may underlie early cognitive deficits and potentially contribute to neuronal vulnerability to degeneration in HD. Development of targeted therapy for the early alterations in synaptic function and plasticity will be discussed.

Funding provided by the Canadian Institutes of Health Research Foundation Grant FDN-143210 and the Huntington Society of Canada Navigator Award.

PL02 Plenary Lecture 2 – Professor Volker Haucke

PL02

Presynaptic function and assembly

**V. Haucke¹, T. Soykan¹, A. Vukoja¹, U. Rey², A. G. Petzoldt²,
D. Puchkov¹, S. Rizalar¹, C. Ott¹, S. J. Sigris²**

¹*Leibniz Institut für Molekulare Pharmakologie, Molecular
Pharmacology & Cell Biology, Berlin, Germany*

²*Freie Universität Berlin, Faculty of Biology, Chemistry, Pharmacy,
Berlin, Germany*

³*Charité Universitätsmedizin, NeuroCure Cluster of Excellence,
Berlin, Germany*

Nervous system function relies on the polarized architecture of neurons, established by directional transport of pre- and postsynaptic cargoes. While delivery of postsynaptic components depends on the secretory pathway, the identity of the membrane compartments that supply presynaptic active zone (AZ) and synaptic vesicle (SV) proteins is unknown. I will discuss recent advances in our understanding of how key components of the presynaptic machinery for neurotransmitter release are transported and assembled focussing

on our recent studies in *Drosophila* larvae and mammalian neurons. These studies have revealed an unexpected function for a lysosome-related organelle as the basic building block for presynaptic biogenesis. In the second part of my lecture I will focus on how SVs cycle once a functional presynaptic compartment has been assembled and how neuronal excitability may be controlled by calcium ion homeostasis. Specifically, I will describe our identification of a clathrin-independent endocytosis (CIE) pathway that serves as the primary route for compensatory membrane uptake following action potential induced exocytic SV fusion. This pathway depends on formin-mediated actin assembly as well as on the orchestrated activity of BAR domain proteins that drive the formation of membrane invaginations from which SVs reform by clathrin/AP-2-mediated budding. Other organelles such as the axonal endoplasmic reticulum may regulate neurotransmission by serving as a homeostatic control system for calcium ions to control neuronal excitability. These studies bear important implications for the ability of neurons to respond to a vast range of stimulation frequencies and to process and store information.

PL03 Plenary Lecture 3 – Professor Li-Huei Tsai

PL03

Single-cell transcriptomic analysis of Alzheimer's disease

L.-H. Tsai^{1,2}

¹MIT, Picower Institute for Learning and Memory, Cambridge, USA

²MIT, Department of Brain and Cognitive Sciences, Cambridge, USA

A number of the genetic variants associated with an increased risk for Alzheimer's disease (AD) in genome-wide association studies are linked to genes with identified or putative roles in myeloid lineage cells, particularly in microglia, the “immune” cell of the brain. We previously showed that increased enhancer activity is associated with upregulated gene expression in AD mouse models and human postmortem AD brains. Nonetheless, much remains to be learned about the molecular changes and dynamic roles of microglia in the AD brain. Using single-cell RNA-sequencing we determined the transcriptome of individual microglia cells isolated from the hippocampus of a mouse model of severe

neurodegeneration with AD-like phenotypes at multiple time points of neurodegeneration. Our analysis identified novel disease stage-specific microglia cell states, revealed the trajectory of cellular reprogramming of microglia in response to neurodegeneration and uncovered the underlying transcriptional programs. Currently, our efforts are directed towards analyzing single-cell transcriptomics of human postmortem brain tissue of subjects with and without AD pathology. The strongest Alzheimer's disease-associated changes appeared early in pathological progression and were highly cell-type specific, whereas genes that were upregulated at late stages were common across cell types and primarily involved in the global stress response. Notably, we found that female cells were overrepresented in Alzheimer's disease-associated subpopulations, and that transcriptional responses were substantially different between sexes in multiple cell types, including oligodendrocytes. Overall, myelination-related processes were recurrently perturbed in multiple cell types, suggesting that myelination may have a key role in the Alzheimer's disease pathophysiology.

PL04 Plenary Lecture 4 – Professor David Attwell

PL04

The role of capillary pericytes in regulating brain energy supply in health and disease

D. Attwell

UCL, Neuroscience, Physiology & Pharmacology, London, UK

Brain blood flow is regulated to ensure adequate power for neuronal computation. Blood flow is increased to areas where neurons are active, and this increase underlies non-invasive brain imaging using BOLD fMRI. I will demonstrate that neuronal activity mainly increases cerebral blood flow by dilating capillaries via pericytes, that this involves signalling via

astrocytes, and that dilation of capillaries and dilation of arterioles are mediated by different messengers. Ischaemia leads to pericytes constricting and dying in rigor, thus producing a long-lasting decrease of blood flow, making pericytes a therapeutic target in stroke. I will show that similar events occur in Alzheimer's Disease: both in humans with dementia who are depositing amyloid beta and in a knock-in model of Alzheimer's disease in mice, capillaries are constricted preferentially at pericyte locations. This constriction is sufficient to approximately halve blood flow and in humans increases with the severity of the disease. Pericyte constriction is a therapeutic target in Alzheimer's disease.

PL05 Plenary Lecture 5 – Professor Fred Meunier

PL05

Unraveling Munc18/Syntaxin1 nanoscale organisation dynamics in health and disease

F. Meunier

University of Queensland, Queensland Brain Institute, Queensland, Australia

Communication between cells relies on regulated exocytosis, a multi-step process that involves the docking, priming and fusion of vesicles with the plasma membrane, culminating in the release of neurotransmitters and hormones. Key proteins involved in exocytosis are subjected to Brownian movement and constantly switch between distinct motion states which are governed by short-lived molecular interactions. Critical biochemical reactions between

exocytic proteins that occur in the confinement of nanodomains underpin the precise sequence of priming steps which leads to the fusion of vesicles. The advent of super-resolution microscopy techniques has provided the means to visualize individual molecules on the plasma membrane with high spatiotemporal resolution in live cells. These techniques are unravelling a surprisingly dynamic nature of the exocytic machinery. In this lecture, I will focus on the nanoscale organisation of soluble *N*-ethylmaleimide-sensitive factor attachment receptor (SNARE) syntaxin-1 and its chaperone Munc18-1, which mediates neurotransmitter release. I will also show that Munc18-1 (AKA STXBP1) nanodomain organisation is greatly affected by mutations associated with early infantile epileptic encephalopathy which may play a pathogenic role.

Marthe Vogt Lecture

MVL01 Marthe Vogt Lecture - Professor Juana Pasquini

MVL01

Marthe Louise Vogt: a leading light in twentieth century neuroscience

J. Pasquini

Universidad de Buenos Aires, Departamento de Química Biológica, Buenos Aires, Argentina

This Marthe Louise Vogt Lecture will highlight Marthe's personality and leadership in the neurochemistry of the twentieth century. We will also discuss her invaluable contributions to science, from the development of transmitter molecules such as

acetylcholine to the treatment of mental illnesses. We will also seize the opportunity to outline the role of women in the progress of neuroscience, and I myself will take you on a tour of the experimental work my students have done over the years, especially in lines related to iron metabolism in the central nervous system.

ISN Young Scientific Lectures

YSLA01 ISN Young Scientific Lecture 1 – Jee Hyun Kim

YSLA01-01

A cross-species approach to understand adolescent vulnerability to methamphetamine use: genetic and cognitive factors**J. H. Kim¹**¹*Florey Institute of Neuroscience and Mental Health, Mental Health, Melbourne, Australia*²*University of Melbourne, Medicine, Dentistry, and Health Sciences, Melbourne, Australia*

Methamphetamine (meth) is an urgent problem with its usage ranking 2nd most prevalent of all illicit substances in the world, with adolescents/young adults being the dominant users. We have established a powerful rat model of adolescent vulnerability to meth use, which shows that meth self-administration during adolescence leads to deficits in inhibition of a meth-associated cue that causes a greater cue induced relapse relative to adults. It also shows that meth self-administration in adolescence leads to an escalated meth intake when the dose is increased. Importantly, our

genome-wide transcriptome analysis (RNAseq) of the dorsal striatum revealed that compared to saline, meth self-administration in adolescence (but not in adulthood) reduces *SLC18A1* expression in the dorsal striatum. *SLC18A1* codes for vesicular monoamine transporter 1 (VMAT1) protein that is critical for cytosolic monoamine uptake and storage. It is well conserved across rodents and humans and is implicated in many types of mental disorders. Therefore, our central hypothesis is that *SLC18A1* mediates vulnerability to meth addiction. We have begun to translate the rodent findings into humans to show that adolescent-onset of meth use leads to deficits in inhibition, and that polymorphisms in *SLC18A1* significantly differ between adolescent- vs adult-onset of meth use in people with current meth use disorder. We are currently manipulating *SLC18A1* expression in the dorsal striatum using CRISPR-dCas9 viral approach to control escalation of meth intake and inhibitory control in rats. The outcomes of these projects may fundamentally change our understanding of addiction and provide novel insight for effective therapeutics and preventative approaches.

YSLA02 ISN Young Scientific Lecture 2 – Taylor Schmitz

YSLA02-01

The cholinergic basal forebrain: Selective neuronal vulnerability in aging and Alzheimer's disease

T. Schmitz

*University of Western Ontario, Physiology and Pharmacology,
London, Canada*

Alzheimer's disease (AD) pathology progresses in stages across anatomically connected regions of the brain, with certain regions affected before others. Why some brain regions are more vulnerable to AD than others has long remained a mystery. However, recent genomics research indicates that neurons with long axonal projections and many arbors exhibit selective neuronal vulnerability (SNV) to AD pathology. If large projection neurons are more vulnerable to AD, they should also be among the earliest affected. The cholinergic neurons of the basal forebrain (BF) are known to have large projections, though

recent work indicates that they are much larger than was previously appreciated: In humans a single cell's full arborisation measures ~100 meters in length. Under the SNV hypothesis, this emerging evidence places the cholinergic BF neurons as likely targets in the earliest stage of AD. In this talk, I will discuss my recent work integrating cerebrospinal fluid biomarkers, multi-modal neuroimaging and genetic data to triangulate Alzheimer's degeneration of human cholinergic BF neurons in vivo. I show that abnormal degeneration within the cholinergic BF system precedes and predicts memory impairment and degeneration in cortical structures. While these data imply a 'clinically silent' phase of cholinergic BF degeneration, I review evidence that this phase might in fact have its own cognitive marker: changes in selective attention. I will discuss why current neuropsychological assessments are poorly optimized to detect this impairment, and how this omission has affected our understanding of the evolution of cognitive dysfunctions in AD.

Symposia

S01 History of Neurochemistry

S01-01

Canadian neurochemists and roles in ISN/ASN

P. Beart

University of Melbourne, Florey Institute of Neuroscience & Mental Health, Parkville, Australia

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

University of Illinois at Chicago, Department of Anesthesiology, Chicago, USA

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

J. Pasquini

Universidad de Buenos Aires, Departamento de Química Biológica, Buenos Aires, Argentina

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 in 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 Joaquín Luco 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

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

J. Coyle¹

¹*McLean Hospital, Mailman Research Center, Belmont, USA*

²*Harvard University, Psychiatry, Cambridge, USA*

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.

S02 Dysfunction at the presynapse

S02-01

Neurodevelopmental synaptopathies: presynaptic dysfunction in intellectual disability

S. Gordon

Florey Institute of Neuroscience and Mental Health, University of Melbourne, Parkville, Australia

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 **K. Smillie¹, R. McAdam¹, S. Gordon², M. Cousin¹**

¹*University of Edinburgh, Centre for Discovery Brain Sciences, Edinburgh, United Kingdom*

²*University of Melbourne, Florey Institute, Melbourne, Australia*

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 **J. Burré¹, N. G. L. Guiberson¹, A. Pineda¹, D. Abramov¹, P. Kharel¹, K. E. Carnazza¹, Rachel. T. Wragg², J. S. Dittman²**

¹*Weill Cornell Medicine, Brain and Mind Research Institute, New York, USA*

²*Weill Cornell Medicine, Biochemistry, New York, USA*

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 **G. Piccoli**

Università degli Studi di Trento, Department of Cellular, Computational and Integrative Biology - CIBIO, Trento, Italy

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

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.

S03 Complement: sculpting the developing and diseased brain

S03-01

Complement and blood-brain barrier integrity

J. Alexander

University at Buffalo, Medicine, CTRC 8030D, USA

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

M. Pekna

University of Gothenburg, Institute of Neuroscience and Physiology, Gothenburg, Sweden

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

T. Woodruff, O. Hawksworth, J. Lee, L. Coulthard

The University of Queensland, Faculty of Medicine, Brisbane, Australia

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 PKC ζ , 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?

C. Lemere^{1,2}

¹*Brigham and Women's Hospital, Ann Romney Center for Neurologic Diseases, Boston, USA*

²*Harvard Medical School, Neurology, Boston, USA*

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 (A β) oligomers in early pre-plaque stages. Therefore, we asked whether complement plays a role in brain aging and/or later stages of AD pathogenesis.

Methods: We crossed an amyloid model of AD, APP^{swe}/PS1^{de9} 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. A β 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) (C3iKO) 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 A β 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.

S04 Glutamate at the crossroad

S04-01

The energetic cost of not resting

C. Rae

*The University of New South Wales, Neuroscience Research
Australia, Sydney, Australia*

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].

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S04-02

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

J. Duarte¹

¹*Lund University, Department of Experimental Medical Science,
Lund, Sweden*

²*Lund University, Wallenberg Centre for Molecular Medicine,
Lund, Sweden*

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 ¹³C tracing experiments *in vivo*, namely for detecting labelling incorporation from [¹³C]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

H. Waagepetersen, L. McNair, Sofie. K. Christensen, Jens. V. Andersen, Blanca. I. Aldana

*University of Copenhagen, Drug Design and Pharmacology,
Copenhagen, Denmark*

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.

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S04 Glutamate at the crossroad

S04-04

Point-Counterpoint: glutamate production and oxidation

D. Rothman

*Yale University, Biomedical Engineering & Radiology and
Biomedical Imaging, New Haven, USA*

The abstract has not been provided.

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

S05-01

Reactive gliosis as a target - why and when

M. Pekny

University of Gothenburg, Department of Clinical Neuroscience, Gothenburg, Sweden

The abstract has not been provided.

S05-02

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

E. Hol

UMC Utrecht Brain Center, Translational Neuroscience, Utrecht, Netherlands

Astrocytes are essential for the maintenance of CNS homeostasis and normal CNS function. In chronic neurodegenerative disorders (e.g. Alzheimer's disease¹) and in acute neurological disorders (e.g. stroke²) 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 disease³⁻⁵. 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.

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S05-03

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

M. Nedergaard

University of Rochester Medical Center, Nedergaard Lab, Rochester, USA

The abstract has not been provided.

S05-04

Astroglia define plasticity responses in the diseased brain

A. Verkhratsky

The University of Manchester, Faculty of Biology, Medicine and Health, Manchester, United Kingdom

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. Astroglipathology 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 astroglipathy), 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).

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

S06-01

Mechanisms controlling GABAergic interneuron plasticity in the adult brain

G. D. Cristo

University of Montreal, CHU Ste Justine Research Centre, Montreal, Canada

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

H. Taniguchi, A. Steinecke

Max Planck Florida Institute for Neuroscience, Development and Function of Inhibitory Neural Circuits, Jupiter, USA

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

M. C. Angulo

INSERM U1266, Institute of Psychiatry and Neuroscience of Paris IPNP, Paris, France

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 postnatal 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.

S06-04

Morphological determinants of cortical gabaergic interneuron myelination**S. Kushner¹, J. Stedehouder¹, D. Brizee¹, J. Slotman², M. Leyrer³, D. Berson³, A. Houtsmuller²**¹*Erasmus MC, Psychiatry, Rotterdam, Netherlands*²*Erasmus MC, Optical Imaging Center, Rotterdam, Netherlands*³*Brown University, Neuroscience, Providence, United States*

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.

S07 RNA Control of Axonal Functions

S07-01

Subcellular localization of RNA-binding proteins for axon growth regulation

M. Fainzilber¹, E. Doron-Mandel¹, I. Koppel¹, Juan. A. Oses-Prieto², R. Kawaguchi³, O. Abraham¹, I. Rishal¹, S. Alber¹, J. Kadlec⁴, P. Jungwirth⁴, G. Coppola³, Alma. L. Burlingame²

¹Weizmann Institute of Science, Dept. of Biomolecular Sciences, Rehovot, Israel

²UCSF, Dept. of Pharmaceutical Chemistry, San Francisco, U.S.A.

³UCLA, Semel Institute for Neuroscience and Human Behavior, Los Angeles, U.S.A.

⁴Czech Academy of Sciences, Institute of Organic Chemistry and Biochemistry, Prague, Czechia

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

J. Twiss

University of South Carolina, Biological Sciences, Columbia, USA

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 **C. Andreassi, A. Riccio**

University College London, MRC Laboratory for Molecular Cell Biology, London, United Kingdom

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 axonally cleaved 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

F. Bronfman, G. Moya-Alvarado, N. Stuardo

Catholic University Chile, Department of Physiology, Santiago, Chile

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

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 TrkB^{BF616A} 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.

S08 The NMDA receptors: from synapse physiology to pathology

S08-01

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

J.-P. Mothet

CNRS - ENS Paris Saclay, Laboratoire Aimé Cotton, UMR9188, Orsay, France

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 D₁ and D₃-receptor activation, respectively. Furthermore, we report that the selective blockade of the D₃-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 D₁R and D₃R 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

N. Empage

University of Oxford, Pharmacology, Oxford, United Kingdom

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

G. Hardingham

University of Edinburgh, UK Dementia Research Institute, Edinburgh, United Kingdom

The GluN2 subtype (2A vs. 2B) determines key biophysical properties of forebrain NMDA receptors. During development, GluN2A becomes incorporated into previously GluN2B-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

K. Dore

UCSD, Neurosciences, La Jolla, USA

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 it 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.

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.

S09 Neuroprotection through Autophagy: the next milestones

S09-01

The molecular interplay between autophagy and proteasome in motoneuron diseases

A. Poletti, R. Cristofani, P. Rusmini, V. Crippa, M. E. Cicardi, V. Ferrari, B. Tedesco, M. Piccolella, M. Galbiati, E. Messi

Università degli Studi di Milano, Center of Excellence on Neurodegenerative Diseases, Milano, Italy

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

Albert Einstein College of Medicine, Institute for Aging Studies, The Bronx, USA

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**

CIB, CSIC, Madrid, Spain

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

P. McPherson

Montreal Neurological Institute, McGill University, Neurology and Neurosurgery, Montreal, Canada

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.

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

O. Reiner, R. Y. Tshuva

Weizmann Institute of Science, Molecular Genetics, Rehovot, Israel

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

M. Johnstone

University of Edinburgh, Division of Psychiatry, Edinburgh, United Kingdom

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 **V. Borrell**

Consejo Superior de Investigaciones Científicas, Institute of Neuroscience, San Juan de Alicante, Spain

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.

S10-04

LGALS3BP modulates local gyrification in the human brain

C. Kyrousi

Max Planck Institute of Psychiatry, Department of Developmental Neurobiology, Munich, Germany

Basal radial glial cells (bRGs) are a neural progenitor type enriched in primates and humans and control the expansion of neurogenesis during cortical development in gyrencephalic species. Shortly after their generation, bRGs delaminate towards the outer subventricular zone, where they divide multiple times before terminal differentiation. The regulation of bRGs generation is essential for the establishment of correct gyrification within the human cortex. Here, we study the role of LGALS3BP, a secreted protein whose RNA is enriched in bRGs. By using cerebral organoids, human fetal tissues and mice, we show that manipulation of *LGALS3BP* regulates bRG generation. Individuals with unique *de novo* variants in *LGALS3BP* demonstrate abnormal gyrification and thickness at multiple sites over the cerebral cortex. Single-cell-RNA-sequencing reveals the extracellular matrix involvement in the *LGALS3BP* mediated mechanism. We find that *LGALS3BP* is required for bRGs delamination and influences cortical development and 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-dependent long term depression

M. Navarrete

Instituto Cajal, CSIC, Functional and Systems Neurobiology department, Madrid, Spain

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

INSERM U1215, Neurocentre Magendie, Bordeaux cedex, France

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

J. Oliveira^{1,2,3}

¹*Life and Health Sciences Research Institute ICVS, School of Medicine, University of Minho, ICVS, Braga, Portugal*

²*ICVS/3B's - PT Government Associate Laboratory, ICVS, Braga, Portugal*

³*IPCA-EST-2Ai, Polytechnic Institute of Cávado and Ave, Applied Artificial Intelligence Laboratory, 2Ai, Barcelos, Portugal*

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

A. Rodriguez-Moreno

Universidad Pablo de Olavide, Departamento de Fisiología, Anatomía y Biología Celular, Sevilla, Spain

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 (A₁R), which also recovers the tonic activation of preNMDARs at P22-P30. By contrast, the

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

induction of t-LTD was prevented at P13-P21 by an agonist of A₁R, 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.

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

A. Taha

University of California - Davis, Food Science and Technology, Davis, USA

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

K. Patten¹, A. E. Valenzuela¹, A. Y. Taha², K. J. Bein^{3,4}, A. S. Wexler^{3,5}, P. J. Lein¹

¹UC Davis, Molecular Biosciences, Davis, USA

²UC Davis, Food Science and Technology, Davis, USA

³UC Davis, Air Quality Research Center, Davis, USA

⁴UC Davis, Center for Health and the Environment, Davis, USA

⁵UC Davis College of Engineering, Mechanical and Aerospace Engineering, Civil and Environmental Engineering, Davis, USA

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

M. Plourde

Universite de Sherbrooke, Medecine, Sherbrooke, Canada

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.

S12-04

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

W. Swardfager¹, D. Yu^{1,2,4}, M. Hennebelle⁵, J. Ramirez^{1,3,4}, P. C. Chan^{6,7}, A. Y. Taha⁵, J. Pettersen⁸, N. Herrmann¹, K. L. Lancôt^{1,2}, M. Masellis^{1,9}, D. J. Sahlas¹⁰, S. E. Black^{1,9,10}

¹Sunnybrook Health Sciences Centre, University of Toronto, Hurvitz Brain Sciences Research Program, Toronto, Canada

²University of Toronto, Department of Pharmacology & Toxicology, Toronto, Canada

³Heart and Stroke Foundation, Canadian Partnership for Stroke Recovery, Sunnybrook RI, Toronto, Canada

⁴Sunnybrook Research Institute, LC Campbell Cognitive Neurology Research Unit, Toronto, Canada

⁵University of California, Department of Food Science and Technology, College of Agriculture and Environmental Sciences, California, USA

⁶Sunnybrook Health Sciences Centre, Department of Clinical Pathology, Toronto, Canada

⁷University of Toronto, Department of Laboratory Medicine and Pathobiology, Toronto, Canada

⁸Sunnybrook HSC, Department of Medicine (Neurology, Toronto, Canada

⁹McMaster University, Department of Medicine (Neurology, Toronto, Canada

¹⁰Toronto Rehabilitation Institute, University Health Network, Toronto, Canada

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.

S13 Emerging biomarker concepts in neurodegenerative diseases

S13-01

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

M. Bähr

Georg-August Universität Göttingen, Neurology, Göttingen, Germany

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

J. Schulz^{1,2}

¹*RWTH Aachen, Dept of Neurology, Aachen, Germany*

²*JARA-Brain Institute Molecular Neuroscience and Neuroimaging, FZ Jülich, Jülich/Aachen, Germany*

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 deterioration¹.

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.

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S13-03

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

L. Kalia

Movement Disorders Centre, Toronto Western Hospital, University of Toronto, Toronto, Canada

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

B. Mollenhauer^{1,2}

¹*University Medical Center Goettingen, Neurology, Goettingen, Germany*

²*Paracelsus-Elena-Klinik, Movement Disorder Center, Kassel, Germany*

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

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 DeNoPa¹ 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.

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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?

A. Sierra

Achucarro Basque Center Neuroscience, Glial Cell Biology Lab, Leioa, Spain

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 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

S. Rivest

CHU de Québec-Université Laval Research Center, Neuroscience, Quebec City, Canada

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 A β levels. In addition, M-CSF treatment resulted in the stabilization of the cognitive decline state in transgenic mice that already had A β 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

S. Koizumi

University of Yamanashi, Department of Neuropharmacology, Interdisciplinary Graduate School of Medicine, Yamanashi, Japan

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

A. Erlandsson

Uppsala university, Department of Public Health and Caring Sciences, Uppsala, Sweden

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

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 ($A\beta$) and alpha-synuclein (α -SYN) that is stored, rather than degraded by the cells. The accumulation of $A\beta/\alpha$ -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 $A\beta/\alpha$ -SYN aggregates in the astrocytes.

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

L. Kaczmarek

Yale University, Pharmacology, New Haven, USA

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

K.-i. Nagata, N. Hamada

Institute for Developmental Research Aichi Human Service Cen, Molecular Neurobiology, Aichi, Japan

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 degraded 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

K. Huber, G. Molinaro, W. Guo, J. Gibson

UT Southwestern Medical Center, Neuroscience, Dallas, USA

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.

S15-04

Molecular mechanisms underlying brain wiring and asd-like behaviours

C. Bagni, N. Dominguez-Iturza

*University of Lausanne, Department of Fundamental
Neurosciences, Lausanne, Switzerland*

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.

S16 Signaling Mechanisms in Cortical Development

S16-01

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

C. Schuurmans^{1,2,3}, S. Han^{1,2}, G. Wilkinson³, I. Faisal^{1,2}, S. Okawa⁴, S. Li³, L. Adnani^{1,3}, V. E. Angarica⁴, D. Zinyk^{1,2,3}, R. Dixit^{1,3}, Y. Illytsky⁵, M. Brooks⁶, E. Raharjo⁷, D. Dennis³, F. Malik⁸, J.-W. Kim⁶, W. Wu¹⁰, W. Rahmani⁷, D. Castro⁹, J. Chan¹⁰, D. Kurrasch⁸, I. Kovalchuk⁵, A. Swaroop⁶, Ad. Sol⁴, J. Biernaskie⁷

¹Sunnybrook Research Institute, Biological Sciences Platform, Toronto, Canada

²University of Toronto, Biochemistry, Toronto, Canada

³University of Calgary, Biochemistry and Molecular Biology, Calgary, Canada

⁴University of Luxembourg, Luxembourg Centre for Systems Biomedicine, Luxembourg, Luxembourg

⁵University of Lethbridge, Biological Sciences, Lethbridge, Canada

⁶National Institutes of Health, National Eye Institute, Bethesda, USA

⁷University of Calgary, Comparative Biology and Experimental Medicine, Calgary, Canada

⁸University of Calgary, Medical Genetics, Calgary, Canada

⁹Instituto Gulbenkian de Ciência, Medicine, Oeiras, Portugal

¹⁰University of Calgary, Pathology and Laboratory Medicine, Calgary, Canada

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

S. Tole

Tata Institute of Fundamental Research, Biological Sciences, Mumbai, India

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

R. Hevner

University of California San Diego, Pathology, La Jolla, USA

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 clonal 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

S. Hippenmeyer

IST Austria, Developmental Neuroscience, Klosterneuburg, Austria

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.

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

M. Mikhaylova

University Medical Center Hamburg-Eppendorf, UKE Center for Molecular Neurobiology, ZMNH, RG Neuronal Protein Transport, Hamburg, Germany

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

Michael. R. Kreutz

Leibniz Institute for Neurobiology, RG Neuroplasticity, Magdeburg, Germany

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 traffic 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

M. Kennedy

University of Colorado School of Medicine, Pharmacology, Aurora, USA

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

C. González, L. Vargas-Saturno, J. Gallardo, N. Venegas, A. Couve

Universidad de Chile, Neuroscience Department and Biomedical Neuroscience Institute BNI, Santiago, Chile

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

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), Galactosyl-transferase (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.

S18 Repeating themes of tandem repeat toxicity in neurological disorders

S18-01

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

A. Hannan¹, T. Renoir¹, G. Kong¹, S. Li¹, C. Love¹, J. Liew¹, C. Mo^{1,2}, C. Gubert¹

¹University of Melbourne, Florey Institute of Neuroscience and Mental Health, Parkville, Australia

²University of Chicago, Department of Neurobiology, Chicago, USA

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 pathogenic 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

M. Pouladi^{1,2,3}

¹National University of Singapore, Department of Medicine, Singapore, Singapore

²National University of Singapore, Department of Physiology, Singapore, Singapore

³Agency for Science, Technology and Research A*STAR, Translational Laboratory in Genetic Medicine TLGM, Singapore, Singapore

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

L. Ranum¹, L. Nguyen¹, F. Montrasio², O. Bardhi¹, S. Guo¹, S. K. Tusi¹, K. Nakamura¹, M. B. Coronel¹, N. Sonenberg³, J. Grimm², T. Zu¹

¹University of Florida, Center for NeuroGenetics, Gainesville, USA

²Neurimmune AG., Schlieren, Switzerland

³McGill University, Biochemistry, Montreal, Canada

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/eIF2 α 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.

S18-04

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

P. Todd

University of Michigan, Neurology, Ann Arbor, USA

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

S18 Repeating themes of tandem repeat toxicity in neurological disorders

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.

S19 Cellular and molecular mechanisms of glial development

S19-01

Role of transcriptional and epigenetic regulation in glial cells

A. Giangrande

IGBMC, Functional Genomics and Cancer, Illkirch, France

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

V. Auld, A. Hunter, M. Gilbert, M. Das

University of British Columbia, Zoology, Vancouver, Canada

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

S. Kucenas

University of Virginia, Department of Biology, Charlottesville, USA

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 this 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.

S19-04

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

M. Wegner

FAU Erlangen-Nürnberg, Institute of Biochemistry, Erlangen, Germany

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.

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

S20-01

Extranuclear protein sumoylation in neurons

J. Henley, K. Talandyte, L. Lee, R. Seager, K. Wilkinson

University of Bristol, School of Biochemistry, Centre for Synaptic Plasticity, Bristol, United Kingdom

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

S. Engelender

Technion-Israel Institute of Technology, Dept. of Biochemistry, Haifa, Israel

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

G. Konopka

UT Southwestern Medical Center, Department of Neuroscience, Dallas, USA

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.

S20-04

Sumoylation impact on synaptic function and alzheimer disease pathology

P. Fraser¹, H. Takamura^{1,2}, S. Matsuzaki^{3,2}, T. Katayama², O. Arancio⁴

¹University of Toronto, Tanz Centre, Toronto, Canada

²Osaka University, United Graduate School of Child Development, Osaka, Japan

³Wakayama Medical University, Department of Pharmacology, Wakayama, Japan

⁴Columbia University, Taub Institute for Research on Alzheimer's Disease and the Aging Brain, New York, USA

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 (A β) 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 A β and plaque density accompanied by increased synaptic loss, more pronounced synaptic and cognitive deficits. These findings suggest a potential impairment in A β 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.

S21 Regulation of Neuronal Development and Plasticity by Palmitoylating Enzymes

S21-01

Post-translational palmitoylation and its regulation of synaptic plasticity

S. Bamji

University of British Columbia, Cellular and Physiological Sciences, Vancouver, Canada

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

G. Thomas^{1,2}

¹Temple University School of Medicine, Shriners Hospitals Pediatric Research Center, Philadelphia, USA

²Temple University School of Medicine, Anatomy and Cell Biology, Philadelphia, USA

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

E. Diaz

UC Davis School of Medicine, Pharmacology, Davis, USA

A key neural mechanism of learning and memory is thought to be activity-dependent changes in synaptic AMPA receptors (AMPA) 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

P. Jenkins^{1,2}, J. Philippe¹

¹University of Michigan Medical School, Pharmacology, Ann Arbor, USA

²University of Michigan Medical School, Psychiatry, Ann Arbor, USA

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

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 β IV 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.

S22 Is Multiple Sclerosis a Primary Cyto-degenerative Disease?

S22-01

Primary neurodegeneration in multiple sclerosis

R. Dutta, B. Trapp

Cleveland Clinic Foundation, Dept Neurosciences NC30, Cleveland, USA

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

K.-A. Nave

Max Planck Institute of Experimental Medicine, Dept. of Neurogenetics, Goettingen, Germany

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^{neomice}*, 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

B. 't Hart, J. Geurts

Vumc, Anatomy & Neurosciences, Amsterdam, Netherlands

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' cyto-degeneration in MS is now up for debate. However, they already seem to provide a tantalizing challenge to the existing dogma of primary autoimmunity.

S22-04

Multiple sclerosis as a protein misfolding disorder

P. Stys

University of Calgary, Clinical Neurosciences, Calgary, Canada

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.

S23 RNA modification in the brain and behaviour

S23-01

RNA modifications and memory

T. Bredy

Queensland Brain Institute, QBI, Brisbane, Australia

In this talk, I will discuss our recent work characterized experience-dependent subcellular compartmentalization of m6A modified RNA and its 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 extinction 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

O. Wang¹, Y. Fujiwara¹, R. Roy¹, M. Sukegawa¹, S. Ramasamy¹, W.-T. Hong¹, I. Oomoto¹, K. Lida³

¹Kyoto University, Institute for Integrated Cell-Material Sciences iCeMS, Kyoto, Japan

²Kyoto University, Graduate School of Biostudies, Kyoto, Japan

³Kyoto University, Medical Research Support Center, Kyoto, Japan

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

B. Walters, S. Josselyn

Hospital for Sick Children Research Institute, Neurosciences & Mental Health Program, Toronto, Canada

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)

X. Zhuang¹, J. Koranda¹, M. J. Patel¹, J. Y. Delgado¹, L. O. Vaasjo¹, J. Thome¹, W. Fu¹, L. Mo¹, H. Shi², C. He²

¹University of Chicago, Department of Neurobiology, Chicago, USA

²University of Chicago, Department of Chemistry, Chicago, USA

N⁶-methyl(m⁶A) is the most abundant mammalian mRNA modification. It pre-regulates RNA splicing, translocation and degradation, and may play a central role in the spatial and temporal control of protein synthesis. The emergence of m⁶A research is largely facilitated by the discovery of its key effector proteins m⁶A "writers" (methyl e.g. METTL14) install m⁶A, "erasers" (demethylase remove m⁶A, and "readers" (e.g. YTHDF1, 2 and 3) recognize and bind to m⁶A to determine the fate of the modified RNA. Different m⁶A readers may mediate different downstream consequences of m⁶A modification of mRNA. Recent data suggest that m⁶A deficiency impairs both neurodevelopment and adult central nervous system function and impairs learning and memory. We have found that conditional deletion of Mettl14 in striatonigral and striatopallidal dopamine neurons impaired reinforcement learning and motor learning, and altered cocaine response. Constitutive deletion of Ythdf1 impaired protein synthesis, altered synaptic transmission and impaired many different forms of learning and memory. Because m⁶A modifies thousands of transcripts and impacts many functions, it's been a challenge to systematically interrogate the dynamic regulation of the m⁶A pathway may control protein synthesis with good spatial and temporal resolution to affect synaptic plasticity, learning and memory. We are using genetic approaches to manipulate the effector proteins. What the targets of these effector proteins are, how the above manipulations may affect transcriptome or transcript splicing, synaptic protein turnover, synaptic transmission plasticity, dendritic morphology and behavior are being systematically examined.

S24 Biological and Therapeutic Roles of Lipids in Neurodegeneration

S24-01

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

E. P. de Chaves, A. Viveiros, K. Smith, A. Mohamed

University of Alberta Faculty of Medicine and Dentistry, Pharmacology, Edmonton, Canada

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 (A β) drives AD pathogenesis. Recent findings indicate that cell to cell transmission of A β by a 'prion-like' spread mechanism contributes to AD progression.

We discovered that A β_{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 β_{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 β_{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 β_{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. A β is released in extracellular vesicles (EVs). Using imaging flow cytometry and nanoparticle tracking analysis we showed that A β -induced autophagy blockade increases EVs secretion favoring cell-to-cell spreading of A β .

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 **S. Sipiione**

University of Alberta, Pharmacology, Edmonton, Canada

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

G. D. Paolo

Denali Therapeutics Inc., Discovery Biology, South San Francisco, USA

Loss of function variants of TREM2, an immune receptor expressed in microglia, increase Alzheimer's disease (AD) risk. TREM2 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

B. Karten

Dalhousie University, Biochemistry and Molecular Biology, Halifax, Canada

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.

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

J. Kittler

UCL, Neuroscience, Physiology and Pharmacology, London, United Kingdom

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_ARs) 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_ARs at synapses and the role played by key inhibitory synaptic components including Neuroligin 2 and the GABA_AR receptor accessory protein LHFPL4/Gar1h. 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

K. Smith, K. Crosby, S. Gookin, J. Garcia, K. Hahm, M. Dell'Acqua

University of Colorado Denver, Pharmacology, Aurora, USA

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_ARs 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

Duke University Medical Center, Cell Biology, Durham, USA

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

INSERM UMR1270, Institut du Fer à Moulin, Paris, France

Upon activation by GABA, GABA_ARs 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. Pharmacoresistant 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 $[\text{Cl}^-]_i$ 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 $\text{Na}^+/\text{K}^+/\text{Cl}^-$ cotransporter NKCC1, which imports chloride into neurons, also increases $[\text{Cl}^-]_i$ 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/T1007. 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 Oxidative 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 $[\text{Cl}^-]_i$. The WNK/SPAK/OSR1 signaling pathway may thus

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.

S26 Emerging pathways in amyotrophic lateral sclerosis

S26-01

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

J. Rothstein, A. Coyne, L. Hayes

Johns Hopkins University, Brain Science Institute; Dept of Neurology, Baltimore, USA

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

L. Van Den Bosch

VIB-KU Leuven Center for Brain & Disease Research, Neurosciences, Leuven, Belgium

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

L. Barbeito

Institut Pasteur Montevideo, Neurodegeneration Lab, Montevideo, Uruguay

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

Laval University, Psychiatry and Neurosciences, Quebec City, Canada

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

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- κ B 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*.

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

W. Macklin¹, H. Hathaway¹, A. Almeida¹, K. Dahl¹, T. Wood²

¹University of Colorado School of Medicine, Department of Cell and Developmental Biology, Aurora, USA

²New Jersey Medical School, Rutgers University, Department of Pharmacology, Newark, USA

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 (PDGFR α)-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 PDGFR α -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?

G. Saretzki

Newcastle University, Institute for Cell and Molecular Biosciences, Newcastle upon Tyne, United Kingdom

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 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

M. Perluigi

Sapienza university of rome, Biochemical science, Rome, Italy

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 A β 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 GSK3 β 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

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 eIF4E-dependent mRNA translation

N. Sonenberg¹, A. Aguilar-Valles^{1,2,3}, A. Skaleka¹, E. Matta-Camacho^{1,3}, M. J. Eslamizade^{1,2}, D. D. Gregorio⁴, A. Torres-Berrio⁵, G. Gobbi⁴, J.-C. Lacaille²

¹McGill University, Department of Biochemistry, Montreal, Canada

²Université de Montréal, Department of Neurosciences, Montreal, Canada

³Carleton University, Department of Neuroscience, Ottawa, Canada

⁴McGill University, Department of Psychiatry, Montreal, Canada

⁵Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, Nash Family Department of Neuroscience, New York, USA

mTOR controls many cellular functions, including mRNA translation through phosphorylation and inactivation of the eukaryotic initiation factor 4E (eIF4E) binding proteins (4E-BPs), which

suppress 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.

Young Investigator Colloquia

YIC01 Dementia, inflammation and Neurodegeneration

YIC01-01

Neuronal and vascular retinal dysfunction on the stages of disease progression in a new experimental model of metabolic syndrome**P. Barcelona***CIBICI CONICET, Department of Clinical Biochemistry, School of Chemical Sciences, UNC, Córdoba, Argentina*

Background: Diabetic retinopathy (DR) is the most serious ocular complication associated with T2DM, which is a metabolic syndrome (MS), and one of the leading causes of secondary blindness. Although animal models of DR have helped to advance in the knowledge of this disease, these models have some limitations to reproduce completely the early stage where take place the beginning of the neuronal and vascular alteration.

Objective: Analyze in early stages of the MS, markers of retinal vascular integrity and neuronal functionality to explore details of the mechanisms of action that command this event.

Materials and Methods: Animals, C57BL/6 (WT) and Apolipoprotein E knockout mice (ApoE-KO) either fed with a normal diet (ND) or a 10% w/v fructose diet (FD) in drinking water from 2 months of age. Time-dependent kinetic studies were done from 4 to 6 months of age analyzing lipid and glucose metabolic parameters, glucose tolerance test (GTT) and insulin tolerance test (ITT), Total, LDL and HDL Cholesterol, Triglycerides, among others. All groups animals ($n = 8$ per group) for GTTs, were fasted 5 h prior to 2 g/kg body weight IP injection of glucose or 0.75U/kg body weight IP of regular human insulin for ITTs. Blood samples were taken from the tail vein at time 0 before injection and at 0.5; 1 and 2 h after this. Retinal functionality was assessed by electroretinography (ERG) at 2, 3 and 4 month of treatment in mice fully dark-adapted overnight (> 15 h). The specificity marker expression of retinal neuronal integrity or degenerative damage was evaluated by Western Blot. Retinal histology and immunofluorescence (IF) analysis were performed in flatmounts and cryosections, whereas vascular permeability and leakage were quantified by Evans Blue extravasations. GraphPad Prism program was employed for statistical analysis.

Results: After 2 month of treatment, ApoE-KO FD mice showed, in addition to dyslipidemic profile, altered GTT and ITT as compared with the other groups. At this time, the ERG a- and b-wave did not show changes but the oscillatory potential (OPs) amplitudes were significantly decrease in retinas of these mice compared to ApoE-KO ND mice (*p*Conclusion: The results showed that ApoE-KO mice after 2 months of taking fructose presented metabolic alterations mimicking some features of human MS at its initial stages of the DR. Thus, this model could offer the benefit to investigate DR at an early stage of the MS, where the beginning of neuronal and vascular retinal dysfunction take place. This represent

a big opportunity to apply different therapeutic strategy at different time point to the traditional.

YIC01-02

Cellular senescence in Parkinson's disease**B. Kolisnyk¹, M. Riessland¹, T. W. Kim², J. Pearson¹, E. Park¹, L. Studer², P. Greengard¹**¹*The Rockefeller University, Laboratory of Molecular and Cellular Neuroscience, New York, USA*²*Memorial Sloan Kettering Cancer Center, Center for Stem Cell Biology, New York, USA*

Cellular senescence is a mechanism used by mitotic cells to prevent uncontrolled cell division. As senescent cells persist in tissues, they cause local inflammation and are harmful to surrounding cells, contributing to aging. The contribution of cellular senescence to neurodegeneration is still unclear. SATB1 is a DNA binding protein associated with Parkinson's disease. We find that SATB1 plays an active role, repressing cellular senescence in post-mitotic dopaminergic neurons. Loss of SATB1 causes activation of a cellular senescence transcriptional program in dopamine neurons, both in human stem cell-derived dopaminergic neurons and in mice. We observed phenotypes which are central to cellular senescence in SATB1 knockout dopamine neurons in vitro and in vivo. We demonstrate that the loss of SATB1 from dopamine neurons in vivo produces local inflammation and active removal of the dopamine neurons by microglia. We have continued this work by exploring the consequences of senescent cells within the local environment of the Substantia Nigra, in additional models of Parkinson's disease. Taken together, our data implicate senescence of dopamine neurons as a contributing factor to the pathology of Parkinson's disease.

YIC01-03

BACE2 inhibits neuronal apoptosis by cleavage of potassium channel KV2.1**Y. Zhang¹, F. Liu², Z. Liang², Q. Sun², H. Liu², J. Zhao², J. Xu², J. Zheng², Y. Yun², X. Yu³, W. Song¹, X. Sun²**¹*The University of British Columbia, Psychiatry, Vancouver, Canada*²*Qilu Hospital, Shandong University, Neurology, Jinan, China*³*Medical College of Shandong University, Physiology, Jinan, China*

Potassium voltage-gated channel subfamily B member one (KCNB1, also known as Kv2.1) is a major voltage-dependent potassium channel in neurons and highly expressed in the cortex and hippocampus. It regulates potassium current in cortical neurons and potassium efflux is necessary for cell apoptosis. As a key component of delayed rectifier current potassium channels, Kv2.1 forms clusters in the membrane of hippocampal neurons and is responsible for the apoptotic current surge in cortical neurons by its phosphorylation at S800. BACE2 is an aspartyl protease to cleave APP to preclude generation of A β , a central component of neuritic plaques in Alzheimer's brain. We identified Kv2.1 as a novel substrate of

BACE2 with three cleavage sites at Thr376, Ala717, and Ser769. The cleavage disrupted Kv2.1 clustering on cell membrane, resulting in decreased I_k of Kv2.1 and a hyperpolarizing shift in primary neurons. Furthermore, we discovered that the BACE2-cleaved Kv2.1 forms, Kv2.1-1-375, Kv2.1-1-716, and Kv2.1-1-768, depressed the delayed rectifier I_k surge and reduced neuronal apoptosis. Our study suggests that BACE2 plays a neuroprotective role by cleavage of Kv2.1 to prevent the outward potassium currents, a potential new target for Alzheimer's treatment.

YIC01-04

Defective hormonal signaling and brain protein synthesis at the basis of memory failure in alzheimer's disease

M. Lourenco

Federal University of Rio de Janeiro, Institute of Medical Biochemistry Leopoldo de Meis, Rio de Janeiro, Brazil

Defects in brain hormonal signaling have been associated with Alzheimer's disease (AD). We and others have further shown that AD pathophysiology involves repression of brain protein synthesis. In AD mouse models, abnormal phosphorylation of the eukaryotic translation initiation factor 2 alpha (eIF2 α -P) attenuates translation and mediates synapse loss and memory failure. These results have raised the notion that the modulating translation could potentially prevent neurodegeneration and memory decline. We have recently investigated whether restoring signaling mechanisms by insulin or irisin, two brain-relevant hormones whose signaling pathways are attenuated in AD, could block eIF2 α -P-linked translational attenuation, and synapse and memory loss. First, we demonstrate that enhancing insulin signaling through labeled anti-diabetic agents prevents eIF2 α -P and memory failure in AD models. We next found that irisin, an exercise-associated hormone, present reduced levels in AD and that replenishing its levels attenuates translational repression in cultured neurons, and memory impairment in mouse models of AD. Taken together, our results indicate that restoration of proper brain hormonal signaling might be able to counteract aberrant signaling and memory failure in AD.

YIC01-05

Baff stimulates Nogo receptor 1 and 3 expressed on b cells within spinal cord follicle-like structures formed during EAE

P. Theotokis^{1, 2}, M. M. Bakhuraysah², J.-Y. Lee², M. J. Kim², W. A. Figgett², F. Mackay², S. Petratos²

¹*Aristotle University of Thessaloniki, B' Department of Neurology, AHEPA University Hospital, Thessaloniki, Greece*

²*Monash University, Department of Neuroscience, Central Clinical School, Melbourne, VIC, Australia*

Despite clear evidence demonstrating that the deletion of Nogo-receptor 1 (NgR1) can protect against axonal degeneration and thus progression of experimental autoimmune encephalomyelitis (EAE), an immunological role for this receptor is yet to yield mechanistic evidence. However, recently NgR has been proposed as an alternate receptor for the B-cell activating factor (BAFF/BlyS) in the central nervous system (CNS). The aim of this study was to define whether NgR and its homologs contribute to immunomodulation during MOG₃₅₋₅₅ and rMOG EAE. Spinal cord, lymph nodes and spleen of *ngR1^{+/+}* wild-type (WT) and *ngR1^{-/-}* mice were analyzed with flow cytometry, immunohistochemistry, western blotting and ELISA. We identified meningeal B-cells expressing NgR1 and NgR3 within the lumbosacral spinal cords of *ngR1^{+/+}* EAE-induced mice at clinical score 1. Furthermore, there were significant increases of secreted immunoglobulins from these NgR1-expressing B-cells. These cells could be directed into the synthesis phase of the cell cycle, after stimulating sorted cells by extracellular BAFF in vitro. However, when BAFF signaling was blocked using either rBAFF-R, or NgR1-Fc, or NgR3 peptides, the cells were observed to be predominately in the G0/G1 phase. Collectively, these data indicate that NgR1 and NgR3 expression is inducible in immune lineage cells upon the induction of EAE, and that the follicular-like NgR1 and NgR3-positive B-cells in the meninges may play an active role during the induction of EAE.

YIC02 Signalling, development and disease

YIC02-01

Quercetin attenuated lipopolysaccharide-induced depressive-like behaviour in mice

A. Eduviere¹, O. Adeoluwa², E. Olonode²

¹University of Medical Sciences, Pharmacology & Therapeutics, Ondo, Nigeria

²Afe Babalola University, Pharmacology & Therapeutics, Ado-Ekiti, Nigeria

Depression is a neuropsychiatric disorder which affects social functions and the quality of life of suffers. Quercetin (QCT) is an antioxidant plant flavonoid which is used as a constituent in dietary products. In this study, the effect of QCT on LPS-induced depression in mice was evaluated. Mice were randomly allotted to groups ($n = 6$): Group 1 and 2 received vehicle (10 mL/kg, i.p.), whilst groups 3-6 received QCT (25, 50 & 100 mg/kg, i.p.) and imipramine (10 mg/kg, i.p.), respectively for 7 days. On day 7, mice in groups 2-6 were treated 30 min prior to LPS (0.83 mg/kg, i.p.) administration and immobility time; a measure of depressive-like symptoms, was assessed 24 h later in the tail suspension test (TST) and forced swim test (FST), respectively. Thereafter, serum corticosterone, brain tumor necrosis factor- α (TNF- α) and interleukin 1 β (IL1 β) levels were measured using ELISA. Also, levels of malondialdehyde, glutathione, super oxide dismutase and nitrite were determined in brain homogenates using UV spectrophotometer. The data obtained showed that QCT (25, 50 and 100 mg/kg) significantly ($p < 0.05$) reversed LPS-induced increase in immobility time in both TST and FST when compared to controls, suggesting antidepressant-like activity. Furthermore, LPS-induced alterations in oxidative stress biomarkers in mice brains were significantly ($p < 0.05$) reversed by QCT. It also significantly ($p < 0.05$) suppressed LPS-induced increase in serum corticosterone, brain TNF α and IL1 β levels in depressed mice, compared to controls. This finding showed that QCT attenuated LPS-induced depressive-like behaviour in mice via mechanisms related to inhibition of oxidative stress and attenuation of inflammatory cytokines.

YIC02-02

Identification of the BDNF prodomain (PBDNF) as a new pathogenic ligand affecting neuronal structure and function

A. A. Gonzalez¹, M. J. Paz¹, J. Wang², J. Giza², F. Lee², B. Hempstead², C. Bracken²

¹Instituto Ferreyra INIMEC-CONICET-Universidad Nacional de Cordoba, Neurobiology, Cordoba, Argentina

²Weill Cornell Medicine, Medicine, New York, USA

Brain-derived Neurotrophic Factor (BDNF) is translated as a precursor protein (proBDNF) that is cleaved to two peptides known as the BDNF prodomain (pBDNF) and mature BDNF (mBDNF). There is a single nucleotide polymorphism (SNP) in the BDNF gene that leads to a valine for methionine substitution (Val66Met) within the pBDNF sequence. The Val66Met SNP is present in more than 25% of the human population, and is associated with increased risk for psychiatric and neurodegenerative disorders. We found, for the

first time, that the Met pBDNF is an abundant active secreted ligand on CNS neurons inducing structural alterations and circuitry remodeling. Here, we demonstrate that the Met and Val pBDNF undergo interaction with zinc at physiologically relevant concentrations. These interactions result in differential aggregate formation between the Met and the Val prodomains, as assessed by cryo-electron microscopy. We have mapped the regions of the Met and Val prodomain that interact with zinc to promote aggregation utilizing point mutagenesis. Most importantly, these aggregation-deficient mutants of the Met prodomain demonstrate a loss of bioactivity, as determined by dendritic spine remodeling. This result documents that zinc-dependent aggregation of the Met pBDNF is required for bioactivity. We identify a mechanism by which the Met pBDNF mediates its biological effects, and propose targets for blocking the deleterious synapse remodeling effects in the human SNP carriers.

YIC02-03

DNA methylation and gene expression of astroglia before, during and after oxygen and glucose deprivation

L. Tovar-y-Romo, I. Ponce-Arias

Universidad Nacional Autónoma de México, Instituto de Fisiología Celular - Department of Molecular Neuropathology, Mexico City, Mexico

The suddenness of changes in vascular and cellular milieu after stroke contributes to modifying genetic expression profiles that might enable the system for adaptive responses, ultimately governing the neurological outcome. Epigenetic mechanisms such as DNA methylation are known to drive strong transcriptional regulation by modifying transcription elongation and splicing in CpG islands located intra- and intergenically. Few studies, however, have addressed at the molecular resolution the overall genetic and epigenetic changes under these circumstances, and in events like the reperfusion damage after ischemic stroke, these processes are practically unknown. We performed whole transcriptome RNA-sequencing and whole genome methylated-DNA immunoprecipitation sequencing analyses in cultured human astrocyte-like cells, derived from non-tumorigenic glioblastoma, subjected to oxygen and glucose deprivation (OGD), to establish a relationship between DNA methylation and gene expression under normoxia, OGD, and recovery. We identified several genomic features, including promoters and enhancers, whose methylation profiles change not only during OGD but also after 8 hours of recovery, and showed significant differences in housekeeping and ubiquitous genes, as well as in cell lineage-specific genes, containing high and low CG promoters, respectively. We also correlated DNA methylation with the expression of several genes under OGD and recovery and found that the overall profiles of transcriptomes and methylomes are different among normoxia, OGD, and recovery, clearly defining at the molecular level 3 states of genetic controlling. Our results contribute to elucidate the overall transformation of cells in terms of transcription and DNA methylation in pathological occurrences involving ischemia and to characterize the damage that occurs during reperfusion at the genomic scale. Supported by DGAPA-PAPIIT IN226617.

YIC02-04

Neuroplastins in synapse formation and memory**R. Herrera-Molina***Leibniz Institute for Neurobiology, Neurochemistry & Molecular Biology, Magdeburg, Germany*

The cell adhesion molecule neuroplastins (Np65 and Np55), highly expressed in the brain and enriched in synapses, are encoded by a single gene. The human *NPTN* gene was shown to be associated with cortical thickness and cognitive abilities in adolescents whereas that, genetically modified neuroplastin mice display severe cognitive alterations like deficits in selective aversive learning and loss of associative memories -retrograde amnesia- depending of hippocampal processing as well as altered cortical-striatal circuit activity and motor functions. Neuroplastin-deficient neurons display profound electrophysiological deficits including reduced short-term plasticity, impaired LTP expression, and altered mEPSCs/mIPSCs transmission confirming that neuroplastin participates in the excitatory/inhibitory synapse balance. Related to this, we reported that Np expression is necessary for a proper postsynapse formation and balance of mature excitatory/ inhibitory synapses in the adult hippocampus and in cultured hippocampal neurons. In search for the underlying mechanism we could show that via its cytoplasmic domain Neuroplastin binds the tumor necrosis factor receptor-associated factor 6 (TRAF6) to trigger the activation of potent synaptogenic signaling cascades to initiate formation of dendritic protrusions and thus promoting excitatory synaptogenesis. Very recently, we and others reported that the expression of the four Plasma Membrane Calcium ATPase paralogs (PMCA1-4) depends on Nps as the membrane proteins form complexes in the adult rodent brain and in mature hippocampal neurons. Our results indicate that Np is essential for PMCA expression which could allow proper $[iCa^{2+}]$ regulation and normal circuit activity in a neuron-type-specific fashion which can be linked to circuit-coded mechanisms involved in cognitive deterioration.

YIC02-05

The role of interneuron and neural precursor communication in oligodendrocyte genesis**A. Voronova***University of Alberta, Medical Genetics Department, Edmonton, Canada*

Background: During development, newborn interneurons migrate from medial ganglionic eminence (MGE) into embryonic cortex. They also associate with cortical neural precursors (NPCs) throughout late embryonic and early postnatal life, when NPCs generate astrocytes and oligodendrocytes in the postnatal cortex and white matter tracts. Here, we have tested the hypothesis that interneurons directly regulate NPC biology.

Methods: We genetically ablated the progeny of MGE interneurons by crossing *Nkx2.1Cre* and *DTA^{stop}* mice. Interneuron-conditioned medium and transcriptomics were used to predict paracrine ligands regulating NPC function. CX3CR1 receptor knockdown in NPCs was used to assess the role of fractalkine signalling in developmental oligodendrogenesis. Infusion of CX3CL1 (fractalkine) was used to assess the role of fractalkine signalling in adult oligodendrocyte genesis.

Results: We show that MGE interneurons secrete factors that promote genesis of oligodendrocytes from glially-biased embryonic cortical NPCs in culture. Moreover, when MGE interneurons were genetically ablated *in vivo* prior to their migration, this caused a deficit in cortical oligodendrogenesis. Modelling of the interneuron-precursor paracrine interaction using transcriptome data identified the cytokine fractalkine as responsible for the pro-oligodendrocyte effect in culture. We show that fractalkine is expressed in interneurons and that fractalkine receptor CX3CR1 is expressed in precursor cells in the developing and adult brain. Knockdown of CX3CR1 in embryonic cortical NPCs caused decreased numbers of OPCs and oligodendrocytes in the postnatal cortex. Our initial data suggests that fractalkine infusion into adult brain may lead to increase in the formation of oligodendrocyte lineage cells from precursor cells.

Conclusions: In addition to their role in regulating neuronal excitability, interneurons act in a paracrine fashion, at least in part through fractalkine signalling, to promote the genesis of oligodendrocytes.

Young Members' Symposia

YMS01 Mechanisms underlying cognition and learning

YMS01-01

Convergence of reward and aversion processing in nucleus accumbens medium spiny neuron subtypes

C. Soares-Cunha^{1,2,3}, N. Vasconcelos^{2,3,4}, B. Coimbra^{2,3}, A. Domingues^{2,3}, N. Sousa^{2,3,5}, A. Rodrigues^{2,3}

¹University of Minho, School of Medicine, Braga, Portugal

²Life and Health Sciences Research Institute ICVS, School of Medicine, University of Minho, Braga, Portugal, School of Medicine, Braga, Portugal

³ICVS/3B's-PT Government Associate Laboratory, Braga/Guimarães, Portugal, School of Medicine, Braga, Portugal

⁴Departamento de Física, Universidade Federal de Pernambuco, 50670-901 Recife, Pernambuco, Brazil, Departamento de Física, Recife, Brazil

⁵Clinical Academic Center-Braga 2CA, NA, Braga, Portugal

Reward is as important as aversion for survival. Deficits in decoding rewarding/aversive signals are present in several neuropsychiatric disorders, such as depression or addiction, emphasising the need to study the underlying neural circuits in detail.

The reward circuit, comprising projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) is crucial for reward/aversion processing. Though the dominant view postulates that NAc D1-MSNs convey reward and D2-MSNs encode aversion, recent results challenged this view.

Here, we show that both MSN populations drive reward and aversion, depending on their pattern of activation. These opposite behaviors result from differential evoked electrophysiological patterns in downstream targets, namely the ventral pallidum (VP) and VTA. Brief MSN optogenetic stimulation of either D1- or D2-MSNs elicited positive reinforcement, in line with the observed decreased VP-to-VTA inhibitory tone, and increased VTA dopaminergic activity. Prolonged activation of either MSN population drove aversion, inducing distinct electrophysiological effects in these target regions.

In addition, we further show that distinct patterns of MSN activation differentially influence cocaine-induced place preference.

In sum, we show that D1- and D2-MSNs bi-directionally control reward/aversion, highlighting that more studies are needed to understand how these two populations interact to modulate behaviour.

YMS01-02

Single-cell RNA-seq of mouse nucleus accumbens reveals a subtype of D1 medium spiny neurons

W. Chen^{1,2,3}, R. Chen^{1,2,3}, M. N. Djekidel^{1,2,3}, A. Bhattacharjee^{1,2,3}, L. M. Tuesta^{1,2,3}, Y. Zhang^{1,2,3}

¹Harvard Medical School, Department of Genetics, Boston, USA

²Boston Children's Hospital, Program in Cellular and Molecular Medicine, Boston, USA

³Howard Hughes Medical Institute, Boston Children's Hospital, Boston, USA

The nucleus accumbens (NAc) is the most important entry point of the basal reward circuitry and has been suggested to play a crucial role in motivational behaviors. However, our knowledge of its cellular heterogeneity is surprisingly limited. Thus, we performed a high-throughput single-cell RNA-seq (10X genomics) and revealed an underestimated cellular complexity of the NAc. Our data revealed a rich cellular heterogeneity of interneurons and medium spiny neurons (MSNs) in the NAc and a tight relationship between transcriptional features and spatial distribution in different neuron subtypes. As a proof-of-concept, we focused on the MSNs and found that the tachykinin 2 (Tac2)-positive neurons in the NAc is a molecularly distinct subtype of D1-MSNs. To characterize the Tac2 clusters as a subtype of D1-MSNs, we combined multi-color FISH (RNAscope) neuronal tracing and behavioral assays. We found that Tac2 is selectively expressed in the *Drd1*⁺ MSNs but not in the *Drd2*⁺ MSNs, and are preferentially projected to the midbrain. In addition, using chemogenetic, we selected manipulated the Tac2 cluster and found that activation of the NAc Tac2 clusters potentiated, while inhibition repressed cocaine sensitization. However, such manipulation had no obvious effects on anxiety- and depression-related behaviors. Furthermore, we investigated the role of the NAc Tac2 clusters in mediating reinforcement in a mouse intravenous self-administration (IVSA) and found that inhibition of the Tac2 clusters significantly reduced cocaine intakes. Collectively, our results suggested that Tac2 clusters in the NAc are a D1 MSN subtype.

YMS01-03

Specific deletion of neuronal MCT2 or astrocytic MCT4 disturbs the hippocampus-dependent acquisition of information

C. Netzhualcoyotzi, L. Pellerin

University of Lausanne, Department of Physiology, Lausanne, Switzerland

The astrocyte-neuron lactate shuttle (ANLS) hypothesis proposes that neuronal glutamatergic activity leads in astrocytes to a large increase in the production of lactate, which is released in the extracellular space through the monocarboxylate transporter 4 (MCT4) to be taken by neurons via MCT2 and used as an energy

substrate to sustain neurotransmission. Lactate released by astrocytes has been suggested to be necessary for working memory. Further, it was demonstrated that MCT1, 2 and 4 are required for the formation of a non-spatial, long-term (24 h) memory and in the expression of plasticity genes. In the present work, we used for the first time the Cre-lox technology to induce the specific deletion of MCT2 in neurons and MCT4 in astrocytes of the dorsal hippocampus to evaluate their requirement for different behavioral tasks. Our results show that the deletion of either MCT2 or MCT4 does not alter innate behavior, but only the acquisition of new information. The short-term storage of information was normal, but long-term memory was significantly affected. However, if the exposition to the new information (training) is sufficiently repeated, it is possible to finally acquire the data and their retrieval in such case is normal. Our results suggest that lactate transport is a critical step in the acquisition of new information, either in the astrocytes or in the neurons. This could be related to the metabolic coupling proposed by the ANLS. Our data also indicate that intense training sessions can induce compensatory responses to overcome MCT deficiencies. Hence, we propose that the ANLS facilitates the acquisition of new hippocampus-dependent information.

YMS01-04

Mesopontine cholinergic signaling influences stress responses affecting behaviour

O. Kljakic^{1,2}, H. Janickova², K. Rosborough^{1,2}, S. Raulic², S. Matovic², R. Gros², L. Saksida^{2,3}, T. Bussey^{2,3}, W. Inoue², M. Prado^{1,2,3}, V. Prado^{1,2,3}

¹University of Western Ontario, Anatomy & Cell Biology, London, Canada

²Robarts Research Institute, Schulich School of Medicine & Dentistry, London, Canada

³Brain & Mind Institute, Schulich School of Medicine & Dentistry, London, Canada

Pedunculopontine and laterodorsal tegmental nuclei (PPT/LDT) are heterogenous brainstem structures that contain cholinergic, glutamatergic and GABAergic neurons. Several neuropsychiatric disorders have been associated with degeneration of the cholinergic neurons in this brain region, however, the importance of PPT/LDT cholinergic signaling for cognitive and non-cognitive functions is poorly understood. Previous work suggested that PPT/LDT cholinergic neurons play a role in attention and other forms of higher-level cognition, however these studies used non-selective methods to kill cholinergic neurons. To test the role of acetylcholine in higher-level cognition, we selectively eliminated the vesicular acetylcholine transporter (VACHT) in the PPT/LDT to generate mice that have impaired cholinergic signaling without interfering with other

brainstem cell types and co-transmitted chemicals. We tested these VACHT-deficient mice using conventional and touchscreen-based cognitive tasks and found that they had little to no impairments in many cognitive functions, including attention, yet failed to perform in the spatial and cued forms of the Morris water maze (MWM). Interestingly, spatial memory and visual spatial learning were intact in VACHT-mutants, but touchscreen performance was affected by a stressor and mice had altered corticosterone levels after the MWM. These results suggest that attention and many other cognitive functions are not affected by the loss of PPT/LDT cholinergic signaling, but an altered stress response can influence cognitive performance in aversive tasks.

YMS01-05

The role of the orexin (hypocretin) system in context-induced relapse to alcohol seeking **E. Campbell¹, M. Hill¹, N. Marchant², A. Lawrence¹**

¹Florey Institute of Neuroscience and Mental Health and The University of Melbourne, Florey, Melbourne, Australia

²VU University Medical Center, Anatomy and Neurosciences, Amsterdam, The Netherlands

Our lab has previously shown a role for orexin signalling via OX1 receptors in cue-induced relapse to alcohol seeking following extinction. However, one shortfall of extinction is that it is experimenter-imposed, and does not model the negative consequences of drug use. Thus, the current study assessed the role of the orexin system in a model of context-induced relapse to alcohol seeking following punishment-imposed voluntary abstinence.

Male iP rats were trained to self-administer 20% alcohol in context A, where alcohol was available without consequence. Subsequently, rats were then trained in a new context (B) where an active lever press resulted in the delivery of an alcohol reward paired with footshock. Footshock was delivered randomly on 50% of lever presses. Shock intensity was increased across day until responding for alcohol ceased, despite the ongoing availability of alcohol. Rats were then tested with either vehicle or SB-334867 (5 mg/kg, ip) in both contexts A and B.

Rats reliably self-administered alcohol in context A and voluntary abstinence was observed in context B. On relapse test, there was a main effect of treatment [$F_{(1,17)} = 24.8$, $p < 0.0001$] and a treatment x context interaction [$F_{(1,17)} = 5.6$, $p = 0.03$]. Vehicle-treated rats showed relapse to alcohol seeking in context A compared to context B. Pre-treatment with SB-334867 reduced alcohol seeking.

The current study further implicates orexin signalling in alcohol seeking using a preclinical model that may be more reflective of the human experience. Ongoing studies will aim to identify the anatomic loci for this effect.

YMS02 Development and disease

YMS02-01

Atoh1 requires primary cilia for the expansion of granule neuron progenitors by modulating centriolar satellites

C. Chang^{1,2,3}, H. S.^{4,5}, M. Zanini^{4,5}, W. Wang⁶, J. Tsai^{3,7}, O. Ayraut^{4,5}

¹*Institute of Biomedical Science, Academia Sinica, Taipei, Taiwan*

²*TIGP in Molecular Medicine, Academia Sinica and National Yang-Ming University, Taipei, Taiwan*

³*Institute of Brain Science, National Yang-Ming University, Taipei, Taiwan*

⁴*Institute of Curie, PSL Research University, Orsay, France*

⁵*Universite Paris Sud, Universite Paris-Saclay, Orsay, France*

⁶*Institute of Biochemistry and Molecular Biology, College of Life Sciences, National Yang-Ming University, Taipei, Taiwan*

⁷*Brain Research Center, National Yang-Ming University, Taipei, Taiwan*

Development of the cerebellum requires the primary cilium to allow the transduction of Sonic Hedgehog (SHH) signaling. Besides, precise regulation of ciliogenesis ensures the proliferation of cerebellar granule neuron progenitors (GNPs). By mosaic manipulation of cerebellum through in vivo neonatal electroporation, we report that Atoh1, a transcription factor required for GNPs formation, controls the presence of primary cilia, maintaining GNPs responsive to the mitogen SHH. Loss of primary cilia abolishes the ability of Atoh1 to keep GNPs in proliferation. Taking advantage of the in vitro GNP purification, we show that Atoh1 promotes ciliogenesis by transcriptionally regulating Cep131, which facilitates centriolar satellite (CS) clustering to the basal body. Importantly, ectopic expression of Cep131 counteracts the effects of Atoh1 loss in GNPs by restoring proper localization of CS and ciliogenesis. Moreover, Atoh1 enhances SHH signaling in GNPs by translocating Smo within the primary cilium, thereby activating the downstream effectors. This Atoh1-CS-primary cilium-SHH pro-proliferative pathway is also conserved in SHH-type medulloblastoma, a pediatric brain tumor arising from the GNPs. Together, our data reveal the mechanism whereby Atoh1 modulates the primary cilium functions to regulate GNP differentiation during cerebellar development.

YMS02-02

Epigenetic control of the RHOA/rock pathway by the histone methyl-transferase G9A promotes neuronal development

C. Wilson¹, L. Giono², V. Rozés-Salvador¹, A. Fiszbein², A. Kornblihtt², A. Cáceres¹

¹*Instituto Ferreyra INIMEC-CONICET-UNC, Neurobiology, Cordoba, Argentina*

²*Instituto de Fisiología, Biología Molecular y Neurociencias IFIBYNE-UBA-CONICET, Neurobiology, Buenos Aires, Argentina*

Neurons are polarized cells characterized by the presence of dendrites and the axon, two highly specialized compartments. Mechanisms controlling polarization have been studied, but its genetic regulation is unknown. In this regard, epigenetics is a global mechanism for gene regulation, based on modifications of histones

and DNA that remodel chromatin conformation. Emerging evidence links epigenetic regulators with neuronal functions in health and disease. Among these, the enzyme G9a, which methylates lysine 9 of histone 3 (H3K9, a repressor code for gene expression), has been associated with broad aspects of neuronal life, ranging from neurogenesis to chronic pain. Nevertheless, its contribution to early neuronal development is missing. In this work, using both in vitro and in vivo neuronal models (cultured neurons and in utero electroporated embryonic brains), we describe that genetic suppression of G9a inhibits polarity acquisition and axonal specification of central neurons. In this regard, our data suggest that the RhoA/ROCK pathway, an inhibitor of neuronal polarity, is regulated by G9a. This hypothesis is based on the following evidence. First, we detected that genes encoding RhoA activators, including the guanine exchange factor Lfc, are bi-methylated at H3K9 and repressed by G9a. Second, G9a suppression increased both RhoA and ROCK activities. Finally, the loss of function of ROCK recovered axonal growth after G9a suppression. Collectively, our data suggest that G9a represses by default the RhoA/ROCK pathway, which is needed for neuronal polarization. In summary, our work reports a novel epigenetic mechanism controlling neuronal polarity acquisition, with implications for growth and function of central and peripheral neurons.

YMS02-03

Recapitulation of familial multiple sclerosis mutation leads to compromised myelin and susceptibility to demyelination insult

A. Ziaei¹, M. Garcia-Miralles¹, C. Radulescu¹, A. Silvin¹, V. Shaygannejad², H. Bae¹, N. Yusof¹, C. Bardile¹, H. Sidik¹, L. Dehghani², C. Bonnard¹, S. Jung¹, B. Reversade¹, F. Ginhoux¹, M. Pouladi¹

¹*A*STAR, BMRC, Singapore, Singapore*

²*MUI, Neurology, Isfahan, Iran*

Efforts are currently underway to uncover factors that trigger the immune system in multiple sclerosis (MS). We sought to identify multi-incidence MS-families for the discovery of genetic background susceptibility. In one such family, inactivating mutations in *ERMN* gene was identified with complete segregation. Ermin is an actin-binding protein found almost exclusively in central-nervous-system myelin-sheath. Although Ermin has been predicted to play a role in the formation and stability of myelin sheaths, this has not been examined. Using Ermin knockout mice, we show that Ermin is essential for myelin sheath integrity and normal saltatory conduction. Loss of Ermin caused non-compacted myelin sheath and myelin fragmentation in electron microscopy imaging, supported by an increase in QD9/MBP ratio, led to slower conduction velocity in the CC and progressive neurological deficits. RNA sequencing of the CC revealed pathways related to axonal degeneration and inflammation in aged Ermin-deficient mice, which were confirmed by immunostaining showing increased axonal damage, microgliosis and astrogliosis. In addition, we observed an increased level of demyelinated-lesion responsive microglia population in the CC also with a higher level of fragmented myelin phagocytosed by these microglia. The inflammatory milieu and microstructural myelin

abnormalities were further associated with increased susceptibility to demyelination in the experimental autoimmune encephalomyelitis model of MS. We hypothesize this non-compact, fragmented myelin and white matter inflammation can expose myelin proteins to the immune system and make individuals susceptible to MS.

YMS02-04

Nickel-induced developmental neurotoxicity in *C. elegans*; neuronal degeneration, altered behaviour, and increased SKN-1 activity

O. Ijomone^{1,2}, M. Miah², G. Akingbade¹, H. Bucinca², M. Aschner²

¹Federal University of Technology Akure, Human Anatomy, School of Health & Health Technology, Akure, Nigeria

²Albert Einstein College of Medicine, Molecular Pharmacology, New York City, USA

Globally, environmental and occupational exposures to heavy metals are an increasing health concern. Nickel (Ni) is one of such metals and has extensive industrial applications. Importantly there is no known physiological role for Ni in humans and other mammals. Brain damage has been severally implicated in Ni overexposure however, published reports are relatively limited. Here, we investigated specific neuronal susceptibility in a *C. elegans* model of acute nickel neurotoxicity. Wild-type *C. elegans* and worms expressing GFP in several neuronal subtypes were treated with NiCl₂ at the first larval (L1) stage. Our results show significantly increasing degeneration of cholinergic, dopaminergic and GABAergic neurons with increasing Ni concentration in worms expressing GFP for these neuronal subtypes. Also, significant functional changes in locomotion and basal slowing response assays reflected impaired cholinergic and dopaminergic neuronal function respectively. Interestingly, a significant effect on number of worms exhibiting shrinker phenotype indicated that function of D-type GABAergic neurons of *C. elegans* may be specifically attenuated while the RME subset of GABAergic neurons is unaffected. GFP expression due to induction of glutathione S-transferase 4 (*gst-4*), a target of Nrf2 homolog *skn-1*, was increased in VP596 (Pgst-4::GFP; P_{dop-3}::RFP) worms highlighting increased SKN-1 activity and consequently, Ni-induced oxidative stress. RT-qPCR verified upregulation of this expression of *skn-1* immediately after exposure. These data suggest that developmental Ni exposure impairs cholinergic, dopaminergic and GABAergic neurotransmitter systems, probably via the generation of oxidative stress. Further studies are ongoing to unravel molecular mechanisms involved in Ni neurotoxicity using *C. elegans* model.

YMS02-05

Alterations in CD300F immunoreceptor are associated to depression in mice and humans

F. N. Kaufmann¹, N. Lago², M. Negro-Demontel^{3,2}, R. López-Vales⁴, A. Abin⁵, R. Silva⁶, D. Lara⁷, G. Ghisleni⁶, M. Kaster¹, H. Peluffo^{2,3}

¹Federal University of Santa Catarina, Santa Catarina, Florianópolis, Brazil

²Institut Pasteur de Montevideo, Montevideo, Montevideo, Uruguay

³Universidad de la Republica, Montevideo, Montevideo, Uruguay

⁴Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain

⁵Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Montevideo, Uruguay

⁶Catholic University of Pelotas, Rio Grande do Sul, Pelotas, Brazil

⁷Pontifical Catholic University of Rio Grande do Sul, Rio Grande do Sul, Porto Alegre, Brazil

Depression is a high prevalent psychiatric disorder, especially among women. Low-grade inflammation has been linked to depression in mice and humans. CD300f immunoreceptor activation leads to further inhibition of inflammatory process and appears to have a protective role against injuries. However, the role of CD300f immunoreceptors in depression was not elucidated yet. Here, we demonstrated that female CD300f knockout mice (CD300f^{-/-}) with 5 months old presented depressive and anhedonic behavior that were persistent at 18 months of age. The 5-month-old CD300f^{-/-} females presented altered IL-6, IL1RN and IL-10 brain gene expression and decreased hippocampal noradrenaline levels. Acute bupropion treatment (noradrenaline/dopamine reuptake inhibitor) improved female mice anhedonic behavior. Moreover, acute lipopolysaccharide treatment exacerbated female mice anhedonic behavior. In humans, the T allele from the polymorphism (rs2034310 C/T) on CD300f immunoreceptors was associated with protection against MDD in women in a cross-sectional population-based study that included 1.110 individuals. In sum, we characterized for the first time the potential role of CD300f immunoreceptors in the regulation of mood and hedonic processes in mice and humans, suggesting it may be useful as diagnostic biomarkers and as new target for pharmacological intervention in depressive patients.

Workshops

W01 How to improve the quality of publications

W01-01

Open science initiative: implementation of incentives for open data reporting

J. Schulz

RWTH Aachen, Dept of Neurology, Aachen, Germany

Many manuscripts are rejected because of an inadequate study design, underpowering, insufficient number of animals, etc. which means that all the experiments must be repeated to achieve robustness. In the end, this may cause unethical waste of resources. As an initiative to improve data quality and foster transparency in data reporting and data sharing, Journal of Neurochemistry adopted “Open Science badges”. These badges mark up manuscripts for which the authors provided source data (“Open Data” badge), share materials (“Open Materials” badge) or have pre-registered their study (“Preregistered” badge) in a public repository, as is standard in clinical research already but not quite common in basic research studies. In clinical studies, a detailed study design, analysis plan, and description of primary and secondary outcomes are commonly pre-registered in a public repository prior to their actual conduct. We feel that pre-registration should likewise be fostered for basic research studies. It also protects the work during the review process.

In addition to discussing the options and potential of Open Science badges, we will therefore discuss pre-registration repositories, and other measures such as Research Resource Identifiers (RRIDs) that help disambiguate materials or animal strains.

W01-02

Adequate methodology reporting in scholarly publications

L. Hausmann

RWTH Aachen University Hospital, Department of Neurology, Aachen, Germany

As important it is to *conduct* a study thoroughly with proper design and in adherence to Good Scientific Practice, it is no less important to appropriately *report* all details necessary for readers to understand and for fellow scientists to reproduce the experiments. Although many journals do not limit space for the Methods descriptions, authors may find it difficult to provide all necessary details in the restricted space of a manuscript. Additionally, terminology may appear ambiguous because, for instance, authors write “subjects were randomized” even if the actual assignment was only pseudo-randomized. As another example, “measures were taken to minimize animals’ suffering” is too unspecific to be informative. In this aspect of the workshop, at the example of the *Journal of Neurochemistry*, we aim to raise awareness for instances in which unclarity in the manuscript phrasing may lead to confusion or even falsification of the reported experiments. We wish to provide young researchers with clear and concrete help to improve the quality of their study designs, analysis and reporting

standards. We will present examples and elucidate common problems in actual manuscripts.

W01-03

Common pitfalls in statistical analysis - make statistics your friend not foe

F. Middleton

SUNY Upstate Medical University, Neuroscience & Physiology, Syracuse, USA

Are you puzzled or concerned about the explosion of questionable findings in your field? Underpowering through insufficient subject numbers, p value fishing by using arrays of tests to evaluate a single hypothesis, and introduction of non-conscious bias due to lack of blinding or true randomization are only a few of the problems encountered in the planning and analysis of basic scientific studies. Such problems have contributed to an exponential increase of irreproducible findings in the biomedical literature. Other factors include the pressure to publish positive findings, the desire to impress funding agencies, and lack of familiarity with the underlying assumptions of different statistical measures. In fact, many researchers and reviewers feel under-prepared in terms of basic knowledge about the best practices and decision processes to follow for experimental design and selecting the most appropriate statistical tests at the end of their study. The purpose of this workshop will be to address some of the most common pitfalls seen in submissions to the *Journal of Neurochemistry* and other scientific journals and to offer specific advice on how to circumvent these through adequate statistical design and decision algorithms. The audience will be welcomed to share their own concerns and experiences, for a lively discussion that should lead to better insights into why and where statistics are needed and how to deploy them and interpret the results that are generated.

W01-04

Repositories and publication accessibility

E. Prager

John Wiley & Sons, Global Research - Life Science Journal, Hoboken, USA

A paradigm shift in many fields of biology, including neuroscience, is leading to increased pressure on researchers to share and archive their data. Funders and an increasing number of publishers are standardizing data sharing policies by encouraging, expecting or mandating data sharing. Sharing data enables researchers to open new collaborations, reuse experimental results and create new work built on previous findings. Sharing data also improves the efficiency of the research process and supports the critical goal of transparency. In this talk we will explore how data sharing works and what data to include in a data repository. We will then discuss the benefits and challenges of sharing, storing and accessing data.

W02 What's Next? - Young Scientist Career Perspectives

W02-01

New platforms to accelerate the development of drugs targeting neurological disorders

M. Magdesian

Ananda Devices, CEO & Founder, Montreal, Canada

Drug development is a slow, expensive and inefficient process. Every new drug is first tested in cell cultures and in animals before clinical trials in humans. Despite the use of over 100 million animals for research each year, over 92% of drugs tested in animals fail in human clinical trials and the main reason for failure is lack of efficacy and toxicity. To accelerate drug development, biopharmaceutical industries need alternative models that better predict the human response to drugs and that are easily scalable to test thousands of drugs in disease relevant assays. Ananda Devices (<http://www.AnandaDevices.com>) is a developer a supplier of unique microchips to grow human nervous system-on-a-chip, enabling scientists to test their products directly on human tissue, up to 50x faster and 90% more cost-effective than current technologies. We have 20 + years of experience in neuroscience and tissue engineering and in collaboration with researchers from McGill University, we have developed technology to grow neurons *in vitro* 60 times faster than *in vivo* (1 mm/h). Our products help customers comply with 3Rs legislation to "Reduce, Refine and Replace" animal experimentation and enable faster and more cost-effective drug development. Our products help scientists to achieve reproducible and impactful results faster. Our technology was recognized with over 25 grants and awards in science and innovation in Canada, USA and France, including 2016 Top 10 Discovery of the year in Quebec; 2017 Hello Tomorrow Challenge in Paris; 2018 Startup Canada Export Challenge and 2019 Cartier Initiative Awards.

W02-02

The serendipitous path to an industry job

S. Jurgensen

Pareto Frontier Consulting, -, Boston, USA

The academic career path is fairly straightforward – in spite of increasing competition, requirements are clear and the rules of the game have been known for a long time. When it comes to industry jobs, the situation is quite different: not only most PhDs are not being prepared for such a transition by their advisors and training programs, but the path is inherently a diverse and serendipitous one. There is currently a wealth of information available online to PhD students and postdocs aspiring to a career in industry. From suggestions on how to craft one's resume to interviewing tips, it is not difficult to take the self-help route. However, two critical components of this process that are not often mentioned are resilience and strategic diversity. In this short talk I will share my perspective as a scientist and lab head in Big Pharma, and attempt to shed light on what helps to increase the odds of recruitment.

W02-03

An academic's perspective on career paths

M. Robinson

University of Pennsylvania School of Medicine, Pediatrics and Pharmacology, Abramson Research Building, Room 502, Philadelphia, USA

I obtained my Bachelor's degree in Chemistry in 1980, a PhD in Biochemistry in 1985, and then joined the faculty at the University of Pennsylvania (Penn) in 1988. I have been continuously funded by the NIH since 1985, I am the Director of an NINDS-funded post-doctoral training grant, and the co-director of an NICHD-funded Intellectual and Developmental Disabilities Research Center. I was Editor-in-Chief of *Neurochemistry International* for seven years. I have published slightly over 130 primary research and review articles that have attracted nearly 10,000 citations. While this sounds great, this wasn't the original plan, and I have been a spectacular failure on many occasions. I went to graduate school because I wanted to be a physician and I did not have good enough grades from my undergraduate work. I was turned down for tenure two years in a row at Penn. I've had to submit most of my grants multiple times, and there have been other tribulations. With this as a background, I will try to provide advice in variety of areas, including 1) defining priorities and remaining focused on the task at hand. As a scientist, we should be creating new knowledge. 2) Making sure you are continuously asking yourself if you are being productive. 3) Creating luck. 4) Getting feedback on your ideas. 5) Being prepared to fail. This is a great career, though not for everybody. Figure out what makes you want to get up and go to work in the morning.

W02-04

On your own

D. Mangoura

Biomedical Research Foundation of the Academy of Athens, Neurosciences Center, Athens, Greece

A successful career in academia starts with choosing the right institute for your strengths, experiences, values, and professional objectives. Having chosen the best fit you may, starting your own lab is an operation full of challenges. We will discuss some aspects of how to face and balance your three main tasks: scholarship, teaching, and service (citizenship). Particular emphasis will be placed on the importance of demonstrating your ability as an independent investigator. We will start with strategies on how your work may give you an identity and have people link a topic to you and your lab, including cons and pros in sticking with a subject or remain flexible during this time that research is mostly driven by fashionable topics. As this main goal is a function of choosing carefully your team -and setting the right pace and excitement for science-, as well as your collaborators and your mentors within your institution and from your field, and, most importantly of publishing early and getting in the funding game, we will discuss some useful approaches and plans.

W02-05

The paper review process: a perspective from the reviewer, editor, and editor in chief points of view **D. Feinstein**

University of Illinois, Dept of Anesthesiology, Chicago, USA

Aside from getting grants, one of the most important goals of most scientists is publishing high quality papers in peer-reviewed journals. While doing excellent science is key to excellent publications, having a good understanding of the review process can help. One of the ways this can be accomplished is by taking on reviewer responsibilities at various stages of your career. In this presentation I'll go through some of the ways a younger investigator can get invited to review for a journal, and some things that may help to get reviews noticed and appreciated. I'll draw from personal experience how one gets invited to join editorial boards, some of the advantages and disadvantages of being a board member, and at which point it may be prudent to remain or to step down. Finally, I'll describe some of the tasks associated with being EIC for *ASN Neuro*, and how some of our priorities may differ from other journals due to its being a society journal.

W02-06

A view from the other side: An insight into the editorial office of journal of neurochemistry **B. Schweitzer**

Uniklinik Aachen, Neurology, Aachen, Germany

"Finish your PhD, complete one or two Postdocs, start your own group, etc." This is what most people say being asked "What is a scientific career?" – but there are other areas in which scientists can contribute to good science! Scientific projects that remain unpublished will not reach the scientific community and thus not help increasing the global knowledge. A thorough peer-review process and quality control of scientific manuscripts is a prerequisite for a trustworthy publication record on which future science is built. I will give some insights into the work within the editorial office of *Journal of Neurochemistry*, what made me work there after finishing my PhD, my motivations and experiences. The editorial office is the first institution receiving your manuscripts, and the bottleneck for initial quality control. The continuous adjustment of reporting guidelines, strategic management, and training of young scientists in particular are important tasks that journals fulfill to help shaping the scientific field. These goals come with very practical duties such as checking for plagiarism or image manipulation, supporting authors in reporting all relevant details of methodology and data, and ensuring that ethical and publication standards are being met in all manuscripts that are published by *Journal of Neurochemistry*. Additionally, working for *Journal of Neurochemistry* allows you to read about latest science at first hand, to interact with a bunch of different people from all over the world (authors, editors, reviewers), to organize and join events like the ISN-JNC Flagshipschool, and to help making such events successful and memorable for everybody.

Poster Sessions Monday/Tuesday

MTU01 Gene regulation & genetics (Session A)

MTU01-01

Allopregnanolone infusion asymmetrically increases mRNA expression of the delta GABA_A receptor subunit in the hippocampus of rats

F. Almeida¹, R. Gomez², A. Hansen², H. Barros¹, M. Nin^{3,2}

¹UFCSA, Departamento de Farmacociências, Porto Alegre, Brazil

²UFRGS, Departamento de Farmacologia, Porto Alegre, Brazil

³Centro Universitário Metodista do IPA, Curso de Farmácia, Porto Alegre, Brazil

Allopregnanolone (ALLO) is a neurosteroid that acts as a positive allosteric modulator on GABA_A receptors (GABA_AR) in the brain. Such modulations appear to be responsible for its antidepressant effects and for modifying the expression of different GABA_AR subunits. The δ subunit, for instance, was found to be upregulated in the hippocampus after ALLO infusions in the prefrontal cortex and nucleus accumbens. Moreover, intra-hippocampal ALLO infusion produces antidepressant-like effects in rats, but its effects on the hippocampal δ GABA_AR subunit expression are still unknown. This study aimed to verify the sub-acute effect of the intra-hippocampal infusion of ALLO (1.25; 2.5; and 5 μ g/rat) on the δ GABA_AR subunit mRNA expression in each hippocampal hemisphere of male rats. An ANOVA detected an interaction between treatment and hemisphere regarding the expression of the δ subunit ($p = 0.010$), and post-hoc analyses showed that the infusion of 5 μ g/rat of ALLO induced a higher δ GABA_AR subunit mRNA expression in the right hemisphere in comparison to the left ($P < 0.001$). Additionally, the mRNA expression in the right hemisphere was higher in the 5 μ g/rat dose when compared to the 1.25 μ g/rat dose ($p = 0.028$). Thus, intra-hippocampal ALLO infusion asymmetrically increased the mRNA expression of the δ GABA_A receptor subunit in the same brain region of rats. These results support the importance of the δ GABA_AR subunit on the antidepressant effects of ALLO, as well as the relevance of exploring inter-hemispheric analysis.

MTU01-02

Gender-specific effect of 5-HT and 5-HIAA on threshold level of behavioral symptoms associated with autism spectrum disorder

B. Chakraborti¹, D. Verma¹, S. Guhathakurta², S. Sinha¹, K. Mohanakumar³, U. Rajamma^{3,1}

¹Manovikas Kendra, Manovikas Biomedical Research and Diagnostic Centre, Kolkata, India

²UCF College of Medicine, Division of Neuroscience, Orlando, Florida

³Inter University Centre for Biomedical Research and Super Speciality Hospital, CDAR, Kottayam, Kerala

ASD is a group of behaviorally defined neurodevelopmental disorders having genetic origin with a distorted sex ratio being observed in the affected. One of the remarkable aspect of this disorder is platelet hyperserotonemia where this imbalanced serotonin level is taken to be the regulating factor for the deficits in their

behavioral presentation. Platelet hyperserotonemia in ASD subsets, efficacy of SSRI in reducing behavioral deficits and gender-bias in normal serotonin synthesis suggest a disruption in stringent regulation of serotonin metabolism in ASD. Therefore, we investigated gender-specific changes in 5-HT and 5-HIAA in ASD to assess its effect on behavior of male and female subjects. ASD cases ($n=215$) were examined using CARS. Platelet 5-HT (104 cases and 26 controls), and platelet/plasma 5-HIAA (73 cases and 17 controls) were estimated using HPLC-ECD. In male probands, we observed increase in platelet 5-HT content in association with increase in the score for adaptive responses and increase in platelet 5-HIAA levels with concomitant decline in the score for intellectual response. Interestingly, platelet/plasma 5-HT and plasma 5-HIAA were higher in female controls and female probands displayed more severe autism-associated behaviors. Overall results indicate a gender-bias in the regulation of 5-HT and 5-HIAA, which probably increases the threshold level of ASD phenotypes in the females, thereby affecting ASD prevalence in a sex-specific manner.

MTU01-03

MAOA and MAOB genes associated with attention deficit hyperactivity disorder in indo-caucasoid population from eastern india

A. Karmakar¹, B. Chakraborti¹, D. Verma¹, S. Sinha¹, K. P. Mohanakumar², U. Rajamma², K. Mukhopadhyay¹

¹Manovikas Kendra, Biomedical Research & Diagnostic Centre, Kolkata, India

²Inter University Centre for Biomedical Research, Centre for Development and Ageing Research, Kerala, India

Attention deficit hyperactivity disorder (ADHD) is a behavioral disorder, characterized by symptoms of inattention, excessive motor activity, and impulsivity, detected mostly during the early childhood. Influence of monoamine neurotransmitters (such as dopamine, serotonin, and norepinephrine) in ADHD associated symptoms is well accepted. Monoamine oxidase A (MAOA) and B (MAOB), mitochondrial outer membrane bound enzymes, catabolize those monoamines and hence regulate neuronal activities. Genetic polymorphisms in MAOA and MAOB showed association with ADHD in different populations. In this study, we have tested association of three polymorphisms in MAO genes (30bp-uVNTR and rs6323 in MAOA, and rs56220155 in MAOB) with ADHD in Indo-Caucasoid population from eastern India. Nuclear families with ADHD-probands ($N=190$) and ethnically matched controls ($N=156$) were recruited in the study following DSM-IV. Genotyping was performed through PCR-based methods/DNA sequencing. Data were analyzed through population based and family based statistical methods. rs6323 'G' allele, rs56220155 'A' allele, 30bp-uVNTR-rs6323 '3R-G' haplotype, and rs6323-rs56220155 'G-A' haplotype showed significant ($p \leq 0.04$) higher frequencies in ADHD-probands as compared to controls. These alleles/haplotypes also revealed significant ($p \leq 0.05$) higher frequencies in male-ADHD-probands as compared to sex-matched controls. Along with these alleles/haplotypes, 30bp-uVNTR-rs56220155 '3R-A' haplotype showed

significant ($p \leq 0.03$) maternal transmission to male-ADHD-probands. rs56220155 'GA' genotype showed significant ($p = 0.03$) higher frequencies in female-ADHD probands as compared to sex-matched controls. It may be inferred that both MAOA and MAOB genes are contributing to the etiology of ADHD in Indo-Caucasoid population from eastern India.

MTU01-04

Association of a coding TLR4 variant with biomarkers of prodromal Alzheimer's disease

J. Miron^{1,2,3}, C. Picard^{1,2,3}, M.-É. Lafaille-Magnan^{1,2,3}, A. Labonté^{1,3}, M. Savard^{1,3,4}, P. Rosa-Neto^{1,4,6}, D. Auld⁵, J. Poirier^{1,3,6}

¹Douglas Mental Health University Institute, Douglas Hospital Research Centre, Verdun, Canada

²McGill University, Neuroscience, Montréal, Canada

³McGill University, STOP-AD Centre, Verdun, Canada

⁴McGill University, Research Centre for Studies in Aging, Verdun, Canada

⁵McGill University, Génome Québec Innovation Centre, Montréal, Canada

⁶McGill University, Psychiatry, Montréal, Canada

Introduction: A coding variant in the TLR4 receptor (the G minor allele of the rs4986790 polymorphism) was associated with increased longevity and protection against Alzheimer's Disease (AD) in two Italian cohorts. Our team further analyzed the association of this variant with biomarkers of prodromal AD in presymptomatic individuals with a familial history of AD (PRE-VENT-AD).

Methods: Genotyping was performed with the Omni2.5 bead-chip. Cognition was assessed using the RBANS. Structural MRI was performed with a 3T Siemens Trio scanner. CSF concentrations of IL-1 β , IL-6, and TNF- α were obtained with Milliplex assays.

Results: Among the different RBANS index scores that were surveyed (including the total score), only the visuospatial constructional index score is significantly higher in rs4986790 G carriers. Analyses of the associations between baseline whole-brain cortical thickness, RBANS visuospatial constructional index scores, and rs4986790 genotypes revealed clusters in the occipital and frontal lobes as well as in the fusiform gyrus. Finally, a significant interaction was observed between rs4986790 genotypes and visits for CSF IL-1 β levels.

Conclusion: The association, in *at risk* presymptomatic subjects, of the TLR4 rs4986790 G variant with a stabilization over time of CSF IL-1 β levels may help maintain their visuospatial and constructional abilities as well as their cortical thickness.

MTU01-05

Role of antioxidants on attention deficit hyperactivity disorder: question of behavior and genetics

M. Moghadas¹, T. Ba-Omar¹, A. Al-Shehi¹, M. M. Essa², E. Eltayeb¹

¹Sultan Qaboos University, College of Science, Department of Biology, Muscat, Oman

²Sultan Qaboos University, College of Agricultural and Marine Sciences, Department of Food Science and Nutrition, Muscat, Oman

Attention Deficit Hyperactivity Disorder (ADHD) is a common behavioral disorder. Several studies have tended to highlight the effects of antioxidants on regulation of ADHD. The present work investigated the effect of Gallic Acid (GA) and Ellagic Acid (EA) in a Trimethyltin Chloride-induced animal model of ADHD with insights from behavioral and genetic aspects. In this study, 60 pregnant Wistar rats were used in six groups (N=10 per group), namely Control (saline only), Sham (TMT 9 mg/kg), Ellagic Acid (TMT 9 mg/kg+ EA 10 mg/kg), and Gallic Acid (TMT 9 mg/kg+ 50, 100, 200 mg/kg) groups. To induce ADHD, TMT was injected to animals on gestational day 15 (G15) intraperitoneally. Treated groups received EA and GA as gavage on G12 to G18. After delivery, 10 pups were selected out of each group to be studied. Then, on day 60 of delivery, Elevated Plus Maze, Open Field and T-Maze were conducted. Following, animals were terminated humanely and prefrontal cortex of their brains was collected for molecular studies followed by SYBER green qPCR to investigate the expression level of DRD4 and DRD5 genes. Behavioral findings revealed that EA and GA could significantly decrease hyperactivity, impulsivity and inattention in rats. The expression level of DRD4 and DRD5 were significantly down-regulated in the sham group as compared to the controls. In addition, EA and GA in all dosages caused an up-regulation of DRD4 and DRD5 expression, although these differences were not significant as compared to the sham group.

MTU01-06

Epigenetic control of the RHOA/rock pathway by the histone methyl-transferase G9a promotes neuronal development

C. Wilson¹, L. Giono², V. Rozés-Salvador¹, A. Fiszbein², A. Kornblihtt², A. Cáceres¹

¹Instituto Ferreyra INIMEC-CONICET-UNC, Neurobiology, Cordoba, Argentina

²Instituto de Fisiología, Biología Molecular y Neurociencias IFIBYNE-UBA-CONICET, Neurobiology, Buenos Aires, Argentina

Neurons are polarized cells characterized by the presence of dendrites and the axon, two highly specialized compartments. Mechanisms controlling polarization have been studied, but its genetic regulation is unknown. In this regard, epigenetics is a global mechanism for gene regulation, based on modifications of histones and DNA that remodel chromatin conformation. Emerging evidence links epigenetic regulators with neuronal functions in health and disease. Among these, the enzyme G9a, which methylates lysine 9 of histone 3 (H3K9, a repressor code for gene expression), has been associated with broad aspects of neuronal life, ranging from neurogenesis to chronic pain. Nevertheless, its contribution to early neuronal development is missing. In this work, using both *in vitro* and *in vivo* neuronal models (cultured neurons and *in utero*

electroporated embryonic brains), we describe that genetic suppression of G9a inhibits polarity acquisition and axonal specification of central neurons. In this regard, our data suggest that the RhoA/ROCK pathway, an inhibitor of neuronal polarity, is regulated by G9a. This hypothesis is based on the following evidence. First, we detected that genes encoding RhoA activators, including the guanine exchange factor Lfc, are bi-methylated at H3K9 and repressed by G9a. Second, G9a suppression increased both RhoA and ROCK activities. Finally, the loss of function of ROCK recovered axonal growth after G9a suppression. Collectively, our data suggest that G9a represses by default the RhoA/ROCK pathway, which is needed for neuronal polarization. In summary, our work reports a novel epigenetic mechanism controlling neuronal polarity acquisition, with implications for growth and function of central and peripheral neurons.

MTU01-07

Hypoxia contributes to Alzheimer's disease by regulating CNTNAP2 gene

Q. Zhang, Y. Zhang, F. Cai, W. Song

The University of British Columbia, Psychiatry, Vancouver, Canada

Stroke is a risk factor for Alzheimer's disease (AD). Hypoxia is a major consequence of stroke. Contactin-associated protein-like 2 (CNTNAP2) functions as a presynaptic cell adhesion molecule, and

plays important roles in neuronal network formation during development. CNTNAP2 rs802571 is a locus for susceptibility to late-onset AD, and abnormal CNTNAP2 expression has been found in the hippocampus of AD patients. However, it remains elusive how CNTNAP2 is regulated at the transcriptional level. In this study, we cloned a 2949 bp 5'-flanking region of the human CNTNAP2 gene. To investigate the activity of human CNTNAP2 gene promoter, thirteen deletion fragments of its 5'-flanking region were cloned into pGL3-Basic vector. Functional analyses showed that the transcriptional start site was located between -524 to -472 upstream of translational start site. The fragment -524 to -81 bp upstream of translational start site had the minimum promoter activity required for transcription. There were five 5'-RCGTG hypoxia-responsive elements (HRE) in the human CNTNAP2 gene promoter. Three putative functional HREs were located between -1322 to -1268 bp upstream of translational start site. The activity of CNTNAP2 promoter was increased to 270% when treated with hypoxia (1% O₂). Co-transfection with hypoxia-inducible factor 1 subunit α (HIF-1 α) expression plasmid significantly increased the CNTNAP2 promoter activity to 133%. This is the first study defining the promoter region of human CNTNAP2 gene. Our results demonstrated that CNTNAP2 is tightly regulated by hypoxia at the transcriptional level. Our study suggests a novel role of hypoxia in contributing to AD pathogenesis by regulating CNTNAP2 gene expression.

MTU02 Signal transduction & synaptic transmission (Session A)

MTU02-01

GSK3-mediated phosphorylation of PI4KII- α regulates ADBE via control of protein interactions

E.-M. Blumrich¹, Jessica. C. Nicholson-Fish¹, D. Kurian², Karen. J. Smillie¹, Michael. A. Cousin¹

¹The University of Edinburgh, Center for Brain Discovery Sciences, Edinburgh, United Kingdom

²The University of Edinburgh, 2The Roslin Institute, Edinburgh, United Kingdom

Activity dependent bulk endocytosis (ADBE) is triggered during high neuronal activity in central neurons. It is a two-step process that generates synaptic vesicles from large bulk endosomes, which are directly invaginated from the presynaptic plasma membrane. The activity of glycogen synthase kinase 3 (GSK3) is essential for ADBE, however how this control is mediated is still incompletely understood. One GSK3 substrate that is present at the synapse is phosphatidylinositol 4-kinase IIa (PI4KII α). Depletion of PI4KII α using shRNA in cultured cerebellar granule neurons (CGNs) arrested ADBE. Delivery of exogenous PI4KII α to these neurons fully restored ADBE, highlighting an important role of PI4KII α . Interestingly, molecular replacement of endogenous PI4KII α with a GSK3- phospho-mimetic mutant failed to rescue ADBE. However, kinase-dead and phospho-null mutants both fully restored ADBE, suggesting that GSK3-dependent phosphorylation of PI4KII α negatively regulates this endocytosis mode. To determine potential phosphorylation-specific interactions, GST-PI4KII α pull downs were performed. Mass spectrometry analysis revealed 5 presynaptic molecules that displayed an increased interaction with the phospho-mimetic PI4KII α mutant, which were confirmed by Western blotting. Truncation and domain swap mutations revealed that mock phosphorylation of Ser-47 on PI4KII α is critical in controlling these interactions. Intriguingly, individual depletion of two of these phospho-dependent interaction partners greatly reduced ADBE in CGNs. These results indicate a key role for PI4KII α in ADBE and confirm the constitutively active GSK3 as a master regulator of this process. We propose that activity-dependent dephosphorylation of Ser-47 on PI4KII α induces the release of key molecules which are crucial for the initiation of ADBE.

MTU02-02

Constitutive neuronal interleukin-1 β release: influence on neuronal excitation

S. Dutta, J. Hewett

Syracuse University, Dept. of Biology, Program in Neuroscience, Syracuse, USA

Interleukin-1 β (IL-1 β) is a pro-inflammatory cytokine of the immune system. While it contributes to various neuroinflammatory and neurodegenerative disease of the central nervous system (CNS), it also modulates several physiological functions. Thus, IL-1 β is a neuromodulator in both dysfunctional and normal CNS. Epilepsy is a brain disorder hallmarked by excessive neuronal firing. IL-1 β 's role has been implicated in seizures, the defining feature of epilepsy. Of relevance to my research, we previously showed that the innate seizure threshold was lowered in mice in which IL-1 β receptor was

genetically disrupted. This provided compelling evidence to support the premise that constitutive IL-1 β production in the normal brain influences neuronal excitability. However, cellular location and regulation of IL-1 β release remain unknown. I hypothesized, i) unlike neuroinflammatory glial release, IL-1 β is released constitutively by neurons and ii) P2X7R activation (a purinergic receptor implicated in the inflammatory release of IL-1 β in periphery and in brain) will be necessary for physiological IL-1 β release in CNS. This possibility was tested both *in vivo* and *in vitro*. *In vivo*, treatment with a selective antagonist of P2X7R, caused buildup of IL-1 β in pyramidal neurons of CA3 region of hippocampus of mice brain. Using the convulsant agent, pentylenetetrazole, to model excessive neuronal excitation, I found that the seizure threshold was lowered in these mice relative to vehicle-treated controls in agreement with receptor knockout mice. Using P2X7R antagonism in primary hippocampal neuronal culture, similar IL-1 β accumulation was found. The possible mechanism of constitutive release of IL-1 β from neurons is under active investigation. Thus, constitutive IL-1 β release from hippocampal neurons, which when restricted from release through P2X7R antagonism, appears to serve as modulated neuronal excitation. My research adds novel information regarding the physiological roles of IL-1 β in CNS.

MTU02-03

Evoked release of soluble amyloid-beta species

R. Hendrix¹, C. Yuede², J. Cirrito¹

¹Washington University in St. Louis, Department of Neurology, St. Louis, USA

²Washington University in St. Louis, Department of Psychiatry, St. Louis, USA

The synaptic activity-driven release of A β and tau to the interstitial fluid (ISF) is a key step in the progression of Alzheimer's disease (AD). A fundamental question regarding this process remains: does activity drive aggregation? Brain regions with the highest neuronal activity correlate with the greatest degree of plaque and tangle pathology. A β and tau pathology spreads along specific neuronal networks in both mice and humans, such as the perforant pathway connecting the entorhinal cortex to the hippocampus. Sustained increase in excitatory synaptic strength, i.e. long-term potentiation (LTP), is impaired in the AD hippocampus. We hypothesize that a threshold of neuronal activity exists in which monomeric release of A β and tau reaches a pro-aggregative level. Though the threshold of activity at which this occurs may differ between A β and tau, both will contribute to the sequence of neuropathogenesis of plaques and tangles. Using a novel micro-immuno-electrode (MIE) allows for detection of A β and tau species every 60 seconds for approximately three hours *in vivo*, or in living acute brain slices. MIEs utilize an antibody-attached electrode to measure the oxidation of tyrosine residues, such as those in human A β or tau. The amount of current detected is proportional to the concentration of target molecule present. Baseline A β was measured in acute slices, followed by high frequency stimulation to induce LTP. There was a rapid increase in A β levels concurrent with the induction of LTP. Greater understanding of these processes may enable interventions aimed at homeostatic control of

hyperexcitability to be developed to prevent the formation and spread of A β and/or tau pathology.

MTU02-04

GM1 oligosaccharide is the active portion responsible for GM1 neuroprotective properties

G. Lunghi¹, M. Maggioni¹, E. D. Biase¹, M. Fazzari¹, G. Tedeschi², E. Maffioli², F. G. Scalvini², S. Sonnino¹, E. Chiricozzi¹

¹University of Milan, Department of medical biotechnology and translational medicine, Segrate, Italy

²University of Milan, Department of Veterinary Medicine, Milano, Italy

GM1 ganglioside has been considered as a master regulator of the nervous system and accumulating evidence is pointing out age-dependent GM1 deficiency as initiator of sporadic Parkinson's disease (PD) pathogenesis. Preclinical data and clinical trials have reported that GM1 administration exerts neuroprotective and neurorestorative properties both in PD animal model and PD patients, although the benefit resulting from GM1 replacement therapy is extremely limited by its amphiphilicity. Recently, we demonstrated in neuronal cell that the oligosaccharide portion of GM1 (OligoGM1) is the actual moiety responsible of GM1 neurodifferentiative properties, by the activation of the TrkA-MAPK pathway. To understand if the exogenous administration of OligoGM1 and the resulting activation of TrkA pathway could account also for GM1 neuroprotective effects we performed a proteomic analysis on N2a cells treated with 50 μ M OligoGM1 for 24 hours. The analysis led to the identification and quantification of more than 3500 proteins, among these 324 proteins were exclusively expressed in OligoGM1-treated cells. Interestingly, the OligoGM1-only proteins are mainly involved in crucial biochemical signaling with a neuroprotective potential, reflecting the GM1 neuroprotective effect. In addition, biochemical analysis showed that OligoGM1 is able to reduce the cellular oxidative stress and to confer a protection against the cell death induced by different toxic molecules (MPTP, glutamate) in N2a cells. Our results suggest that the molecular mechanisms underlying the GM1 protective role, as described in the past, depend on its oligosaccharide chain, making the OligoGM1 a promising therapeutic molecule.

MTU02-05

Neuroplastin-plasma membrane Ca²⁺ ATPases complexes: a new team regulating Ca²⁺ clearance, signaling, and synaptic plasticity

A. Malci^{1,2}, M. Naumann³, Eckart. D. Gundelfinger^{2,4}, Constanze. I. Seidenbecher^{2,4}, R. Herrera-Molina²

¹Otto-von-Guericke-University Magdeburg, Faculty of Natural Sciences, ESF Graduate School ABINEP, Magdeburg, Germany

²Leibniz Institute for Neurobiology, Department of Neurochemistry and Molecular Biology, Magdeburg, Germany

³Otto-von-Guericke-University Magdeburg, Institute of Experimental Internal Medicine, Magdeburg, Germany

⁴Center for Behavioral Brain Sciences, ^{CBS}, Magdeburg, Germany

Neuroplastins, type-1 transmembrane proteins with extracellular Ig-like domains, play a crucial role in synapse architecture and plasticity. We and others have shown that neuroplastins bind all four

isoforms of the plasma membrane Ca²⁺ ATPase (PMCA) to regulate expression levels and Ca²⁺-extruding activity of these pumps. Indeed, we have also shown that in neuroplastin-deficient neurons with altered long-term potentiation, the protein levels of PMCA are reduced. A crucial Ca²⁺-dependent downstream signaling pathway implicated in synaptic plasticity is via extracellular signal-regulated kinases (ERK). Thus, we hypothesize that neuroplastin-PMCA-modulated Ca²⁺ signals may regulate ERK activation during synaptic plasticity. Our preliminary results indicate that ERK phosphorylation and PMCA abundance are drastically altered in brain homogenates from neuroplastin-deficient mice. Using super resolution STED microscopy and FLIM/FRET-based biosensors, we monitor ERK activation and Ca²⁺ dynamics in synapses of cultured hippocampal neurons. We assessed the contribution of neuroplastin to PMCA levels and activity-dependent plastic mechanisms using lentivirus-driven overexpression and small extracellular peptides targeting neuroplastin and PMCA. We have observed that pharmacological inhibition of PMCA activity alters the normal ERK activation in electrically stimulated neurons. Also, we employ high-frequency stimulation protocols to study the link between neuroplastin/PMCA with ERK activation in synaptic junction fractions from hippocampal slices. Taken together, considering Ca²⁺-dependent ERK activation as a prominent mechanism underlying activity-dependent synaptic plasticity, we propose neuroplastin-PMCA complexes as major regulators of synaptic Ca²⁺ clearance during synaptic plasticity.

MTU02-06

A distinct neurodevelopmental disorder is caused by mutations in synaptotagmin-1 that alter neurotransmitter release dynamics

H. Melland¹, F. Bumbak¹, S. L. Leech¹, D. J. Scott¹, K. Baker², S. L. Gordon¹

¹The University of Melbourne, The Florey Institute of Neuroscience and Mental Health, Melbourne, Australia

²MRC Cognition and Brain Sciences Unit, Executive Processes Group, Cambridge, UK

Synaptotagmin-1 (sytl) is an essential synaptic vesicle protein that acts as the Ca²⁺-sensor for fast, synchronous neurotransmitter release, with additional roles in other aspects of synaptic physiology. We have recently characterised the first known cases of human mutations in sytl, revealed through whole exome sequencing of 11 individuals with neurodevelopmental impairments not typical of any recognised disorder. 5 discrete *de novo* missense mutations have been identified in these individuals who exhibit a phenotypic spectrum of common symptoms including motor delay, intellectual disability, movement disorders and behavioural abnormalities. Given all human mutations cluster within the C2B domain, a Ca²⁺-binding region crucial for sytl function, we investigated whether these sytl variants perturb the dynamics of neurotransmitter release. The homologous mutations were induced in pHluorin-tagged rat sytl and expressed in cultured mouse hippocampal neurons, establishing that expression level and synaptic vesicle localisation of sytl variants were equivalent to that of the WT protein, with the exception of one mutant (p<0.01, n=3-4). pHluorin imaging revealed that sytl mutants slow the rate of evoked exocytosis in a dominant-negative manner (p<0.05, n=5-7), and notably this slowing was less pronounced for the variant presenting with a less severe clinical profile. These results suggest that

impairment of Ca^{2+} -dependent neurotransmitter release is likely an important pathophysiological mechanism underpinning this disorder and, together, our findings demonstrate that *sytl* variants with mutation-specific impacts on protein functionality give rise to a distinct and recurrent neurodevelopmental disorder.

MTU02-07

Alpha 2 adrenergic receptor agonist guanabenz directly inhibits HCN channels

S. Oh^{1,2}, J. Won², P. R. Lee²

¹School of Dentistry, Seoul National University, Department of Neurobiology and Physiology, Seoul, Korea South

²College of Natural Sciences, Seoul National University, Department of Brain and Cognitive Sciences, Seoul, Korea South

Alpha 2 (α_2 -) adrenergic receptor agonists, such as clonidine or dexmedetomidine, have been found to inhibit hyperpolarization-activated, cyclic nucleotide-modulated (HCN) channels, not only by reducing intracellular cyclic AMP levels but also by directly blocking HCN channels. In this study, we examined the inhibitory effect of guanabenz, a centrally acting α_2 -adrenergic receptor agonist with high specificity for α_{2A} -subtype, on HCN channels in mesencephalic trigeminal nucleus (MTN) neurons which robustly express HCN channels and have been suggested to coexpress α_{2A} -adrenergic receptors. By performing whole-cell patch-clamp recording on MTN neurons in brainstem slices, hyperpolarization-activated inward current (I_h) was examined during guanabenz treatment. Guanabenz inhibited I_h in a dose-dependent manner, which was likely to be ZD7288-sensitive HCN current as it did not affect barium-sensitive inward rectifying potassium current. Guanabenz not only inhibited I_h but also shifted the voltage-dependent activation curve to hyperpolarizing potentials. Interestingly, I_h inhibition by guanabenz was not reversed by α_2 -adrenergic receptor antagonist atipamezole treatment or by intracellular cyclic AMP perfusion, suggesting that the inhibition may not result from α_{2A} -adrenergic receptor signalling pathway but from direct inhibition of HCN channels. Coherent to our electrophysiological results, single-cell RT-PCR revealed that most MTN neurons lack α_{2A} -adrenergic receptor mRNA. Our study demonstrates that guanabenz can directly inhibit HCN channels in addition to its primary role of activating α_{2A} -adrenergic receptors.

MTU02-08

EM17 - a new kainate receptor selective antagonist: pharmacology and X-ray crystallography

D. Pickering¹, S. Møllerud¹, K. Frydenvang¹, J. Kastrup¹, J. Andreassen¹, R. Hansen¹, M. Diaz-delCastillo¹, A. Paternain², T. Johansen¹

¹University of Copenhagen, Drug Design & Pharmacology, Copenhagen, Denmark

²CSIC-UMH, Institute of Neuroscience, San Juan de Alicante, Spain

Most fast excitatory neurotransmission in the mammalian CNS is mediated by glutamate acting through ionotropic glutamate receptors (iGluRs). This receptor family includes the kainate receptors (KAR) consisting of the subunits GluK1-5, located both pre- and

postsynaptically. KAR are believed to modulate the activity of neuronal networks by regulating neurotransmitter release. This modulatory role of KAR provides a therapeutic target for fine-tuning the balance of excitatory and inhibitory signaling. The physiological functions of KAR are incompletely understood, partially due to a lack of selective KAR pharmacological compounds. Extensive effort has thus been put into development of KAR selective antagonists but there still exists an unmet need for subunit-selective KAR ligands. 2,3-Quinoxalinediones were among the first antagonists applied in non-NMDA receptor research. Nonetheless, these initial compounds were not optimal for elucidating the functions of KAR as they are also antagonists of AMPA receptors. We have recently published on 2,3-quinoxalinediones substituted at the N1-, 6- and 7-positions which demonstrated GluK3 preference. Here, a new series of 2,3-quinoxalinedione analogues with substitutions in the 7-position have been characterized. One compound (EM17) showed high affinity at GluK3 ($K_i = 78$ nM) and was further characterized by patch clamp electrophysiology in HEK293 cells expressing GluK3 ($K_B = 39$ nM) and *in vivo* in the mouse tail flick test where it was efficacious as an analgesic. Finally, to understand the molecular interactions of this series of 2,3-quinoxalinediones we report a crystal structure of EM17 in complex with the GluK1 ligand-binding domain.

MTU02-09

Actin regulation by non-prenylatable RHOA and RAC1 in neurite outgrowth and cell clustering

N. Raut, D. Hynds, J. Reddy

Texas Woman University, Biology, Denton, USA

Encouraging axon regeneration after traumatic lesions to the central nervous system (CNS) or the onset of neurodegenerative conditions like Alzheimer's disease (AD) may increase the functional recovery. Unfortunately, there is no effective treatment that promotes axon regeneration or synaptic plasticity. Both regeneration and synapse formation involve dynamic rearrangements of the growth cone actin cytoskeleton. For process extension, actin monomers polymerize to filaments near the leading edge and monomers are removed at the transition to the axon. Actin polymerization and depolymerization are controlled in large part by Rho guanosine triphosphatases (GTPases). These proteins are active when bound to guanosine triphosphate (GTP) and inactive when bound to guanosine diphosphate (GDP). Rho GTPases are targeted to the plasma membrane by addition of a 20-carbon lipophilic geranylgeranyl isoprene. It is not known how Rho and RhoA geranylgeranylation affects the location and activity of downstream effectors to facilitate either polymerization or depolymerization of actin. We used non-geranylgeranyllatable RhoA and Rac1 constructs to test how inhibiting geranylgeranylation affects morphology, localization of activation of RhoA and Rac1 cell signaling pathways. Western blot analysis and confocal microscopy show that expressing non-geranylgeranyllatable constructs increases cortical actin filament content in growth cones, but have differential effects on process outgrowth from neuroblastoma cells and rat primary cortical neurons. Expressing non-geranylgeranyllatable Rac1 decreased neurite initiation and elongation, while expressing non-geranylgeranyllatable RhoA increased neurite elongation. Furthermore, expressing non-geranylgeranyllatable RhoA or Rac1 significantly altered formation of the actin nucleation complex of WAVE with the Arp2/3 complex. Elucidating the signaling cascades

of the aberrantly-localized active Rho GTPases and the effect on actin may identify the downstream effectors that can be used as a novel targets to facilitate axon regeneration in traumatic or degenerative neurological conditions.

MTU02-10

Inhibition of MMP-9 enhances cholinergic-induced synaptogenesis in hippocampal CA1 pyramidal neurons **A. Salamian, A. Beroun, L. Kaczmarek**

Nencki Institute of Experimental Biology, Molecular and Cellular Neurobiology, Warsaw, Poland

Matrix metalloproteinases (MMPs) are enzymes modifying synaptic function via remodeling the milieu surrounding chemical synapses. Matrix metalloproteinase-9 (MMP-9) is of particular importance because of being able to change dendritic spine morphology, suggesting its contribution to structural synaptic plasticity. One potential way to trigger synaptic plasticity changes is activation of cholinergic system – a model that has been recently extensively studied. Little is known, however, regarding the involvement of MMP-9 in cholinergic activation in the hippocampus. Therefore, using organotypic hippocampal slice, we investigated the role of MMP-9 in synaptic plasticity evoked by carbachol – a cholinergic agonist. We found the elevation of MMP-9 activity following 1 h of carbachol treatment, with a peak at 24 h after stimulation. We tested the hypothesis that elevated MMP-9 activity contributes to modifying synaptic function evoked by cholinergic activation. We assessed excitatory and inhibitory synaptic transmission of the CA1 pyramidal neurons after carbachol induction using whole-cell patch-clamp electrophysiology technique. Data revealed an enhancement of the frequency of miniature inhibitory postsynaptic currents (mIPSCs) a few hours after carbachol, which was impaired by blocking the MMP-9 activity. We also found an increase in the frequency of miniature excitatory postsynaptic currents (mEPSCs) evoked by carbachol within a longer time after carbachol treatment. Interestingly, blocking MMP-9 further increased the frequency of mEPSCs evoked by carbachol. Evaluation of size and distribution of excitatory presynaptic (VGLUT) and postsynaptic (PSD-95) markers revealed that the enhancement of excitatory postsynaptic currents is mediated by the increase in the number of synapses. Our data emphasize the contribution of MMP-9 to the balance between excitatory and inhibitory synaptic transmission affecting excitatory synaptic contacts in the hippocampus, induced by cholinergic activation.

MTU02-11

Subcellular localization of sphingosine 1-phosphate receptors in synapses of the mouse cortex **C. Skoug¹, A. Meissner^{1,2}, J. Duarte^{1,2}**

¹Lund University, Dep. Experimental Medical Science, Lund, Sweden

²Lund University, Wallenberg Centre for Molecular Medicine, Lund, Sweden

Sphingosine 1-phosphate (S1P) has pleiotropic biological functions in the regulation of proliferation, survival, migration, inflammation or angiogenesis. S1P acts as intracellular second messenger, as well as extracellular receptor ligand via five G-protein coupled

receptors (S1PR1-5). In the brain, S1P regulates neuronal proliferation or apoptosis, excitatory neurotransmission and neuroglia activation, and S1P metabolism alterations have been associated to neurodegenerative disorders. Interestingly, an agonist targeting S1PR1,3,4,5 (FTY720) shows neuroprotective properties through mechanisms that are not fully unveiled, but might include the control of neuroinflammation, vascular deterioration and synaptic dysfunction. The subcellular distribution of S1PRs in nerve terminals is hitherto unknown. The present study aimed at determining the synaptic localisation of S1PRs in the cortex of adult male mice. Synaptosomes were purified from mouse cortex homogenates using a sucrose density gradient centrifugation, and further fractioned into pre-, post- and extrasynaptic zones using a series of pH shifts that allow successive solubilisation of synaptic components (Phillips et al., Neuron 32:63, 2001). Western blot analysis of the obtained fractions, as well as total protein extracts from cortex revealed that S1PR1 is present in similar amounts in total extracts, synaptosomes and the extrasynaptic fraction, but absent from the pre- and postsynaptic fractions. S1PR2 was ubiquitously distributed, showing 3-fold higher levels in the presynaptic zone than the post- ($P<0.05$) and extrasynaptic ($P<0.05$) fractions. Similarly, S1PR4 was also distributed across all synaptic fractions but 4-fold more enriched presynaptically. S1PR3 and S1PR5 were not efficiently detected by immunoblotting in synaptosomes and synaptic fractions. Altogether, these results point towards S1PR2 and S1PR4 being particularly well poised to directly modulate synaptic transmission and plasticity upon S1P activation.

MTU02-12

The sonic hedgehog and WNT/beta-catenin signalling pathways under the chronic stressful condition is influenced by nicotine

M. Tayyab, S. Farheen, M. P. M Mubeena, N. Khanam, M. Mobarak Hossain, M. Shahi

Aligarh Muslim University, Interdisciplinary Brain Research Centre (IBRC), J.N. Medical College, Faculty of Medicine, Aligarh, India

Background: Sonic hedgehog (Shh) is a member of the hedgehog family and regulates embryonic development. Recently, various studies recommended its significant role in adult neural tissues through its distinctive mechanisms such as hippocampal neurogenesis, anti-oxidation and anti-inflammation. On the other hand, Shh is a critical target in various neurological diseases like brain cancer and depressive disorders. Moreover, Brain-derived neurotrophic factor (BDNF) and Wnt/ β -catenin signalling also plays important role in the neuropathology of depression. In our study, we focused on the role of Shh and Wnt/ β -catenin signalling pathways in chronic stress-induced depression including loss of learning and memory caused by depression and the influence of nicotine. Nicotine is the most common and highly addictive drug of abuse. Interestingly, studies suggested that nicotine abuse is the self-effort to feel reward and fight depression in depressed individuals.

Method: Twenty-four male Wistar rats were randomly divided into four groups; Control (saline), Nicotine (NIC 0.3mg/kg), chronic unpredictable mild stress (CUMS) and CUMS+NIC. Forced swim test, open field test and Morris water maze were performed and the hippocampal mRNA expression of BDNF, Shh, GLI1/2, NKX2.2, PAX6 and β -catenin were observed.

Results: We observed that nicotine reversed CUMS induced depressive behaviour as well as CUMS associated cognitive deficits. The mRNA expression of BDNF, Shh, GLI1/2, NKX2.2 and β -catenin were decreased in CUMS, and these decreased mRNA levels were recovered by nicotine. To conclude our study, we suggest that BDNF, Wnt/ β -catenin and Shh signalling pathways play important role in the pathophysiology of depression and in providing the nicotine-mediated antidepressant effect.

MTU02-13

TGF β 1-signaling importance in insulin-sensitive GLUT4 trafficking to membrane in the cortex of mice with acute liver failure

M. Popek, M. Zielinska

Mossakowski Medical Research Centre Polish Academy of Sciences, Neurotoxicology, Warsaw, Poland

Hepatic encephalopathy is a neuropsychiatric syndrome caused by liver failure (ALF) with multifactorial pathomechanism in which ammonia and neuroinflammation play a key role. TGF- β 1, a pleiotropic cytokine controls different cell functions, among other glucose metabolism-dependent on insulin. TGF- β 1 signaling is

impaired in ALF mouse model. In the presented study the role of TGF- β 1 signaling in insulin-sensitive glucose 4 transporter (Glut4) trafficking through PI3K/Akt/PKC ζ signaling pathway in cerebral cortex of ALF mice was measured. C57Bl6 mice with ALF induced by AOM injection (i.p.100 mg/kg), and mice with TGF- β 1 neutralization (i.p.ab-TGF- β 1, 1 mg/kg) were used. The reduction in TGF- β 1 level by $\sim$ 200% and $\sim$ 100% in serum and cortex homogenates were observed. The expression of Insulin Receptor β and Insulin Receptor Substrate 1 were unaltered in both models. In addition, decreased expression of TGF- β Receptor 2 by $\sim$ 15% in mice after neutralization of the cytokine in the absence of changes in ALF mice was observed. The subtle changes in the expression of p-PDK1 and p-Pi3K proteins were demonstrated in ALF brain homogenates, accompanied with reduction in p-PKC ζ by $\sim$ 15% in mice after neutralization and decrease in p-AKT by $\sim$ 50% in ALF mice were observed. In both groups the increase in Glut4 protein by $\sim$ 100% and $\sim$ 80% in the cytosolic fraction was observed, without changes in the membrane fraction. The results indicate that ALF alters brain glucose metabolism, however the mechanism of Glut4 translocation to the membrane in ALF mice might be not solely associated with TGF- β 1 signaling. Particular mechanism requires additional research. Supported by the grant Prelludium10 2015/19/N/NZ5/02249.

MTU03 Neuroinflammation & neuroimmunology (Session A)

MTU03-01

N-butanol fraction of *OLAX subscorpioidea* attenuates lipopolysaccharide-induced depression by inhibiting NF-KB in mice

O. Adeoluwa

Afe Babalola University Ado Ekiti Ekiti State, Pharmacology and Therapeutics, Ado Ekiti, Nigeria

Background: The leaves of *Olox subscorpioidea* (OS) is a mainstay in the management of mental illness in folkloric medicine in Nigeria. Studies have shown its antidepressant, and anti-inflammatory properties in animals. However, with emanating evidences suggesting link between immuno-inflammatory pathways and depression, there is dearth of information linking or attributing its antidepressant effect to its anti-inflammatory mechanism. We thus evaluated antidepressant effect of n-butanol fraction (BF) of OS on LPS-induced depressive behaviours with respect to its action on inflammation.

Methods: Sixty mice were randomly assigned into six groups (n=10): group1 (vehicle), group2 (BF/5mg/kg), group3 (BF/10mg/kg), group 4 (BF/20 mg/kg), group5 (Imipramine/10 mg/kg), group6 (vehicle). Mice were treated with vehicle or BF or imipramine for seven days. Thirty minutes after treatment on day seven, animals were injected with LPS (0.83mg/kg,i.p.) except group1 (vehicle only). Twenty-four hours after LPS injection, animals were assessed for depressive symptoms using sucrose preference test, locomotor and exploratory activity and immobility using tail suspension test. Brain levels of Interleukin-1 β , TNF- α , malondialdehyde, reduced glutathione and corticosterone were measured by ELISA technique. Expressions of Indolamine-2,3-Dioxygenase(IDO), inducible-nitric-oxide synthase (iNOS) and nuclear factor-kappa B(NF-kB) were quantified by immunohistochemistry.

Results: LPS increased immobility of mice in TST and decreased sucrose preference indicating of depressive-like behaviours compared to controls. These behaviours were attenuated by BF compared to control. The altered levels of MDA, GSH, corticosterone, TNF- α , and IL-1 β were significantly reversed by BF. Induction of IDO, iNOS and NF-kB translocation were also reversed by BF.

Conclusion: Attenuation of LPS-induced depression may be attributed to its inhibitory effect on immunoinflammatory pathways.

MTU03-02

Ultrastructural study of mature and immature corpora amylacea in human brain

E. Augé^{1,2}, M. Riba^{1,2}, J. Duran-Vilaregut¹, I. Bechmann³, N. Llor¹, M. Krueger³, C. Pelegrí^{1,2}, J. Vilaplana^{1,2}

¹*Universitat de Barcelona, Facultat de Farmàcia i Ciències de l'Alimentació, Barcelona, Spain*

²*Universitat de Barcelona, Institut de Neurociències, Barcelona, Spain*

³*University of Leipzig, Institute for Anatomy, Leipzig, Germany*

Corpora amylacea (CA) are spherical bodies of unknown origin and function, which accumulate in the human brain during the aging process and neurodegenerative disorders. CA have been associated

with PAS granules, which are degenerative granular structures that appear progressively with age in the mouse brain. Although immature PAS granules have been described, the formation of CA is an unknown process. The aim of the present study is to identify CA during their genesis and describe them at ultrastructural level. We show that most CA, which are mature CA, consist of a core or compacted mass of randomly oriented short linear structures surrounded by some cytoplasmic organelles such as mitochondria and all encircled by a plasma membrane. Moreover, we observed some CA in early stages. These immature CA contain an inner region that is less compact than that of mature CA, and this inner region contain mitochondria, cellular debris and membranous blebs. All these findings support the correspondence between human CA and PAS granules and reinforce the hypothesis that CA, as PAS granules, are involved in the entrapment of damaged and non-degradable products and have a role in protective or cleaning mechanisms.

MTU03-03

Cytokine profile of patient with major depressive disorder **E. Babusikova¹, I. Ondrejka², I. Hrtanek², D. Dobrota¹**

¹*Comenius University in Bratislava, Department of Medical Biochemistry, Jessenius Faculty of Medicine in Martin, Martin, Slovakia*

²*Comenius University in Bratislava, Clinic of Psychiatry, Jessenius Faculty of Medicine in Martin, Martin, Slovakia*

Major depressive disorder (MDD) is a common mental disorder associated with significant negative impact on quality of life. Two percentages of Slovak population undergo treatment but 10 – 20% of population is not diagnosed. MDD is a complex disorder in which psychological, biological, genetic and environmental factors are affected by the onset and development of disease. The dominant hypothesis of MDD described abnormalities in interactions between neurotransmitters and hormones in the brain. Nowadays it is believed that a number of complex metabolic pathways will be involved in etiology and pathogenesis: oxidative damage, inflammation, neuro-immune pathways, serotonergic system, and tryptophan catabolism. In the present study, we examined the hypothesis that increased inflammation is associated with MDD. All patients have endogenous MDD, 96% of patients have severe MDD. We analysed concentration of 12 cytokines: interleukins (IL-1 α , 1 β , 2, 4, 6, 8, 10) and growth factors (vascular endothelial growth factor, tumor necrosis factor α , epidermal growth factor, interferon gamma, and monocyte chemoattractant protein 1). Concentration of six interleukins (IL-1 α , 1 β , 2, 4, 8, 10) was increased in patients with depression (5 – 60%). Concentration of TNF α was decreased in 55% of patients and 90% of patients have decreased epidermal growth factor concentration. Basic clinical biochemical parameters were in physiological ranges in all patients. There is a lack of precisely characterised populations of MDD patients. Deeper analysis of interleukins together with polymorphisms of interleukins could help explain effects of interleukins changes in MDD patients. *The project was supported by: VEGA 1/0266/18.*

MTU03-04

Vascular and neurogenic rejuvenation in aging mice by modulation of ASM

J.-S. Bae¹, M. H. Park¹, K. H. Park¹, I. K. Jung¹, M. S. Jeong², B. J. Choi², H. K. Jin²

¹ School of Medicine, Kyungpook National University, Department of Physiology, Daegu, Korea South

² College of Veterinary Medicine, Kyungpook National University, Department of Laboratory Animal Medicine, Daegu, Korea South

Although many reports have revealed dysfunction of endothelial cells in aging, resulting in blood-brain barrier (BBB) breakdown, the underlying mechanism(s) remain to be explored. Here, we find that acid sphingomyelinase (ASM) is a critical factor for regulating brain endothelial barrier integrity. ASM is increased in brain endothelium and/or plasma of aged humans and aged mice, leading to BBB disruption by increasing caveolae-mediated transcytosis. Genetic inhibition and endothelial specific knock-down of ASM in mice ameliorated BBB breakdown and neurocognitive impairment during aging. Using primary mouse brain endothelial cells, we found that ASM regulated the caveolae-cytoskeleton interaction through protein phosphatase 1-mediated ezrin/radixin/moesin (ERM) dephosphorylation, as well as apoptosis. Moreover, mice with conditional ASM overexpression in brain endothelium accelerated significant BBB impairment and neurodegenerative change. Overall, these results reveal a novel role for ASM in the control of neurovascular function in aging, suggesting that ASM may represent a new therapeutic target for anti-aging.

MTU03-05

Characterization of neurotropic virus-induced acute flaccid paralysis and motor neuron death in an experimental model

A. Basu, M. Bhaskar, S. Mukherjee, I. Akbar

National Brain Research Center, Molecular and Cellular Neurosciences, Gurugram, India

Poliomyelitis like illness is a common manifestation associated with neurotropic virus infection. Functional loss and death of motor neurons in spinal cord often lead to reduced muscle tone and paralysis, which subsequently result in clinical symptoms like movement disorders, cognitive impairment and long term neurological sequelae amongst the survivors. Despite of several reports on molecular basis of encephalopathy, the pathogenesis of flaccid paralysis upon viral infection remained largely unknown. The present study aims to elucidate the mechanism responsible for limb paralysis by studying clinical isolates of Japanese encephalitis virus (JEV) and Chandipura virus (CHPV) causing clinical-AFP (Acute flaccid paralysis) in vast region of south-east Asia. Experimental model for studying virus-induced AFP was generated by intraperitoneal infection of 10-day old BALB/c mice. Mice were subjected to a series of behavioural tests to assess gait, neurodegeneration and locomotory behaviour. Progressive decline in motor performance of infected animal was found when compared with mock. Paralysis was correlated with death of motor neuron (MN) by studying various cell death-assays both *in vivo* and *in vitro*. Furthermore, this study demonstrate that upon viral infection MN trigger type-I interferon production through RIG-I dependent pathway via activation of transcription factor IRF-3 and IRF-7. Once activated, this pathway in turn leads to interferon-induced

extrinsic apoptosis of MN. Both, gene silencing using specific RIG-I siRNA and INFAR receptor blocking abrogate MN apoptosis *in vitro*, thus validating the important role of RIG-I and interferon in MN death upon viral infection. Hence, we are hypothesizing that host innate antiviral response is critical in deterioration of motor functioning and pathogenesis of flaccid paralysis upon neurotropic virus infection.

MTU03-06

Neuroimmune interactions mediated by TNF- α -mediated in the induction of rapid plasticity after CNS injury

L. Chagas¹, P. Trindade^{2,3}, R. Antonioli-Santos⁴, A. Rodrigues¹, T. Mázala-de-Oliveira¹, C. A. Serfaty¹

¹Federal Fluminense University, Neurobiology, Niteroi, Brazil

²Unirio, Biochemistry, Rio de Janeiro, Brazil

³IDOR: Instituto D'OR de Pesquisa e Educação, Neuroscience, Rio de Janeiro, Brazil

⁴Instituto de Pesquisas Biomédicas. Hospital Naval Marcílio Dias, Biomedical science, Rio de Janeiro, Brazil

Introduction: CNS lesions are often folby structural reorganization within incircuits of the brain. In para focal lesions also induce neuroreaction that encourages the under-ing of the mechashared beneuroplasticity and immune activation, within CNS injury context. TNF- α is a key procyto that exerts its effects via TNF receptor subtype-1 (TNFR1) or -2 (TNFR2). Calcineurin (CaN) is a phosrelated to synaptic pruning and immune func that mediates microactivation and TNF- α release.

Goal: Here we evaluthe role of TNF- α and microon the sproof inretinotectal axons folmonocular enucleation (ME) and the tempo-ral expreof TNFR1/2 and Arg-1 after lesion.

Results: Lister Hooded rats were submitted to ME at P10 and evaluin different survival times. Animals also received systemic injections of cyclosporin A (50mg/kg,sc) or minocycline (125 mg/kg,sc) 3h folME. A third group received local delivery (ELVAX) of a TNF- α neutralizing anti3 days before. Neurotracers mapped structural plasticity while immunoand western blot were used to study microglia morphology, TNF- α , TNFR1/2 and CaN content. A proinof activated microin the contralateral supercolliculus (SC) 24h after ME, peaking at 72h prea temporal corwith an inin TNFR1 and TNFR2 immunoreactivity, that ceased 7d after. Inhibition of microor TNF- α resproof intact uncrossed retinotectal axons, amoe-boid microand TNF- α exssion. We also oba transinof Arg-1, a anti-inmarker of microreactivity, 24h and 7days after lesion. Inibitors decreased the Iba-1 and iNOS levels, 72h after lesion sugan anti-effect.

Conclusion: Data support the hypothat TNF- α signalling is reguduring a micro-deneuroplasticity induced by lesions during early brain de Approbly local animal care committee (CEUA/UFF: pro0015109).

MTU03-07

Combined administration of dopaminergic and nondopaminergic drugs reverses neuroinflammation in a rat model of parkinson's disease**G. Costa¹, M. Serra¹, M. Morelli^{1,2,3}, A. Pinna³**¹*University of Cagliari, Department of Biomedical Sciences, Section of Neuroscience, Cagliari, Italy*²*National Institute of Neuroscience, University of Cagliari, Cagliari, Italy*³*National Research Council of Italy, Institute of Neuroscience, Cagliari, Italy*

A previous study of our laboratory demonstrated an improved motor performance in 6-hydroxydopamine (6-OHDA) unilaterally lesioned rats, a model of Parkinson's disease (PD), that were treated with the combination of L-dopa, the serotonin 5-HT_{1A/1B} receptor agonist eltoprazine, and the adenosine A_{2A} receptor antagonist preladenant. Starting from these findings, and from evidences that implicates neuroinflammation in PD progression, the present study investigated whether counteraction of neuroinflammation participated in the motor effects of the L-dopa+eltoprazine+preladenant combination.

6-OHDA-lesioned rats were chronically treated with L-dopa+eltoprazine+preladenant. Then, we evaluated in the denervated caudate-putamen (CPu) and substantia nigra pars compacta (SNc) the immunoreactivity (IR) for the glial fibrillary acidic protein (GFAP), and the co-localization of the ionized calcium binding adaptor molecule 1 (IBA1), with interleukin (IL)-1 β , tumor necrosis factor- α (TNF- α) and IL-10. Finally, the IR for tyrosine hydroxylase (TH) and the dopamine (DA) transporter (DAT) was quantified.

Combined treatment with L-dopa+eltoprazine+preladenant induced a reduction of basal GFAP and IBA1 IR in both CPu and SNc. Moreover, a reduction of IL-1 β in IBA1-positive cells both in CPu and SNc and of TNF- α in IBA1-positive cells in SNc was observed. Besides, a significant increase in IL-10 in IBA1-positive cells was also observed in SNc. Finally, a significant reduction of DAT and TH IRs was found in all the experimental groups.

The present findings indicate that the combined administration of L-dopa+eltoprazine+preladenant reduced the inflammatory and neurodegenerative responses in the nigrostriatal system of 6-OHDA-lesioned rats.

MTU03-08

Systemic LPS induces vesicular co-expression of rage and LC3 in dopaminergic neurons of the substantia nigra
D. Gelain, J. Gasparotto, C. Tiefensee-Ribeiro, N. Somensi, P. Brum, D. Peixoto, J. C. Moreira*Universidade Federal do Rio Grande do Sul, Departamento de Bioquímica, Porto Alegre, Brazil*

The receptor for advanced glycation endproducts (RAGE) is a pattern-recognition receptor that triggers pro-inflammatory gene transcription mainly through NF- κ B activation. Induction of RAGE is observed in several non-infectious pathologies with a pro-inflammatory characteristic, including diabetes, atherosclerosis, cancer and Alzheimer's disease. Disruption of autophagy flux and induction of macroautophagy are observed in α -synuclein mutants and are suggested to be a key step in Lewy's bodies formation. Here we used a rat model of long-term dopaminergic neurodegeneration induced by systemic LPS injection to evaluate the involvement of

RAGE in α -synuclein and LC3-I/II expression and progression of dopaminergic cell death. Adult Wistar rats were subjected to systemic LPS injection (5 mg/kg, i.p.) and the substantia nigra was isolated at 6 and 10 months after injection for IF. RAGE, α -synuclein and LC3-I/II staining were all increased in dopaminergic neurons, although the number of TH-positive cells in the substantia nigra decreased in the course of 10 months. Co-localization of RAGE and LC3-I/II staining is observed in vesicles of TH-positive cells. Systemic (previous to LPS) or intranigral (2 months after LPS) injection of the RAGE inhibitor FPS-ZM1 inhibited RAGE and LC3-I/II expression, α -synuclein staining and preserved the number of TH-positive cells. These data indicate an important role for RAGE in dopaminergic neurodegeneration triggered by systemic inflammation, and also suggest that changes in the regulation of autophagic flux are a key step in the mechanism of neurodegeneration associated to RAGE. Financial support: CNPq, FAPERGS, CAPES and Propesq-UFRGS.

MTU03-09

Smoking mice: the effects of sub-chronic cigarette smoke exposure on microglia**F. G. Ibáñez¹, M.-K. St-Pierre¹, M. Carrier¹, J. Savage¹, M. Morissette², M.-É. Tremblay¹**¹*Université Laval CRCHU de Québec, Axe Neurosciences, Québec, Canada*²*CRIUCPQ, Pneumologie, Québec, Canada*

According to World Health Organization, in 2015, there were 1.1 billion smokers worldwide. Smoking is responsible for 7 million deaths per year and constitutes an important risk factor for several diseases, including mental diseases. Animal studies on cigarette smoke exposure have shown increased levels of inflammatory markers and oxidative stress in several organs including the brain. Microglia are the resident immune cells of the brain. They are required for the proper functioning of the brain and are equipped with a myriad of receptors that allow them to monitor their environment, recognize damage, eliminate cells and debris. They also have a role in plasticity by promoting the growth or directly eliminating synapses by phagocytosis, making them active modifiers of the neuronal network circuitry. Microglia are able to respond to inflammation as well as to external environmental changes, suggesting possible alteration of their physiological functions by cigarette smoke exposure. Using a model of sub-chronic cigarette exposure, this project aims to study the effects that cigarette smoke has on hippocampal microglia of 4-month-old male mice. The hippocampus is essential for memory and learning, in addition to being a niche for adult neurogenesis. With the use of immunohistochemistry against the microglial marker IBA1, we have analyzed microglial density, distribution, and morphology. Using array tomography and scanning electron microscopy, we are currently characterizing ultrastructural changes of microglial morphology, phagocytic activity and interactions with synaptic elements. Overall, this study will unravel the consequences of cigarette smoke on microglia and shed light on a possible mechanism by which smoking could affect brain health.

MTU03-11

Attenuation of acute inflammatory pain following a surgical incision by neuropeptide y in rats

S. Gupta, R. Kumar, M. Gautam, S. B. Ray

All India Institute of Medical Sciences, Anatomy, New Delhi, India

Aim: Postoperative pain continues to be an important health care problem. Pain signals are modulated at spinal level by various neurotransmitters and neuropeptides. Neuropeptide Y (NPY) is abundantly distributed in the mammalian nervous system. Several reports have shown its involvement in various pain models but its role in postoperative pain is poorly understood. In the current study, on the hind paw incision model temporospatial change in expression of NPY in the spinal cord was observed.

Methods: Male Sprague-Dawley rats were subjected to hind paw incision. Immunohistochemical localization of NPY was performed at the spinal level (L4-L5). Another set of animals (n=12) were administered NPY or saline through an intrathecal catheter. Finally, NPY antibody (n=6) was administered through catheter followed by NPY and the effect observed. Three nociceptive assays were used to evaluate the antinociceptive effect starting from 2h post-incision until postoperative day 7.

Results: NPY immunoreactivity was observed as punctate varicosities in the superficial laminae of the dorsal horn. On the contrary, neurons positive for NPY, was observed in the deeper laminae. NPY immunostaining decreased after incision at 3 h followed by an increase at 12 h. At day 1, it decreased again. This variable pattern of expression suggested the involvement of NPY in postincisional nociception. Subsequently, on intrathecal administration, nociception was significantly decreased between 2 h to day 2, which was reversed by antibody to NPY.

Conclusion: Neuropeptide Y likely acts as an antinociceptive factor in the spinal modulation of pain. This information could have clinical relevance.

MTU03-12

Complement mediates dysfunction and neurodegeneration in amyloidosis and tauopathy models and is activated in Alzheimer's disease

J. Hanson

Genentech Inc., Neuroscience, South San Francisco, USA

Complement pathway overactivation can lead to neuronal damage in a variety of neurological diseases. While Alzheimer's disease (AD) is characterized by both amyloid-beta plaques (amyloidosis) and Tau tangles, previous work examining complement has largely focused on amyloidosis models. We find that in mouse models of amyloidosis (PS2APP) or tauopathy (TauP301S), glial cells show increased expression of complement classical pathway components including C1q and the central component C3; however, complement proteins accumulate more extensively in TauP301S mice. Blocking complement function by knockout (KO) of C3 not only rescued the plaque-associated synapse loss in PS2APP mice, but also ameliorated neuron loss and brain atrophy in TauP301S mice. Neuroprotection in TauP301Sx3C3KO mice was accompanied by improvements in neurophysiological and behavioral measurements. We also find that C1q and C3 protein are elevated in AD patient brains, including at synapses. In AD patient CSF we find that levels and processing of C3 are increased and correlate with tau but not amyloid-beta. Together these results

demonstrate that complement activation can contribute to neurodegeneration caused by tau pathology and suggest that blocking C3 function might be protective in AD and other tauopathies.

MTU03-13

N^ω-NITRO-L-arginine methyl model of pre-eclampsia elicits differential IBA1 and EAAT1 expressions in brain

O. Ijomone, P. Shallie, A. Naicker

University of KwaZulu-Natal, Optics and Imaging Centre, Durban, South Africa

Pre-eclampsia (PE) is a pregnancy syndrome associated with an increased risk of both the mother and the baby developing cardiovascular disorders later in life. It is widely accepted that women with severe PE develop a neurological impairment however studies have revealed that the mother and baby are at jeopardy for a neurological deficit later in life. The present study examined expression of Iba1 and EAAT1 as neuroinflammatory markers in an N^ω-nitro-L-arginine methyl (L-NAME) model of early- and late-onset PE-like syndrome in rat models. Forty-five adult nulliparous pregnant Sprague-Dawley rats were used for this experiment. They were divided into Control, EOPE and LOPE groups. Administration of L-Name was done between gestational days 8-17 for the treated groups. Animals were sacrificed at GD 19, PND 1 and 60 and the brain excised for further analysis. Our study confirmed L-NAME induced PE-like symptoms in rat models as evidenced by significant increase in systolic blood pressure and urine protein compared with Control. There was upregulation of IBA1 expression and increased microglial activation in the brain of PE rat models assessed at gestational day 19, post-natal day 1 and 60. Also, IBA1 expression is up regulated in the pups at post-natal day 1 and 60. Contrastingly, EAAT1 expression is down-regulated in the brain of PE rat models assessed at gestational day 19, post-natal day 1 and 60, as well as offspring at post-natal day 1 and 60. These results demonstrate likely neuro-inflammation within the brain of PE mothers during pregnancy, that persist into later life, as well as possible neuro-inflammation in brains of offspring of PE mothers.

MTU03-14

Neuronal SPHK1 acetylates COX2 and contributes to pathogenesis in a model of Alzheimer's disease

H. K. Jin¹, M. H. Park², B. J. Choi¹, M. S. Jeong¹, K. H. Park², I. K. Jung², J.-s. Bae²

¹*College of Veterinary Medicine, Kyungpook National University,*

Dept. of Laboratory Animal Medicine, Daegu, Korea South

²*School of Medicine, Kyungpook National University, Dept. of Physiology, Daegu, Korea South*

Although many reports have revealed the importance of defective microglia-mediated amyloid beta phagocytosis in Alzheimer's disease (AD), the underlying mechanism remains to be explored. Here we demonstrate that neurons in the brains of patients with AD and AD mice show reduction of sphingosine kinase1 (SphK1), leading to defective microglial phagocytosis and dysfunction of inflammation resolution due to decreased secretion of specialized proresolving mediators (SPMs). Elevation of SphK1 increased SPMs secretion, especially 15-R-Lipoxin A4, by promoting acetylation of serine residue 565 (S565) of cyclooxygenase2 (COX2)

using acetyl-CoA, resulting in improvement of AD-like pathology in APP/PS1 mice. In contrast, conditional SphK1 deficiency in neurons reduced SPMs secretion and abnormal phagocytosis similar to AD. Overall, these results reveal a novel mechanism of SphK1 pathogenesis in AD that leads to defective microglial phagocytosis due to impaired SPMs secretion, and suggests that SphK1 in neurons has acetyl-CoA dependent cytoplasmic acetyltransferase activity towards COX2.

MTU03-15

TRPV4 activation contributes more to inflammation and endothelial damage rather than calcium after spinal cord injury

H. Kumar, I. Han

CHA University, Neurosurgery, Seongnam-si, Korea South

Importunate activation of transient receptor potential vanilloid type 4 (TRPV4) is associated with cellular toxicity and might contribute to the degeneration of neural tissue after spinal cord injury (SCI). We examined the TRPV4 role and its involvement in major biological cascades in the pathology of SCI. We studied expression at 3 hours, 8 hours, 1, 3, 5, 7, 14, 21 and 28 day (s) in a clinically relevant model of moderate compression (35 g for 5 min at T10 level in rats) for SCI. Also, we checked the TRPV4 expression in injury dependent manner (compression using 20 g, 35 g and 50 g for 5 min) and transaction model of SCI. We quantitatively estimate Ca^{2+} at the same time points using two-photon microscopy and co-related the TRPV4 expression with Ca^{2+} after SCI. Additionally, we used a specific TRPV4 antagonist (RN-1734 5 mg/kg, i.p.) and TRPV4 KO mouse to elucidate the role of TRPV4 in SCI pathology. TRPV4 inhibition using specific antagonist (RN-1734 5 mg/kg, i.p.) attenuated the inflammatory cytokines, chemokines, promotes vascular stabilization prevented the tight junctions protein degradation and blood-spinal cord barrier (BSCB) break down after SCI. Likewise, TRPV4 KO mouse showed reduced inflammation and prevented the tight junctions protein degradation, BSCB breakdown, and neuropathic pain after SCI (20 g for 1 min). Thus, our result suggests that increased TRPV4 expression was associated with the early inflammatory phase of SCI, tissue damage, vascular destabilization, BSCB breakdown, and cell injury. Inhibiting TRPV4 significantly attenuated SCI-induced inflammation, BSCB breakdown, and cell injury. Additionally, TRPV4 inhibition serves as a promising therapeutic strategy to attenuate neuropathic pain, secondary damage and promoting vascular stabilization after SCI.

MTU03-16

Evaluation of role of somatostatin and somatostatin type-2 receptor in post-incisional nociception in rats

R. Kumar, P. Prasoon, S. Gupta, S. B. Ray

All India Institute of Medical Sciences, Anatomy, New Delhi, India

Background: Somatostatin (SST) is widely expressed in mammalian central and peripheral nervous system. It has significant modulatory effect on the release of neurotransmitters. Several studies have observed the anti-nociceptive effect of its analogue octreotide. However, its expression at the spinal level following an acute nociceptive stimulus is not well known. Moreover, its involvement in mediating nociception at the periphery is also not well established. In the present study, the spatio-temporal expression of SST and its receptor (type-2) was observed at the spinal level. Thereafter, antinociceptive effect of somatostatin was assessed by behavioural assays.

Methods: Male Sprague-Dawley rats ($N = 88$) were subjected to hind paw incision. The expression study of SST and its receptor type-2 was performed by Immunohistochemistry and Western blot at different post-incisional time points (2 h, 8 h, day 1, day 3). Comparison of anti-nociceptive effect of intra-wound (10, 30, 100mcg) and systemic (400 μ g/Kg i.p.) SST administration was evaluated by 3 different behavioural assays. Blood glucose level was examined. c-Fos expression in the spinal cord was also studied.

Results: Expression of SST showed an upregulation at 2 h, which decreased at 8 h and on day1. SSTr2 was also upregulated at 2 h and 8 h but decreased by day1. Repeated systemic administration relieved mechanical allodynia from 2 h to day 3. Intra-wound SST relieved guarding pain between 2 h to day 3 and mechanical allodynia from day 4 onwards. Blood glucose level remained unaltered. c-Fos positive nuclei were significantly less after SST administration.

Conclusion: Somatostatin is involved in nociceptive modulation at both central as well as peripheral levels. This information could have clinical significance.

MTU03-17

Novel curcumin derivatives and carotenoids as inhibitors of amyloid- β aggregation and inflammation in Alzheimer's drug discovery

J. L. Beitia¹, P. Llanes¹, O. Larionov², A. Durant¹, K. S. Jagannatha Rao¹

¹Institute of Scientific Research and High Technology Services
INDICASAT, Centre for Biodiversity and Drug Discovery,
Panama, Panama

²University of Texas at San Antonio, Chemistry, San Antonio, USA

Alzheimer's disease (AD) is the most common neurodegenerative disorder affecting the elderly population worldwide. Brain inflammation plays a key role in the progression of AD. Deposition of senile plaques in the brain stimulates inflammatory response with the overexpression of pro-inflammatory mediators as IL-6. Drug discovery based on nutraceutical molecules for prevention and treatment of AD is a growing topic. In this sense, carotenoids and polyphenols such as curcumin have been reported benefits for human health. Cryptocapsin showed the highest bioactivity, while cryptocapsin-5,6-epoxide and zeaxanthin exhibited similar activity on anti-aggregation assays. Meanwhile, curcumin has revealed to be a potential compound for treating of AD following different neuroprotective mechanisms, such as inhibition of aggregation and

decrease in brain inflammation. Its low bioavailability, and susceptibility to degradation in biological systems and poor solubility in plasma has, however, prevented the curcumin as drug. We synthesized new curcumin derivatives with the aim of providing good anti-aggregation capacity but also improved anti-inflammatory activity. Nine curcumin derivatives were synthesized by etherification and esterification of the aromatic region. Compound 4 exhibited a strong anti-aggregation effect higher than curcumin. Monofunctionalized curcumin derivatives showed better bioactivity than difunctionalized compounds. Moreover, the presence of bulky groups in the chemical structure of curcumin derivatives decreased bioactivity. Molecular docking analysis revealed that carotenoids and curcumin derivatives might follow two mechanisms for inhibiting A β aggregation: one by preventing the formation of the fibril and second through disruption of the A β aggregates.

MTU03-18

Alterations in CD300F immunoreceptor are associated to depression in mice and humans

F. N. Kaufmann¹, N. Lago², M. L. Negro-Demontel^{3,2}, R. López-Vales⁴, A. Abin⁵, R. Silva⁶, D. Lara⁷, G. Ghisleni⁶, M. Kaster¹, H. Peluffo^{2,3}

¹Federal University of Santa Catarina, Santa Catarina, Florianópolis, Brazil

²Institut Pasteur de Montevideo, Montevideo, Montevideo, Uruguay

³Universidad de la Republica, Montevideo, Montevideo, Uruguay

⁴Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain

⁵Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Montevideo, Uruguay

⁶Catholic University of Pelotas, Rio Grande do Sul, Pelotas, Brazil

⁷Pontifical Catholic University of Rio Grande do Sul, Rio Grande do Sul, Porto Alegre, Brazil

Depression is a high prevalent psychiatric disorder, especially among women. Low-grade inflammation has been linked to depression in mice and humans. CD300f immunoreceptor activation leads to further inhibition of inflammatory process and appears to have a protective role against injuries. However, the role of CD300f immunoreceptors in depression was not elucidated yet. Here, we demonstrated that female CD300f knockout mice (CD300f^{-/-}) with 5 months old presented depressive and anhedonic behavior that were persistent at 18 months of age. The 5-month-old CD300f^{-/-} females presented altered IL-6, IL1RN and IL-10 brain gene expression and decreased hippocampal noradrenaline levels. Acute bupropion treatment (noradrenaline/dopamine reuptake inhibitor) improved female mice anhedonic behavior. Moreover, acute lipopolysaccharide treatment exacerbated female mice anhedonic behavior. In humans, the T allele from the polymorphism (rs2034310 C/T) on CD300f immunoreceptors was associated with protection against MDD in women in a cross-sectional population-based study that included 1.110 individuals. In sum, we characterized for the first time the potential role of CD300f immunoreceptors in the regulation of mood and hedonic processes in mice and humans, suggesting it may be useful as diagnostic biomarkers and as new target for pharmacological intervention in depressive patients.

MTU03-19

Induction of cerebral hyperexcitability by peripheral viral challenge is mediated by CXCL10

T. Petrisko¹, S. Srinivas², J. Bloemer², Y. Du², S. Setti², V. Suppiramaniam², M. Reed², G. Konat¹

¹West Virginia University, Biochemistry, Morgantown, USA

²Auburn University, Drug Discovery and Development, Auburn, USA

Peripheral viral infections are potent comorbid factors that exacerbate neurodegeneration, albeit, the underlying mechanisms have not been defined. In a quest to elucidate these mechanisms, we have developed a preclinical model, in which a viral mimetic, polyinosinic-polycytidylic acid (PIC) is injected intraperitoneally to simulate peripherally-restricted viral challenge. We have demonstrated that PIC challenge elicits robust hyperexcitability of cerebral networks as seen from the development of seizure hypersusceptibility, increased basal synaptic transmission (BST), and the enhancement of long-term potentiation (LTP). Because neuronal hyperexcitability is a causative factor in neurodegeneration, our finding buttresses the contention that the enhancement of neuronal hyperexcitability is the putative mechanistic link between peripheral viral infections and exacerbations of neurodegeneration. At the molecular level, PIC challenge-induced hyperexcitability is concurrent with robust generation of cerebral CXCL10, a chemokine known to modulate neuronal activity. The present study was undertaken to determine the involvement of CXCL10 and its cognate receptor, CXCR3 in the development of neuronal hyperexcitability. Briefly, 8-week old female C57BL/6 mice were ip injected with 12 mg/kg PIC or equivolume saline, and after 24 h, the brains were analyzed. Confocal microscopy revealed CXCL10 to be generated primarily by neurons and astrocytes in the hippocampus and cortex. No CXCL10 generation was found in microglia. The expression of CXCR3 was confined to neurons. Blockage of CXCR3 through intracerebroventricular injection of an inhibitor, AMG-487 (3 mg/kg), abolished PIC-induced increase of BST and LTP. Based on these results, we posit that the activation of neuronal CXCL10/CXCR3 axis drives the development of hyperexcitability instigated by PIC challenge.

MTU03-20

Development of novel therapeutics against Alzheimer's disease by targeting neuroinflammation in SH-SY5Y cells

K. Rafi¹, S. S. Hussain², S. Faizi², S. U. Simjee^{1,2}

¹ICCBS, Dr. Panjwani Center for Molecular Medicine and Drug Research, Karachi, Pakistan

²ICCBS, H.E.J. Research Institute of Chemistry, Karachi, Pakistan

Neuroinflammation plays an important role in pathogenesis and progression of Alzheimer's disease (AD), mainly characterized by the presence of senile plaques and neurofibrillary tangles. The production of inflammatory mediators, ROS and RNS causes synaptic dysfunction that is responsible for AD associated memory decline. To understand the role of inflammation in AD pathogenesis, we have developed an *in vitro* model of AD using Phytohaemagglutinin (PHA) to target AD at early stage by suppressing neuroinflammation and delay its onset and progression. Initially plaques formation was observed using Phytohaemagglutinin (PHA). SH-SY5Y cells were incubated with 5-40 μ g/ml PHA for 24 hours and cellular morphology was observed by microscopy. Oxidative

stress was analyzed at same concentrations by fluorimetric method using 2',7'-dichlorodihydrofluorescein diacetate. Presence of A β plaques upon PHA stimulation was confirmed by immunocytochemistry. RT-qPCR was performed to analyze the gene expression of inflammatory markers (TNF- α , IL-1 β , iNOS, P38- α and P38- β) and secretases involved in plaques formation. Morphologically no prominent changes appeared at 5 μ g/ml PHA while visible aggregates were observed at 10, 20 and 40 μ g/ml concentrations. Oxidative stress analysis demonstrated significant increase in ROS levels at 10 μ g/ml PHA. Immunocytochemistry at 10 μ g/ml PHA showed significant increase in A β expression than unstimulated cells. Gene expression analysis showed altered expression of genes in PHA stimulated cells. Further we have screened different compounds for their neuroprotective effect and found that quinic acid and N-(2-hydroxyphenyl) acetamide increased cell viability. Next, we will test these compounds and determine their molecular mechanism in reducing PHA-induced neuroinflammation and A β generation.

MTU03-21

Differential effects of age and cytokines between brain areas: when histology meets biophysics

P. Sanchez-Molina¹, N. Benseny-Cases³, B. González¹, B. Castellano¹, A. Perálvarez-Marín², B. Almolda¹

¹Autonomous University of Barcelona, Cell Biology, Physiology and Immunology, Barcelona, Spain

²Autonomous University of Barcelona, Biochemistry and Molecular Biology, Barcelona, Spain

³ALBA Synchrotron Light Source, MIRAS, Barcelona, Spain

Microglial cells become hyper-activated during physiological aging. This activation varies among brain areas, being higher in white matter (WM) compared to grey matter (GM). Previous results from our laboratory show that microglial cells expressed phagocytic receptors involved in myelin recognition solely in WM areas during aging, indicating aging-derived myelin damage. Moreover, transgenic (Tg) animals overproducing IL-6 and IL-10, two altered cytokines during aging, show differences in the expression of these myelin-recognition receptors. Thus, our aim is to evaluate whether the specific microglial changes observed in WM areas of aged and transgenic mice are related to alterations in myelin composition. We used the synchrotron- μ FTIR as a highly sensitive method to assess lipid and protein composition in tissues. Our results indicate that there are regional differences between GM and WM regardless of age and genotype. Low lipid:protein ratio and high oxidation are found on GM compared to WM. We observe decreased lipid:protein ratio and lipid oxidation in WM areas of WT aged compared to adult animals. We also detect lower lipid:protein ratio and higher oxidation in Tg adults compared to WT in WM, but no significant difference is observed between WT and Tg aged. In GM, we observe lower lipid oxidation in Tg aged animals compared to WT. The present study shows that aging correlates with a loss of lipid in WM, supporting our aging-related myelin damage/deterioration hypothesis. Besides, changes in the cytokine microenvironment modify lipid composition in aging and adulthood.

MTU03-22

Inflammation contributes to greater visual pathway dysfunction in animal models of multiple sclerosis

M. Sekyi, K. Atkinson, C. Diaz, M. Feri, S. Noori, W. Rincon, S. Tiwari-Woodruff

University of California - Riverside, School of Medicine - Biomedical Sciences, Riverside, USA

Multiple sclerosis (MS) is an inflammatory, demyelinating and neurodegenerative disease with impaired visual function, a prevalent feature in the majority of patients. Considering optic neuritis is an early marker of MS, we hypothesize that the presence of inflammation throughout the visual pathway combined with demyelination plays a significant role in visual dysfunction. To understand the role of inflammation and chronic demyelination on visual dysfunction in MS, two commonly used mouse models for MS, the lymphocyte-mediated chronic experimental autoimmune encephalomyelitis (EAE) and the non-lymphocyte mediated chronic cuprizone diet demyelination model were used to assess and compare visual pathway pathology. Longitudinal *in vivo* electroretinograms and visually evoked potentials (VEP) were used to assess visual function in EAE (1, 3, 5, and 8 weeks) and cuprizone (3, 6, 9, and 12 weeks) mice. Five mice were euthanized at denoted time points for immunohistochemistry analysis correlating to *in vivo* assessments. Myelination, inflammation, and neurodegeneration in visual pathway structures was assessed by immunohistochemistry. Electroretinograms and VEPs for both EAE and cuprizone animals exhibited changes in latency and/or amplitude. Response time to light stimulus stabilized with disease progression, however magnitude of the visual responses did not. IHC analysis revealed differences in EAE and cuprizone groups. Inflammation, demyelination and neurodegeneration was substantial in EAE thought the visual pathway, however, cuprizone showed significantly less inflammation and neurodegeneration, but exhibited localized structural demyelination as compared to EAE. In summary, our results reveal a significant role of inflammatory demyelination in causing significant visual pathway neurodegeneration in EAE as compared to minimal axon damage with cuprizone demyelination, mimicking diverse pathology observed in MS patients.

MTU03-23

TGF- β associated MAPK pathway: a possible approach to halt pentylenetetrazole-induced epileptogenesis in mice

M. Shahid¹, U. Nisar¹, F. Shaheen¹, A-u. Rahman^{1,2}, M. I. Choudhary^{1,2}, M. Askani¹, S. U. Simjee^{1,2}

¹ICCBS, H.E.J. Research Institute of Chemistry, Karachi, Pakistan

²ICCBS, Dr. Panjwani Center for Molecular Medicine and Drug Research, Karachi, Pakistan

Epilepsy is illustrated by persistent predisposition of the brain to generate seizures and considered as one of the most common neurological disorder affecting 1% of the individuals worldwide. A growing body of advanced researches now points a link between inflammation and various epilepsy syndromes, reflecting both an inflammatory state inside the epileptic brain along with increased BBB permeability, heading towards enhanced neuronal excitability. The probable contribution of TGF- β in epileptogenesis is reinforced by animal studies viewing TGF- β up-regulation as measure of inflammatory reaction in the brains of kindled animals that are exposed to status epilepticus. The main focus of this study was to

investigate the potential relationship between the TGF- β associated MAPK pathway and epilepsy which will aid in confirming that up regulation of TGF- β genes might be one of the underlying cause of epilepsy. A novel anticonvulsant [E/Z] isoxylitones was used to treat epileptic seizures in pentylenetetrazole-induced kindling model of mice. To confirm aforementioned evidences, expression levels of TGF- β , TRAF6, and JNK3 with inflammatory cytokine IL-1 β were analyzed. It was observed that as compared to the PTZ-control group, there was a significant decrease in the response of seizures observed in [E/Z] isoxylitones treated group. Furthermore, expressions of these genes were significantly reduced in [E/Z] isoxylitones treated groups. It is concluded that, TGF- β signaling pathway can be a potential subcellular target for reducing seizure duration and [E/Z] isoxylitones is an effective way to achieve this therapeutic target.

MTU03-24

The effect of guarana (*paullinia cupana mart.*) in a LPS-induced inflammation rat model

A. Silveira, A. Kunzler, C. T. Ribeiro, J. Gasparotto, P. Brum, D. Gelain, J. C. Moreira

Federal University of Rio Grande do Sul, Biochemistry, Porto Alegre, Brazil

Neuroinflammation is present in several neurodegenerative disorders. Polyphenols have been proposed to be useful as adjuvant therapy in inflammatory process, because of their anti-inflammatory effects. Besides, epidemiological evidence suggests that caffeine consumption reduces the risk of several neurological and neurodegenerative diseases. Guarana (*Paullinia cupana* Kunth var. *sorbilis* (Mart.) Ducke) is a nontraditional medicinal plant, which effects are mainly related to the high polyphenol content and large amount of caffeine. The effects of Guarana supplementation on a neurological and systemic inflammation state are still poorly understood. In this work, we investigate the role of Guarana supplementation in a systemic inflammation induced by lipopolysaccharide (LPS). Wistar rats received oral supplementation of guaraná (42 mg/Kg/day) for 28 days prior to an intraperitoneal LPS injection (5 mg/Kg). Immunostaining of Iba-1 and GFAP demonstrated that LPS modulates glial activation in the substantia nigra 24 h after LPS stimulus, effect that was prevented by Guarana supplementation. However, ELISA analyses revealed that guarana was not able to prevent the LPS-induced increase of IL-1 β in the substantia nigra. In fact, guarana slightly increased the amount of this proinflammatory cytokine. In serum, guarana supplementation did not present any effect on IL-1 β levels. Guarana pre-treatment reduced TNF- α levels in serum in healthy conditions, but it had no protective effect against LPS insult. Spleen flow cytometry shown that guarana did not induce an antibody immune response through CD3 + antibodies activation. Therefore, these results indicate Guarana supplementation had an effect on glial modulation, without had an effect on proinflammatory cytokines release nor on antibody immune response.

MTU03-25

Ameliorative potential of furanocoumarin for acute and chronic pain studies: synthesis, molecular docking analysis and biological

G. Singh, R. Bhatti

Guru Nanak Dev University, Amritsar-143005, Punjab, India, Department of Pharmaceutical Sciences, AMRITSAR, India

Objective: Furanocoumarin are substituted 6,7-furlycoumarins with wide range of pharmacological activities. In this study, synthesis and investigation biological activity of FCs an acute and chronic inflammatory pain has been undertaken.

Methods: A series of FCs were synthesized (Gs₁-Gs₅) and docked at the sites of iNOS, COX-2 and NF κ B. Acute toxicity of most active compound was carried as per OECD guidelines. In acute study, analgesic and anti-inflammatory activity of compounds were tested using acetic acid writhing, formalin induced nociception and carrageenan test in mice. In chronic studies, vincristine induced neuropathic pain was employed. Post mortem studies including biochemical analysis for inflammatory mediators and immunohistochemical examinations were performed.

Results: Docking studies on the active sites of COX-2, iNOS and NF κ B indicated good binding of compound Gs₄ with appreciable docking score. Acute toxicity studies revealed largely unremarkable visceral organs including heart, liver and kidney. Pharmacological studies indicated significant analgesic effect and anti-inflammatory activity of different compounds with maximum activity of compound Gs₄. In neuropathic pain, marked reduction in pain behavior was observed in compound treated group. Compound Gs₄ also attenuated expression of COX-2, iNOS and NF κ B, as well as inflammatory cytokines and oxidative stress.

Conclusion: It based on the results of the current investigation, it may be concluded that FCs nucleus provide interesting leads for an molecules with promising analgesic & anti-inflammatory potential.

MTU03-26

Esculetin ameliorates poly(I:C)-induced autism spectrum disorder in mice by impeding neuroinflammation and improving BDNF signaling

K. Sulakhia¹, V. Paramanik²

¹*Indira Gandhi National Tribal University IGNTU, Department of Pharmacy, Amarkantak, India*

²*Indira Gandhi National Tribal University IGNTU, Department of Zoology, Amarkantak, India*

Maternal immune activation (MIA) in pregnant mother causes autism spectrum disorder (ASD) in offspring by stimulating immuno-inflammatory and oxido-nitrosative pathway while inhibiting serotonergic neurotransmission and brain derived neurotrophic factor (BDNF) level. Esculetin (ESC) possesses antioxidant, anti-inflammatory and neuroprotective activities. The study is designed to investigate the effect of ESC against poly(I:C)-induced ASD in mice and biochemical changes in placenta, fetal brain and adult mouse brain. Female C57BL/6 mice ($n = 4-5$) were pre-treated with esculetin (25 & 50 mg/kg, p.o.) from E0.5 to E12.5 and injected poly(I:C) (20 mg/kg, i.p.) on E12.5 to induce MIA. After 4 h of poly(I:C) injection mice were sacrificed to measure cytokines (IL-17 α , IL-6 & IL-10), NO, and BDNF level in placenta and fetal brain. Other pregnant mice were allowed to deliver pups, and these offspring were subjected to behavioural testing in EPM & 3 chambered test at

5 & 12 weeks of age to assess anxiety and social interaction, and then sacrificed for cytokines, serotonin and BDNF analysis. Findings demonstrated that poly(I:C) significantly decreased both open arms entries and duration in EPM ($p < 0.01$) and decreased time spent with novel mouse in 3 chambered test ($p < 0.001$) which was significantly ($p < 0.01$) ameliorated by ESC pre-treatment. Cytokines and NO level in mice were increased significantly ($p < 0.001$) after poly(I:C) injection which were reversed by ESC pre-treatment. Furthermore, ESC pre-treatment attenuated poly(I:C)-induced decrease in IL-10, 5-HT & BDNF level in mice. In summary, results suggested that ESC provided ameliorating effect against poly(I:C)-induced neurobehavioral and neurochemical alterations by impeding neuroinflammation, nitrosative stress, and up-regulating serotonin & BDNF signaling mechanism.

MTU03-27

DA attenuates LPS-induced cytokine expression by inhibiting the microtubule-dependent nuclear transport of NF- κ B p65 in BV-2 cells

Y. Yoshioka, Y. Sugino, F. Shibagaki, A. Yamamuro, Y. Ishimaru, S. Maeda

Setsunan University, Pharmaceutical Sciences/
Pharmacotherapeutics, Hirakata, Japan

We recently reported that dopamine (DA) attenuated lipopolysaccharide (LPS)-induced mRNA expression of cytokines by inhibiting the nuclear translocation of NF- κ B p65 in mouse microglial cell line BV-2, and that the covalent modification of proteins by dopamine quinone might be involved in its inhibition. It has been reported that NF- κ B p65 is microtubule-dependently transported to nuclei in neuronal cells and that β -tubulin, a component of microtubules, is one of the proteins that are susceptible to covalent modification by dopamine quinone. To further investigate the mechanism by which DA inhibited the nuclear translocation of NF- κ B p65, in the present study, we examined the involvement of the microtubule-dependent transport system in the nuclear translocation of NF- κ B p65 in BV-2 cells by using vinblastine, a microtubule-disrupting agent. Vinblastine (0.3 μ M) inhibited the LPS (10 μ g/mL)-induced increase in the NF- κ B p65 level in the nuclear fraction, but not affect the LPS (10 μ g/mL)-induced decrease in the I κ B α level in the whole cell lysate. Immunocytochemistry revealed that the treatment with vinblastine (0.3 μ M) disrupted microtubules, changed the morphology of BV-2 cells, and blocked the LPS (10 μ g/mL)-induced nuclear translocation of NF- κ B p65. On the other hand, although DA (30 μ M) inhibited the LPS (10 μ g/mL)-induced nuclear translocation of NF- κ B p65, it did not affect the morphology of BV-2 cells or the structure of the microtubules. These results indicate that NF- κ B p65 was transported to the nuclei via the microtubule-dependent transport system in BV-2 cells and that DA inhibited the microtubule-dependent transport system.

MTU03-28

Motor and synaptic deficit in 5-lipoxygenase knockout mice

M. C. Silva¹, R. M. Campos¹, J. França², P. Frost², J. Clarke², V. T. R. de Resende¹, C. Canetti¹

¹Federal University of Rio de Janeiro, Institute of Biophysics, Rio de Janeiro, Brazil

²Federal University of Rio de Janeiro, School of Pharmacy, io de Janeiro, Brazil

The 5 (5-LOX) is an essential enzyme in the synthesis of leukotrienes and lipoxins. It is widely expressed in cells involved in the regulation of inflammation, allergies and other immune responses. However, recent works show that central nervous system (CNS) neurons express high levels of 5-LOX, although the physiological role of neuronal 5-LOX remains unclear. The present work aims to evaluate how the absence of 5-LOX enzyme can influence synaptic plasticity, microglial activation and regeneration. For this purpose, 129/sv male adult mice knockout for 5-LOX (5-LO^{-/-}) or wild type (5-LO^{+/+}) were used. The basal levels of synaptophysin and PSD95 were evaluated by western blot analysis in the motor cortex and hippocampus of both groups. Synaptophysin levels were significantly higher both in motor cortex and hippocampus of 5-LO^{-/-} animals, when compared to WT animals ($n = 6$; $p < 0.01$). Moreover 5-LO^{-/-} animals show a lower baseline motor performance, assessed by the rotarod test, when compared to WT animals ($n = 10$, $p < 0.01$). In spite of the results obtained in the motor analysis, no differences were observed in the sensorial tests (Von frey hair test, formalin test and hot plate test). Microglial quantification and morphology was evaluated by immunofluorescence through labeling Iba-1 protein in the motor cortex and hippocampus, this quantification showing similar results for both 5-LO^{-/-} and WT group.

MTU03-29

Conditional knockout of LKB1 from astrocytes increases inflammatory activation and metabolic dysfunction: effects on EAE disease

D. Feinstein^{1,5}, S. Kalinin¹, G. Meares², S. Lin¹, L. Pietruchyk¹, G. Saher³, K. Nave³, E. Benveniste⁴

¹University of Illinois, Anesthesiology, Chicago, USA

²West Virginia University, Microbiology, Immunology and Cell Biology, Morgantown, USA

³Max Planck Institute of Experimental Medicine, Neurogenetics, Gottingen, Germany

⁴University of Alabama at Birmingham, Cell, Developmental, and Integrative Biology, Chicago, USA

⁵Jesse Brown VA Medical Center, Research, Chicago, USA

The consequences of astrocyte metabolic dysfunction in EAE disease are not well characterized. Liver Kinase B1 (LKB1) is a ubiquitously expressed kinase involved in regulation of cell metabolism, growth, and inflammation. We previously reported that a single nucleotide polymorphism in the gene encoding LKB1 is a risk factor for multiple sclerosis (MS). We now examined the consequences of LKB1 conditional knockout (cKO) from astrocytes in the MOG peptide chronic MS model. While disease incidence was similar, disease severity was worsened in cKO mice. RNAseq analysis identified KEGG pathways enriched in cKO mice relating to mitochondrial function, confirmed by alterations in mitochondrial

complex proteins and reductions in mRNAs related to astrocyte metabolism. Enriched pathways also included major histocompatibility class II genes, confirmed by increases in MHCII protein in spinal cord and cerebellum of cKO mice. We observed increased presence of CD4⁺ Th17 cells and increased neuronal damage in spinal cords of cKO mice, associated with reduced expression of choline acetyl-transferase, accumulation of immunoglobulin-g, and reduced expression of factors involved in motor neuron survival. In vitro, LKB1-deficient astrocytes showed reduced metabolic function and increased inflammatory activation. These data suggest that metabolic dysfunction in astrocytes, in this case due to LKB1 deficiency, exacerbates demyelinating disease by loss of metabolic support and increase in the inflammatory environment.

MTU03-30

Digoxin regulates oligodendrocyte number, function, and myelin structure

H. Titus, S. Miller

Northwestern University, Microbiology-Immunology, Chicago, USA

Demyelination and neurodegeneration are part of the pathology in the CNS autoimmune disease of Multiple Sclerosis (MS). At present, all clinical trials of potential myelin repair therapies have failed. As MS is a chronic disease that often presents in young adulthood, there is a need for both halting progression and repairing

existing damage. In a collaborative effort, the NIH library of oral FDA approved drugs was screened for potential myelin repair therapy candidates. The Na⁺/K⁺ ATPase inhibitor Digoxin was a top candidate and our studies revealed it promoted an increase in the oligodendrocyte cell lineage in vitro and in vivo in C57BL/6 mice as well as in the LPC spinal cord model of demyelination/remyelination, in the non-T cell-mediated Cuprizone model of demyelination/remyelination promoted a quicker restoration of myelin integrity in the corpus callosum, and improved clinical score throughout the autoreactive Th1/Th17 driven C57BL/6 Chronic experimental autoimmune encephalomyelitis (EAE) time course. MS patients currently have access to disease-modifying therapies that are global immunosuppressants with limited efficacy and a wide range of side effects. Our lab is able to induce immune tolerance to selectively target the immune system in relapsing-remitting (RREAE) and chronic progressive (C-EAE) experimental autoimmune encephalomyelitis murine models of MS using an i.v. infusion of nanoparticles coupled with or encapsulating myelin peptides (Ag-PLG) that prophylactically prevent disease and therapeutically stop disease progression. Our hypothesis was that a combination of selective immune regulation and myelin repair therapy is required to effectively target disease course and severity in MS. Combination therapy with PLG-MOG and Digoxin at peak of C-EAE disease completely ameliorated clinical disease severity. These promising pre-clinical findings steer toward future clinical trials using combination therapy in MS.

MTU04 Molecular basis of disease (Session A)

MTU04-01

Role of acetamide analogue in arthritic rat model

Z. A. Razzak, Shabana. U. Simjee

International Center for Chemical and Biological Sciences ICCBS, Dr. Panjwani Center for Molecular Medicine and Drug Research, Karachi, Pakistan

Instruction: Rheumatoid Arthritis (RA) is considered as persistent inflammation of multiple joints, however it may affect the other parts of the body as well. Due to the production of matrix metalloproteinases (MMPs) enzymes, destruction of extracellular matrix (ECM) proteins occur. These MMPs are rate-limiting factor in the degradation of collagen a major part of ECM. Autoimmunity is also link along with inflammation and has an ample societal effect in cost, disability, and productivity loss. Severity of disease can be lessen but not completely cure by certain pharmacologic interventions. The aim of this proposed study is to target the production of MMPs to find the new and potent and safe therapeutic moiety for the treatment of RA.

Material and Method: In the present study collagen induced arthritis (CIA) was develop on female rats. Severity of arthritis was check by paw edema test, blood was collected for serum separation and TNF, GSH, NO and PO were assayed. Brain samples are processed to check gene expression profiling of certain inflammatory markers on Real Time PCR.

Results and Conclusion: Based on our data, we found that the group who were treated with Acetamide analogue shows significant decrease in paw edema. SH, NO, and PO assay results show decrease in the level, by comparing treated and non-treated groups. Hence we concluded that, Acetamide analogue has the potential anti-inflammatory activity in joints. These results will be further confirmed by ELISA experiments specifically for MMPs and immunohistochemistry of joints. In future, this will lead to new drug development for rheumatoid arthritis.

MTU04-02

PI3K inhibition reduces mechanical allodynia and sensitization of spinal TRPV1 in a model of paclitaxel-induced neuropathy

P. Adamek^{1,2}, M. Heles^{1,2}, J. Palecek¹

¹ *Institute of Physiology of the Czech Academy of Sciences, Dept. of Functional Morphology, Prague, Czech Republic*

² *Faculty of Science, Charles University, Dept. of Animal Physiology, Prague, Czech Republic*

Peripheral neuropathy is a major adverse effect of paclitaxel chemotherapy. We have reported previously that paclitaxel increased TRPV1 sensitivity to repeated capsaicin application via TLR4-mediated mechanism. The results presented here describe the role of phosphatidylinositol 3-kinase (PI3K) in the paclitaxel-induced signaling between TLR4 and TRPV1. Neuropathy was induced by a single application of Paclitaxel (Mylan, 8 mg/kg, *i.p.*) in adult male mice C57BL/6. Mechanical allodynia was evaluated by paw withdrawal threshold measurement. Whole-cell patch-clamp recordings of miniature excitatory postsynaptic currents (mEPSC) were made from superficial dorsal horn spinal neurons. Paclitaxel-

induced robust mechanical allodynia was prevented for up to eight days by PI3K antagonist wortmannin pretreatment *in vivo*. Both *in vitro* and *in vivo* paclitaxel treatments enhanced capsaicin-evoked responses recorded as an increased mEPSC frequency in dorsal horn neurons. Acute co-application of PI3K antagonist wortmannin or LY-294002 with paclitaxel attenuated this effect of paclitaxel. Acute *in vivo* paclitaxel administration also increased phosphorylation of Akt kinase, a marker of enhanced PI3K signaling, in rat L5 DRG neurons. Wortmannin pretreatment prevented this increase in pAkt expression. We showed that PI3K plays an important role in the early development and maintenance of mechanical allodynia and in the modulation of TRPV1 function after paclitaxel treatment. We suggest that inhibition of PI3K may help alleviate pathological pain in the paclitaxel-induced neuropathy. New data focused on paclitaxel-induced changes of inhibitory synaptic transmission will be presented on site. Grant Support: Czech Science Foundation 18-09853S, LQ1604 BIOCEV-FAR, BIOCEV CZ.1.05/1.1.00/02.0109, RVO:67985823, GAUK 734218.

MTU04-03

Neuroprotective influence of luteolin and gallic acid on cobalt-induced behavioural and biochemical alterations in rats

A. Akinrinde, O. Adebisi

University of Ibadan, Nigeria, Department of Veterinary Physiology and Biochemistry, Ibadan, Nigeria

Cobalt (Co) intoxication arising from occupational exposures and ion release from metal implants has been associated with neurological alterations such as cognitive decline, incoordination and depression. The present study evaluated the mechanisms of neuroprotection by Luteolin (100 mg/kg) and Gallic acid (120 mg/kg) in Wistar rats exposed to cobalt chloride (CoCl₂) at 150 mg/kg for 7 consecutive days. Cognition, grip strength and motor coordination were assessed with the Morris water maze, hanging wire and Open Field tests, respectively. Rat's whole brain samples were processed for biochemical analyses of markers of oxidative damage and acetylcholinesterase activity. Immunohistochemistry was used to measure the immuno-reactivity of glial fibrillary acidic and calbindin D-28k proteins in brain tissues. Results indicate that CoCl₂ induced neuro-behavioural deficits, specifically producing decreased exploratory activities, increased anxiety and significant reduction in hanging latency. Co-treatment with luteolin or gallic acid, however, restored these parameters to values near those of normal controls. Moreover, Luteolin and Gallic acid prevented CoCl₂-induced increases in hydrogen peroxide, malondialdehyde and nitric oxide in the brain, while increasing the activities of acetylcholinesterase, glutathione S-transferase and superoxide dismutase. Furthermore, Luteolin or Gallic acid treatment produced increased astrocytic expression of glial fibrillary acidic protein (GFAP), with intense calbindin (CB) staining and pronounced dendrites in the Purkinje cells. Taken together, luteolin and/or gallic acid exerted protection against Co neurotoxicity by restoring Ca²⁺ homeostasis, acetylcholinesterase and antioxidant enzyme activities, while also inhibiting lipid peroxidation in the brain.

MTU04-04

Several disease-associated properties of the beta-amyloid peptide are neutralized by its phosphorylation

E. Barykin¹, I. Petrushanko¹, S. Kozin¹, V. Mitkevich¹, S. Radko², A. Makarov¹

¹ EIMB RAS, Laboratory of protein conformational polymorphism in health and disease, Moscow, Russia

² IBMC RAS, Laboratory of transcriptomic analysis, Moscow, Russia

Zinc-induced oligomerization of A β represents a potential seeding mechanism for the formation of neurotoxic A β oligomers and aggregates in Alzheimer's disease (AD). Phosphorylation of A β by Ser8 (pS8-A β), found *in vivo*, is localized inside the zinc-binding domain of the peptide and may significantly alter its zinc-induced oligomerization and related pathogenic properties. Indeed, using dynamic light scattering (DLC) and complementary methods we have shown that phosphorylation by Ser8 dramatically reduces zinc-induced aggregation of A β , and moreover, pS8-A β suppresses zinc-driven aggregation of non-modified A β in an equimolar mixture. We have further analyzed the effect of pS8-A β on the progression of cerebral amyloidosis with serial retro-orbital injections of the peptide in APPSwe/PSEN1dE9 murine model of AD, followed by histochemical and immunohistochemical analysis of amyloid burden in the hippocampus. Unlike the non-modified A β that has no influence on the amyloidosis progression in murine models of AD, pS8-A β injections reduced the number of amyloid plaques in the hippocampus of mice by one-third. Recently shown inhibition of Na⁺,K⁺-ATPase activity by A β is prevented by phosphorylation of the peptide. We showed that the binding of A β to Na⁺,K⁺-ATPase creates a seed for A β oligomerization, which leads to the inhibition of Na⁺,K⁺-ATPase. Such Na⁺,K⁺-ATPase-based oligomerization is not observed for pS8-A β . Moreover, the presence of Na⁺,K⁺-ATPase in solution hastens the zinc-dependent aggregation of A β ; the aggregation of pS8-A β in presence of Na⁺,K⁺-ATPase does not change. Thus, several AD-associated pathogenic properties of A β are neutralized by its phosphorylation.

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MTU04-05

Modulation of the hyaluronan-based extracellular matrix in mouse models of epilepsy

A. Blondiaux¹, S. Jia², R. Frischknecht^{3,1}, A. Fejtova⁴, A. Dityatev², E. Gundelfinger¹, C. Seidenbecher¹

¹ Leibniz Institute for Neurobiology, Neurochemistry and Molecular Biology, Magdeburg, Germany

² German Center for Neurodegenerative Diseases DZNE, Molecular Neuroplasticity, Magdeburg, Germany

³ University of Erlangen-Nuremberg, Biology, Erlangen, Germany

⁴ Erlangen University Clinic, Molecular neurobiology, Erlangen, Germany

The composition of the brain extracellular matrix (ECM) can affect neuronal activity via changes to neuronal properties. In turn, neuronal activity regulates the ECM composition. This balance is disrupted in epilepsy as neuronal activity is not properly regulated.

We hypothesize that there is a correlation between the severity of epileptic phenotypes and the composition and integrity of the hyaluronan-based ECM. This ECM consists of proteoglycans (like brevican or aggrecan), which bind to hyaluronan with the help of link proteins (like HAPLN1) and glycoproteins (like tenascins).

To investigate this correlation, we use new models of epilepsy: three mouse lines, mutant for the presynaptic scaffolding protein Bassoon (*Bsn*). In the same animals, we recorded EEGs and extracted the forebrain to quantify ECM proteins in different sub-cellular fractions, including synaptosomes.

We show that lack of functional *Bsn* causes frequent seizures in adult mice. A survival study suggests that epilepsy could be present already in early life. Interestingly, some ECM proteins are correlated with seizure parameters. Brevican strongly correlates with seizures, making it a protein of interest for further analysis.

Our work suggests that the ECM composition in the epileptic brain may be of interest for diagnostic purposes and as a potential therapeutic target.

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MTU04-06

Impaired synaptic vesicle recycling in a rat model of fragile X syndrome

K. Bonnycastle, P. Kind, M. Cousin

University of Edinburgh, Centre for Discovery Brain Sciences, Edinburgh, United Kingdom

Fragile X syndrome (FXS) is a leading monogenic cause of intellectual disability, autism spectrum disorder, and epilepsy. It results from the loss of the fragile X mental retardation protein (FMRP). Although historically considered a disorder of the postsynapse, there is evidence for presynaptic defects in mouse models of FXS, including accelerated synaptic vesicle (SV) recycling and an increased number of SVs in the recycling pool. Due to this, we aimed to characterise SV exocytosis and endocytosis through clathrin-mediated endocytosis (CME) and activity-dependent bulk endocytosis (ADBE) in a novel rat model of FXS. We hypothesise that a deficit in SV recycling may be an important feature of the presynaptic phenotype in *Fmr1*^{-/-} rats. Using live-cell imaging in hippocampal neurons, we observed that the rate of CME was not altered in *Fmr1*^{-/-} compared to wildtype littermates at either low frequency or high frequency stimulation. Interestingly, there were fewer nerve terminals undergoing ADBE, the predominant endocytosis mode during elevated neuronal activity, in *Fmr1* KO hippocampal neurons compared to WT. Additionally, using electron microscopy, we observed fewer bulk endosomes in *Fmr1* KO nerve terminals although the number of SVs was unchanged across genotypes. These results taken together suggest insufficient SV endocytosis in this model. This SV endocytosis deficit could in turn lead to neurotransmission failure which may, in part, underlie the deficits observed in FXS.

MTU04-07

Regulation of KCNQ genes as a mechanism underlying epileptogenesis**R. Butler-Ryan***University of Leeds, Faculty of Biological Sciences, Leeds, United Kingdom*

Epilepsy is a common and debilitating neurological disorder which is often associated with ion channel dysfunction. The M-current released by Kv7 voltage-gated potassium channels helps to control hyperexcitability within the neuron, and mutations in the KCNQ genes encoding this channel result in a form of epilepsy. An insult such as an initial seizure can alter gene expression patterns and cell function which drives the neuron toward hyperexcitability and epileptogenesis, making the individual more susceptible to further seizures. Organotypic hippocampal slice cultures provide a convenient method for development of an epileptogenic model which retains a high degree of structural and functional similarity to the brain *in vivo*. They also provide an easy medium for applying treatments and analysing the effects through various techniques. Presented are details surrounding the technicalities of developing a robust organotypic system for this type of work, and data showing successful adenoviral infection for manipulation of gene expression in the organotypic hippocampal cultures. Previous research has shown the KCNQ2 and KCNQ3 genes encoding the Kv7 channel subunits to be downregulated by the transcription factors REST, but this mechanism is not well understood. Using adenoviral gene transfer, electrophysiology, qRT-PCR and immunohistochemistry, current work is focussing on elucidating this mechanism to highlight key areas for therapeutic targeting to prevent epileptogenesis in people who have suffered an initial brain trauma.

MTU04-08

Ibogaïne downregulates CREB1 and GRIA1 mRNA expression in the dorsal hippocampus**T. Calvey¹, J. Woolf¹, C. Dickens²**¹ *University of the Witwatersrand, Anatomical Sciences, Johannesburg, South Africa*² *University of the Witwatersrand, Internal Medicine, Johannesburg, South Africa*

The glutamatergic system of the hippocampus plays a central role in learning and memory of drug-related associations. Ibogaïne is an African psychedelic medicine that has shown promise in treating substance use disorder (SUD) and opioid withdrawal. As ibogaïne is a NMDA receptor antagonist, many of its therapeutic effects may be due to its ability to modulate the glutamatergic system.

The aim of this research was to assess changes in GRIA1 and CREB1 mRNA expression in the dorsal (dHPC) and ventral (vHPC) hippocampus during withdrawal from chronic morphine administration with and without ibogaïne treatment.

Male Sprague-Dawley rats were divided randomly into 6 test groups of $n = 10$ for chronic morphine administration (daily morphine sulphate 10 mg/kg s.c. for 10 days), 3 day withdrawal from chronic morphine administration, ibogaïne HCl (single i.p. 50 mg/kg), chronic morphine with ibogaïne and the relevant saline control groups. Upon termination, right dHPC and vHPC were dissected for qPCR analysis.

Ibogaïne reduced CREB1 expression in dHPC relative to control ($p = 0.051$). A highly significant reduction in GRIA1 expression

was found in dHPC of the ibogaïne treatment group relative to saline control ($p = 0.003$). Differences in GRIA1 expression between dHPC and vHPC were highly significant. No significant differences in expression were found between withdrawal groups and combined morphine-ibogaïne treatment.

The results indicate that ibogaïne decreases CREB1 and GRIA1 mRNA expression in the dHPC which highlights a novel mechanism of action in treating SUD and opioid withdrawal. The lack of any significant changes in CREB1 and GRIA1 expression in combination morphine-ibogaïne treatment highlights the role of the mu-opioid receptor in glutamatergic regulation.

MTU04-09

Beneficial effects of the regulation of miRNAs by dimethyl fumarate via NRF2 in tauopathies**S. C. Sánchez^{1,2,3}, I. Lastres-Becker^{1,2,3}**¹ *Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas CIBERNED, Biochemistry, Madrid, Spain*² *Instituto de Investigación Sanitaria La Paz IdiPaz, Neuroscience, Madrid, Spain*³ *Instituto de Investigaciones Biomédicas Alberto Sols UAM-CSIC, Biochemistry, Universidad Autónoma de Madrid, Madrid, Spain*

miRNAs regulate gene expression controlling physiological and pathological processes. Redox stress can alter miRNA biogenesis and processing pathways, suggesting an involvement of the transcription factor NRF2, master regulator of redox homeostasis. We first assessed whether NRF2 could modulate miRNA biogenesis. Bioinformatics analysis revealed antioxidant response elements in the promoters of miRNA processing proteins. This was corroborated *in vitro* showing that the main miRNA processing proteins were modulated in a NRF2-dependent way. Then, we determined whether treatment with dimethyl fumarate (DMF), an NRF2 inducer, is able to modulate miRNA expression and its potential therapeutic value in tauopathies. For this, we performed luciferase activity assays using the 3'UTR-NRF2-LUC reporter. Next, we performed a microarray assay to determine which miRNAs were altered by TAU overexpression and if this effect could be reversed by DMF treatment, in a murine model of tauopathy. It was observed that the overexpression of TAU increases the levels of miR-142-3p/5p and DMF treatment is capable of reversing this effect. Bioinformatic analyses show that the miR-142-3p/5p is involved in the processing pathways of RNA binding and regulation of its stability and vesicle-mediated and intracellular transport. One of the main genes that regulates miR-142 is staufen1 and 2, implicated in synaptic plasticity and memory formation, of great relevance in the pathological processes associated with TAU. Taken together, our study suggests that the modulation of miRNAs by regulation of NRF2 by DMF treatment is an effective therapeutic target for the treatment of tauopathies.

MTU04-10

Radiation exposure induces acute trafficking of excitatory and inhibitory receptors in cultured hippocampal neurons
C. Cronkite*Baylor College of Medicine, Department of Biochemistry and Molecular Biology, Houston, USA*

Radiation-induced cognitive impairment (RICI) is a well-characterized consequence of cranial radiation therapy for brain tumors that includes chronic learning disability, executive dysfunction, and behavioral and mood disorders. Interestingly, a single radiation insult can lead to chronic and progressive neuropsychiatric sequelae, suggesting that there are long-term changes in neuronal function from a single inciting event. While most research into RICI has focused on the effects of radiation on neural progenitor cells, recent work in our lab has shown that there are structural and functional changes in dendritic spines minutes after a radiation insult. Dendritic spines are highly dynamic structures that are able to respond to activity, inducing long lasting changes in synaptic and neuronal function. For example, high activity leads to reinforcement of synaptic transmission through an increase in surface expression of receptors, and these changes are mediated primarily by NMDA receptor activity. We have been able to visualize the acute trafficking of NMDA and GABA receptors after radiation in dissociated hippocampal cell cultures by fusing the pH-sensitive GFP derivative superecliptic pHluorin to obligatory receptor subunits. Additionally, we have used NMDA receptor inhibitors specific for localization and receptor subtype in order to determine the activity dependence of these changes. Through serial live imaging, we have shown that NMDA and GABA receptors are differentially trafficked following a radiation insult in an NMDA receptor activity-dependent manner. Additionally, radiation leads to neurotoxic transcriptional changes through the inactivation of CREB that can be prevented by the administration of the extrasynaptic NMDA receptor inhibitor memantine. Together, these findings suggest that radiation leads to acute changes in synaptic function that in turn adversely and chronically affect neuronal health.

MTU04-11

A glucocorticoid receptor-dependent mechanism of bile acid action with therapeutic impact in polyglutamine disease**J. D. Silva^{1,2}, S. Duarte-Silva^{1,2}, A. Neves-Carvalho^{1,2}, C. Soares-Cunha^{1,2}, J. Correia^{1,2}, G. Nogueira-Gonçalves^{1,2}, S. Oliveira^{1,2}, A. Teixeira-Castro^{1,2}, P. Maciel^{1,2}**¹ *University of Minho, School of Medicine, Braga, Portugal*² *ICVS/3B's, Neurosciences, Braga/Guimarães, Portugal*

Spinocerebellar ataxia type 3 (SCA3) is an autosomal dominant neurodegenerative disorder caused by a polyglutamine expansion within the protein ataxin-3, leading to protein dyshomeostasis and ultimately neuronal demise. Clinically, it is characterized by gait imbalance, with a mid-life onset. No directed treatments are currently available for this invariably fatal disease. In this work we tested bile acids as potential therapeutic agents for SCA3, since these molecules have been shown to be neuroprotective in other conditions. Using a *C. elegans* model of SCA3 we observed that tauroursodeoxycholic acid (TUDCA) was the most efficient bile acid in improving the animals' motor phenotype. A significant improvement was also observed in a pre-clinical trial using

CMVMJD135 mice: chronically treated mice showed markedly improved performance motor behavior tests, reduced neuropathology and neuroinflammation markers. Using the *C. elegans* SCA3 model, we dissected the mechanism of action of this drug. Surprisingly, we observed that the effect of TUDCA was independent of its canonical nuclear receptor, the farnesoid X receptor (FXR), but fully dependent on the glucocorticoid receptor (GR). Moreover, GR protein levels were markedly decreased in the CMVMJD135 mouse model, and fully recovered upon acute treatment with TUDCA. Finally, and most importantly, we observed a decrease in GR levels in the pons, a highly disease-affected brain region, of SCA3 patients. In sum, we identified TUDCA, a drug with a high translational potential, as a contender compound for the treatment of SCA3, and propose a novel mechanism of action that could be of interest in the future, including for other neuromuscular disorders currently treated with glucocorticoids.

MTU04-12

Aberrant regulation of monoubiquitination via E3 ubiquitin ligase RNF20 confer Gbm cancer stem-like cells survival and maintenance**K. Daun¹, N. Morimura¹, K. Nozaki², K. Tanigaki³, S. Hitoshi¹**¹ *Shiga University of Medical Science, Integrative Physiology, Otsu, Shiga, Japan*² *Shiga University of Medical Science, Department of Neurosurgery, Otsu, Shiga, Japan*³ *Research Institute, Shiga Medical Center, Moriyama, Shiga, Japan*

A quiescent slow-growing state of GSCs subpopulation in GBM is thought to underlie the tumor propagation, drug resistance, and relapse. However, the underlying mechanism of how epigenetic modification controls stemness features in GSCs remain poorly understood. Therefore, we postulate that a strong relationship between genetic changes with epigenetic modification via RNF20 (E3 ubiquitin ligase) by monoubiquitinating histone H2B (H2Bub1) may contribute to the maintenance of GSCs. We first examined whether RNF20 is expressed in GSCs lines. We confirmed that the RNF20-positive cells overlap with the cancer stem cells marker CD133. We then established a clone which is stably over-expressing RNF20 using the tetracycline-inducible system and designed two shRNAs for RNF20 knockdown targeting at the coding region. The H2Bub1 positively correlated with the level of RNF20. The RNF20 over-expression exhibit higher mRNA expression of stemness (SOX2, OCT4) and CD133 markers while RNF20 knockdown down-regulate SOX2. The proliferation rate of over-expressing RNF20 cells is higher than the control. In contrast, the RNF20 knockdown has shown small morphological GBM spheres and suppressed proliferation. Since GSCs exhibit specific gene expression signatures to control cell fate during differentiation, we emphasize that RNF20 through H2Bub1 may involve in GSCs differentiation. As a result, RNF20/H2Bub1 regulates GSCs differentiation into astrocyte and oligodendrocyte. Moreover, RNF20 overexpression can enhance the therapeutic effect of Temozolomide while RNF20 knockdown leads to Temozolomide resistance. Taken together, our findings suggest that RNF20 is required as an epigenetic regulator for maintenance of GSCs.

MTU04-13

Palmitate increases microglia-derived TNF-alpha levels and impairs hippocampal insulin signaling**H. D. Melo¹, G. Sd. Silva², B. Melo¹, J. Fortuna¹, V. Coreixas¹, S. Ferreira^{1,3}, F. D. Felice^{1,4,5}**¹ Federal University of Rio de Janeiro, Institute of Medical Biochemistry Leopoldo de Meis, Rio de Janeiro, Brazil² Federal Institute of Education Science and Technology of Rio de Janeiro, Department of Biochemistry, Rio de Janeiro, Brazil³ Federal University of Rio de Janeiro, Institute of Biophysics Carlos Chagas Filho, Rio de Janeiro, Brazil⁴ Queen's University, Centre for Neuroscience Studies, Kingston, Canada⁵ Queen's University, Department of Psychiatry, Kingston, Canada

Unhealthy diets are related to an increasing burden of metabolic disorders worldwide, including type 2 diabetes (T2D) and obesity. T2D and obese patients exhibit cognitive impairment and increased risk of developing dementia. Elevated levels of free fatty acids in the circulation is linked to peripheral insulin resistance, especially saturated fatty acids (SFA), such as palmitate. Interestingly, brain palmitate uptake is increased in obese patients, with a positive correlation with aging. Thus, to understand how excessive levels of SFAs could impact brain function, we investigate the impact of palmitate, the most abundant circulating SFA, on the hippocampus, important region for learning and memory. Interestingly, intracerebroventricular infusion of palmitate led to microglial activation and increased TNF- α levels in the mouse hippocampus. In addition, palmitate reduced insulin expression and induced neuronal insulin receptor substrate 1 (IRS-1) phosphorylation at multiple inhibitory serine residues in primary hippocampal cultures. Importantly, palmitate failed to cause insulin signaling impairment in the presence of minocycline or infliximab, which inhibits microglial activation and neutralizes TNF- α , respectively. Altogether, our results delineate a pro-inflammatory mechanism underlying the deleterious effects of palmitate on neuronal insulin signaling, a pathway centrally involved in the learning and memory.

MTU04-14

Neuropharmacological prospective of urena sinuata (borss) l**T. Emran, Md. Atiar Rahman**

BGC Trust University Bangladesh, Pharmacy, Chittagong, Bangladesh

Urena sinuata (Borss) L. is a wild shrubby plant with some folk medicinal use. To our knowledge, the biological importance of this plant has not been investigated yet. The present study aimed to determine the neuropharmacology, antinociceptive, anti-inflammatory and antipyretic effects of the chloroform extract of *Urena sinuata* leaves (CEUS) in rodents and to elucidate the possible mechanism of antinociception involved with its acute toxicity and phytochemical studies. Neuropharmacological activities of CEUS were conducted by hole cross, open field test, elevated plus-maze test and thiopental induced sleeping time test. For the analgesic activity of CEUS different methods like hot plate test, acetic acid induced test, formalin-induced test, tail immersion test and glutamate-induced nociception were used. Additionally, the possible mechanism of nociception is identified by cyclic guanosine monophosphate (cGMP) and ATP-sensitive K⁺-channel pathway

analysis. Carrageenan-induced rat paw edema and cotton pellet-induced granuloma test also were used to detect anti-inflammatory activity and brewer's yeast induced pyrexia test for antipyretic activity. The extract (200 and 400 mg/kg) was administered orally 60 min prior to subjection to the respective test. The results obtained demonstrated that CEUS produced significant ($p < 0.05$) neuropharmacological, anti-inflammatory and antipyretic activity with low or no toxicity. The extract also exerts antinociceptive response in all the chemical and thermal-induced nociception models. Furthermore, it involves cyclic guanosine monophosphate (cGMP) and ATP-sensitive K⁺-channel pathway mediated antinociceptive effect. These data show for the first time that CEUS has significant neuropharmacological, anti-inflammatory and antipyretic effects which appear to be related to the inhibition of the glutamatergic system and rationalized the traditional use of the leaf in the treatment of different types of inflammation in intestines and bladder. Thus the leaves of *Urena sinuata* could be used in the treatment of several types of inflammation in intestines and bladder.

MTU04-15

Glast activity is modified by acute manganese exposure in bergmann glial cells**M. E. Lopez, L. C. R. Hernandez-Kelly, A. Ortega**

Cinvestav, Toxicology, Mexico City, Mexico

Manganese (Mn) is an essential trace element, serving as a cofactor for several enzymes involved in various cellular and biochemical reactions. However, chronic overexposure to Mn from occupational or environmental sources induces a neurological disorder, characterized by psychiatric, cognitive, and motor abnormalities, known as manganism. Astrocytes, the most abundant non-neuronal glial cells in the brain, play a critical role in glutamate homeostasis. The fine regulation of extracellular glutamate in the brain is accomplished by two major glutamate transporters (GLT-1 and GLAST) that are predominantly expressed in astrocytes. Excitotoxicity has been highlighted as a critical mechanism in Mn neurotoxicity and is also involved in the pathology of multiple neurodegenerative diseases including ALS, AD and PD. Recent studies show that Mn accumulates in different brain regions, including the cerebellum. Bergmann glial cells (BGC) are radial glial cells prevalent in the adult cerebellum and represent the most abundant glia within this structure. This characteristic localization is related to their involvement in neurotransmitter uptake and turnover, K⁺ homeostasis, lactate supply and pH regulation. Despite these well-known facts, there is no evidence about the acute effect of Mn exposure in BGC physiology. To this end, in this contribution we focused on the molecular mechanisms induced by Mn affecting GLAST in BGC. A time and dose-dependent increase in GLAST activity was found upon acute Mn exposure. This augmentation might be explained as a complex interaction between Mn and GLAST since its maximal transport capacity was affected after Mn exposure. This effect is accompanied by a reduced glucose uptake, that in the long term could contribute to the transporter dysfunction. These results strengthen the notion of the critical involvement of radial glia in glutamatergic neurotransmission.

MTU04-16

The oligosaccharide portion of ganglioside GM1 as mitochondrial regulator**M. Fazzari¹, G. Lunghi¹, E. D. Biase¹, M. Audano², N. Mitro², S. Sonnino¹, E. Chiricozzi¹**¹ *University of Milano, Department of Medical Biotechnology and Translational Medicine, Segrate, Italy*² *University of Milano, Department of Pharmacological and Biomolecular Sciences, Milano, Italy*

Functional data and clinical studies suggest the existence of a positive loop between the age-dependent GM1 deficiency and alpha-synuclein (α S) accumulation determining the neurodegeneration onset of sporadic Parkinson's Disease (PD). This loop is triggered by the plasma membrane GM1 deficiency, which leads to a failure of trophic signaling and to the α S accumulation, increasing the susceptibility to neuronal death. Recently we shed new light on the molecular basis underlying GM1 effects highlighting that GM1 oligosaccharide (OligoGM1) directly binds TrkA receptor, triggering TrkA-MAPK pathway activation which leads to neuronal differentiation and protection. Following its administration to B4galnt1^{+/-} PD mouse model, OligoGM1 was found to completely rescue the physical symptoms, reduce α S aggregates and restore tyrosine-hydroxylase neurons. Since the mitochondrial dysfunction plays a central role in the exacerbation of nigrostriatal degeneration in PD, we decide to evaluate the putative OligoGM1 mitochondrial modulation in murine neuroblastoma cells, N2a. Following its exogenous administration, proteomic analysis revealed an increased expression of proteins involved in mitochondrial bioenergetics and in oxidative stress protection. By biochemical studies we found that OligoGM1 protects N2a cells from MPTP toxic effect as well as from mitochondrial oxidative stress. Moreover, by immunoblotting we identified an increased expression of TOM20/HtrA2 mitochondrial proteins, whose reduced expression has been associated with PD. At functional level, we found increased basal and uncoupled mitochondrial respiration following OligoGM1 administration. Collectively our data indicate a possible role of OligoGM1 as mitochondrial regulator that by inducing mitochondriogenesis and enhancing mitochondrial activity could determine mitochondrial restoration in PD neurons.

MTU04-17

Reductive reprogramming: a not-so-radical hypothesis of neurodegeneration**T. Foley***University of Scranton, Chemistry, Scranton, USA*

Free radical-mediated oxidative stress, neuroinflammation, and excitotoxicity have long been hypothesized to contribute to the progression of Alzheimer's disease and other aging-related neurodegenerative disorders (NDD). Among these phenomena, the significance of oxidative stress and, more generally, redox perturbations, for NDD remain ill-defined and unsubstantiated. Here, I argue that (i) free radical-mediated oxidations of biomolecules can be dissociated from the progression of NDD, (ii) oxidative stress fails as a descriptor of cellular redox states under conditions relevant to disease, and (iii) aberrant upregulation of compensatory reducing activities in neural cells, resulting in reductive shifts in thiol-based redox potentials, may be an overlooked and paradoxical contributor

to disease progression. In particular, I summarize evidence, from *in vitro* studies, which supports the view that reductive shifts in the extracellular space can occur in response to oxidant and inflammatory signals and that these have the potential to reduce putative regulatory disulfide bonds in exofacial domains of the N-methyl-D-aspartate (NMDA)-subtype of glutamate-gated receptors as well as other synaptic regulatory proteins, leading potentially to aberrant increases in neuronal excitability and, if sustained, excitotoxicity. Moreover, I provide data from my laboratory which establishes the presence in the brain, *in vivo*, of disulfide bonds in the NMDA receptor as well as other glutamate and non-glutamate-gated receptors. All of these are potential targets of reductive stress. This novel reductive reprogramming hypothesis of neurodegeneration provides an alternative view of redox perturbations in NDD and links these to both neuroinflammation and excitotoxicity.

MTU04-18

The role of monocarboxylate transporter-1 on cognitive deficits development during NAFLD**A. Hadjichambi¹, P. Hosford², L. Pellerin¹**¹ *UNIL, Department of Physiology, Lausanne, Switzerland*² *UCL, Neuroscience, physiology and pharmacology, London, UK*

Non-alcoholic fatty liver disease (NAFLD) is a major complication of obesity. Certain observations regarding NAFLD induced neuropsychiatric alterations have been reported but mechanisms are unknown. Monocarboxylate transporter-1 (MCT1) haploinsufficient mice, which resist high fat diet (HFD) induced hepatic steatosis represent an interesting model. Using a mouse model of NAFLD (HFD+high fructose/glucose in water [HF/HG]) we investigated the development of cognitive deficits and state of cerebral oxygenation and cerebrovascular reactivity.

Behavioural tests (open field/novel object recognition/forced swimming test [FST]) were performed in mice fed control diet (NC; WT/MCT1 +/- +NC) or HFD HF/HG (WT/MCT1 +/- +HFD HF/HG) for 16 weeks. Baseline cortical PO_2 and in response to systemic hypercapnia (10% CO_2) was monitored under anaesthesia by a fluorescence method. Microelectrode biosensors were used for lactate measurements by cortical slices. EchoMRI was performed to assess lean/fat mass.

Increased fat mass was observed in WT and MCT1 +/- mice on HFD HF/HG compared to NC controls. Liver mass was only significantly higher in WT+HFD HF/HG mice compared to controls. Behavioural tests revealed no significant differences between groups except for FST, which indicated a depression-related behaviour in the WT+HFD HF/HG group compared to controls. This was not observed with MCT1 +/-+HFD HF/HG mice. WT+HFD HF/HG mice had a lower cerebral PO_2 baseline and hypercapnia-induced PO_2 response compared to controls, while MCT1 +/- groups remained unchanged. Tonic lactate release was unaltered between all groups although the MCT1 +/-+HFD HF/HG group indicated a decreased lactate tone trend.

Our results suggest that NAFLD is associated with a depression-related behaviour and decreased cerebral PO_2 baseline. MCT1 haploinsufficient mice were resistant to the reported phenotypes, suggesting a link between liver metabolism and neuropathophysiological alterations.

MTU04-19

Quantitative proteomic analyses of dynamic signalling events associated with neuronal death in excitotoxicity
M. A. Hoque^{1,2,3}, Nicholas. A. Williamson³, C.-S. Ang³, J.S. Oakhill¹, H.-C. Cheng²

¹ St Vincents Institute of Medical Research, Metabolic Signaling Laboratory, Protein Chemistry and Metabolism, Fitzroy, Victoria, Australia

² University of Melbourne, Department of Biochemistry and Molecular Biology, Parkville, Victoria, Australia

³ University of Melbourne, Bio21 Molecular Science and Biotechnology Institute, Parkville, Victoria, Australia

Excitotoxicity, caused by over-stimulation or dysregulation of ionotropic glutamate receptors (iGluRs), is a major pathological process directing neuronal death in both acute and chronic neurological disorders. The aberrantly stimulated iGluRs direct massive influx of calcium ions into the affected neurons, leading to changes in expression and phosphorylation of specific proteins to modulate their functions and direct their participation in the signalling pathways that induce excitotoxic neuronal death. To define these pathways, herein we utilised quantitative proteomic and phosphoproteomic approaches to identify neuronal proteins associated with excitotoxic cell death. We identified > 150 neuronal proteins with significant dynamic temporal changes in abundance and/or phosphorylation levels at different time points (5-240 min) following glutamate overstimulation in cultured primary cortical neurons. Bioinformatic analyses predicted that many of them are components of signalling networks directing defective neuronal morphology and functions. Biochemical approaches confirmed the findings of the proteomic analysis for Erk1/2, GSK3 and Tau. Bioinformatic analysis further predicted Akt, JNK, Cdk5, MEK, CK2, Rock and SGK1 as the potential upstream kinases phosphorylating some of these perturbed proteins and biochemical studies confirmed our predictions. We also defined > 40 significantly changed neuronal (phospho)proteins including CK2 and AMPK that are downstream of neurotoxic GluN2B-containing extra-synaptic NMDA receptors. Our predicted signalling networks and signalling dynamics of neuronal protein kinases form the conceptual framework for future investigation to define the spatial and temporal organisation of cell signalling pathways governing neuronal death in excitotoxicity.

MTU04-20

PPV-6 suppresses amyloid beta-induced cell cycle reentry in differentiated primary cortical neurons
B.-Y. Hou, D.-I. Yang

National Yang-Ming University, Institute of Brain Science, Tainan, Taiwan

Amyloid beta-peptide (A β) is the main neurotoxic component of senile plaque, which is the pathological hallmark of Alzheimer's disease. In addition, A β is also known to trigger cell cycle reentry in post-mitotic neurons followed by cell death. Many studies have reported that polysaccharides from medicinal plants may carry therapeutic potential in AD. However, the mechanisms underlying polysaccharide-mediated inhibition of A β neurotoxicity is still unclear. Therefore, this study was designed to explore the neuro-protective mechanisms of polysaccharides extracted from a perennial vine (PPV-6). We hypothesized that PPV-6 may suppress cell

cycle reentry and subsequent cell death induced by A β in the fully differentiated post-mitotic neurons. To test this hypothesis, post-mitotic primary cortical neurons were subjected to cotreatment with A β and PPV-6. Western blotting, immunocytochemistry, and flow cytometry were conducted to assess the extents of neuronal cell cycle reentry. MTT assay was performed to determine cell viability. Compared with A β alone, cell viability and morphology were recovered by cotreatment with PPV-6. Further, A β -induced upregulation of G1-phase markers including cyclin D1 and phosphorylated retinoblastoma protein (pRb), G2-phase marker such as proliferating cell nuclear antigen (PCNA), and mitotic marker histone H3 phosphorylated at Ser-10 were all reversed by PPV-6. Similar results were obtained with flow cytometry. Taken together, our finding indicated that the neuroprotective mechanisms of PPV-6 involve suppression of A β -induced neuronal cell cycle reentry and subsequent cell death.

MTU04-21

Mechanism underlying age of disease onset in familial amyloid polyneuropathy (ATTR-FAP)

R. Ibrahim¹, T.-Y. Yu³, C.-C. Chan², J.-W. Tsai¹

¹ National Yang Ming University, Brain Science, Taipei, Taiwan

² National Taiwan University, Physiology, Taipei, Taiwan

³ Academia Sinica, Atomic and Molecular sciences, Taipei, Taiwan

Familial amyloid polyneuropathy (FAP) is a progressive neurodegenerative and systemic disease caused by the deposition of amyloid fibrils of misfolded transthyretin (TTR) origin.

Although over 100 disease causing point mutations have been identified in the TTR gene, the effects of different mutations are highly variable reflected by heterogeneous phenotypes seen in affected human. To understand these phenotypic differences, we generated TTR mutants with different disease onsets and study their amyloidogenic properties using cell lines, biophysical approaches and *Drosophila melanogaster* as a model. We analysed secretory patterns and cytotoxic potentials of TTR mutants in HEK 293 and IMR-32 neuroblastoma cells respectively. Differential scanning calorimetry (DSC) was also used to determine the stabilities of TTR mutants. To model TTR associated amyloid disease, we employed the *Drosophila* as a disease model. We generated transgenic flies overexpressing amyloidogenic TTR mutant variants and the wild-type protein. We analysed the effect of mutant TTR on *drosophila*'s climbing activity, and lifespan. Our results reveal differences in the secretory efficiencies and stability of TTR mutants corresponding to their age of disease onset in patients. Importantly, our data reveal that stability of TTR monomers and their interaction with ER chaperone GRP78 determines age of onset in ATTR-FAP. In our *Drosophila* model, late onset TTR mutants results in shortened lifespan while early onset TTR mutants result in earlier reduced climbing activities mimicking phenotypes seen in human patients.

MTU04-22

Involvement of mitochondria mediated oxidative stress dependent cell signaling events and SYK tyrosine kinase activation in tumor

A. Kanthasamy, S. Kanuri, H. Jin, V. Anantharam, A. Kanthasamy

Iowa State Univ., BMS, Ames, USA

Neuro-inflammation, mitochondrial dysfunction, and impaired clearance of aggregate prone proteins are implicated in Parkinson's Disease (PD) pathogenesis; however, the mechanism by which inflammatory mediators increase the vulnerability of dopaminergic neurons to oxidative stress-induced apoptotic cell death remain poorly characterized. Therefore, the goal of the present study was to investigate the cell signaling events that underlie TWEAK induced apoptotic cell death using an *in vitro* dopaminergic cell culture model, N27 cells. Herein, we show that N27 cells express both TWEAK and its receptor Fn14 and that TWEAK-elicited dose dependent apoptotic cell death in N27 cells. Exposure of N27 cells to TWEAK evoked dissipation of mitochondrial membrane potential (MMP), suppression of GSH levels, activation of caspase-8 and 3 and concomitant up regulation of Phospho-Tau and Phospho-NF- κ B P65 levels. Moreover, these changes were accompanied by the down regulation of Phospho-AKT, P-GSK3 β (Ser9) and LC3 levels in TWEAK treated dopaminergic neuronal cells. Intriguingly, upregulation of TWEAK was evidenced in MPP⁺ treated dopaminergic neuronal cells. Likewise TWEAK was upregulated in the SNpc of MPTP treated mice. Consistent with the role of TWEAK in the induction of oxidative stress response, pretreatment of N27 cells with varying concentration of quercetin, a bioflavonoid abrogated TWEAK-induced apoptotic cell death. In a similar fashion NF κ B inhibitor, SN50 ameliorated TWEAK-induced loss of dopaminergic cell viability. Together, these data suggest that TWEAK exerts deleterious effects on dopaminergic neuronal survival via aberrant activation of NF- κ B and impaired mitochondrial function in an oxidative stress dependent manner.

MTU04-23

Pre-ischemic administration of nutraceutical offers neuroprotection against stroke injury by attenuating mitochondrial dysfunction

P. Kaushik¹, H. Tabassum², S. Parvez¹

¹*Jamia Hamdard, Department of medical elementology and toxicology, New delhi, India*

²*Indian Council of Medical Research, Division of Biomedical Sciences, New delhi, India*

Stroke is the worldwide threat that causes death and disability in adults. The dynamic nature of mitochondria is related to cellular survival, growth and death. Previous literature have reported that ischemic stroke (IS) reduces mitochondrial respiration, enhances production of reactive oxygen species (ROS) and triggers apoptotic cell death, suggesting a prominent role of mitochondria in IS pathophysiology. The selected nutraceutical, PIP, has been reported to have anti-inflammatory and anti-oxidant properties. Pre-treatment of PIP has been found to be neuroprotective in IS. The present study emphasises on the possible neuroprotective role of PIP via mitochondria as therapeutic target for stroke treatment. After PIP administration (10 mg/kg b.wt. once daily, p.o. for 15 days), the male wistar rats underwent the tMCAO surgery. The right middle cerebral artery was occluded for 1 h followed by 23 h of

reperfusion. Behavioural assessment was done after 24 h. The brain samples for analysis of mitochondrial impairment were extracted after behavioural assessment. The results showed that PIP significantly reduced the infarct volume, mitochondrial ROS and restored complexes activity, mitochondrial membrane potential and cytochrome c release, thereby, attenuated the mitochondrial wreckage. Taken together, our results are the first to demonstrate that PIP has the potential to constrict the mitochondrial dysfunction in IS.

MTU04-24

Investigation of immune modulators produced by hipsc-derived astrocytes from schizophrenic patients

P. L. Cardozo¹, P. Trindade², J. Alves-Silva³, L. Chicaybam⁴, M. Bonamino⁴, S. Rehen^{2,5}, F. Ribeiro¹

¹*Federal University of Minas Gerais, Biochemistry and Immunology, Belo Horizonte, Brazil*

²*D'or Institute for Research and Learning, National Laboratory of Stem Cells, Rio de Janeiro, Brazil*

³*Federal University of Minas Gerais, Morphology, Belo Horizonte, Brazil*

⁴*Cancer National Institute, Experimental Medicine Division, Rio de Janeiro, Brazil*

⁵*Federal University of Rio de Janeiro, National Laboratory of Stem Cells, Rio de Janeiro, Brazil*

Schizophrenia (SCZ) is a neuropsychiatric disorder, caused by genetic and environmental factors. Schizophrenic individuals exhibit cognitive deficits, positive (psychosis, hallucinations and delusions) and negative symptoms (depression, avolition and anhedonia), as well as reduced gray matter volume. Microanatomical analyses suggest that this gray matter reduction is due to diminished dendritic spine density, which likely happens as a result of exaggerated synaptic pruning. Recently, it has been shown that the classical complement cascade and CX3CL1/CX3CR1 pathway play a direct role in this process, triggering synaptic engulfment by microglia. In addition, astrocytes secrete cytokines capable of modulating the complement cascade. Taking into account that pre-natal infection acts as a risk factor for SCZ, this work aimed at analyzing complement components, proinflammatory cytokines and CX3CL1 production employing induced pluripotent stem cells (iPSCs)-derived astrocytes from schizophrenic individuals after stimulation with TNF- α . The results demonstrate that TGF- β 3 and IL-1 β transcripts are altered in SCZ-derived astrocytes relative to healthy control-derived (HC) astrocytes. In contrast, C3, C4 and CX3CL1 show similar mRNA expression patterns in both groups. Finally, non-secreted CX3CL1 levels seems to be increased in SCZ astrocytes, suggesting problems in the release of the soluble form. Altogether, these results indicate that SCZ astrocytes produce immunomodulators in a distinct manner compared to HC astrocytes.

MTU04-25

A transposon-mediated somatic mutagenesis screen identifies new genes associated with malformations of cortical development**I.-L. Lu^{1,2}, C. Chen^{3,5}, C.-Y. Tung⁴, H.-H. Chen^{3,5}, J.-P. Pan⁴, J.-W. Tsai²**¹*Academia sinica, Institute of Biomedical Sciences, Taipei, Taiwan*²*National Yang-Ming University, Institute of Brain Sciences, Taipei, Taiwan*³*Taipei Veterans General Hospital, Neurological Institute, Taipei, Taiwan*⁴*National Yang-Ming University, VYM Genome Research Center, Taipei, Taiwan*⁵*Taipei Veterans General Hospital, Department of Pediatrics, Taipei, Taiwan*

Malformations of cortical development (MCDs) are heterogeneous neurodevelopmental disorders that often result in epilepsy and developmental delays in children. However, many genetic mutations involved in MCD pathogenesis remain unidentified. To identify new genes potentially involved in cortical development and the pathogenesis of MCDs, we took advantage of forward genetic screening by transposon somatic mutagenesis during brain development. Here we developed a genetic screening paradigm by combining transposon-based somatic mutagenesis with *in utero* electroporation in the developing mouse cortex. We identified 33 potential MCD genes, several genes have been previously implicated in neuronal development and disorders. Consistent with the screening results, functional disruption of these genes by RNA interference or using CRISPR/Cas9 causes alterations in the distribution of cortical neurons that resemble human cortical dysplasia. To verify potential clinical relevance of these candidate genes, we analyzed somatic mutations in brain tissue from patients with focal cortical dysplasia type II (FCDII) and found mutations enriched in these candidate genes. These results demonstrate that the approach is able to identify potential novel genes involved in cortical development and MCD pathogenesis.

MTU04-26

Hypoxia or nicotine- which is worse on the infant brain? from neurotransmitters, growth factors, to apoptosis and microglia**R. Machaalani***University of Sydney, Faculty of Medicine and Health, University of Sydney, Australia*

A respiratory hypoxic environment and cigarette smoke exposure around babies have long lasting effects on the brain such as decreased neuroprotection, decreased IQ, and long-term increased addictive behaviours (cigarette smoke exposure). Extensive studies of the effects of such exposures to the developing brain on the expression of neurotransmitters, receptors, growth factors, markers of apoptosis and microglia in the brain have been undertaken in our laboratory over the past decade and will be presented herein. Our results are from two brain tissue datasets: 1- infants who died suddenly and unexpectedly, and 2- piglet models of intermittent hypercapnic hypoxia (IHH) and postnatal nicotine exposure. Brain tissue was subjected to immunohistochemistry for apoptotic markers (caspase-3 & TUNEL), NMDA receptor 1, brain derived neurotrophic factor (BDNF) and its receptor TrkB, serotonin receptor

1A (5HT1A), pituitary adenylate cyclase activating polypeptide (PACAP) and its receptor PAC1, orexin, nicotinic acetylcholine receptors (nAChRs) and microglia. Staining was quantified and compared between exposures to control (non-exposures). We found that across the studies, the IHH exposure induced greater expression changes than nicotine, and many changes equated between the piglet models and the infant findings when stratified for hypoxia related conditions of prone sleeping, bedsharing, and cigarette exposure, including the syndrome of Sudden Infant death (SIDS). These changes predominated in the brainstem medulla, a region containing nuclei of importance in cardiac and respiratory regulation.

MTU04-27

Sphingosine-1-phosphate signaling in stroke - a potential role for astrocytes**H. Matuskova^{1,2}, F. Matthes³, G. Petzold^{1,2}, A. Meissner^{2,3,4}**¹*DZNE, Neurovascular Diseases, Bonn, Germany*²*University Hospital Bonn, Department of Neurology, Bonn, Germany*³*Lund University, Department of Experimental Medical Sciences, Lund, Sweden*⁴*Lund University, Wallenberg Centre for Molecular Medicine, Lund, Sweden*

Stroke is a leading cause of long-term disability worldwide. Due to its complexity, treatment options are sparse. Recently, the bioactive signaling molecule, phospholipid sphingosine-1-phosphate (S1P), has gained increasing attention in cardiovascular disease due to its involvement in both vascular function and immune cell responses. As astrocytes play a critical role in the injured brain, we sought to determine a potential contribution of astrocytic S1P signaling to a stroke disease progression. In a mouse model of transient middle cerebral artery occlusion (MCAo), we first investigated the expression pattern of S1P-generating enzyme 1 (SphK1) and S1P receptor 3 (S1PR3) in response to ischemia in wild type mice, followed by an analysis of gene expression in astrocyte RiboTag transgenic mice. Additionally, the concentration of S1P in plasma and brain samples by mass spectrometry was analyzed to complement the data. 24 hours following MCAo, the SphK1 mRNA expression significantly increased in the ischemic hemisphere. This effect was abolished 72 hours post MCAo, indicative of a transient SphK1 response following ischemia. Moreover, similar results were obtained from the analysis of the astrocytic ribosome-associated mRNAs, suggesting a critical involvement of astrocyte in the SphK1 response to ischemia. Interestingly, the S1PR3 expression was markedly reduced 24 hours, as well as 72 hours post-ischemia in the contralateral hemisphere, while astrocytic ribosome-associated mRNA revealed a significant S1PR3 upregulation in the ischemic hemisphere. In conclusion, our findings point to an important astrocyte-specific contribution in the activation of the S1P/S1PR3 signaling axis post-stroke.

MTU04-28

Amyloid-beta alone induces changes in hippocampal GABAergic synapses

M. Mayer¹, Katalin. E. Sos¹, Virág. T. Takács¹, Z. Bardóczi¹, Á. Major¹, B. Béres¹, T. Szeles¹, Takaomi. C. Saido², T. Saito², Tamás. F. Freund¹, G. Nyiri¹

¹*Institute of Experimental Medicine, Laboratory of Cerebral Cortex Research, Budapest, Hungary*

²*RIKEN Brain Science Institute, Laboratory for Proteolytic Neuroscience, Wako, Japan*

Alzheimer's disease (AD) is a neurodegenerative disorder, characterized partly by amyloid-beta (A β) depositions in cortical areas. Several transgenic mouse lines were created to examine the effect of A β , however, most of them employed strong non-specific promoters to drive A β expression. We used APP-NL-F mice that are unique, as they express A β driven by the natural promoter of amyloid precursor protein (APP), the latter of which is a mutated version of the human APP in this strain. We investigated subcellular effects of A β in the hippocampus of APP-NL-F mice. We found that A β alone caused typical plaque formation, glial activation and malformation of neurites. It induced the formation of significantly larger synapses on axon initial segments of pyramidal cells and caused impairment in natural anxiety in the elevated plus maze. However, these mice lack some changes typical in AD model animals, including the degeneration of septo-hippocampal cholinergic and parvalbumin positive pathways and changes in the number of hippocampal parvalbumin and somatostatin positive interneurons. These results suggest that upregulation of A β expression alone can induce changes in the inhibitory balance of hippocampal pyramidal cells, which may contribute to disease progression during the preclinical phase of AD.

MTU04-29

An implantable microelectrode array for simultaneous *in vivo* recordings of glutamate, Gaba and neural activity
E. Mitricheva, R. Kimura, L. P. Mansilla, E. Krampe, A. Oeltermann, N. Logothetis, Hamid. R. Noori

Max Planck Institute for Biological Cybernetics, Department of Physiology of Cognitive Processes, Tuebingen, Germany

Glutamate and γ -aminobutyric acid (GABA) are the most common neurotransmitters in the central nervous system. By exciting, inhibiting, and modulating neural elements and microcircuits, these chemicals critically regulate brain information processing and energy metabolism at different spatiotemporal scales. However, the exact relationship between the extracellular concentration of these molecules and emergence of specific patterns in neuronal ensemble activity remains elusive. Partly this is due to the fact that recording of the mean extracellular field potentials (mEFP) concurrently with a quantitative assessment of alterations in the concentration of such neurochemicals are currently unavailable. Here, we present a silicon-based implantable ultrafine microelectrode array (35 μ m diameter) composed of several iridium-stabilized electrochemical and electrophysiological contacts. The electrophysiological electrodes have an average impedance of 0.5 M Ω at 1 kHz. The amperometric electrochemical channels, divided into two groups of glutamate- and GABA-responsive electrodes, show a sensitivity of 0.39 nA/ μ M for glutamate and 0.38 nA/ μ M on the adjacent channel for GABA. This novel multimodal microelectrode

was used to simultaneously monitor extracellular glutamate and GABA concentrations, spikes, multi-unit neuronal activity (MUA) and local field potentials (LFP) in the lateral geniculate nucleus (LGN) of anaesthetized rats ($n = 5$). Retinal stimulation with flickering monochromatic light, emphasizing the simplest form of feedforward processing in thalamus, induced neuronal response patterns in LGN that were highly correlated with the temporal alterations in glutamate concentrations. GABA responses, while similar in profile to MUA and LFP recordings, were found to be event-selective, suggesting network-level processes. Our findings suggest that this multimodal method may greatly contribute into our understanding of microcircuit organization, by reducing the inherent ambiguity in the mEFP through neurotransmitter-release-tracking.

MTU04-30

Dysfunction of SV2A elicits dopaminergic hyperactivity via interacting accumbal gabaergic neurons in rats

Y. Ohno, K. Tokudome, A. Ikari, Y. Iguchi, T. Sugahara, N. Kunisawa, M. Kato, T. Serikawa, S. Shimizu

Osaka University of Pharmaceutical Sciences, Pharmacology, Osaka, Japan

Synaptic vesicle protein 2A (Sv2A) regulates action potential-dependent synaptic release of neurotransmitters in the brain. To explore the role of SV2A in modulating CNS functions, we have recently created SV2A-mutant (*Sv2a*^{L174Q}) rats carrying a missense mutation (L174Q) in *Sv2a* gene and demonstrated that the *Sv2a*^{L174Q} mutation disrupts synaptic GABA release and facilitates epileptogenesis (Sci. Rep., 6, 27420, 2016; Front. Pharmacol., 7, 210, 2016). Here, we performed behavioral and neurochemical studies using SV2A-mutant rats to clarify the role of SV2A in modulating psychotic disorders. In SV2A-mutant rats, methamphetamine (MAP)-induced hyperactivity was significantly augmented as compared to the control (F344) rats. Development of MAP reverse tolerance (supersensitivity) with repeated treatments was also enhanced by the *Sv2a*^{L174Q} mutation. In addition, social isolation stress-induced aggressive behaviors were significantly enhanced in SV2A-mutant rats. *In vivo* microdialysis study revealed that dopamine release induced by high K⁺ or MAP was markedly enhanced in SV2A-mutant rats, as compared to F344 rats. When bicuculline (BIC, 100 μ M) was applied to the nucleus accumbens through the dialysis probe to block GABA_A receptors, enhanced dopamine release by the *Sv2a*^{L174Q} mutation was reversed to the control level. In addition, high K⁺-induced GABA release in the nucleus accumbens was significantly decreased in SV2A-mutant rats compared to F344 rats. The present study shows that dysfunction of SV2A by the *Sv2a*^{L174Q} mutation augments the MAP susceptibility and aggressive behaviors by enhancing dopamine release in the nucleus accumbens. Our results suggest that SV2A play an important role in regulating the vulnerability to psychotic disorders via the SV2A-GABA interaction.

MTU04-31

Pilot human study to define the impact of vascular and inflammatory risk factors in Alzheimer's disease
F. Prestia¹, P. Galeano¹, M. Dalmasso¹, E. Castaño¹, D. Politis², S. Kochen³, L. Brusco⁴, L. Morelli¹

¹*Leloir Institute Foundation, IIBBA-CONICET, Buenos Aires, Argentina*

²*HIGA, CONICET, Buenos Aires, Argentina*

³*El Cruce Hospital, CONICET, Buenos Aires, Argentina*

⁴*School of Medicine, UBA, Buenos Aires, Argentina*

Clinical evidence suggests a leading role of vascular and inflammatory risk factors in Alzheimer's disease (AD). Angiogenic factors, chemokines and pro-inflammatory cytokines were evaluated in plasma of control (CTR, n = 20) and sporadic AD (n = 18) patients recruited from hospitals in Argentina. Statistical comparisons were carried out by Student's t or Mann-Whitney tests. Moreover, cognitive performance was evaluated with the MMSE test and, as expected, significant differences were observed between groups. Levels (pg/mL) of 41 analytes were determined by multiplex ELISA (V-PLEX Human Biomarker kit-MSD Technology). From the 41 analytes, 33 were detected in most of the patients and significant differences were observed in the levels of 16 of them (GM-CSF; IL-16; VEGF; IL-8; TNF α ; EOTAXIN; EOTAXIN3; IP10; MDC; MIP-1 α ; MIP-1 β ; sVCAM1; Fit1; PIGF; VEGFA). It is of note that IL-1 β was only detected in AD patients. A discriminant analysis performed with all of the data revealed that 81.6% of subjects (AD: 83.3%; CTR: 80%) were correctly classified. To determine whether APOe4, the most relevant genetic risk factor for AD, was associated with particular analytes, AD patients were divided into two groups: APOe4(+) and APOe4(-). GM-CSF was the only analyte that was significantly different between groups (0.068 ± 0.006 vs. 0.030 ± 0.007). These results suggest that a set of circulating vascular and inflammatory factors is able to discriminate between AD and CTR patients, while further studies are required to determine the relationship between genetic risk factors and plasma biomarkers.

MTU04-32

Dysregulation of autophagy and stress granule-related proteins in stress-driven tau pathology
J. Silva¹, S. Rodrigues¹, P. Gomes¹, A. Takashima², B. Wolozin³, I. Sotiropoulos¹

¹*Life and Health Sciences Research Institute, School of Health Sciences, Braga, Portugal*

²*Gakushuin University, Department of Life Science, Tokyo, Japan*

³*School of Medicine, Boston University, Department of Pharmacology & Experimental Therapeutics, Boston, USA*

Consistent with suggestions that lifetime stress may be an important AD precipitating factor, and knowing that imbalance in neuronal proteostasis associated with Tau misfolding and aggregation is a common feature between AD and other Tauopathies, we previously demonstrated that chronic stress and high glucocorticoid (GC) levels induce accumulation of aggregated Tau; however, the molecular mechanisms for such process remain elusive. Hereby, we monitor a novel interplay between RNA-binding proteins (RBPs) and autophagy has underlying mechanisms through which chronic stress and high GC levels impact on Tau proteostasis precipitating Tau aggregation. Using molecular, pharmacological and behavioral

analysis, we demonstrate that chronic stress and high GC trigger an mTOR-dependent inhibition of autophagy, leading to accumulation of Tau and cell death in P301L-Tau expressing mice and cells. In parallel, we found that environmental stress and GC disturb cellular homeostasis and trigger insoluble accumulation of different RBPs, such as PABP, G3BP1, TIA-1 and FUS, shown to form Stress granules (SGs) and Tau aggregation. Interestingly, using an mTOR-driven pharmacological stimulation of autophagy (CCI-779) attenuated the GC-driven Tau and SG-related proteins accumulation, as well as the related cell death, suggesting a critical interaction between autophagy and SG response in chronic stress and GC driven neurodegeneration. Moreover, *in vivo*, this compound also reverted some of the previously observed behavioral deficits, acting as an anti-depressant and reverting short-term memory deficits. These studies provide novel insights into the RNA-protein intracellular signaling regulating the precipitating role of environmental stress and GC on Tau-driven brain pathology.

MTU04-33

Prenatal hypoxia-induced alterations are accompanied with malfunction of glutamatergic system in rat hippocampus
V. Stratilov^{1,2}, O. Vetrovoy^{1,2}, E. Tyulkova¹

¹*Pavlov Institute of Physiology, Laboratory of regulation of brain neuronal function, St. Petersburg, Russia*

²*St. Petersburg State University, Department of Biochemistry, St. Petersburg, Russia*

Prenatal hypoxia (PH) is one of the most common causes of developing brain pathologies. This study was aimed to analyze the characteristics of the glutamate system and behavior during early (2-week), adult (3-month) postnatal ontogenesis and in the process of aging (18-month) of rats subjected to hypoxic stress (5% O₂, 3 h) during 14-16 days of prenatal development. We have shown progressive with age decrease in the amount of glutamate in the hippocampus of rats subjected to PH, which is accompanied by a decrease in the number of NeuN+ cells, as well as a decrease in long-term memory and learning ability in the Morris water maze. A gradual decrease in the amount of glutamate inversely correlates with, apparently, a compensatory increase in the levels of mGluR1, IP3R1 and polyphosphoinositides. At the same time, the use of mGluR1 agonists normalizes the cognitive ability of rats subjected to PH. 18-month animals subjected to PH demonstrate decreased activity of liver glucose-6-phosphatase, the product of glucocorticoid-dependent transcription. This enzyme contributes to increase of glucose blood level and thus to reaction of glutamate synthesis in the brain. Glucocorticoid receptor levels, similarly, decrease with age in rats subjected to PH. These results indicate a significant contribution of the dysfunction of the glutamatergic system to the formation of early aging caused by PH. The mechanism of glutamatergic deficit can be glucocorticoid-dependent.

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MTU04-34

Synapse formation and remodeling unveil a glutamatergic/GABAergic imbalance in hippocampal neurons in the VPA model of autism**M. Traetta^{1,2}, M. Codagnone^{1,2}, N. Uccelli¹, S. Zárate¹, A. Reinés^{1,2}**¹*Instituto de Biología Celular y Neurociencia, IBCN-UBA-**CONICET, Buenos Aires, Argentina*²*Cátedra de Farmacología, FFyB-UBA, Buenos Aires, Argentina*

Autism spectrum disorders (ASD) are characterized by impairments in social interaction and repetitive-stereotyped behaviors. Although increased cortical excitatory-inhibitory (E-I) ratio has been described in ASD patients, results in the hippocampus are not conclusive. Using the valproic acid (VPA) model of ASD, we reported in the hippocampus of VPA rats a reduction in the synaptic marker synaptophysin along with an increased adhesive/non-adhesive form expression ratio of the neural cell adhesion molecule (NCAM). This study aimed to evaluate E-I balance, synapse formation and remodeling in primary hippocampal neurons either from VPA or control male pups. Synaptic markers were evaluated by immunocytochemistry and western blot. At DIV14, hippocampal neurons from VPA animals displayed a reduced dendritic tree (reduced MAP-2 area), a reduced number of glutamatergic synapses (decreased vGLUT puncta number and area) and NMDA receptor clusters (decreased NR1 puncta number and individual puncta area) but no changes in gabaergic synapses (conserved GAD-67 puncta number). These neurons also exhibited reduced number of functional synapses (FM4-64 labelling) which contained smaller vesicular pools with preserved unloading kinetics; total NCAM expression increased while its non-adhesive form (PSA-NCAM) decreased. While in neurons from control animals glutamate exposure (5 μ M-3 min) induced an NMDA-dependent dendritic retraction and synapse number reduction, neurons from VPA animals only exhibited dendritic retraction. Our results indicate that neurons from VPA animals form fewer glutamatergic synapses with a more adhesive and resistant profile to synaptic remodeling, suggesting an underlying mechanism that would contribute to reduced structural synaptic plasticity in the hippocampus.

MTU04-35

Short and long non-coding RNA interactions in trauma and fatty liver disease**Y. Tzur***Hebrew university of Jerusalem, Biological chemistry, Kibbutz Shamir, Israel*

Trauma-related and metabolic impairments are notably linked, but the underlying mechanisms are incompletely understood. Here, we report that long non-coding RNAs and microRNAs (lncRNAs, miRs) might have co-evolved to ameliorate trauma, metabolic syndrome (MetS) and sepsis conditions. In diet-induced obese mice, antisense AM132 oligonucleotide suppression of the trauma-inducible miR-132 led to liver deregulation of MetS-associated miRs including miR-122-5p and miR-26a-5p, which were down-regulated in human fatty liver tissues. Both these miRs may be targeted by the lncRNA MIAT, known to be deregulated in MetS, myocardial and diabetic disorders. Supporting functional relevance, we found MIAT upregulation in human fatty liver samples. Next, we pursued miR-target associations in the web-available CAPSOD transcriptomic dataset of human liver and blood cell biopsies from

sepsis and MetS patients compared to controls. Searching the 1,152 subjects' CAPSOD dataset identified the sepsis-related histocompatibility antigen HLA-DRA and the brain-expressed pseudogene PGOHUM-565 as targets of the primate-specific miR-608, compatible with the reduced risks of both sepsis and trauma in human carriers of single nucleotide polymorphisms interrupting miR-608-target interactions. Our findings support causal involvement of lncRNA-miR interactions in stress-related metabolic imbalances and predict that these interactions might have provided survival advantages to evolving primates in both contexts.

MTU04-36

Targeting FTD/ALS: udca prevents CHMP2B-intron5 induced neurodegeneration, revealing a novel drug target for dementia research**C. Ughode, R. West, S. Sweeney, F.-B. Gao***University of York, Biology, York, United Kingdom*

Frontotemporal Dementia (FTD) is the second most prevalent form of early-onset dementia. The pathological mechanisms driving neuronal atrophy in FTD remain poorly understood and no therapeutic interventions exist. The FDA approved drug Ursodeoxycholic acid (UDCA), normally used to treat biliary cirrhosis, shows both cytoprotective and anti-apoptotic activity, however its mechanism of action remains unknown. Mutations in the FTD gene CHMP2B (CHMP2B^{Intron5}) cause severe neurodegeneration and apoptosis. Our data shows UDCA can alleviate these neurodegenerative phenotypes and provides novel insights into the mechanism of action of UDCA. Given that UDCA has just entered clinical trials in Europe for the treatment of Amyotrophic Lateral Sclerosis (ALS) our data suggests that UDCA has a broader neuroprotective effect across the FTD/ALS spectrum. Our lab has previously characterised the effect of the mutant CHMP2B^{Intron5} protein in both *Drosophila* and mammalian neuron models. Ectopic expression of CHMP2B^{Intron5} in cortical neurons causes dendritic collapse associated with autophagosome accumulation. Transgenic mouse models expressing CHMP2B^{Intron5} globally or in forebrain neurons display neurodegeneration and behavioural deficits. Also prominent are accumulations in p62 and ubiquitin positive inclusions in neurons and glia, mirroring events in CHMP2B^{Intron5} and other FTD/ALS (GRN, C9ORF72, MAPT) patient tissue. We show that UDCA alleviates apoptotic cascades, dendritic collapse and synaptic aberrations in both *Drosophila* and mammalian models of CHMP2B^{Intron5} induced FTD. In addition, we identify a novel "orphan" receptor as a potential target of UDCA and demonstrate genetic manipulation of this receptor is sufficient to alter CHMP2B^{Intron5} associated phenotypes. UDCA represents a compound with potential to be repurposed for the treatment of FTD associated with the CHMP2B^{Intron5} disease causing mutation.

MTU04-37

Neurodevelopmental deficits in human isogenic fragile x syndrome neurons

K. Utami¹, N. H. Skotte², A. R.-F. Colaco², N. A. B. M. Yusof¹, B. Sim¹, X. Y. Yeo³, H.-G. Bae³, M. Garcia-Miralles¹, C. Radulescu¹, S. Jung^{3,4}, M. Mann², M. Pouladi^{1,5}

¹Translational Laboratory in Genetic Medicine, Neurodegenerative Disease Program, Singapore, Singapore

²Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, Proteomics and Signal Transduction, Copenhagen, Denmark

³Singapore Bioimaging Consortium SBIC, A*STAR, Neuromodulation and Neurocircuitry group, Singapore, Singapore

⁴Yong Loo Lin School of Medicine, Department of Physiology, Singapore, Singapore

⁵Yong Loo Lin School of Medicine, Department of Medicine, Singapore, Singapore

Fragile X Syndrome (FXS) is a neurodevelopmental disorder caused by epigenetic silencing of *FMR1* and loss of FMRP expression. Here, we describe the generation of an isogenic human pluripotent embryonic stem cell (hPSC) model of FXS. Using CRISPR/Cas9 to introduce indels in exon 3 of *FMR1* resulting in complete loss of FMRP (*FMR1*KO), we show that FMRP-deficient neurons exhibit a number of phenotypic abnormalities, including neurite outgrowth and branching deficits, and impaired electrophysiological network activity as measured by multi-electrode arrays. RNA-Seq and proteomic analysis of FMRP-deficient neurons revealed dysregulation of pathways related to neurodevelopment, neurotransmission, and cell cycle. These changes were paralleled by abnormal neural rosette formation and neural progenitor proliferation. Of note, our transcriptional and proteomic analyses identified marked deficits in a key enzyme involved in the metabolism of catecholamines (such as dopamine) and that has been linked to a number of neuropsychiatric disorders. Using isogenic *FMR1*KO hPSCs as a model to investigate the pathophysiology of FXS in human neurons, we reveal key neural abnormalities arising from loss of FMRP, including some with potential for therapeutic intervention.

MTU04-38

Recapitulation of familial multiple sclerosis mutation leads to compromised myelin and susceptibility to demyelination insult

A. Ziaei¹, M. Garcia-Miralles¹, C. Radulescu¹, A. Silvin¹, V. Shaygannejad², H. Bae¹, N. Yusof¹, C. F. Bardile¹, H. Sidik¹, L. Dehghani², C. Bonnard¹, S. Jung¹, B. Reversade¹, F. Ginhoux¹, M. Pouladi¹

¹A*STAR, BMRC, Singapore, Singapore

²MUI, Neurology, Isfahan, Iran

Efforts are currently underway to uncover factors that trigger the immune system in multiple sclerosis (MS). We sought to identify multi-incidence MS-families for the discovery of genetic background susceptibility. In one such family, inactivating mutations in

ERMN gene was identified with complete segregation. Ermin is an actin-binding protein found almost exclusively in central-nervous-system myelin-sheath. Although Ermin has been predicted to play a role in the formation and stability of myelin sheaths, this has not been examined. Using Ermin knockout mice, we show that Ermin is essential for myelin sheath integrity and normal saltatory conduction. Loss of Ermin caused non-compacted myelin sheath and myelin fragmentation in electron microscopy imaging, supported by an increase in QD9/MBP ratio, led to slower conduction velocity in the CC and progressive neurological deficits. RNA sequencing of the CC revealed pathways related to axonal degeneration and inflammation in aged Ermin-deficient mice, which were confirmed by immunostaining showing increased axonal damage, microgliosis and astrogliosis. In addition, we observed an increased level of demyelinated-lesion responsive microglia population in the CC also with a higher level of fragmented myelin phagocytosed by these microglia. The inflammatory milieu and microstructural myelin abnormalities were further associated with increased susceptibility to demyelination in the experimental autoimmune encephalomyelitis model of MS. We hypothesize this non-compact, fragmented myelin and white matter inflammation can expose myelin proteins to the immune system and make individuals susceptible to MS.

MTU04-39

Binding of ethanol in the C1 domain of presynaptic munc13-1: A molecular dynamics simulation study
J. Das, Y. You

University of Houston, Department of Pharmacological and Pharmaceutical Sciences, Houston, USA

Identifying molecular targets of alcohol and understanding the molecular mechanism of alcohol actions are necessary to develop effective therapeutics for Alcohol Use Disorder (AUD). Munc13-1 is a presynaptic protein involved in the vesicle priming and neurotransmitter release in the brain. Our earlier studies identified Glu-582 as the alcohol-binding residue in the activator (diacylglycerol/ phorbol ester)-binding C1 domain of Munc13-1. Here we describe a 250 ns molecular dynamics simulation study on the interaction of ethanol and the activator-bound C1 domain of Munc13-1 in the presence of varying concentrations of phosphatidylserine. Our results suggest that phorbol 13-acetate forms fewer number of the hydrogen bond with the C1 domain in the ethanol solvent than in water. Ethanol does not change the protein structure significantly and it forms hydrogen bonds with the Glu-582 at 45.61 ns. When Glu-582 was mutated to alanine, ethanol molecules were not observed in the vicinity (5Å) of Ala-582 at this time point. This study is important in providing structural basis of ethanol's action in presynaptic proteins.

MTU05 Brain development & cell differentiation (Session A)

MTU05-01

Association between demethylation and differentiation of neural cells by mammalian GCM1 and GCM2

A. Abdullah, Y. Hayashi, S. Fuke, T. Fuchigami, N. Morimura, N. Koyama, S. Hitoshi

Shiga University of Medical Science, Integrative Physiology, Otsu, Japan

Glial cells missing (Gcm) gene was discovered in *Drosophila* and thought to act as binary switch in determining the fate of neuron and glia. We reported that orthologs of *gcm* in mammals, *Gcm1* and *Gcm2*, were associated with *Hes5* expression in neural precursor cells (NPCs) of early embryos with active demethylation of *Hes5* promoter (Hitoshi et al., 2011). To determine the differentiation fate of *Gcm1* or *Gcm2* overexpressed NPCs, we performed *in utero* electroporation (IUEP) to an embryonic brain at E14.5. Our results showed that *Gcm1* promotes differentiation of NPCs into GFAP and S100 β positive astrocytes 72 hours after IUEP. *Gcm2* gene have polymorphism in the coding region between C57B6 and ICR mice, and IUEP of *Gcm2* from ICR promotes differentiation of NPCs into NeuN positive neurons, which were detected in the ventricular zone/subventricular zone rather than cortical plate. We could not observe any changes in the *Gcm2* gene from C57B6. These results suggest that *Gcm2* from ICR has stronger function than C57B6. To determine the association between these phenotypes and DNA demethylation, we performed *in vitro* assay using Neuro2a cells. To reveal the function of demethylation by *Gcm* genes, we performed sanger-bisulfite sequencing analysis. Since the *Gcm1*-overexpressing Neuro2a upregulated *Vegfa* expression, which have CpG islands in the first intron, we are analyzing its methylation percentage. Because *Vegfa* intron 1 is already hypomethylated in the control, we could not detect changes by *Gcm1* overexpression. Now we are looking for other candidate genes in appropriate cell line and the best system to clarify the function of *Gcm1* and *Gcm2*.

MTU05-02

HIF-1A inhibition impairs neurodifferentiation induced by retinoic acid in SH-SY5Y cells

P. Brum, C. Girardi, C. T. Ribeiro, N. Somensi, J. C. Moreira, D. Gelain

UFRRGS, Department of Biochemistry, Porto Alegre, Brazil

Several studies indicate hypoxia as a key player in neuronal stem cell differentiation and proliferation. Low oxygen concentration increases the survival and proliferation rate of neuronal precursors as well as the differentiation to a dopaminergic phenotype when compared to normal oxygen concentrations. Hypoxia leads to the accumulation of the hypoxia inducible factor-1 α (HIF-1 α). As *in vitro* studies have shown, there is a correlation between its up regulation and the increase of neuronal markers. Therefore, HIF-1 α emerges as a possible regulatory step of dopaminergic differentiation. This work aims to investigate the effects of the selective inhibition of HIF-1 α on the differentiation induced by retinoic acid in human neuroblastoma cells from the SH-SY5Y lineage with the purpose of elucidating its role in the dopaminergic differentiation. siRNA reverse transfection was performed to inhibit the expression

of HIF-1 α and was followed by a 7 days differentiation protocol utilizing retinoic acid as a differentiation promoter. HIF-1 α silencing efficiency was assessed by western blot and RT-qPCR and differentiation markers were analyzed by immunofluorescence and RT-qPCR. Neuron average length and total number of neurites per cell were assessed utilizing NeuronJ. Our results indicate that HIF-1 α inhibition is capable of reducing the neuron-like phenotype, the immunoccontent, and the expression of neuronal markers indicating the regulatory role of this transcription factor in neuronal differentiation.

MTU05-03

Distribution, density, and morphology of peripheral myeloid cells invading the murine brain during normal postnatal development

M. Carrier¹, M.-È. Robert¹, J. C. Savage¹, F. Geissmann², M.-È. Tremblay¹

¹*Université Laval, Axe neurosciences, Centre de recherche du CHU de Québec-Université Laval, Québec, Canada*

²*Memorial Sloan Kettering Cancer Center, Immunology Program, New York, USA*

Microglia are the resident immune cells of the brain that exclusively derive from the embryonic yolk sac. During trauma or disease, bone marrow-derived cells (BMDC) can also invade the brain, infiltrating through the blood-brain barrier, to accomplish neuroinflammatory roles. Preliminary result from our team showed that these cells were present in the brain during normal development, leaving the question of where and why they invade the brain without insult. We have described at the ultrastructural level a new phenotype of brain myeloid cells that is highly prevalent upon chronic stress, aging, and neurodegenerative disease. Recently, we also found these cells to be abundant during normal development. These 'dark microglia' are tightly associated with blood vessels. They also interact extensively with synapses, suggesting their possible implication in the remodeling of neuronal circuits. To study BMDC in the context of normal development and determine the origin of dark microglia from the bone marrow or embryonic yolk sac, this study was conducted using Flt3^{cre}RFPllox mouse model in which BMDC are selectively labelled, without radiation or chemotherapy that can affect the BBB permeability. The animals were sacrificed under steady-state conditions at different postnatal ages from birth until adulthood. Serial sections providing a non-biased representation of the brain were then imaged with a slide scanner to analyze the distribution, density, and morphology of FLT3-positive cells across development. 3D electron microscopy with immunostaining (array tomography technology) experiments are now underway to determine the origin of dark microglia.

MTU05-04

ATOH1 requires primary cilia for the expansion of granule neuron progenitors by modulating centriolar satellites**C.-H. Chang^{1,2,3}, H. Shirvani^{4,5}, M. Zanini^{4,5}, W.-J. Wang⁶, J.-W. Tsai^{3,7}, O. Ayrault^{4,5}**¹*Institute of Biomedical Science, Academia Sinica, Taipei, Taiwan*²*TIGP in Molecular Medicine, Academia Sinica and National Yang-Ming University, Taipei, Taiwan*³*Institute of Brain Science, National Yang-Ming University, Taipei, Taiwan*⁴*Institute of Curie, PSL Research University, Orsay, France*⁵*Universite Paris Sud, Universite Paris-Saclay, Orsay, France*⁶*Institute of Biochemistry and Molecular Biology, College of Life Sciences, National Yang-Ming University, Taipei, Taiwan*⁷*Brain Research Center, National Yang-Ming University, Taipei, Taiwan*

Development of the cerebellum requires the primary cilium to allow the transduction of Sonic Hedgehog (SHH) signaling. Besides, precise regulation of ciliogenesis ensures the proliferation of cerebellar granule neuron progenitors (GNPs). By mosaic manipulation of cerebellum through *in vivo* neonatal electroporation, we report that Atoh1, a transcription factor required for GNPs formation, controls the presence of primary cilia, maintaining GNPs responsive to the mitogen SHH. Loss of primary cilia abolishes the ability of Atoh1 to keep GNPs in proliferation. Taking advantage of the *in vitro* GNP purification, we show that Atoh1 promotes ciliogenesis by transcriptionally regulating Cep131, which facilitates centriolar satellite (CS) clustering to the basal body. Importantly, ectopic expression of Cep131 counteracts the effects of Atoh1 loss in GNPs by restoring proper localization of CS and ciliogenesis. Moreover, Atoh1 enhances SHH signaling in GNPs by translocating Smo within the primary cilium, thereby activating the downstream effectors. This Atoh1-CS-primary cilium-SHH pro-proliferative pathway is also conserved in SHH-type medulloblastoma, a pediatric brain tumor arising from the GNPs. Together, our data reveal the mechanism whereby Atoh1 modulates the primary cilium functions to regulate GNP differentiation during cerebellar development.

MTU05-05

A *de novo* mutation of CEP170 leads to neuronal migration defects and human lissencephaly**N.-H. Chao¹, Y.-S. Chang¹, M.-H. Tsai², J. Tsai¹**¹*National Yang Ming University, Institute of Brain Science, Taipei, Taiwan*²*Kaohsiung Chang Gung Memorial Hospital, Department of Neurology, Kaohsiung, Taiwan*

During cortical development, postmitotic neurons migrate along the radial fiber to organize the six-layered neocortex. In the process of neuronal migration, centrosome moves ahead of the nucleus into the leading process by the pulling forces of cytoplasmic dynein through the microtubule network. Defects in neuronal migration have been found to cause human brain disorder, lissencephaly (smooth brain). Here we identified a *de novo* mutation in the centrosomal protein CEP170 in a lissencephaly patient. CEP170 is localized on the subdistal appendage of the mother centriole; however, its role in brain development and how the mutation caused lissencephaly are still unclear. To investigate the function of

CEP170 in migrating neurons, we delivered CEP170 shRNA into the embryo mice by *in utero* electroporation to knock down CEP170 expression in progenitor cells. CEP170 dysfunction led to neuronal migration delay and presented the abnormal morphology in their leading process at postnatal day 6. The mutant CEP170 showed decreased localization to the centrosomes in culture cells. Immunoprecipitation also showed less interactions of CEP170 mutant with CCDC120 and CCDC68, two proteins that have been shown to recruit CEP170 to the subdistal appendage hierarchically. Since CEP170 has been shown to play a key role in microtubule organization, these results suggest that failure of CEP170 in centrosomal localization may lead to migration defect. Our findings reveal the role of CEP170 in cortical development and provide novel mechanisms of the pathogenesis of lissencephaly.

MTU05-06

Acute and chronic neurological consequences of neonatal Zika virus infection in mice**I. N. D. O. Souza¹, P. Frost^{1,2}, J. França², J. Nascimento-Viana¹, R. Neris³, C. Nogueira¹, G. Neves², L. Chimelli⁴, F. De-Felice^{5,6}, S. Ferreira^{5,7}, I. Assunção-Miranda³, C. Figueiredo¹, A. D. Poian⁵, J. Clarke¹**¹*Federal University of Rio de Janeiro, Faculty of Pharmacy, Rio de Janeiro, Brazil*²*Federal University of Rio de Janeiro, Institute of Biomedical Sciences, Rio de Janeiro, Brazil*³*Federal University of Rio de Janeiro, Institute of Microbiology, Rio de Janeiro, Brazil*⁴*State Institute of Brain, Laboratory of Neuropathology, Rio de Janeiro, Brazil*⁵*Federal University of Rio de Janeiro, Institute of Medical Biochemistry, Rio de Janeiro, Brazil*⁶*Queen's University, Department of Biomedical and Molecular Sciences, Kingston, Canada*⁷*Federal University of Rio de Janeiro, Institute of Biophysics, Rio de Janeiro, Brazil*

Prenatal Zika virus (ZIKV) infection is associated to several birth defects. However, how ZIKV affects the developing brain long term is poorly understood. This study investigates whether neonatal ZIKV infection leads to neurological changes in immunocompetent mice throughout their lifespan. For such, Swiss mice were infected subcutaneously with a Brazilian ZIKV strain at P3. ZIKV-infected group showed brain viral replication, persistent lower body weight and mortality rates of ~60%. Cytokine expression indicated extensive proinflammatory profile, further characterized through immunohistochemistry. Moreover, ZIKV caused postnatal microcephaly and motor deficits throughout the lifespan. During the acute phase of infection, mice developed seizures, which were reduced by TNF- α inhibition. During adulthood, ZIKV replication persisted and the animals showed increased susceptibility to chemically induced seizures, neurodegeneration and behavioural deficits. Altogether, we show that neonatal ZIKV infection has long-term neurological complications in mice and that early inhibition of TNF- α prevent the development of some chronic neurological abnormalities.

MTU05-07

VPA treatment in neurosphere culture: an approach towards *in vitro* modelling of autism**S. Dwivedi, Y. Perumal***Birla Institute of Technology and Sciences BITS- Pilani Hyderabad Campus, Department of Pharmacy, Hyderabad, India*

Autism Spectrum Disorder (ASD) is a complex neurodevelopmental disorder of early onset, highly variable in its clinical presentation. Although animal models for autism already exist, but gives little idea about the mechanism of disease progression during brain development. *In utero* valproic acid (VPA) exposure in rodents leads to behavioral phenotypic changes related to ASD in their offspring. Therefore, we have tried similar approach of VPA exposure to neurospheres (*in vitro*). We have investigated whether direct VPA exposure on neurospheres may recapitulate the molecular alterations seen *in vivo*. Neuronal precursor cells isolated from time pregnant SD rats were allowed to generate free floating neurosphere. Neurosphere were treated with VPA (0.5 mM, 1 mM and 2 mM) for 7 days with daily observation. The neurospheres were investigated for size and proliferation, LDH release, gene expression and differentiation studies. VPA exposure in neurospheres do not cause alteration in LDH release indicating no cytotoxicity of VPA (up to 2 mM), however a decrease in proliferation of neurospheres followed by disrupted differentiation pattern, and significant alteration in expression level of high risk genes for autism was observed with 3 days of VPA treatment. We have also observed the improvement in neurosphere proliferation and differentiation by co-treatment of VPA with few herbal drugs, which supports use of neurosphere for preliminary screening of novel candidate molecules. This approach requires one or two animals at a time, thus, reduces the number of animals in an experiment. Although, our study gives an insight to understand the mechanism of VPA induced molecular changes in ASD, still the approach needs further exploration to validate utility of neurospheres as a high throughput screening tool for target specific molecules.

MTU05-08

Temporal changes in the brain in neonatal hydrocephalic mice: structural and neurobehavioural findings**O. Femi-Akinlosotu¹, A. Naicker², T. Shokunbi¹**¹*University of Ibadan, Department of Anatomy, Ibadan, Nigeria*²*University of KwaZulu-Natal, Optics & Imaging Centre, Durban, South Africa*³*University of Ibadan, Department of Surgery, Ibadan, Nigeria*

In hydrocephalus, there is accumulation of cerebrospinal fluid in the ventricles and subarachnoid space. The impact of this on neurobehaviour and structure of cellular organelles of pyramidal neurons and their synapses in neonatal hydrocephalic mouse brain overtime are not fully understood.

Hydrocephalus was induced in day-old mice by intra-cisternal injection of sterile kaolin suspension. The pups were tested for reflex developments prior to sacrifice on postnatal days 7,14,21. Cortical thickness and neuronal density in the sensorimotor cortex were evaluated using hematoxylin and eosin and Nissl stains while ultrathin stained sections were also assessed.

Surface righting reflex (3.08 ± 0.48 vs 1.27 ± 0.16 ; 2.49 ± 0.10 vs 1.06 ± 0.05) and cliff avoidance activities (17.15 ± 2.18 vs 10.50 ± 2.00) were significantly impaired in hydrocephalic pups. The cortical thickness (μm) of hydrocephalic mice was significantly reduced on PND7 (2409 ± 43.37 vs 3752 ± 65.74), PND14 (2035 ± 322.10 vs 4273 ± 67.26) and PND21 (1676 ± 33.90 vs 4945 ± 81.79) compared to controls. Compared with age-matched controls ($129.60 \pm 3.72 \times 10^{-6} \mu\text{m}^2$; $230.0 \pm 44.1 \times 10^{-6} \mu\text{m}^2$), the neuronal density of the sensorimotor cortex in hydrocephalic mice was significantly increased on PND14 ($157.70 \pm 21.88 \times 10^{-6} \mu\text{m}^2$) and PND21 ($373.20 \pm 21.54 \times 10^{-6} \mu\text{m}^2$). The TEM of the hydrocephalic mice brains showed loss of structural integrity of cellular organelles and depletion of synaptic junctions. The synaptic densities (per $\mu\text{m}^2 \times 10^{-5}$) of hydrocephalic mice were significantly lower (188.0 ± 22.67 ; 120.0 ± 21.68 ; 72.0 ± 0.66) than their age-matched controls (336.0 ± 37.09 ; 486.0 ± 18.60 ; 600.0 ± 17.61) on days 7, 14 and 21 respectively.

The quantitative changes and ultrastructural findings seen in the neuronal population of the hydrocephalic mice may provide supportive data for the structural basis of the neurological disabilities associated with neonatal hydrocephalus.

MTU05-09

A link between temporal competence and reprogramming**M. Fries^{1,2}, P. Mattar^{1,3}, M. Cayouette^{1,2}**¹*IRCM, Cellular Neurobiology, Montreal, Canada*²*University of Montreal, Molecular Biology, Montreal, Canada*³*Ottawa Hospital Research Institute, Regenerative Medicine, Ottawa, Canada*

The production of desired cell types via cellular reprogramming has generated intense interest among researchers. Many reprogramming phenomena depend on transcription factors with unusual potency, but it remains largely unclear why particular transcription factors possess reprogramming activity while others do not. We have previously shown that the transcription factor *Ikzf1* encodes the early competence of neural progenitors in the retina and neocortex. Since *Ikzf1* was sufficient to expand the potential of neural progenitors, we wondered whether the induction of competence shared properties with reprogramming. To test this idea, we compared the activities of *Ascl1* (*Mash1*) and *Pou3f2* (*Brn2*) to *Ikzf1*. Surprisingly, like *Ikzf1*, overexpression of *Ascl1* or *Pou3f2* reset the developmental clock of transfected progenitors, causing the production of early-fate retinal ganglion cells at inappropriate postnatal stages. The induction of ganglion cell production occurred in opposition to the natural roles of *Ascl1* and *Pou3f2* in retinal development, and was dependent on high-level overexpression. Similarly, like *Ascl1* and *Pou3f2*, when *Ikzf1* was expressed in fibroblasts, they were directly reprogrammed to the neuronal fate. These data demonstrate that artificial reprogramming phenomena can also occur within lineages, perhaps by co-opting natural programs for regulating neural progenitor potential. Our study also points to the requirement for careful interpretation of gain-of-function data in developmental studies.

MTU05-10

Glial cells missing 1 promote cell differentiation and angiogenesis by growth factor expression**Y. Hayashi¹, S. Fuke¹, Y. Go², A. Abdullah¹, T. Fuchigami¹, N. Morimura¹, N. Koyama¹, S. Hitoshi¹**¹*Shiga University of Medical Science, Integrative Physiology, Shiga, Japan*²*National Institutes of Natural Sciences, Exploratory Research Center on Life and Living Systems, Aichi, Japan*

Glial cell missing (*gcm*) plays a critical role in glial cell development in *Drosophila*. Overexpression of *Gcm1* in the mammalian embryonic brain was shown to promote the differentiation of neural precursor cells into astrocytes. On the other hand, when the brain was injured, a lot of astrocytes proliferate and play a key role in the repair. Here, we show that the *Gcm1* was upregulated in the brain 3 days after cold injury. To determine the function of *Gcm1*, we performed *in utero* electroporation studies to overexpress *Gcm1* together with *Gfp* in neural precursor cells at E14.5 and analyzed at E17.5. The *Gcm1* significantly promoted the emergence of GFAP(+) and S100β(+) astrocytes. Next, we investigated the differentiation into oligodendrocyte lineage cells by immunostaining the *Gcm1* electroporated brains. The number of Olig2(+) cells, an oligodendrocyte lineage marker, increased both in GFP(+) and in GFP(-) populations. In the *Gcm1* electroporated brain at postnatal day 14, a large number of cells positive for GST-π, a marker of mature oligodendrocytes, were observed. These results suggested *Gcm1* overexpression promoted both astrocytic and oligodendrocytic differentiation in the embryonic brain. Furthermore, we also noticed that *Gcm1* overexpression resulted in robust angiogenesis. Interestingly, the astrocytic differentiation and angiogenesis were regulated by LIF, VEGFA, and VEGFC secretion. Thus, considering that brain injury requires gliogenesis and angiogenesis for the repair, our results suggest that modification of *Gcm1* expression could be a new therapeutic strategy for the perinatal brain injury.

MTU05-11

Nigella sativa oil ameliorates the effects of early weaning on the cerebellum of wistar rats**R. Jaji-Sulaimon, M. Adekunle, I. Gbadamosi, G. Omotoso***University of Ilorin, Anatomy, Ilorin, Nigeria*

Early weaning has become a common practice among nursing mothers in different communities around the world and may be one of the leading causes of neuronal degeneration. This study aimed at investigating the effects of *Nigella sativa* oil (NSO) on the cerebellum of early weaned Wistar rats. We hypothesize that NSO will attenuate the effects of early weaning on the cerebellum of wistar rats.

Rats were divided into normal weaned (NW) and Early Weaned (EW) groups, weaned on post natal day (PND) 28 and 18 respectively and EW+NSO group, weaned on PND 18 and administered 25 ml/kg NSO. Exploratory activities were tested using the open field test (OFT). All experimental animals were sacrificed on PND 35. Cerebelli were processed for light microscopy. We estimated levels of malondialdehyde (MDA), Glutathione peroxidase (GPx) and Superoxide dismutase (SOD). Data were analysed using one-way analysis of variance. All animals received humane care in compliance with the regulations of the

University of Ilorin Ethical Review Committee and best international practice.

Exploratory activities of the EW rats were significantly pronounced when compared to rats in the NW and EW+NSO groups. Biochemical analysis showed that NSO ameliorated early weaning-induced oxidative stress as indicated by increased GPx and SOD levels in the NSO treated group. Lipid peroxidation was also attenuated as evidenced by reduced expression of MDA. Early weaning caused several alterations in cerebellar cytoarchitecture. However, the group treated with NSO showed less alterations and a uniform cytoarchitecture similar to the control NW group.

Data from this study showed that oral administration of NSO attenuated early weaning-induced anxiety, cerebellar oxidative stress and neural tissue damage.

MTU05-12

LIS1 alterations drive distinct epigenetic, post-transcriptional & chromatin accessibility modes to resolve lineage commitment**A. Kshirsagar, T. Olender, J. Hanna, O. Reiner***Weizmann Institute of Science, Department of Molecular Genetics, Rehovot, Israel*

LIS1 mutations and deletions have been associated with Lissencephaly; a condition wherein the cerebral cortices of patients assume smooth shape. The *LIS1* protein is involved in several key functions including cell proliferation and neuronal migration, and is involved in the regulation of the molecular motor cytoplasmic dynein and the cytoskeleton. Increase in the dosage of the *LIS1* gene also causes mild brain malformations and developmental delay. During early development, following embryonic day 3.5, regulation of RNA at the transcriptional and post transcriptional levels plays a pivotal role in the regulation of pluripotency and differentiation. *Lis1* knockout mice are early embryonic lethal and the role of this protein during early development together with its function in the nucleus still remains to be elusive.

Immunostaining of mouse wild type blastocysts affirmed that *LIS1* co-localizes predominantly in inner cell mass cells. Here, in this study, *LIS1* is detected in the nucleus of mammalian embryonic stem cells in association with chromatin embedded proteins, and most notably, chromatin modifiers, the RISC complex and splicing factors. Multiomic studies using *Lis1* mutant mouse ES cells (mESCs) showed that *LIS1* together with ribonucleoprotein complexes physically associate with chromatin accessibility, alternative splicing and non-coding RNA regulation. Further, *LIS1* mutant human embryonic stem cell (hESCs) lines were generated using CRISPR/Cas9 genome editing. We developed a novel on-chip platform to grow 3D cortical organoids from mutant hESCs and modelled growth with reduced folding. Extra cellular matrix (ECM) related genes were differentially expressed when wild-type and *LIS1* +/- organoids were compared at different growth stages.

Our study reveals novel molecular roles of *LIS1* in ESCs and during early stages of brain development, and provide a model system to understand the crucial mechanism associated with Lissencephaly.

MTU05-13

The function of KLF5 gene in adult brain**A. Kuroda¹, T. Fuchigami¹, N. Morimura¹, Y. Hayashi¹, N. Koyama¹, M. Ema², S. Hitoshi¹**¹*Shiga University of Medical Science, Integrativephysiology, Otsu, Japan*²*Shiga Medical University, Depart of Stem Cells and Human Disease Models, Otsu, Japan*

Klf5, one of the *Krüppel*-like factor (*Klf*) family genes, is an ortholog of *Drosophila melanogaster* gene *Krüppel*. It is a transcription factor involved in cellular differentiation or proliferation during mammalian development. *Klf5* deficient embryos are lethal at blastocyst stage showing defective trophectoderm development and implantation failure. Our previous data suggested that *Klf5* gene promotes the cell proliferation of neural precursor cells (NPCs) in the developing mice brain. In this study, we analyzed the function of *Klf5* in adult NPCs. Adult neural stem cells (NSCs) reside only in the subependymal zone (SEZ) and the subgranular zone of the dentate gyrus (DG). In these regions, the adult NSCs, which have the self-renewal and multipotent capabilities, are maintained in quiescent state. To investigate roles of *Klf5* in the adult brain, Nestin-Cre::CAGstop*Klf5* mice were generated, in which neural precursor-specific recombination induces the *Klf5* overexpression. We perform a neurosphere assay using cells derived from the SEZ of *Klf5* overexpressing mice's brain. Contrary to our expectation, the number of neurosphere was decreased. We evaluated the incorporation of BrdU at DG and found that incorporated BrdU was decreased in *Klf5* overexpressing mice's brain. *Klf5* overexpression mice showed small body weight and behavioral abnormality. Now, we are trying to evaluate the molecular mechanisms underlying these phenotypes.

MTU05-14

A hierarchy of beta-spectrins is required for maintenance, but not assembly, of axonal sodium channels clustering
C.-H. Liu¹, S. Ha², R. Seo³, P. Mohler⁴, J. Noebels³, M. Stankewich⁵, M. Rasband^{1,2}¹*Baylor College of Medicine, Program in Developmental Biology, Houston, USA*²*Baylor College of Medicine, Department of Neuroscience, Houston, USA*³*Baylor College of Medicine, Department of Neurology, Houston, USA*⁴*The Ohio State University College of Medicine, Departments of Physiology and Cell Biology and Internal Medicine, Columbus, USA*⁵*Yale University, Department of Pathology, New Haven, USA*

Highly-concentrated ion channels at axonal excitable domains including axon initial segments (AIS) and nodes of Ranvier are necessary to transmit action potentials. Cytoskeletal protein β IV-spectrin is proposed to stabilize voltage-gated sodium (Nav) channels at nodes, and β I-spectrin is reported to preserve this function after losing β IV-spectrin. However, patients carrying β IV-spectrin mutants showed epilepsy and intellectual disability, while their peripheral sensory functions were intact, suggesting β I-spectrin plays distinctive compensatory roles in the CNS and PNS. Furthermore, the necessity of β -spectrins for Nav channels clustering at axons are unknown. To determine the function of β I and β 4-

spectrin in the nervous system, we generated mice lacking these proteins in the CNS and PNS neurons, respectively. Mice lacking both β I/ β 4-spectrin in PNS showed ataxia and impaired action potential conduction. With increasing age, there was progressive loss of nodal Nav channels. Losing β I-spectrin in the CNS showed normal AIS integrity while losing β 4-spectrin reduced Nav channel intensity. Unexpectedly, β I-spectrin was only detected at the AIS of parvalbumin interneurons in β 4-spectrin deficient mice. Mice lacking both β I/ β 4-spectrin showed severe seizures. These data suggest that β -spectrins are necessary to maintain Nav channels at axonal excitable domains. Furthermore, β 4-spectrin is the primary stabilizer, while β I-spectrin performs secondary functions in a context-dependent manner.

MTU05-15

Cannabidiol, capsaicin and the multiple fates of neural stem cells**D. Lourenco^{1,2}, R. Soares^{1,2}, R. Rodrigues^{1,2}, A. Sebastião^{1,2}, S. Xapelli^{1,2}**¹*Instituto de Farmacologia e Neurociências, FMUL, Lisboa, Portugal*²*iMM - JLA, FMUL, Lisboa, Portugal*

Adult neural stem/progenitor cells (NSPC) with multipotent and self-renewing properties can be mostly found in two neurogenic niches, the Subventricular Zone (SVZ) and the Dentate Gyrus (DG) of the hippocampus. Cannabinoids have been shown to play pivotal roles in different neurogenic stages, namely in differentiation and maturation of NSPC. Cannabidiol (CBDV), a non-psychoactive phytocannabinoid, homolog of cannabidiol, with high affinity for the vanilloid receptor 1 (TRPV1), is a potential candidate for therapeutic use. Therefore, we aimed at unravelling the role of CBDV on SVZ postnatal neurogenesis. SVZ neurospheres were prepared from C57BL/6J (WT) mice pups (P1-3) and were incubated for 2 days with CBDV and Capsaicin (TRPV1 agonist), according to the experimental condition. Three groups were tested: 1) control (no drugs); 2) CBDV (100 nM; 300 nM; 1 μ M); 3) Capsaicin (3 μ M; 10 μ M; 30 μ M). Immunocytochemistry against mature neurons (NeuN) and oligodendrocyte progenitor cells (NG2) was used to evaluate the effect of CBDV and Capsaicin on cell differentiation. Here we show that SVZ neurospheres treated for 2 days *in vitro* with 1 μ M of CBDV have a tendency ($p = 0.07$) for an increase in the number of NeuN-positive cells and a significant increase ($p < 0.05$) with 30 μ M Capsaicin. Regarding NG2-positive cells, only SVZ neurospheres treated with 10 μ M Capsaicin showed a tendency ($p = 0.08$) for an increase in number of oligodendrocyte progenitor cells. These results show that CBDV and capsaicin have differential effects on SVZ cell differentiation. This work will allow determining, *in vitro*, whether the activation of TRPV1, through CBDV or Capsaicin, can modulate postnatal neurogenesis and will be important for future brain repair strategies.

MTU05-16

Analysis of TRPC5 expression in developing retina**O. Mai, I. Yasuki, S. Koji***Gunma University Graduate School of Medicine, Department of Molecular and Cellular Neurobiology, Maebashi, Japan*

Transient receptor potential canonical 5 (TRPC5) is a non-selective cation channel, which is activated by various stimuli. TRPC5 suppresses axonal outgrowth through its activation in hippocampal neurons. On the other hand, we previously reported that transient receptor potential vanilloid 2 (TRPV2) promotes axonal outgrowth in developing sensory neurons. Thus, opposing effects of TRPV2 and TRPC5 might modulate sensory nerve growth as a positive and a negative regulator, respectively.

In this study, we examined whether TRPC5 activation is involved in axonal outgrowth as in the case of hippocampal neurons. We first determined the timing of TRPC5 expression using *in situ* hybridization (ISH) with retinal tissue sections from E12.5 to adult. The expression was not detected at E12.5, but started at E14.5. The signal of TRPC5 mRNA became strong in differentiated cell layers such as the ganglion cell layer from E16.5 to adult. By utilizing double-immunostaining of TRPC5 and retinal cell type specific markers for retinal ganglion cells (RGC), amacrine cells (AC), bipolar cells (BP), horizontal cells (HC) and Müller cells, we next identified which cell type expresses TRPC5 protein. Consequently, the protein expression was consistent with the mRNA expression pattern, indicating that our ISH probe specifically detected TRPC5. Among retinal cells, RGC and AC selectively expressed the TRPC5 throughout the development. Since the peak timing of RGC axonal elongation is from E11.5 to P0, and TRPC5 expression started from E14.5, we hypothesize that the TRPC5 activation regulates the RGC axonal outgrowth during retinal development. Currently, we analyze whether TRPC5 regulates axonal outgrowth in embryonic retinal explants treated with TRPC5 antagonist. Possible mechanism of TRPC5-regulated optic nerve growth during development will be discussed.

MTU05-17

Cerebellar development and function in neonatal rats following intrauterine and postnatal exposure to caffeine
F. Olopade¹, T. Shokunbi^{1,2}¹*University of Ibadan, Department of Anatomy, Ibadan, Nigeria*²*University of Ibadan, Department of Surgery, Ibadan, Nigeria*

Caffeine is commonly consumed in pregnancy and also used therapeutically in the management of apnea in preterm babies. Moderate to high maternal consumption of caffeine is associated with detrimental neurological effects in the newborn. However, there is insufficient information on its effects on the structural and functional development of the cerebellum. This study investigates the effect of perinatal caffeine consumption in rat dams, on neurobehavioral and structural cerebellar development of their offsprings.

Pregnant rats received 50 or 100 mg/kg day of caffeine (CAF50, CAF100) by gavage throughout pregnancy and three weeks postnatal while the controls had sterile water. Post-delivery, the dams nursed their pups and after three weeks, the pups underwent neurobehavioral tests: cliff aversion for sensorimotor reflex development, negative geotaxis for motor coordination, and forearm grip strength test for muscular strength. By serial sacrifice from Day 19

to 21, morphological assessment of the cerebellum's development was assessed by measuring the external granular layer (EGL) thickness and the cellular density within the molecular layer.

Development of motor reflexes and coordination appeared slightly earlier in CAF50 pups than controls, but significantly delayed in CAF100 pups. Muscular strength in CAF50 pups was comparable to controls, but reduced in CAF100 pups. The EGL was consistently thicker and number of transiting cells in the molecular higher in CAF100 pups than controls, from Day 19-21.

High maternal caffeine consumption delays the migration of the cells of the EGL into the granular layer and therefore retards the development of this layer in their offsprings. We propose there is a relationship between this developmental delay and retarded neurobehavioural functions observed.

MTU05-18

Cannabinoids, adenosine A2A receptors and postnatal neurogenesis**R. Rodrigues^{1,2}, A. Armada-Moreira^{1,2}, F. Ribeiro^{1,2}, A. M. Sebastião^{1,2}, S. Xapelli^{1,2}**¹*Instituto de Farmacologia e Neurociencias, FMUL, Lisboa, Portugal*²*IMM - JLA, FMUL, Lisboa, Portugal*

Postnatal neurogenesis operates in specialized niches of the mammalian brain in a process modulated by cannabinoid type 1 and 2 receptors (CB1R and CB2R). Recent evidence sheds light on the interaction of adenosine A2A receptors (A2AR) with cannabinoid receptors. Herein, we aimed at understanding the putative role of A2AR on cannabinoid-mediated cell fate, cell proliferation and neuronal differentiation of rat neonatal subventricular zone (SVZ) and dentate gyrus (DG) neurospheres. CB2Rs or A2AR activation was found to promote self-renewing divisions of DG cells. Importantly, A2AR antagonist blocked the effect mediated by CB2R activation, while CB1R or CB2R antagonists blocked A2AR-mediated effect. SVZ cell proliferation was only affected by CB1R activation, an effect blocked in the presence of an A2AR antagonist. Although CB1R, CB2R or A2AR activation alone did not alter DG cell proliferation, CB1R or CB2R co-activation with A2ARs promoted a significant increase in DG cell proliferation. Lastly, CB1R and/or CB2R activation promoted SVZ and DG neuronal differentiation, while A2AR activation only promoted DG neuronal differentiation. In both cases, the proneurogenic effect mediated by CB1R or CB2R agonists was blocked by an A2AR antagonist, while in DG the A2AR-mediated actions on neuronal differentiation were blocked by CB1R or CB2R antagonists. Taken together, our findings suggest an interaction between the adenosinergic and cannabinergic systems, cross-antagonism being evident, responsible for controlling early stages of postnatal neurogenesis.

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MTU05-19

PI3K signalling in trans-resveratrol mediated prevention of monocrotophos damaged neuronally differentiating human stem cells

S. Singh, S. Jahan, A. B. Pant

CSIR-Indian Institute of Toxicology Research, Developmental Toxicology Division, Lucknow, India

The role of Resveratrol(RV) as a neuroprotectant is well recognized and cellular molecules involved in imparting the physiological effect have been well illustrated. However, some ambiguity still prevails as the specific receptor and downstream signaling molecules are not yet clearly stated. So, we investigated the signaling pathway(s) involved in its cellular protection in the human umbilical cord blood mesenchymal stem cells (hUCB-MSCs) derived neuronal cells. The mesenchymal stem cells were exposed to various concentrations (10, 100, 1000 μ M) of Monocrotophos (MCP), a known developmental neurotoxic organophosphate pesticide, for a period of 24 h. The MAPK signaling pathways (JNK, p38, and ERK) known to be associated with MCP induced damages were also taken into consideration to identify the potential connection. The biological safe dose of RV (10 μ M) shows a significant restoration in the MCP induced alterations. Under the specific growth conditions RV exposure was found to promote neuronal differentiation in the hUCB-MSCs. The exposure of cells to a specific pharmacological inhibitor (LY294002) of PI3K confirms the significant involvement of PI3K mediated pathway in the ameliorative responses of RV against MCP exposure. Our data identifies the substantial role of RV in the restoration of MCP induced cellular damages, thus proving to have a therapeutic potential against organophosphate pesticides-induced neurodegeneration.

MTU05-20

The role of NPRL2 and NPRL3 in neural development and disorders

S.-Y. Yeh¹, Y.-T. Liu², J.-W. Tsai¹

¹*National Yang-Ming University, Institute of Brain Science, Taipei City, Taiwan*

²*Taipei Veterans General Hospital, Neurological Institute, Taipei City, Taiwan*

The neurodevelopmental disorder focal cortical dysplasia (FCD) is the most common cause of medically refractory epilepsy in children. Several genes have been identified to be involved in the pathogenesis of this disease, including NPRL2, NPRL3 and DEPDC5, the components of GATOR1 complex. GATOR1 complex acts as a negative regulator of mTORC1 in mTOR signaling pathway, which regulates cell growth, metabolism, autophagy, and proliferation. Although mutations in these genes have been reported to cause FCD and focal epilepsy, the functions of NPRL2/3 in neural development is still not fully understood. To investigate the roles of NPRL2/3 in cortical development, we delivered shRNA by *in utero* electroporation (IUE) to knock down NPRL2/3 in neural progenitors of mouse embryos. We found that NPRL2/3 knockdown during development caused neuronal migration delay. Furthermore, we observed morphological changes of dendritic spines in the NPRL2/3-knockdown neurons in postnatal mice. Meanwhile, we identified potential novel mutations on NPRL2 and NPRL3 in patients with focal epilepsy. To study whether these mutations may cause neuronal defects, we electroporated wild type or mutant NPRL2/3 into mouse embryonic neural progenitors. However, expression of these mutants did not cause apparent neural migration defects. Our study may help us understand the roles of NPRL2/3 in neuronal development and provide information for developing effective treatment to NPRL2/3-related neural developmental disorders.

MTU06 Bioenergetics & metabolism (Session A)

MTU06-01

ATP-citrate lyase (ACLY) is a key element of brain energy metabolism

L. Achanta¹, D. Thomas², G. Housley¹, C. Rae¹

¹UNSW, SOMS, Sydney, Australia

²UNSW, MWAC, Sydney, Australia

ATP-citrate lyase (ACLY) is the key enzyme generating cytosolic acetyl-CoA and oxaloacetate from citrate. Widely expressed in the brain, its role in healthy brain energy metabolism needs elucidation. We inhibited ACLY with BMS-303141 (3,5-Dichloro-2-hydroxy-N-(4-methoxy[1,1'-biphenyl]-3-yl)-benzene-sulfonamide), at 1 μ M (IC₅₀) and 10 μ M applied to guinea pig cortical brain tissue slices that were incubated with 2 mM [2,3-¹³C] pyruvate, 2 mM [3-¹³C]lactate, or 0.25 mM [U-¹³C] β -hydroxybutyrate (β OHB) for 60 minutes, or with 5 mM [1-¹³C]glucose alone, or 0.5 mM [1,2-¹³C]acetate and 5 mM [1-¹³C]glucose for 90 minutes. The brain slices were extracted with methanol/chloroform, lyophilised and reconstituted in D₂O. ¹H, {¹³C}-decoupled ¹H, and {¹H}-decoupled ¹³C NMR spectra were acquired from each sample (N = 4). The resultant isotopomers and metabolite pools were quantified. Inhibition of ACLY using 1.0 μ M BMS-303141 with pyruvate, lactate, or β OHB as substrates resulted in increased incorporation of label into Krebs cycle intermediates and glycolytic by-products, showing that normal ACLY activity results in a significant efflux of label from the Krebs cycle. When [1-¹³C]glucose was the sole substrate, total metabolite pools and net flux of ¹³C into Krebs cycle intermediates and the glycolytic by-products lactate and alanine were significantly reduced. This effect was "rescued" by 0.5 mM [1,2-¹³C]acetate, where 1.0 μ M BMS-303141 resulted in increased incorporation of label from both glucose and acetate. BMS-303141 at 10 μ M had limited further effects in all cases, suggesting that the ability of the system to accommodate further ACLY inhibition is limited. These results indicate that the impact of ACLY activity on Krebs cycle flux is significant, although it is currently not a part of most models of brain metabolism.

MTU06-02

Metabolic impairments in neurons and astrocytes derived from human induced pluripotent stem cells of Alzheimer's disease patients

B. A. García¹, C. Salcedo¹, P. Hyttel², H. Waagepetersen¹, K. Freude²

¹University of Copenhagen, Drug Design and Pharmacology, Copenhagen, Denmark

²University of Copenhagen, Veterinary and Animal Sciences, Copenhagen, Denmark

Metabolic impairments are one of the earliest cerebral pathogenic events in Alzheimer's disease (AD). In order to investigate the underlying mechanisms of these metabolic alterations, we studied cellular energy and amino acid metabolism in neurons and astrocytes derived from human induced pluripotent stem cells (hiPSC) obtained from AD patients and their respective CRISPR/Cas9 gene edited controls or age-matched controls. Cultures of hiPSC-derived neurons and astrocytes from AD patients and

isogenic or aged-matched controls were incubated with ¹³C-labeled energy substrates, enrichment in metabolites was determined using gas chromatography coupled to mass spectrometry, followed by metabolic mapping and Western blot analyses. Mitochondrial function was assessed via the Seahorse XFe96 Analyzer. AD neurons displayed changes in enzymes associated with glutamine and glutamate processing. Specifically, phosphatase-activated glutaminase (PAG) and aspartate amino transferase (AAT) were up regulated in AD neurons. The observed increase of PAG resulted in increased levels of glutamate converted from glutamine, which is expected to trigger excitotoxicity, one of the early pathological hallmarks in AD neurons. Similarly, increased levels of ATT resulted in increased conversion of aspartate to oxaloacetate fuelling the tricarboxylic acid cycle (TCA). Interestingly, a decreased mitochondrial respiratory function was observed in AD hiPSC-derived neurons, suggesting that an overactive TCA cycle might be a compensatory mechanism. The metabolic studies of astrocytes revealed increases in Lactate production, which could point towards a compensatory mechanism to counteract the increased glutamate levels secreted from AD neurons and thereby combatting excitotoxicity.

MTU06-03

Menadione-mediated WST 1 reduction as indicator for the metabolic potential of cultured astrocytes

E. Ehrke¹, J. Steinmeier^{1,2}, C. Protzel¹, R. Dringen^{1,2}

¹University of Bremen, Centre for Biomolecular Interactions, Bremen, Germany

²University of Bremen, Center for Environmental Research and Sustainable Technology, Bremen, Germany

The water-soluble tetrazolium salt 1 (WST1) is frequently used as indicator of the metabolic potential of cultured cells and to determine cell viability. The presence of the membrane-permeable electron cyclor menadione caused an almost linear increase in WST1 formazan with maximal WST1 reduction in the presence of 0.5 mM glucose that was not further stimulated by increasing glucose concentrations up to 10 mM after 30 min, while hardly any reaction was observed in the absence of glucose. Only the hexose mannose was able to fully replace glucose as substrate for WST1 formazan generation but not other sugars nor mitochondrial substrates. Intracellular menadione reduction is catalyzed by a cytosolic enzyme that uses efficiently both NADH and NADPH as electron donor, indicated by similar K_M- and v_{max}- values for both cofactors. Accordingly, application of WST1 and menadione to astrocytes led to rapid depletion of NADH and NADPH. The menadione-mediated WST1 reduction was highly sensitive towards dicoumarol as demonstrated for the enzyme in lysates by an inhibitor constant of approximately 2 nM and by the half-maximal inhibition of the cell-dependent WST1 reduction at around 45 nM dicoumarol. These data demonstrate that the NAD(P)H:quinone acceptor oxidoreductase 1 (NQO1) is involved in cellular menadione reduction which in turn facilitates extracellular WST1 reduction. Interestingly, the amount of WST1 formazan generated matched well with the decrease observed in the lactate release, suggesting that electrons from glycolysis-derived NADH contribute to the menadione-

dependent WST1 reduction. In conclusion, the menadione-dependent WST1 reduction can be used as valuable tool to study the metabolism of cultured brain cells.

MTU06-04

NNT is required for brain mitochondrial redox balance and is highly expressed in nitric oxide synthase and serotonergic neurons

A. Francisco¹, D. Engel¹, J. Ronchi¹, T. Figueira², F. Rogério¹, A. de Bem³, R. Castilho¹

¹University of Campinas, UNICAMP, Campinas, Brazil

²University of São Paulo, USP, Ribeirão Preto, Brazil

³University of Brasília, UnB, Brasília, Brazil

Nicotinamide nucleotide transhydrogenase (NNT) is a protein located in the inner mitochondrial membrane that catalyzes the reduction of NADP⁺ at the expense of NADH oxidation coupled to inward proton translocation from the intermembrane space to the matrix. NNT is known to support NADPH-dependent processes such as peroxide detoxification and reductive biosynthesis. Considering the scarce knowledge about NNT roles in brain, we aimed at investigating NNT activity, distribution and contribution to brain physiology by using congenic mice carrying mutated *Nnt* alleles from the C57BL/6J strain (*Nnt*^{-/-}) or the wildtype *Nnt* (*Nnt*^{+/+}). Biochemical analyses of isolated brain mitochondria showed that the lack of NNT resulted in lower total NADP⁺-reducing activity. In the absence of NNT, a higher mitochondrial H₂O₂ production was detected when the metabolism of respiratory substrates did not favor the flux through other mitochondrial NADPH sources or when the respiratory chain was inhibited. Concerning the spatial distribution of NNT in mouse brain, we observed a higher NNT expression and activity in the pons with increased NNT labeling of neurons in raphe and hindbrain nuclei. Most of the neurons exhibiting strong NNT labeling were also endowed with enzymes involved in biosynthetic pathways for 5-hydroxytryptamine and nitric oxide production, which require NADPH. Behavioral evaluations showed NNT absence is associated with impaired locomotor activity and depressive-like behavior in aged mice, but not in adults. These results indicate that NADPH from NNT activity has relevance for brain mitochondrial redox homeostasis and neurotransmission.

MTU06-05

Integration of microRNAome and metabolomics to dissect cerebral disease progression in X-linked adrenoleukodystrophy

J. Singh, L. Poisson, S. Giri

Henry Ford Health System, Dept. of Neurology, Detroit, USA

Introduction: X-linked adrenoleukodystrophy (X-ALD) is a progressive neurodegenerative disease caused by mutations in peroxisomal ABCD1 gene. X-ALD males develop fatal cerebral demyelinating disease (ALD) the mechanism for which remains unknown. We took a novel multi-omics approach of untargeted metabolomics and next generation sequencing (HiSeq) to find regulatory (microRNA) and active (metabolite) pathways underlying the fatal neuroinflammation in ALD.

Methods: Postmortem brain tissue from healthy controls and ALD patients were processed for microRNA (miRNA) and

metabolite extraction and analysis. Data analysis was performed by “MetaboAnalyst 2.5” for GC-MS and Bioconductor for miRNA.

Results: Each measured miRNA and metabolite was screened using appropriate ANOVA models. Thresholds for significance were set to control the estimated false discovery rate, per platform, at 5%. We compared postmortem brain white matter of healthy controls (CTL) with normal looking area (NLA) and periphery of plaque/lesion (PLS) regions within the ALD brain white matter. Analysis of variance ($p < 0.05$) and Post-hoc t-tests identified nineteen miRNA and eleven metabolites that significantly differed across the three groups (control, NLA and PLS). Of the nineteen miRNA seventeen were increased (PLS > NLA > CTL) and two were decreased (CTL > NLA > PLS). Seven metabolites were upregulated (PLS > NLA > CTL) and four were downregulated (CTL > NLA > PLS). We calculated the Pearson's correlation coefficient between the expression of these nineteen miRNA and the metabolite intensities of eleven metabolites for putative links between the global gene expression modulators (miRNAs), and metabolites.

Conclusion: Our novel “transomic” modeling identifies, for the first time, integrated miRNA and metabolite pathways underlying demyelination in X-ALD.

MTU06-06

Axonal metabolic support and energy dynamics in active white matter tracts

A. Trevisiol¹, K. Kusch¹, J. Hirrlinger^{2,1}, Klaus. A. Nave¹

¹Max Planck Institute for Experimental Medicine, Neurogenetics, Goettingen, Germany

²Carl-Ludwig-Institute for Physiology, Hirrlinger, Leipzig, Germany

In white matter, axonal energy homeostasis critically depends on glial support. Failure in glial-mediated delivery of metabolic substrates into the axonal compartment results in axonal energy deficit and may anticipate the axonal degeneration described in several myelin disorders and neurodegenerative diseases. In mice, neuronal transgenic expression of an ATP-fluorescent sensor allowed us to visualize axonal energy content in acutely isolated optic nerves while simultaneously performing electrophysiological compound action potentials (cAP) recordings. The real-time monitoring of activity-dependent axonal ATP revealed a strong correlation between axonal energy metabolism and nerve conduction. Further on, to determine possible metabolic consequences of myelin defects we monitored ATP and cAP in Plp1^{null/y} optic nerves. Genetic ablation of Plp1, encoding a myelin membrane protein, serves as a model of spastic paraplegia type-2, where an impaired axo-glia unit leads to secondary axonal loss. We found that the energy metabolism of myelinated axons of Plp1^{null/y} optic nerves is perturbed long before the onset of clinical symptoms and major pathological changes. To understand further the role of oligodendroglia and myelin formation in the white matter energy balance, we focused on the metabolic properties of spinal cord sensory fibres *in vivo*, following a long-term FLIM analysis in a model of MS where we could determine the axonal metabolic changes induced by demyelination and remyelination. The parallel monitoring of axonal ATP and cAP is therefore a powerful tool to study white matter metabolism and metabolic support mechanisms under physiological conditions and in models of neurodegenerative disorders.

MTU06-07

The neuroprotective role of 5-methoxyindole-2-carboxylic acid in ischemic stroke injury in rat brain**L.-J. Yan***University of North Texas Health Science Center, Pharmaceutical Sciences, Fort Worth, USA*

This presentation summarizes our findings that 5-methoxyindole-2-carboxylic acid (MICA) can serve as a chemical conditioning agent in neuroprotection against stroke injury and the corresponding underlying mechanisms. MICA is a reversible inhibitor of mitochondrial dihydrolipoamide dehydrogenase that is involved in bioenergetics and metabolism. We performed both MICA preconditioning and postconditioning in the rat brain using an ischemic stroke model. For preconditioning studies, MICA (200 mg/kg body

weight) was mainly administered via diet intake for 4 weeks. For postconditioning studies, MICA (also 200 mg/kg body weight) was injected intraperitoneally at the onset of 24 h reperfusion following 1 h ischemia. Our results indicate that stroked animals treated with MICA either in preconditioning or postconditioning studies showed less brain infarction volume than that of vehicle-treated animals. Common protective mechanisms in both MICA preconditioning and postconditioning studies involve Nrf2 up-regulation of NQO1 expression, decreased oxidative stress, increased mitochondrial membrane integrity, and decreased cell death. Our findings demonstrate that MICA can produce an effective pre- and post-conditioning effects in the ischemic brain of rat and the underlying mechanism likely involves preservation of mitochondrial function, upregulation of cellular antioxidative capacity, and attenuation of oxidative stress.

MTU07 Neuronal plasticity & behavior (Session A)

MTU07-01

Methyl jasmonate mitigates cognitive impairment and loss of neuronal dendritic spines in the brain of chronically stressed mice

O. Aluko¹, A. Pignataro², S. Umukoro¹, M. Ammassari-Teule²

¹University of Ibadan, Department of Pharmacology and Therapeutics/Neuropharmacology, Ibadan, Nigeria

²National Research Council, Institute of Cell Biology and Neurobiology, Rome, Italy

Morphological changes such as retraction of neuronal dendrites and spine loss have been identified as a common consequence of chronic stress on the brain. Methyl jasmonate (MJ) is a bioactive compound and naturally occurring anti-stress plant hormone previously reported with ameliorative effect against acute and chronic stress in mice. This study investigated the effect of MJ on cognitive impairment, dendritic spines, and density of mice subjected to unpredictable chronic mild stress (UCMS).

Mice were chronically stressed for 10 days and intraperitoneally treated with either vehicle or 50 mg/kg MJ. Thereafter, mice were assessed for freezing time in fear conditioning test as a measure of cognitive function. Dendritic morphology of brains using Golgi-Cox staining procedure followed by Sholl analysis and immunohistochemical expression of nrf2 and parvalbumin were also assessed.

Our results revealed that UCMS triggered a significant increase in freezing duration, a decrease in neuronal dendritic spine density, intersection, and length of hippocampal CA1, basolateral amygdala, and prefrontal cortex, with concomitantly increased nrf2 and decreased parvalbumin expressions. MJ significantly attenuated UCMS-induced cognitive dysfunction, alterations in dendritic arborization and protein expression, indicating less susceptibility to stressors and increased neuronal communication.

Due to the safety profile of MJ, it could have a therapeutic outcome in stress-induced cognitive deficits and neuronal alterations. We are currently investigating the molecular mechanisms underlying these effects using western blotting techniques for quantification of BDNF, HSP 70, CREB, and Glut1.

MTU07-02

The role of the orexin (hypocretin) system in context-induced relapse to alcohol seeking

E. Campbell¹, M. Hill¹, N. Marchant², A. Lawrence¹

¹Florey Institute of Neuroscience and Mental Health and The University of Melbourne, Florey, Melbourne, Australia

²VU University Medical Center, Anatomy and Neurosciences, Amsterdam, The Netherlands

Our lab has previously shown a role for orexin signalling via OX1 receptors in cue-induced relapse to alcohol seeking following extinction. However, one shortfall of extinction is that it is experimenter-imposed, and does not model the negative consequences of drug use. Thus, the current study assessed the role of the orexin system in a model of context-induced relapse to alcohol seeking following punishment-imposed voluntary abstinence.

Male iP rats were trained to self-administer 20% alcohol in context A, where alcohol was available without consequence.

Subsequently, rats were then trained in a new context (B) where an active lever press resulted in the delivery of an alcohol reward paired with footshock. Footshock was delivered randomly on 50% of lever presses. Shock intensity was increased across day until responding for alcohol ceased, despite the ongoing availability of alcohol. Rats were then tested with either vehicle or SB-334867 (5 mg/kg, ip) in both contexts A and B.

Rats reliably self-administered alcohol in context A and voluntary abstinence was observed in context B. On relapse test, there was a main effect of treatment [$F_{(1,17)} = 24.8$, $p < 0.0001$] and a treatment x context interaction [$F_{(1,17)} = 5.6$, $p = 0.03$]. Vehicle-treated rats showed relapse to alcohol seeking in context A compared to context B. Pre-treatment with SB-334867 reduced alcohol seeking.

The current study further implicates orexin signalling in alcohol seeking using a preclinical model that may be more reflective of the human experience. Ongoing studies will aim to identify the anatomic loci for this effect.

MTU07-03

Single-cell RNA-SEQ of mouse nucleus accumbens reveals a subtype of D1 medium spiny neurons

W. Chen^{1,2,3}, R. Chen^{1,2,3}, Mohamed. N. Djekidel^{1,2,3}, A. Bhattacharjee^{1,2,3}, Luis. M. Tuesta^{1,2,3}, Y. Zhang^{1,2,3}

¹Harvard Medical School, Department of Genetics, Boston, USA

²Boston Children's Hospital, Program in Cellular and Molecular Medicine, Boston, USA

³Howard Hughes Medical Institute, Boston Children's Hospital, Boston, USA

The nucleus accumbens (NAc) is the most important entry point of the basal reward circuitry and has been suggested to play a crucial role in motivational behaviors. However, our knowledge of its cellular heterogeneity is surprisingly limited. Thus, we performed a high-throughput single-cell RNA-seq (10X genomics) and revealed an underestimated cellular complexity of the NAc. Our data revealed a rich cellular heterogeneity of interneurons and medium spiny neurons (MSNs) in the NAc and a tight relationship between transcriptional features and spatial distribution in different neuron subtypes. As a proof-of-concept, we focused on the MSNs and found that the tachykinin 2 (Tac2)-positive neurons in the NAc is a molecularly distinct subtype of D1-MSNs. To characterize the Tac2 clusters as a subtype of D1-MSNs, we combined multi-color FISH (RNAscope) neuronal tracing and behavioral assays. We found that Tac2 is selectively expressed in the *Drd1*⁺ MSNs but not in the *Drd2*⁺ MSNs, and are preferentially projected to the midbrain. In addition, using chemogenetic, we selected and manipulated the Tac2 cluster and found that activation of the NAc Tac2 clusters potentiated, while inhibition repressed cocaine sensitization. However, such manipulation had no obvious effects on anxiety- and depression-related behaviors. Furthermore, we investigated the role of the NAc Tac2 clusters in mediating reinforcement in a mouse intravenous self-administration (IVSA) and found that inhibition of the Tac2 clusters significantly reduced cocaine intakes. Collectively, our results suggested that Tac2 clusters in the NAc are a D1 MSN subtype.

MTU07-04

Functional and molecular markers of vulnerability towards stress in a rat model of PTSD**A. Datusalia, N. Sala, L. Musazzi, P. Maurizio***Università degli Studi di Milano, Dipartimento di Scienze Farmacologiche e Biomolecolari, Milan, Italy*

Stressful events represent a major risk factor for the development of neuropsychiatric disorders such as depression and PTSD. Although genetic vulnerability may represent a moderating factor, it is not clear what mechanisms address the individual responses towards pro-adaptive or maladaptive course. Here we aimed to investigate short/long-term cellular/molecular changes in the aftermath of acute stress to understand better the dynamics of the stress response and identify key factors that may trigger stress-related psychopathology. We adapted sucrose intake (SI) test to identify foot shock (FS)-stress-induced anhedonic phenotype. Baseline sucrose intake was established for 5 weeks in rats. Rats were randomly assigned to FS-stress/control groups and subjected to FS-stress. After 24 h in SI test, anhedonic animals (showing at least 25% within-subject decrease in SI) were classified as vulnerable (FS-V), while others as resilient (FS-R). We demonstrated that FS-stress increased basal glutamate release only in FS-V, while depolarization-evoked glutamate release was increased in both FS-V and FS-R. In Further, we observed significant increase in the nuclear expression of MR only in FS-R while GR expression remains unaltered. Recent studies have demonstrated multiple roles of miRNAs in governing functional and structural synaptic plasticity as well as in pathophysiology of psychiatric disorders. Currently we are investigating key miRNAs responsible for resilience and vulnerable response after single acute stress episode and their target genes at short- and long-term after stress. We have also investigated plasticity related protein like ERK1/2, CREB etc. expression and found increased nuclear translocation of pERK in FS-V rats. This study results will help to identify key molecules associated with stress-induced maladaptive (vulnerable) response and novel targets for therapy of stress-related neuropsychiatric disorders.

MTU07-05

Neonatal nicotine exposure primes midbrain neurons to a dopaminergic phenotype and increases adult drug consumption**D. Dulcis¹, B. Romoli¹, I. M. Sandoval², F. Manfredsson², D. K. Berg³, A. F. Lozada³, T. Hnasko⁴**¹UCSD, University of California San Diego, Psychiatry, La Jolla, USA²Michigan State University, Translational Science & Molecular Medicine, Grand Rapids, USA³UCSD, University of California San Diego, Neurobiology, La Jolla, USA⁴UCSD, University of California San Diego, Neuroscience, La Jolla, USA

Nicotine is a psychoactive substance that induces addiction through neuroplasticity affecting the function of the Ventral Tegmental Area (VTA) and dopamine release in the reward circuitry. We have previously shown that altered neuronal activity can change dopamine (DA) expression both in the developing and

adult brain. Here, we investigated the effect of neonatal nicotine (NN) exposure on DA plasticity of VTA neurons.

Osmotic pumps were implanted in lactating mice to deliver 2 mg nicotine/Kg/day to P2-P16 pups. Adult mice underwent nicotine and ethanol 2 bottle-choice test. Brains were processed for tyrosine hydroxylase and nuclear receptor related-1 protein (Nurr1) immunohistochemistry and *in situ* hybridization. Calcium spike activity was measured via *ex-vivo* calcium imaging. We overexpressed Nurr1 and altered neuronal activity (DREADDs) in non-DAergic VTA neurons using VGLUT2- & VGAT-cre mice.

NN exposure potentiated nicotine preference in adult (P90) mice, increased nicotine-induced responses, induced ectopic Nurr1 expression within VTA glutamatergic neurons. Adult nicotine exposure increased both the total number of DA neurons and DA co-expression with glutamate in the VTA of NN-exposed mice. Overexpression of Nurr1 induced an increase in nicotine preference when paired to DREADDs-mediated activity boost. Downregulation of Nurr1 in glutamatergic neurons revealed its expression is necessary for non-DAergic neurons to acquire a dopaminergic identity. These findings may provide a new critical link between developmental exposure to drugs of abuse and adult vulnerability to drug addiction.

This research was supported by the Tobacco-Related Disease Research Program Grant #271R-0020.

MTU07-06

Rauwolfia vomitoria AFZEL. root bark extract adversely affects behaviour and brain microstructures**M. Ekong***University of Uyo, Department of Anatomy, Faculty of Basic Medical Sciences, Uyo, Nigeria*

Rauwolfia vomitoria (RV) Afzel. is a medicinal plant of the Apocynaceae family, widely used locally in the management of psychiatry conditions. The plant is beneficial, especially when applied during diseased state, and as antipsychotics and antipyretics. Adverse effects have also been ascribed, especially to its constituents, reserpine and yohimbine. Thus, the present study evaluated the effect the root bark extract has on some behaviour and brain microstructures. Eighteen adult male Wistar rats of 220 g average body weight, were divided into three groups (n = 6); control (distilled water), 200 mg/kg and 400 mg/kg body weight of RV root bark extract. The administration was orally and lasted seven days. On day 8, the open field and Morris water mazes tests, as well as test for olfaction were carried out and the animals sacrificed. Their brains were processed for histology and immunoreactivity. Results showed that learning and memory were not affected, but there was freezing, sedation, and inhibition of locomotion and olfaction. The histology showed degenerative features in the olfactory bulb, hippocampus, dentate gyrus and cerebellum. Immunohistochemically, neuron specific enolase (NSE) expression was increased in the hippocampus and cerebellum of the 200 and 400 mg/kg RV groups; dentate gyrus of the 200 mg/kg RV group; and olfactory bulb of the 400 mg/kg RV group. NSE expression was decreased in the olfactory bulb of the 200 mg/kg RV groups and dentate gyrus of the 400 mg/kg RV group. Expressions of glial fibrillary acidic protein (GFAP) was increased in the olfactory bulb, hippocampus, dentate gyrus and cerebellum of the 200 mg/kg RV group, but decreased in the 400 mg/kg RV group, compared to the control. At these given doses, RV may be deleterious to the brain; in cognitive behaviour and microstructure, and these effects were dose dependent.

MTU07-07

Double hit of perinatal stress evokes depressive behavior and affects midbrain levels of dopamine, serotonin and their metabolites**M. Ferraz, F. Rito-Costa, T. Ferraz, S. Pereira, M. Cavalleira, A. C. Carvalho, U. Araújo***Universidade do Estado do Rio de Janeiro, Pharmacology and Psychobiology, Rio de Janeiro, Brazil*

Perinatal stress is a susceptibility factor for the development of mental disorders, whose etiology is in possible changes in the integrity or functionality of neurobiological circuits. The aim of our study is to investigate the effects of double perinatal stress, consisting of the association of prenatal hypoxia ischemia and short maternal separation, on behavior depressive-like and levels of dopamine, serotonin and their metabolites in basal midbrain. Hypoxia Ischemia was induced by clamping the uterine arteries of pregnant rats for 45 minutes, on the 18th day of gestation. The same conditions were applied to form the SHAM group only without clamping the arteries. Maternal separation was performed from the first to the sixth day of birth. The anxiety-like and depressive-like behavior were analyzed by the open field, plus maze, forced swim and head shaking tests. Nociception response was evaluated by hot plate test. Analysis of the serotonin, dopamine and their metabolites contents in basal midbrain and prefrontal cortex were done by high performance liquid chromatography. Data were compared with a two-way ANOVA, expressed by mean $\pm$ SEM. Double perinatal stressed rats showed increase of depressive-like behavior and mesencephalic serotonergic hypoactivity. These results are consistent with the hypothesis of increased vulnerability of the serotonergic system for perinatal stress.

This work was supported by FAPERJ grants.

MTU07-08

The role of kinesin 1 isoform, KIF5B, in dendritic spine plasticity**A. H. K. Fok, K.-O. Lai, J. Huang, C. S. W. Lai***The University of Hong Kong, School of Biomedical Sciences, Hong Kong, Hong Kong*

Regulation and localization of plasticity-related proteins (PrPs) are important for proper synapse maturation and function. Kinesin 1 motor protein family is known to be important in carrying and distributing PrPs and mRNAs in dendrites. The three members of kinesin 1 family, namely KIF5A, B and C, were traditionally thought to be functionally redundant. However, previous studies found different phenotypes in knockout mouse models of each kinesin 1 family member. It therefore suggests that each kinesin 1 member has specific functions. In order to test this hypothesis, a KIF5B conditional knockout mouse model was generated, in which CRE recombinase expression was driven by CaMKII promoter to remove *kif5b* exon 2 endogenously. We used western blot and immunohistochemical staining to confirm the reduced protein level of KIF5B in the conditional knockout mouse model, while there was no significant effect on other isoforms of the kinesin 1 family. We also found that KIF5B conditional knockout mice had increased protein level of PSD95, NR2B, and GluR2. Next, we performed a series of behavioral tests to evaluate the cognitive functions of KIF5B conditional knockout mice. We found that they showed no

significant different in anxiety-related behaviors, but they showed learning deficits in auditory-cued fear conditioning, novel object recognition test, three-chamber social interaction test, and Barnes maze. To investigate the cause of behavior deficit, we carried out chronic two-photon intravital imaging. We found that KIF5B conditional knockout mice showed an increase of dendritic spine turnover rate over a 7-day observation period. We will further investigate the role of KIF5B in learning-induced dendritic spine plasticity by *in vivo* imaging.

MTU07-09

Adult hippocampal neurogenesis impairment at pre-plaque stage in a transgenic rat model of Alzheimer's-like amyloid pathology**P. Galeano¹, M. Dalmasso¹, F. Prestia¹, E. Castaño¹, C. Cuello², L. Santín³, L. Morelli¹**¹*Institute Leloir Foundation, IIBBA-CONICET, Buenos Aires, Argentina*²*Department of Pharmacology and Therapeutics, McGill University, Montreal, Canada*³*School of Psychology, Malaga University-IBIMA, Malaga, Spain*

The contribution of adult hippocampal neurogenesis (AHN) impairment on cognitive decline in early Alzheimer's disease (AD) remains poorly understood. This can be ascribed to the technical difficulties to measure AHN in post-mortem brains and patients. Furthermore, most animal models of AD exhibit an aggressive neuropathology at early age and harbor gene mutations and express transgenes that disrupts AHN by pathways not directly involved in AD pathology. To overcome some of these limitations, we studied AHN at pre-plaque stage (6-month-old) in hemizygous (Tg+/-) and homozygous (Tg+/+) McGill-R-Thy1-APP transgenic rats. This model exhibits a much less aggressive neuropathology that nevertheless is associated with a marked cognitive impairment from early age. Our results revealed that Tg+/+ rats showed a reduced number of PCNA+ cells, DCX+ immature neurons and BrdU+/NeuN+ colabeled neurons in dorsal and ventral dentate gyrus. Moreover, dendritic arborization was less developed. AHN was not impaired in Tg+/- rats, although dendritic arborization was slightly decreased. On the other hand, both hemizygous and homozygous rats exhibited spatial memory impairments in the Morris water maze. These results suggest that: 1) AHN is dysregulated from the pre-plaque stage in homozygous rats; 2) AHN impairment is dependent on APP transgene copy numbers since hemizygous rats did not show it; 3) Dysregulation of AHN is not directly associated with spatial memory impairments since hemizygous rats exhibited spared neurogenesis despite showing spatial memory deficits. Funding: International Society for Neurochemistry CAEN Grant and Andalucía TECH-ICE (PG), and PICT-2015-0285 (LM).

MTU07-10

Selective long term memory impairment in transgenic McGill-R-Thy1-APP rat model of Alzheimer's disease
M. Habif¹, F. Filippin¹, D. Salas¹, M. Cercato¹, N. Coletti¹, C. Cuello², E. Kornisiuk¹, D. Jerusalinsky¹

¹IBCN - UBA/CONICET, Lab. of Neuroplasticity & Neurotoxins
 LaNyN -, Buenos Aires, Argentina

²McGill University, Dept Pharmacology and Therapeutics,
 Montreal, Canada

Memory impairment in early Alzheimer Disease (AD) is hypothesised to rely on the initial increase in soluble A β -oligomers, potent neurotoxins altering synaptic plasticity.

McGill-R-Thy1-APP Wistar-transgenic (Tg) rats, bearing human Amyloid Precursor Protein with Swedish and Indiana mutations of familial AD, offer a singular opportunity for testing learning and memory abilities at AD onset. Homozygous Tg rats show cognition deficits at 3 months; human A β accumulates intra-neuronally from first postnatal week and develops extracellular amyloid pathology by 6-9 months. Hemizygous Tg (He) show a more subtle phenotype and do not develop extracellular plaques even at 20 months.

13 month old He rats and their wild type litter-mates (WT) were left to freely explore an open field (OF) for 5 min and tested 24 hr later (long-term-memory, LTM); bi-dimensional exploration was quantified, being significantly lower in test than in training for both groups.

Rats were then trained in a two object recognition task (OR); both WT and He discriminated new vs. known object 1 hr later (short-term-memory, STM), while He rats did not show LTM.

They were then trained in an inhibitory avoidance (IA) to a mild foot-shock task. Latencies to go from an enlightened to a dark compartment where they get the shock, were recorded. Test latencies 24 hr later, were significantly higher than training latencies for WT, while remained unchanged for He.

Therefore, unlike WT, He evidenced deficits in memory formation for object discrimination and for an associative memory involving aversive and spatial components.

MTU07-11

Mesopontine cholinergic signaling influences stress responses affecting behaviour

O. Kljakic^{1,2}, H. Janickova², K. Rosborough^{1,2}, S. Raulic², S. Matovic², R. Gros², L. Saksida^{2,3}, T. Bussey^{2,3}, W. Inoue², M. Prado^{1,2,3}, V. Prado^{1,2,3}

¹University of Western Ontario, Anatomy & Cell Biology, London, Canada

²Robarts Research Institute, Schulich School of Medicine & Dentistry, London, Canada

³Brain & Mind Institute, Schulich School of Medicine & Dentistry, London, Canada

Pedunculopontine and laterodorsal tegmental nuclei (PPT/LDT) are heterogenous brainstem structures that contain cholinergic, glutamatergic and GABAergic neurons. Several neuropsychiatric disorders have been associated with degeneration of the cholinergic neurons in this brain region, however, the importance of PPT/LDT cholinergic signaling for cognitive and non-cognitive functions is poorly understood. Previous work suggested that PPT/LDT cholinergic neurons play a role in attention and other forms of higher-level cognition, however these studies used non-selective methods to kill

cholinergic neurons. To test the role of acetylcholine in higher-level cognition, we selectively eliminated the vesicular acetylcholine transporter (VACHT) in the PPT/LDT to generate mice that have impaired cholinergic signaling without interfering with other brainstem cell types and co-transmitted chemicals. We tested these VACHT-deficient mice using conventional and touchscreen-based cognitive tasks and found that they had little to no impairments in many cognitive functions, including attention, yet failed to perform in the spatial and cued forms of the Morris water maze (MWM). Interestingly, spatial memory and visual spatial learning were intact in VACHT-mutants, but touchscreen performance was affected by a stressor and mice had altered corticosterone levels after the MWM. These results suggest that attention and many other cognitive functions are not affected by the loss of PPT/LDT cholinergic signaling, but an altered stress response can influence cognitive performance in aversive tasks.

MTU07-12

Enhancing adult neuroplasticity by epigenetic regulation of PV interneuron

M. L. Jolin^{1,2}, B. Chattopadhyaya², F. Dumouchel^{1,2}, A. V. Varlan^{1,2}, G. D. Cristo^{1,2}

¹Université de Montréal, Neurosciences, Montréal, Canada

²CHU Sainte-Justine, Research center, Montréal, Canada

Parvalbumin-positive cells (PV), the major source of GABAergic inhibition in the brain, innervate hundreds of postsynaptic targets with multiple perisomatic synapses. They are important for the regulation of multiple cognitive functions and developmental cortical plasticity. Although PV cell function is being explored extensively, the mechanisms that control their development and plasticity have not been entirely resolved. Molecular mechanisms involved in synapse formation and strengthening include the regulation of specific subsets of genes by epigenetic modifications. Histone Deacetylase 2 (HDAC2) regulates excitatory synapse plasticity and memory formation. However, whether and how HDAC2 affects PV cell synapse development and brain plasticity is unknown. Here, we show that HDAC2 is expressed by PV neurons. In order to dissect its role in PV cell, we used the conditional KO mice (PV_Cre;HDAC2^{lox/lox}), which express Cre selectively in PV cells after P14. We found that adult PV_Cre;Hdac2^{lox/lox} mice show enhanced fear memory extinction, along with a reduction of perineural nets (PNN) around PV cells somas in the prefrontal cortex and the basolateral amygdala as well as an increase in perisomatic PV synapse remodeling after fear extinction. Finally, the direct role of Hdac2 in PNN formation and PV cell synapse plasticity will be tested by viral overexpression of Hdac2 in prefrontal cortex of PV_Cre;Hdac2^{lox/lox} mice. All together, our work supports the model in which PV cells and PNN play a pivotal role in brain plasticity and suggests that modulation of Hdac2, in combination with behavioral therapy can improve the treatment of post-traumatic stress disorder (PTSD).

MTU07-13

In silico characterization and functional analysis of non-synonymous polymorphisms present in gpm6a's extracellular coding regions**A. León, G. Aparicio, C. Frasch, C. Scorticati***Instituto de Investigaciones Biotecnológicas IIB-UNSAM-CONICET, Laboratorio de Neurobiología Molecular y Celular, San Martín, Argentina*

Membrane glycoprotein M6a is mainly expressed in neurons of the central nervous system and it is involved in neuronal plasticity. M6a promotes neurite and axonal outgrowth, filopodia/spines formation and synaptogenesis in primary neuronal cultures and neuronal cell lines. Recently, altered expression or polymorphisms in the human M6a gene have been associated with neurological disorders such as schizophrenia, bipolar disorder, depression, claustrophobia and Alzheimer's disease. However, the molecular mechanisms underlying the development of such pathologies remain unknown. M6a, together with M6b and PLP/DM20, belongs to the tetraspan proteolipid protein family. According to its structure, we speculate that certain amino acids within M6a's extracellular loops mediate specific interactions with other proteins and those contribute to its function. Thus, out of more than a hundred submitted entries, we selected 13 non-synonymous SNPs from the NCBI dbSNP database located at the coding sequence of GPM6a's extracellular loops (EC1 and EC2). The selected nsSNPs had to be validated by frequency, cluster and/or 1000G. *In silico* analysis –using -Polyphen and I-mutant 2.0- predicted that all nsSNPs might decrease protein stability and have a moderate to strong functional damaging effect. In the case of SNPs located in the EC1, none of them modify M6a neuronal membrane distribution and topology, however, some of them rs375144137 (G69E), rs370813625 (T71P) and rs747244424 (T76I) impair M6a neurite extension in N2a cell line. We speculate that these variants blocked M6a's neurite extension because of the drastic single amino acid substitution (non polar to acid or basic residue and polar to non polar) that might affect extracellular loops interactions.

MTU07-14

Adult neurogenesis in the paleognathous birds: the common ostrich (*Struthio camelus*) and emu (*Dromaius novaehollandiae*)**P. Mazenganya, A. Bhagwandin, A. O. Ihunwo***University of Witwatersrand, Anatomical sciences - Faculty of Health Sciences, Johannesburg, South Africa*

Adult neurogenesis is a process occurring in varying magnitudes in different species ranging from invertebrates to vertebrates. We examined adult neurogenesis throughout the brains of the common ostrich (*Struthio camelus*) and emu (*Dromaius novaehollandiae*) using immunohistochemistry for the endogenous markers proliferating cell nuclear antigen, which labels proliferating cells, and doublecortin, which stains immature and migrating neurons. The distribution of PCNA and DCX labelled cells was widespread throughout the brain of both species. The highest density of cells immunoreactive to both markers was observed in the olfactory bulbs and the telencephalon, especially the subventricular zone of the lateral ventricle. The density of PCNA immunoreactive cells was less exuberant in the telencephalon of the emu compared to the common ostrich. Substantial numbers of PCNA immunoreactive

cells were observed in the diencephalon and brainstem, but DCX immunoreactivity was weaker in these regions. PCNA and DCX immunoreactive cells were observed in moderate density in the cortical layers of the cerebellum of both species. Columns of migrating cells were observed at three distinct points extending from the lateral wall of the lateral ventricle into parenchyma of the telencephalon at rostral levels in both species. The distribution of putative proliferating cells and immature neurons in the brain of the common ostrich and the emu is widespread, far more so than in mammals, and compares with the neognathous birds, and suggests that brain plasticity and neuronal turnover is an important aspect of cognitive brain functions in these birds.

MTU07-15

Behavioral, cytoarchitectural, and neurochemical changes in the offspring of methylazoxymethanol treated in mice**O. Nakagawasai¹, K. Takahashi¹, W. Sakuma¹, W. Nemoto¹, T. Odaira¹, L. Jia-Rong¹, H. Onogi², L. K. Srivastava³, K. Tan-No¹**¹*Tohoku Medical and Pharmaceutical University, Dept of Pharmacology, Sendai, Japan*²*Tohoku Fukushi University, Faculty of Health Science, Sendai, Japan*³*McGill University, Douglas Mental Health University Institute, Montreal, Canada*

Methylazoxymethanol (MAM) treated pregnant rats at gestation day (GD) 17 has been shown to be a valuable animal model for schizophrenia. However, this model is still not established in mice models. To examine face, construct and predictive validities, we observed behavioral, cytoarchitectural, and neurochemical changes in the offspring of MAM treated mice. We found in contrast to a single injection of MAM to dams at GD 15, 16 or 17, its daily administration from GD 15 to 17 led to deficits in prepulse inhibition (PPI) of startle in the post-pubertal offspring. Moreover, we observed behavioral deficits such as increasing locomotor activity to NMDA antagonist MK-801, working memory and social interaction. These animals also showed a reduction of volume at the prefrontal cortex (PFC) and hippocampus, and neuroanatomical changes such as discontinuities and heterotopias in the hippocampus. Atypical antipsychotic drugs clozapine, risperidone, and aripiprazole, but not the typical drug haloperidol, reversed the deficit in PPI. Therefore, the treatment of pregnant mice with MAM during GD 15-17 MAM offers a new procedure to research neurobiological mechanisms involved in the pathogenesis of schizophrenia.

MTU07-16

Expression of molecular signatures during long-term memory consolidation, established by behavioural tagging model**M. Naseem***Jamia hamdard university, Department of toxicology, Delhi, India*

Memory is one of the most fundamental processes of brain and learning and memory are one of those frontier areas of neurobiology which attract us to investigate the intricacy of this process. Here we aimed to investigate the general mechanism of "Behavioural

Tagging" in long term memory (LTM) formation. Long term potentiation (LTP) is a form of synaptic plasticity and it is considered as a cellular model of learning and memory. One of the LTP specific PRPs, PKM- ζ , is required for the formation of LTM as well as for the maintenance of LTP. In our study, we have shown that for the consolidation of LTM, in addition to LTP-specific PRPs, synaptic tags are also required to interact with each other. In the present study, we investigated the involvement of LTP-specific PKM- ζ and learning tags within a critical time window, which are required for the formation of LTM without affecting STM. Behavioural tagging is an established model for the assessment of some forms of learning and memory. Despite being studied for LTM formation for many years, no studies investigated the role of PKM- ζ in Behavioural tagging model. Hence, by using these two different memories based tasks (Inhibitory avoidance and Novel object recognition tasks), we observed how PKM- ζ activated by exposing a novel arena after a weak training and led to the consolidation of memory. These findings thus show how the process of behavioural tagging activates LTP-specific PKM- ζ for the formation of LTM.

MTU07-17

Antinociceptive effect of diminazene aceturate, an angiotensin-converting enzyme 2 activator, in the mouse formalin test

W. Nemoto, R. Yamagata, O. Nakagawasai, S. Michimata, K. Nakagawa, K. Tan-No

Tohoku Medical and Pharmaceutical University, Department of Pharmacology, Faculty of Pharmaceutical Sciences, Sendai, Japan

We have reported that intrathecal (i.t.) administration of angiotensin (Ang) II into mice produces a nociceptive behavior accompanied by the phosphorylation of p38 MAPK in the spinal cord, which was mediated through AT1 receptors. In addition, both the p38 MAPK phosphorylation and subsequent nociceptive behavior were attenuated by the i.t. co-administration of Ang (1-7), an N-terminal fragment of Ang II formed by angiotensin-converting enzyme 2 (ACE2), that acted via Mas receptors. However, the role of ACE2 on spinal nociceptive transmission remains unknown. Therefore, in the present study, we examined the effect of diminazene aceturate (DIZE), an ACE2 activator, on the formalin-induced biphasic nociceptive behavior. When administered i.t. 1 hr prior to the intraplantar injection of 2% formalin, DIZE dose-dependently attenuated the nociceptive behavior during the second phase, but not the first phase. The inhibitory effect of DIZE was prevented by i.t. administration of A779, a Mas receptor antagonist. The i.t. administration of DIZE also dose-dependently attenuated the Ang II-induced nociceptive behavior, which was prevented by i.t. administration of A779. Although phosphorylation of p38 MAPK was observed in the lumbar dorsal spinal cord after the injection of formalin or Ang II, these phosphorylation were attenuated by DIZE, which was inhibited by A779. In addition, DIZE significantly increased the ACE2 activity in the lumbar dorsal spinal cord. These results suggest that DIZE attenuates the formalin-induced nociceptive behavior during the second phase through the increase in Ang (1-7) generated from Ang II by the activation of ACE2 and subsequent inhibition of p38 MAPK phosphorylation via Mas receptors.

MTU07-18

Pain behavioural response in plasmodium berghei-induced malaria

A. Oyewole¹, O. Akinola¹, B. Owoyale^{1,2}

¹*University of Ilorin, Physiology, Ilorin, Nigeria*

²*Massachusetts General Hospital & Harvard Medical School, Dept of Neurology, Boston, US*

Several forms of pain are reported by patients with *Plasmodium falciparum* and are mostly described as one of the symptoms for malarial infection. Neural processes that influence pain perception in malaria-infected persons and its contributions to malaria related mortality are poorly understood. In the current studies, *Plasmodium berghei* (Pb) infected Swiss mice exhibited attenuated behavioural responses to noxious chemical without compromising the motor function. This parasite-induced analgesia improved with increase daily proliferation of erythrocyte stage Pb and it appeared to be synergistically mediated by opioidergic (via μ -opioid receptor) and serotonergic (via 5HT2A receptor) system. Systemic intersection study among drugs of varying mechanisms capable of eliciting anti- or pro- nociception showed little or no contribution of pain system in malaria related death. Though there were mild morphological changes in brainstem cells and Nissl substance of infected mice, neural secretory activity (of serotonin, noradrenaline and ATPase) was preserved. These results indicated that animal model for malaria is not a potential model to unravel mechanism surrounding malaria parasite-induced pain reported in human. This however is the first report demonstrating that malaria parasite causes analgesic-like effects in mice.

MTU07-19

Lipid raft dynamics in adolescent brain: alcohol-stimulant co-use

D. Rhoads, C. O'Shea, M. Telatin

Monmouth University, Biology, West Long Branch, USA

Co-use of alcohol and stimulants, including caffeine and amphetamine, is a growing concern, particularly among adolescents already prone to binge alcohol consumption. We are modeling chronic co-use of alcohol and stimulants in adolescent Long-Evans rats. Previous behavioral work in our lab has shown that chronic administration of caffeine or amphetamine with alcohol resulted in decreased severity of alcohol withdrawal symptoms. Alcohol withdrawal is considered a hyperglutamatergic state. Regulation of glutamate activity may occur in part through lipid raft structures. The goal of the present study was to characterize glutamatergic receptor components of lipid rafts as a way of determining if raft dynamics were a target for alcohol-stimulant interactions. Adolescent rats were fed ethanol, amphetamine, caffeine, or combinations of ethanol and amphetamine or caffeine as part of a liquid diet. Detergent-resistant membranes were isolated by ultracentrifugation and raft fractions identified by Western blotting with the raft marker flotillin. Higher (HBR) and lower (LBR) buoyancy raft fractions were identified. Compared to control rats fed liquid diet without drug, there was a 2-3 fold increase in the LBR fraction from rats consuming alcohol. Western blotting showed that this fraction was rich in NMDA-subtype of glutamate receptors which may account in part for upregulation of NMDA receptors during chronic alcohol consumption. Other components of glutamatergic transmission

(mGluR1 and 5; Homer) also appeared in raft fractions. When rats consumed ethanol along with caffeine, there was no increase in the LBR fraction. Similar results were obtained after co-consumption of ethanol and amphetamine. Thus, a change in lipid raft dynamics was correlated with alcohol withdrawal symptoms but not under conditions where stimulant co-consumption attenuated withdrawal severity. Lipid rafts may be a site wherein stimulants antagonize adaptive responses of the brain to chronic ethanol.

MTU07-20

Comorbidity between stress and cocaine: role of cofilin in nucleus accumbens during the acquisition of cocaine self-administration

D. Rigoni¹, María. P. Avalos¹, Andrea. S. Guzmán¹, M. Julieta Boezio¹, Liliana. M. Cancela¹, F. Bollati¹, M. Bisbal²

¹Universidad Nacional de Córdoba, IFEC- CONICET. Departamento de Farmacología, Facultad de Ciencias Químicas, Córdoba, Argentina

²Instituto de Investigación Médica M. y M. Ferreyra, -, Córdoba, Argentina

The development of addictive behavior is associated with functional and structural plasticity in the mesocorticolimbic pathway. Animal models have demonstrated that exposure to stress predisposes to developing substance use disorders. Our laboratory has shown that repeated stress alters the capacity of a subsequent cocaine injection to modulate dendritic spine morphology and actin dynamics. These findings demonstrate that the pharmacological inhibition of actin polymerization in the nucleus accumbens (NA) prevents stress cross-sensitization with cocaine and influences actin cytoskeleton remodeling in the NA. Thus, the main goal of this project is to evaluate the impact of the actin cytoskeleton in the changes underlying the facilitatory influence of stress in the acquisition of cocaine self-administration (SA). For this purpose, we have generated a lentivirus containing a short hairpin RNA (shRNA) specific to cofilin, to inhibit its expression in NA, and explore its function during the acquisition of cocaine SA. Thus, Sprague dawley rats pre-exposed to chronic restraint stress, will be administered intra-accumbens with shRNA of cofilin, and later they will undergo surgery for implantation of catheters in the jugular vein one week before SA sessions. Our results reveal that the inhibition of cofilin prevents the stress-induced sensitization to cocaine and reverts the facilitation of the acquisition of cocaine self-administration induced by stress, suggesting that cofilin regulation is crucial for the stress-induced facilitation on the vulnerability to develop cocaine addiction.

MTU07-21

Outgrowth of filopodia is associated with intracellular trafficking of GPM6A

N. Rosas, A. C. Frasch, B. Fuchsova

Instituto de Investigaciones Biotecnológicas/Universidad de San Martín IIB-UNSAM, Laboratorio de Neurobiología, San Martín, Argentina

Gpm6a is a neuronal membrane glycoprotein that functions in the processes of neuronal development and its overexpression leads to the extensive formation of filopodia. Neuropsychiatric disorders and

chronic stress exposure have been linked to the alterations in Gpm6a expression levels or sequence. However, the mechanism of action of Gpm6a is not clearly understood. Previously, we identified K250, K255, and E258 in the C-terminal of Gpm6a as key functional residues for the formation of filopodia. Subsequent bioinformatic analysis revealed that K250, K255, and E258 are predicted as part of sorting signals of transmembrane proteins. Colocalization assay showed that deletion of the C-terminus diminishes the association of Gpm6a with clathrin in hippocampal neurons implying involvement of clathrin-mediated trafficking events. Moreover, using flow cytometry we found that substitution of K250, K255, and E258 with alanine diminishes the amount of Gpm6a on cell surface and in case of K255 and E258 also leads to the lower amount of total expressed protein. Here using confocal microscopy we analyze the subcellular localization of the mutant forms of Gpm6a that fail to induce filopodia formation. K250A and E258A display increased intracellular accumulation of the protein and a preferential localization to Lamp1-positive structures. To determine if K250, K255 and E258 substitutions leads to an increased protein degradation we use flow cytometry to quantify the Gpm6a expression levels upon treatment with different protease inhibitors. The localization of Gpm6a mutants to LC3- and Calnexin- positive structures was also evaluated.

MTU07-22

Convergence of reward and aversion processing in nucleus accumbens medium spiny neuron subtypes

C. Soares-Cunha^{1,2,3}, N. Vasconcelos^{2,3,4}, B. Coimbra^{2,3}, A. V. Domingues^{2,3}, N. Sousa^{2,3,5}, A. J. Rodrigues^{2,3}

¹University of Minho, School of Medicine, Braga, Portugal

²Life and Health Sciences Research Institute ICVS, School of Medicine, University of Minho, Braga, Portugal, School of Medicine, Braga, Portugal

³ICVS/3B's-PT Government Associate Laboratory, Braga/ Guimarães, Portugal, School of Medicine, Braga, Portugal

⁴Departamento de Física, Universidade Federal de Pernambuco, 50670-901 Recife, Pernambuco, Brazil, Departamento de Física, Recife, Brazil

⁵Clinical Academic Center-Braga ^{2CA}, NA, Braga, Portugal

Reward is as important as aversion for survival. Deficits in decoding rewarding/aversive signals are present in several neuropsychiatric disorders, such as depression or addiction, emphasizing the need to study the underlying neural circuits in detail.

The reward circuit, comprising projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) is crucial for reward/aversion processing. Though the dominant view postulates that NAc D1-MSNs convey reward and D2-MSNs encode aversion, recent results challenged this view.

Here, we show that both MSN populations drive reward and aversion, depending on their pattern of activation. These opposite behaviors result from differential evoked electrophysiological patterns in downstream targets, namely the ventral pallidum (VP) and VTA. Brief MSN optogenetic stimulation of either D1- or D2-MSNs elicited positive reinforcement, in line with the observed decreased VP-to-VTA inhibitory tone, and increased VTA dopaminergic activity. Prolonged activation of either MSN population drove aversion, inducing distinct electrophysiological effects in these target regions.

In addition, we further show that distinct patterns of MSN activation differentially influence cocaine-induced place preference.

In sum, we show that D1- and D2-MSNs bi-directionally control reward/aversion, highlighting that more studies are needed to understand how these two populations interact to modulate behaviour.

MTU07-23

Mild ketogenic diet as promising approach for cognition enhancement: medium-chain triglyceride supplement improves memory in rats

A. Trofimov¹, A. Schwarz^{2,1}, D. Krytskaya¹, V. Shavva^{3,1}, E. Veniaminova⁴, V. Sizoff¹, A. Shcherbakova^{5,1}

¹Institute of Experimental Medicine, Pavlov Department of Physiology, Saint Petersburg, Russia

²Sechenov Institute of Evolutionary Physiology and Biochemistry, Multidisciplinary Laboratory of Neurobiology, Saint Petersburg, Russia

³Karolinska Institute, Department of Medicine, Stockholm, Sweden

⁴Sechenov First Moscow State Medical University, Institute of Molecular Medicine, Moscow, Russia

⁵Para Impacto, Department of Adaptive Learning, Tokyo, Japan

Ketogenic diet is known to be a viable approach for correction of brain disorders. The strict ketogenic diet is difficult to adhere to. Search for an intervention both beneficial and not too restrictive appears important. Intermittent mild ketosis by using medium-chain triglycerides (MCT: C8–C10) may be such intervention. The study was aimed at the investigation of effects of MCT-enriched diet on the memory of adult intact animals. Adult Wistar males kept on standard diet (SD) were tested in Y-maze, Open field, Object recognition. Then, 2 groups were formed: MCT (chow excluded for 6 h/day, MCT intragastric, 2 ml/kg) and SD control (water intragastric). After 2 weeks of diet, the tests were repeated, adding Morris water maze. Statistics: rm-ANOVA, *post hoc* Sidak; Student's, Mann-Whitney, $p < 0.05$. In the Y-maze, MCT group demonstrated better working memory: more spontaneous alternations compared to control. In the Open field, MCT animals showed decreased exploration than SD when normalized to pre-diet trials, indicating

better memory of environment. No differences were found in the Object recognition. In the probe trial of Morris water maze, MCT animals spent more time in the target quadrant than SD group, indicating better spatial memory. MCT-enriched diet is shown to be a promising non-drug approach to improve cognitive functioning.

MTU07-24

Discovery of a key missing signaling between RHOA/RHO-kinase and ras underlying spine enlargement and LTP

M. Wu¹, Y. Funahashi¹, T. Takano², D. Tsuboi¹, R. Ahammad¹, M. Amano¹, K. Kaibuchi¹

¹Nagoya University, Cell pharmacology, NAGOYA, Japan

²Duke University, Cell Biology, Duke, USA

The small GTPase RhoA and its downstream effector Rho-kinase are considered as one of the key regulators in dendritic spine formation and synaptic plasticity. However, how RhoA/Rho-kinase signaling involved in modulating synaptic plasticity still remains unknown. We have recently developed a phosphoproteomic analysis method that uses affinity beads coated with 14-3-3 proteins to enrich phosphorylated proteins and established the kinase-associated neural phosphosignaling (KANPHOS) database that provides the phosphorylated sites identified by our phosphoproteomic approaches. Using the KANPHOS database, we identified SynGAP1, which is a synaptic Ras-GTPase activating protein, as a novel Rho-kinase substrate. In this study, we found that phosphorylation of SynGAP1 by Rho-Kinase increased its interaction with 14-3-3 but decreased with PSD-95, which is a major scaffolding protein in the postsynaptic densities of dendritic spines. SynGAP1 was dispersed from spines upon long-term potentiation (LTP) induction in cultured neurons, and this dispersion depends on phosphorylation of SynGAP1 by Rho-kinase. Moreover, we found that Rho-kinase increased Ras and ERK activity through phosphorylation of SynGAP1. Thus, the synaptic dispersion of SynGAP1 which phosphorylated by Rho-kinase during LTP represents may be a key signaling link element that transduces RhoA/Rho-kinase activity to Ras-ERK signaling-mediated spine enlargement, AMPA receptor (AMPA) synaptic incorporation, and synaptic potentiation.

MTU08 Clinical studies, biomarkers & imaging (Session A)

MTU08-01

Sensitive and stable quantitation of endogenous oxytocin in mice using reduction/alkylation approach for elisa **S. Cherepanov, M. Gerasimenko, T. Yuhi, S. Yokoyama, H. Higashida**

Kanazawa University, Center for Child Mental Development, Kanazawa, Japan

Oxytocin (OT) is a nonapeptide essential for the social brain. Several studies have reported the ability of plasma OT levels to be a neurological biomarker of autism, anxiety and other mental disorders. One of the main methods of OT determination - is immunosorbent assay (ELISA). Specificity of this method is criticized. Matrix interference and binding of OT with plasma albumins make data of direct determination doubtful. An established way to solve this problem - is a measurement of only the free OT fraction, employing solid phase extraction (SPE). However, SPE required a significant amount of plasma which made it difficult to use in research employing rodents.

Recently, Brandtzaeg group discovered reduction/alkylation followed by proteins precipitation (RAPPT) method that enables to save a sufficiently high amount of OT released from plasma proteins as well as provide a reduction of matrix interference. Importantly, the required volume of plasma is just 100 µl. This method was implemented for the Mass Spectrometry platform.

We aim to adopt this approach for ELISA. We have performed RAPPT prior to ELISA measurement using ICR mice plasma pool. OT levels after RAPPT were lower than in diluted plasma, but significantly higher compared with SPE samples. RAPPT samples demonstrate linearity within dilution, while samples after dilution only - not. Protein precipitation only leads to a dramatic loss of OT from the sample. This data indicated essential roles of RAPPT to release OT from binding with plasma albumins. Finally, we validated RAPPT sample treatment in comparison of plasma pools of ICR and CD38KO mice (a model with disrupted central OT release). Obtained data confirmed lower levels of OT in CD38KO plasma.

MTU08-02

Traumatic brain injury and risk of dementia: A meta-analysis of cohort studies using real-world data **M. S. Hussain¹, S. O. Rahman¹, A. K. Najmi²**

¹*Jamia Hamdard, Department of Pharmaceutical Medicine, New Delhi, India*

²*Jamia Hamdard, Department of Pharmacology, New Delhi, India*

Introduction: Published epidemiological studies found positive association between TBI and dementia risk. However, there are studies which found no association. So, this study is aimed to assess the association between TBI and dementia risk.

Methods: Articles were retrieved from PubMed and Embase database by running the keywords related to TBI and dementia till February 2019. We included all the cohort studies which assessed dementia risk due to TBI. Newcastle-Ottawa scale was used to assess the study quality. The primary outcome of this study was to compute the pooled dementia risk due to the TBI. Secondary

outcomes include dementia risk based on subgroups like dementia subtypes, geographic region, and sex. Statistical analysis was performed using Review Manager software.

Results: A total of sixteen articles qualified the inclusion criteria and comprised of 5,429,711 patients with mean age of 62.28 ± 11.4 years. Majority of the studies were of high quality. Pooled relative risk found that TBI significantly increased the dementia risk with a relative risk (RR) of 1.61 (95% CI: 1.39 – 1.86), $p < 0.00001$. Subgroup analysis also revealed significant Alzheimer's risk due to TBI with RR of 1.17 (95% CI: 1.12 – 1.22), $p < 0.00001$. Studies conducted in US ($n = 6$) and other parts of the world ($n = 10$) also found significant dementia risk due to TBI with RR of 1.54 (95% CI: 1.14 – 2.08), $p = 0.005$ and 1.67 (95% CI: 1.38 – 2.01), $p < 0.00001$. No significant association was observed with gender.

Conclusion: The finding of this study suggests that TBI is significantly associated with dementia as well as Alzheimer's risk.

MTU08-03

Importance of the existence of salivary proteins for stress biomarkers founded by proteome after mental or physical stress loading

S. Oshiro¹, T. Kaburagi², N. Nishiyama¹, S.-y. Kadowaki¹, T. Suagasa³, M. Morioka⁴

¹*Graduate School of Sports and Health Science, Daito Bunka University, Cell Biology, Higashi-matsuyama, Saitama, Japan*

²*Graduate School of Sports and Health Science, Daito Bunka University, Nutrition Science, Higashi-matsuyama, Saitama, Japan*

³*Graduate School of Comprehensive Human Science, University of Tsukuba, Sport Medicine, Higashi-matsuyama, Saitama, Japan*

⁴*RIKEN ACCC, Preventive Medicine and Applied Genomics Unit, Tsukuba, Ibaraki, Japan*

Saliva is a useful sample non-invasively and repeatedly collected from body fluid. Our objective in the present study is to find salivary biomarker proteins for mental and/or physical stress for quality of life. Quite recently, we investigated rat saliva marker proteins for mental or physical stress by proteome using rat stress models. The increased proteins by mental stress were subjected to liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS). We detected the known enzymes and secretory proteins with MW of 20-70 kDa in rat saliva proteins. Furthermore, we analyzed the biomarkers for physical stress by proteome after treadmill running loading to rats. After the separation by SDS-PAGE, the increased proteins by physical stress were used for LC-MS/MS and comprehensive proteome analysis (isobaric Tags for Relative and Absolute Quantitation, iTRAQ). We might find biomarkers for mental and/or physical stress. In the present study, we discussed on the importance of the existence of salivary proteins as stress biomarkers of mental and/or physical stress loading to rats.

MTU08-04

RAGE-associated serum markers along with motor and cognitive clinical parameters as predictors of parkinson's disease

N. Somensi¹, J. Gasparotto¹, C. T. Ribeiro¹, N. M. Soares^{2,4}, A. C. Panzenhagen¹, J. C. F. Moreira¹, A. Hilbig^{3,5}, C. R. de Mello Rieder^{2,4}, D. P. Gelain¹

¹Universidade Federal do Rio Grande Sul, Bioquímica, Porto Alegre, Brazil

²Universidade Federal do Rio Grande Sul, Ciências Médicas, Porto Alegre, Brazil

³Universidade Federal de Ciências da Saúde de Porto Alegre, Neurologia, Porto Alegre, Brazil

⁴Hospital de Clínicas de Porto Alegre, Clínica Médica, Porto Alegre, Brazil

⁵Santa Casa de Misericórdia de Porto Alegre, Neurologia, Porto Alegre, Brazil

Parkinson's disease (PD) affects nearly 10 million people globally. The course of disease is highly variable and there are no established biomarkers with diagnostic value or predictive models. The receptor for advanced glycation end products (RAGE) is crucial in the propagation of inflammatory events, exerting a major role in neuroinflammation and dopaminergic denervation. We evaluated the correlation of inflammatory cytokines with RAGE agonists in serum, in parallel with cognitive (MoCA) and motor/non-motor (UPDRS) clinical parameters of PD. Blood samples were collected from 51 cases and 37 controls. Serum parameters were measured by Multiplex. PD-patients had increased concentration of serum HMGB1 and decreased IL-1 β , TNF- α , IL-8, RANTES and IL-6. HMGB1 is correlated with other RAGE agonists, such as nitrotyrosine, 4-HNE, CML along with S100B, but when analyzed together they do not predict the outcome. RANTES is negatively correlated to MoCA and TNF- α and together they show to be good predictors of PD. Although α -synuclein does not differ between control and PD, it is positively correlated to TNF- α in PD, and together they are factors that predict the disease. More parameters should be developed in order to discover RAGE-associated potential molecular markers that may aid in clinical diagnostic.

MTU08-05

Frontal theta asymmetry changes while watching emotional film clips and role of difference pair of frontal electrodes

W. Suwannapu¹, N. Kraiwattanapirom¹, S. Prayoonsak², V. Siripornpanich¹

¹Institute of Molecular BiosciencesMB, Research Center for Neuroscience, Mahidol University, Nakhonpathom, Thailand

²Faculty of Education, Educational Psychology and Guidance, Mahasarakham University, Mahasarakham, Thailand

The brain hemispheric asymmetry has been hypothesized associated with emotional processing related emotional valence. Amygdala and prefrontal cortex are the important brain structures involved in emotional processing. Frontal alpha (8.5-13 Hz) asymmetry (FAA) has been received most attention to be a neuro-biomarker of emotion, but the brain asymmetry on other spectral frequencies, particularly in frontal theta (4.5-8 Hz) asymmetry (FTA), are needed to be clarified. This study aimed to investigate the FTA in response to emotional clips and the influence of difference pairs of frontal sites (F4/F3: medial-frontal vs. F8/F7: lateral-frontal). The participant were 10 healthy females (aged 20-32 years; M = 25.80, SD = 4.85). Two sets of emotional clips (4 clips per set) were used to elicit target emotional states (sadness, fear, happiness, and neutral). The FTA was calculated using the equation: $\ln[\text{right}] - \ln[\text{left}]$ theta power. The results found the significantly decreased FTA at F4/F3 pair in response to negative and neutral clips when compared to resting-states. The significant reduction of FTA among types of emotional clips was obviously demonstrated at F4/F3 pair. However, only one pair of emotional clip (fear and happiness) can be revealed with FTA at F8/F7 pair. These findings indicated that FTA can be used for studying emotional response, especially with negative stimuli, and may be a valuable tool as a neuro-biomarker of emotion apart from the FAA. However, the selection of frontal electrode pairs is important. The FTA between medial-frontal electrodes seem to be sensitive with emotional response than the FTA between lateral-frontal sites.

MTU09 Neurodegeneration and mental health (Session A)

MTU09-01

Effects of garlic constituents in a rat model of reserpine-induced depression

Khushboo, B. Sharma

University of Allahabad, Biochemistry, Allahabad, India

Commonly available chemical drugs used for nervous system disorders have various unfavorable side effects. Due to this, herbal medicines are being prescribed as an alternative therapy. In the present study, we have investigated the effect of antidepressant and neuroprotective effect of garlic extract against reserpine-induced depression in the rat model. Rats were divided into four groups: 1) control, 2) reserpine, 3), reserpine with garlic extract and 4) reserpine with fluoxetine. The forced swimming test was used to evaluate the antidepressant activity of garlic extract. The levels of antioxidant enzymes including SOD, GST and CAT and some metabolic enzymes such as LDH and MDH were significantly decreased in depressed rat brain. The levels of serotonin and acetylcholinesterase activity were also altered which suggested the abnormal dopamine cycle in the brain. A histological study shows significant malformations in brain parts. Reserpine-induced a significant increase in the immobility time of rats in the forced swimming test and treatment with garlic extract ameliorated the reserpine-induced changes. Reserpine-induced reduction in brain marker enzymes was improved using garlic extract. Also, it reduces the MDA level in the brain. The study suggests the antidepressant activity of garlic extract against the reserpine-induced depression.

MTU09-02

Potential effects of genistein on human amniotic mesenchymal stem cells for cholinergic neuronal differentiation

M. Ahmad-Mahidi¹, W. Thangnipon¹, T. Phermthai², S. Julavijitphong²

¹*Institute of Molecular Biosciences, Research Center for Neuroscience, Nakhon Pathom, Thailand*

²*Faculty of Medicine Siriraj Hospital, Department of Obstetrics and Gynecology, Bangkok, Thailand*

Alzheimer's disease (AD) is an irreversible neurodegenerative disorder that worsens without receiving proper treatment. AD is widely known for causing dementia but it is also characterized by loss of cholinergic neurons and high production of reactive oxygen species (ROS). Mesenchymal stem cells (MSCs) became a recent area of interest as a treatment of AD, because under specific conditions MSCs can differentiate into cholinergic neurons. Previous studies have shown that pretreating amniotic fluid mesenchymal stem cells with an antioxidant called *N*-benzylcinamide (PT-3) aided in preventing cell death during cholinergic neuronal induction. In the present study, human amniotic mesenchymal stem cells (hAMSCs) were retrieved with consent after full-term labor and prepared for cell culture (PSC5 cells). Additionally, phytoestrogens with antioxidative properties are also an upcoming candidate for the treatment of AD as low estrogen was found to be related to AD progression. Genistein (GEN), an isoflavone, binds to estrogen receptor α and even more estrogen receptor β (ER α and ER β) and it

is present in some legumes such as soy beans and red beans. GEN was found to be beneficial to the nervous system because of its estrogen-like properties but at the same time possessed low oncogenicity. Treatment of PSC5 cells with GEN for 48 h showed that it was able to protect cells from cell death via cell viability assay (MTT), and decreased the amount of ROS production measured by ROS assay.

MTU09-03

DY-9836 AS calmodulin inhibitor ameliorates cognition via inhibiting nitrosative stress and NLRP3 signaling in mice model of BCAS

M. M. Ahmed^{1,2}, W. Rui^{2,3}, Y. Yi-Xuan², S. Andleeb¹, X. Juan², I. Ch¹, K. Fukunaga⁴, Y.-Z. Du², S. Nh. Shah¹, F. Han²

¹*Bahauddin Zakariya University, Faculty of Pharmacy, Multan, Pakistan*

²*Zhejiang University, College of Pharmaceutical Sciences, Hangzhou, China*

³*Ningxia Medical University, Department of Pharmacology, Key Laboratory of Ministry of Education in Fertility Preservation and Maintenance, Yinchuan, China*

⁴*Tohoku University, Department of Pharmacology, Graduate School of Pharmaceutical Sciences, Sendai, Japan*

Vascular dementia (VaD) is a heterogeneous brain disorder or which there are no effective approved pharmacotherapy available. The aim is to elaborate the effect of calmodulin inhibitor, DY- 9836, and its loaded nano drug carrier system on cognitive impairment and gain a better understanding of the protective mechanisms in mice with bilateral carotid artery stenosis (BCAS). DY- 9836 (0.5 or 1 mg/kg) or DY-9836 (0.25 mg/kg)-encapsulated polysialic acid-octadecylamine (PSA-ODA) micelles (PSA- ODA/DY) were given to BCAS mice for 4 weeks. Administration of DY- 9836 or PSA-ODA/DY reduced escape latency in space exploration and working memory test compared with vehicle group. Vehicle treated mice showed reduced phospho- CaMKII (Thr286/287) levels in the hippocampus, whereas partially restored by DY- 9836 (1 mg/kg) or PSA- ODA/DY (0.25 mg/kg) treatment. In accordance with the pharmacological profile of DY- 9836 observed during behavioral studies, experimental molecular and biochemical markers induced by CAS, such as protein tyrosine nitration, Nod- like receptor protein 3 (NLRP3), caspase- 1, and interleukin- 1 β , were reduced by DY- 9836 and PSA- ODA/DY treatment. This study discloses the novel therapeutic potential of DY-9836, and its encapsulated nanodrug delivery system significantly enhanced the cognitive function in mice model of Vascular dementia.

MTU09-04

Kolaviron mitigates rotenone-induced behavioural incompetence and nigrostriatal degeneration**I. Awogbindin**

University of Ibadan, Ibadan Nigeria, Molecular Drug Metabolism and Toxicology Laboratory, IBADAN, Nigeria

Parkinson's disease (PD) is the most prevalent movement disorder. Currently, therapies are palliative with associated irreversible behavioural incompetence. Here, we investigated the ability of kolaviron (KV), an anti-inflammatory agent, to rescue nigrostriatal damage and redo-inflammation in rats exposed to rotenone (ROT). Aged rats exposed to 11 days of rotenone intoxication were treated with KV either concurrently or for 18 days (7 days of pre-treatment prior 11-day concurrent ROT-KV treatment). ROT-exposed rats lost weight appreciably and travelled less distance with reduced speed, decline efficiency to maintain a straight path, enhanced freezing, increased immobile episodes and poor hole recognition. The motor incompetence was attributed to enhanced nigrostriatal degeneration and increased alpha synuclein formation. ROT resulted in reduced tyrosine hydroxylase (TH) intensity in substantia nigra (SNc; 50%) and striatum (75%), and depletion of SNc TH-positive cells (50%). ROT intoxication significantly elicited reactive species production, induction of striatal antioxidant system and damage to biomolecules. ROT increased COX-2 expression, myeloperoxidase activity and secretion of striatal interleukin-6 (IL-6), IL-1 β and tumour necrosis factor (TNF- α). KV treatment reversed the rotenone-associated locomotor impairment, exploratory deficits and motor/neuromuscular incompetence. KV-treated rats showed improved capacity to maintain efficient gait with minimal rigidity and enhanced coordination. KV pre-treatment preserved more than 70% striatal dopaminergic terminal and 75% SNc TH-positive neurons. KV significantly attenuated ROT-induced neuro-biochemical imbalance, altered antioxidant defence system, reduced DJ-1 secretion, neuroinflammation and enhanced striatal infiltration of CD45R⁺ cells. Taken together, kolaviron treatment mitigated the molecular processes and pathological features associated with PD via mechanisms related to its antioxidant and anti-inflammatory properties. Thus, kolaviron may be beneficial in the management of PD.

MTU09-05

Adenosine A1 and A2A receptors modulation by atorvastatin: neuroprotective and antidepressant-like effects**L. B. Binder¹, F. K. Ludka², N. F. Marques¹, L. C. Constantino¹, C. I. Tasca¹**

¹Universidade Federal de Santa Catarina, Departamento de Bioquímica, Florianópolis, Brazil

²Universidade do Contestado, Curso de Farmácia, Canoinhas, Brazil

Adenosinergic system is involved in key neuromodulatory processes. The purpose of this study was to evaluate the modulation of this system on neuroprotective and antidepressant-like effects of atorvastatin. Adults male Swiss mice received an acute administration of atorvastatin (0.1 or 0.01 mg/kg, p.o.). CHA (0.05 mg/kg, i.p., A₁R agonist) or DPCPX, (2 mg/kg, i.p. A₁R antagonist) was administered 30 min before atorvastatin. Animals received dipyrindamole (0.1 μ g/site, i.c.v., adenosine transport inhibitor) or

SCH5621 (0.05 mg/kg, i.p., A_{2A}R antagonist) 45 min after atorvastatin. Mice were subjected to tail suspension test (TST) and open field test (OFT), 1 h after Atorvastatin. For *ex vivo* evaluations mice received sub-effective atorvastatin for 7 days, once a day (10 mg/kg). Hippocampal slices were pre-incubated with DPCPX (250 nM) or SCH5621 (SCH, 100 nM) and subjected to glutamate toxicity protocol (10 mM) for 1 hour and cellular viability was evaluated. The coadministration of atorvastatin and CHA produced an additive effect, reducing the immobility time in TST. DPCPX administration prevented the immobility time reduction induced by an effective dose of atorvastatin. Similarly, atorvastatin antidepressant-like effect was blocked by SCH administration. To evaluate adenosine transport, dipyrindamole was administered but no alterations were observed in TST and OFT. In neuroprotective *ex vivo* evaluations, atorvastatin treatment prevented glutamate-induced cellular viability decrease, however, pre-incubation with DPCPX, or SCH prevented atorvastatin effect. This set of results suggests a dependence on A₁R and A_{2A}R activation for the antidepressant-like and neuroprotective atorvastatin effects, as well as, a correlation between these mechanisms.

MTU09-06

Aging-induced neurodegeneration in relation to brain regional A β deposition, locomotor and cognitive function: role of carnosine**S. Banerjee**

Jadavpur University, Pharmaceutical Technology, Kolkata, India

Aging develops positive association of biological disability to the stress related physiological conditions to culminate into the risk of disease with the deterioration of different antioxidant system. Carnosine, an endogenous antioxidant dipeptide biomolecule present in different tissues including brain. The present study focuses on central amyloid beta (A β) and carnosine in relation to neurodegeneration, locomotor activity (LA) and cognitive function (CF) in young (4 months) and aged (18 and 24 months) male albino Wistar rats. Results revealed that aging significantly (1) enhanced brain regional A β deposition (as plaque) in the order of hippocampus > cerebral cortex > hypothalamus > pons-medulla, without its any existence in cerebellum in spite of having its (A β) highest level among the brain regions, (2) reduced (a) steady state level of carnosine and neuronal cell count of the brain regions studied and (b) LA and CF. Carnosine (2.0 μ g/Kg/day, i.t for 21 consecutive days) attenuated this aging-induced (a) brain regional increase of A β levels and plaques along with their neuronal cell loss and reduction in endogenous carnosine level, (b) decrease in LA and CF towards the results that were observed in young rats. Thus it may be concluded that (a) aging-induced up regulation of brain regional A β in association with their reduction in carnosine content may be correlated with the neurodegeneration and down regulation of both LA and CF, (b) carnosine attenuated the above mentioned aging-induced changes in brain regulated behavior, possible by *in vivo* up regulation of antioxidant system.

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MTU09-07

The characterisation of oligodendrocytes derived from iPSC from ALS patients harbouring point mutations in the TDP-43 gene

S. Barton^{1,2,3}, D. Magnani^{2,3}, B. Selvaraj^{2,3,4}, M. Livesey^{2,4,5}, E. Cleary³, N. Vasistha³, K. Burr^{2,3,4}, D. Story^{2,3,4}, R. Carter⁶, N. Morton⁶, G. Hardingham^{2,4,5}, D. Wyllie^{4,5}, S. Chandran^{2,3,4}

¹Florey Institutes, University of Melbourne, Melbourne, Australia

²Euan MacDonald Centre for MND, University of Edinburgh, Edinburgh, UK

³UK DRI, University of Edinburgh, Edinburgh, UK

⁴Centre for Discovery Brain Sciences, University of Edinburgh, Edinburgh, UK

⁵Centre for Cardiovascular Science, University of Edinburgh, Edinburgh, UK

TDP-43 pathology is common to > 95% of ALS patients and has been identified in glia, including oligodendrocytes. Oligodendrocytes have two main roles in the brain, which are myelination and providing metabolic support and both are critical for maintaining neuronal health and function. The effect of TDP-43 pathology on oligodendrocyte function remains unknown. We derived oligodendrocytes from induced pluripotent stem cells (iPSC) from patients harbouring separate point mutations in the *TDP43* gene, namely G298S and M337V. We investigated the effect of these mutations on oligodendrocyte TDP-43 subcellular localization, cell morphology, and function. Using advanced CRISPR-Cas9 technology we generated an isogenic control line for comparison and also generated oligodendrocytes from an unrelated control. We assessed cellular development and morphology as well as metabolic capacity of diseased oligodendrocytes compared to controls using a 'disease in a dish' approach. For the first time, we demonstrated pathogenic TDP-43 protein mislocalization in iPSC-derived oligodendrocytes that was not present in the isogenic control or unrelated control. Despite this TDP-43 pathology, the oligodendrocytes did not have a developmental or morphological deficit and there was no effect of the *TDP43* mutations on the oligodendrocytes' metabolic capacity.

MTU09-08

Intranasal delivery of insulin for the restoration of memory signaling in Alzheimer disease

M. Bhargava^{2,1}

¹Himalayan University, Pharmacy, Kanpur, India

²GTB Hospital, R&D, Kanpur, India

Alzheimer's disease (AD), a form of dementia, is progressive, degenerative brain disease characterized by marked atrophy of cerebral cortex and loss of cortical and sub-cortical neurons. Weakening of insulin receptor signaling is involved in ageing-related brain degeneration like AD. Objective of study is to develop delivery-system to overcome BBB by employing novel, non-invasive approach via nasal route i.e. delivery of antibody appended Insulin encapsulated carrier, PEGylated nanoparticle coated with chitosan to facilitate nasal absorption for efficient transfer to brain. PEGylated-PLGA nanoparticles were prepared by modified Double Emulsification method and coated with chitosan by freeze drying. Characterization was done by FTIR, NMR and *in-vitro* parameters. *In-vivo* study comprised biodistribution in various organs and fluorescence microscopy, estimation of Anti-A β antibody, PET-

Imaging of Brain, Hemolytic Toxicity studies, Histopathology of Nasal Mucosa and Brain with periodic Blood Glucose Level Monitoring. Degree of hemolysis showed PEGylated (PEG-NP's) and chitosan coated nanoparticles (cPEG-NP's) were less toxic. Blood glucose monitoring indicates reduction in blood glucose level in cPEG-NP's. Biodistribution assessment suggests nanoparticles showed maximum availability at olfactory bulb entrance. Chitosan coating increased CSF availability of drug even at initial period of administration. Uptake study shows intense fluorescence in brain revealing higher uptake of nanoparticles. These studies highlight possible biological significance of cPEG-NP's for delivery to brain. Results from various studies suggest nanoparticles are effective delivery system for targeted delivery of insulin in brain for extended period. Coating with chitosan elicits associated benefits in addition to prolonging uptake via intranasal route. This project may provide sound platform towards employment of this modified nanoparticle carrier for brain delivery of proteins and peptides towards intranasal delivery of insulin for restoration of memory signaling in Alzheimer patients.

MTU09-09

Novel molecular-genetic probe for visualizing protein aggregation in neurodegenerative diseases by 3d electron microscopy

D. Boassa¹, J. Hu¹, S. Phan¹, M. Mackey¹, R. Ramachandra¹, S. Adams², J. Ngo³, M. Ellisman¹

¹University of California San Diego, Neuroscience, La Jolla, USA

²University of California San Diego, Pharmacology, La Jolla, USA

³Boston University, Department of Biomedical Engineering, Boston, USA

We engineered a protein-fragment complementation assay for detecting and localizing protein aggregates associated with neurodegenerative diseases, including the homomeric aggregation of alpha-synuclein (α Syn) and tau proteins. This tool was built by bisection of miniSOG, a fluorescent flavoprotein derived from the light, oxygen, voltage (LOV)-2 domain of *Arabidopsis* phototropin. When brought together by interacting proteins, the fragments reconstitute a functional reporter that permits tagged protein complexes to be visualized by fluorescence light microscopy, and then by standard as well as "multicolor" electron microscopy (EM) via the photooxidation of 3-3'-diaminobenzidine and its derivatives. Unlike previous EM-compatible PCAs, the split-miniSOG fragments are relatively small, and display low affinity for each other, minimizing perturbation of the native dynamics of the tagged interacting partners. These experiments show the general utility of the system for detection of spatial organization of molecular complexes in mammalian cells at nanometer resolution. Using tagged monomers of α Syn, we carried out correlative analyses and observed the self-aggregation of the protein within neuronal cells for the first time. Applying 3D EM and electron tomography, we observed both straight and twisted α Syn filaments with a diameter ranging from 4 to 15 nm. In dendrites of expressing neurons, these aggregates were contained in membrane-limited organelles, fusing to the plasma membrane and suggesting release to the extracellular compartment. We further extended the split miniSOG proximity indicator/protein aggregation detection strategy to Tau proteins. This approach allows to clearly visualize how the aggregation occurs in the context of the intracellular milieu.

MTU09-10

Proteomic profiling of exosomes derived from brain microvascular endothelial cells under hypoxia: Potential role in remyelination**A. Campero-Romero¹, E. Ríos-Castro², A. Cárdenas-Rivera¹, Luis. B. Tovar-y-Romo¹**¹*Universidad Nacional Autónoma de México, Instituto de Fisiología Celular, Molecular Neuropathology, Mexico City, Mexico*²*IPN-CINVESTAV, Unidad de Genómica, Proteómica y Metabólica, Mexico City, Mexico*

Brain endothelium plays critical roles in the modulation of responses to injury, such as regulation of blood-brain barrier dynamics, neuroinflammatory processes, and reperfusion after stroke, and the molecular characterization of exosomes released by endothelial cells might enable the identification of adaptive signaling responses to stress. Here we profiled the protein content of brain microvascular endothelial cells (BMEC)-derived exosomes shed by primary BMEC harvested from adult rat brains that were subjected to 6 h of hypoxia followed by 18 h of recovery. We also characterized exosomes released by BMEC cultured under normoxic conditions. Exosome molecular signatures and structure were confirmed by transmission electron microscopy, NanoTracking assays and the presence of exosome markers. Proteomic analyses were carried out with ESI-IMS-MS. We identified 262 high-quality hits with BMEC features such as molecules involved in focal adhesion, prostaglandin synthesis, and integrin signaling and molecular regulators that target recovery mechanisms after an injury to the CNS such as remyelination. We tested the potential of BMEC-derived exosomes to restore lipophosphatidylcholine-demyelinated corpus callosum in the rat, and we found that intracerebral injection of these extracellular vesicles improved the rate of remyelination as compared to non-treated animals. The proteomic characterization of extracellular vesicles released by BMEC under hypoxic stress enabled us to discover the participation of vascular endothelium in repairing processes like remyelination that could constitute new therapeutic targets to repair brain damage. This work was supported by DGAPA-PAPIIT grant IN226617.

MTU09-11

Agathisflavone binds to estrogen and retinoic receptors and drives remyelination in a demyelination-induced model**M. M. Carneiro^{1,2}, F. Pieropan², A. Rivera², L. Oliveira³, M. S. Junior³, C. Souza¹, A. Butt², S. Costa¹**¹*Federal University of Bahia, Department of Biochemistry and Biophysics, Salvador, Brazil*²*University of Portsmouth, School of Pharmacy and Biomedical Sciences, Portsmouth, United Kingdom*³*State University of Feira de Santana, Department of Health, Feira de Santana, Brazil*

Myelin dysfunction plays a significant role in the pathogenesis of various neurodegenerative diseases. Therefore, there is a critical need to develop more effective therapies for demyelinating disorders. Here, we have examined the protective effect of the flavonoid agathisflavone (FAB 5 and 10 μ M) in the lyssolecithin (LCT) model of demyelination, in mouse cerebellar slice organotypic culture. Treatment with LCT (0.5 mg/mL) resulted in significant

demyelination, determined by loss of MBP immunostaining, and a significant increase in PLP1-DsRed⁺ mature oligodendrocytes and NG2⁺ oligodendrocyte progenitor cells (OPCs). Treatment with FAB significantly increased MBP immunostaining and the number and proliferation of OPCs. In addition, LCT induced astrogliosis (GFAP immunostaining) and microglial proliferation (IBA1 + Ki67⁺), as well as a shift in microglia to a more inflammatory phenotype (M1/M2 ratio: CD16/32 + /CD206 +). These astroglial and microglial changes were reverted by FAB, which also reduced *Tnfa*, *Il1b* and *Nos2* and increased *Arg1*, *Tgfb* and *Acrv1b* expression, as determined by RT-qPCR. Molecular docking analysis demonstrated intermolecular interactions between FAB and α /b-estrogen receptors (ER α /b), retinoic acid receptors (RAR), and α / γ retinoic X receptors (RXR α / γ), which are involved in myelination and neuronal survival. Furthermore, blockade of ER α reduced FAB-induced promotion of remyelination. Together these findings provide evidence that FAB has a significant protective effect against demyelination and implicates ER, RAR and RXR in these effects. Supported by CAPES/CNPq (SLC), BBSRC/MS (AMB), MS (FP).

MTU09-12

Hippocampus metabolic changes and memory impairment in mice under high fat-sucrose diet for 4 months are reversed by normal diet**A. G. Serrano¹, J. Duarte¹**¹*Lund University, Experimental Medical Science, Lund, Sweden*²*Lund University, Wallenberg Centre for Molecular Medicine, Lund, Sweden*

Type 2 diabetes (T2D) increases dementia risk through mechanisms that not fully understood. Brain metabolic dysregulation plays a role in T2D-induced memory dysfunction. We investigated the potential for reversibility of brain metabolic alterations and memory impairment in male and female C57B16/J mice fed a high-fat and high-sucrose diet (HFHSD) that develop T2D. Age matched control mice were fed a 10%-fat diet (CD). Diabetic mice were exposed to HFHSD (60%-fat, plus 20% sucrose in drinking water) for 6 months. A group of mice under HDHSD for 4 months was reversed to CD for 2 months (RD). Metabolic profiles in hippocampus and cortex were measured longitudinally by magnetic resonance spectroscopy (MRS) at baseline, and after 4 and 6 months. Memory performance was assessed at 6 months using object relocation and novel object recognition tasks. HFHSD-fed mice developed overweight, glucose intolerance and insulin resistance, which recovered to control values after diet reversing (all $p < 0.01$, RD vs. HFHSD). HFHSD-fed mice showed poor memory performance in object recognition tasks (both $p < 0.001$ vs. CD), but not mice in RD. At 4 months of HFHSD, the hippocampal metabolic profile showed a prominent increment in taurine concentration ($p < 0.01$ vs. baseline). This change persisted at 6 months in HFHSD mice ($p < 0.01$ vs. baseline), but recovered to baseline levels in RD mice ($p < 0.05$ vs. HFHSD). Metabolic changes were not observed in cortex. We conclude that HFHSD leads to T2D and T2D-induced memory impairment, and increased hippocampal taurine concentration. T2D and brain alterations were all reversed upon dietary switch from HFHSD to a regular diet.

MTU09-13

Ascorbic acid augments nicotine neuromodulatory roles in transferrin-mediated cortico-hippocampal neuropathology in wistar rats**I. Gbadamosi, O. Olayemi, G. Omotoso***University of Ilorin, Anatomy, Ilorin, Nigeria*

Controlled activation of nAChRs in *invivo* model of neurodegeneration is a remarkable way of combatting molecular aberrations in Alzheimer's. Nicotine, being an allosteric modulator of these receptors, exacerbates production of reactive species thereby compromising its candidacy as a drug target for the management of Alzheimer's disease. We characterized the behavioral outcome and molecular fingerprints in transferrin-mediated neuroinflammation while exploring the potentials of nicotine in mitigating molecular aberrations in the presence of ascorbic acid.

Following due ethical approval, five groups (A-E) of Wistar rats ($n = 8/\text{group}$) were used for this study. Group A was treated with distilled water daily for 8 week. Transferrin-mediated neuroinflammation was achieved in groups B-E through daily oral infusion of 100 mg/kg of AlCl_3 for four weeks. Groups C-E were then post treated with ascorbic acid (100 mg/kg daily), nicotine (10 mg/kg daily) and nicotine (10 mg/kg daily) + ascorbic acid (100 mg/kg daily), for four weeks. Following behavioral assessments, prefrontal cortex (PFC) and hippocampus were prepared for biochemical analyses, histology and immunohistochemistry.

Nicotine+ascorbic acid significantly reversed reduction of working memory, cognitive decline and special memory dysfunction. These correlated with nicotine-dependent modulation of Trp-1 expression that was complemented by significant reversal of neural oxidative and nitrosative stress by antioxidant properties of ascorbic acid. Furthermore, nicotine+ascorbic acid treatment regimen further inhibited neural dysfunction within PFC and hippocampus correlating with increased glucose-6-phosphate dehydrogenase. Nissl staining and immunohistochemical profiling of thin sections corroborated roles of nicotine+ascorbic acid in reversing AlCl_3 -induced neuropathology.

Summarily, we have showed the role of ascorbic acid in enhancing nicotine neuromodulatory activities in transferrin-mediated behavioral decline and molecular aberration in the frontal cortex and hippocampus of Wistar rats.

MTU09-14

Cholinergic regulation of plaque pathology in Alzheimer's disease knock-in mouse models**L. German-Castelan^{1,2}, T. Saito³, T. Saido³, M. Prado^{1,2,4}, V. Prado^{1,2,4}**¹*Western University, Neuroscience, London, Canada*²*Robarts Research Institute, London, Canada*³*RIKEN Brain Science Institute, Wako-shi, Japan*⁴*Western University, Physiology/Pharmacology, London, Canada*

Cholinergic deficiency is characteristic of many neurodegenerative disorders including Alzheimer's disease (AD). Decreased levels of the vesicular acetylcholine transporter (VACHT) have been detected in AD patients, and previous work suggested that cholinergic deficiency increase AD-like pathology in mouse models. In humans, plaque pathology has been linked to the loss of VACHT; however, whether changes in VACHT have a causal relationship with plaque accumulation is unknown. To study this aspect of AD,

we crossed a humanized APP-knock-in mouse carrying 3 AD-associated mutations ($\text{App}^{\text{NL-G-F/NL-G-F}}$) with mice overexpressing VACHT using a BAC transgene. We analyzed the number and area populated by A β -plaques in the cortex, as well as Iba1 marker (for microglia) and GFAP (for reactive astrocytes). Our preliminary results show a significant decrease in the number of reactive astrocytes and the number of microglia associated with plaques at 2 months of age. Likewise, cortical plaque area was significantly decreased at 2 months, but not at 3 or 6 months. Remarkably, we observed a sharp decrease in the levels of VACHT in $\text{App}^{\text{NL-G-F/NL-G-F}}$ -VACHT-BAC mice at 6 months, effectively reducing the overexpression of VACHT. Accordingly, $\text{App}^{\text{NL-G-F/NL-G-F}}$ mice presented age-decreased VACHT levels at 3 and 6 months when compared to 2-months-old. Moreover, elimination of cortical VACHT increased the number of plaques in $\text{App}^{\text{NL-F/NL-F}}$ mice, a humanized model with less aggressive pathology. These results suggest a causal relationship between cholinergic tone and plaque accumulation in a humanized AD mouse model and that amyloid plaques can interfere with cholinergic tone by decreasing VACHT levels.

MTU09-15

Nickel-induced developmental neurotoxicity in c. elegans; neuronal degeneration, altered behaviour, and increased SKN-1 activity**O. Ijomone^{1,2}, M. Miah², G. Akingbade¹, H. Bucinca², M. Aschner²**¹*Federal University of Technology Akure, Human Anatomy, School of Health & Health Technology, Akure, Nigeria*²*Albert Einstein College of Medicine, Molecular Pharmacology, New York City, USA*

Globally, environmental and occupational exposures to heavy metals are an increasing health concern. Nickel (Ni) is one of such metals and has extensive industrial applications. Importantly there is no known physiological role for Ni in humans and other mammals. Brain damage has been severally implicated in Ni overexposure however, published reports are relatively limited. Here, we investigated specific neuronal susceptibility in a *C. elegans* model of acute nickel neurotoxicity. Wild-type *C. elegans* and worms expressing GFP in several neuronal subtypes were treated with NiCl_2 at the first larval (L1) stage. Our results show significantly increasing degeneration of cholinergic, dopaminergic and GABAergic neurons with increasing Ni concentration in worms expressing GFP for these neuronal subtypes. Also, significant functional changes in locomotion and basal slowing response assays reflected impaired cholinergic and dopaminergic neuronal function respectively. Interestingly, a significant effect on number of worms exhibiting shrinker phenotype indicated that function of D-type GABAergic neurons of *C. elegans* may be specifically attenuated while the RME subset of GABAergic neurons is unaffected. GFP expression due to induction of glutathione S-transferase 4 (*gst-4*), a target of Nrf2 homolog *skn-1*, was increased in VP596 (Pgst-4::GFP; Pdp-3::RFP) worms highlighting increased SKN-1 activity and consequently, Ni-induced oxidative stress. RT-qPCR verified upregulation of this expression of *skn-1* immediately after exposure. These data suggest that developmental Ni exposure impairs cholinergic, dopaminergic and GABAergic neurotransmitter systems, probably via the generation of oxidative stress. Further studies are ongoing to unravel molecular mechanisms involved in Ni neurotoxicity using *C. elegans* model.

MTU09-16

Isolation and neuroprotective effect of ethyl acetate fraction of terminalia macroptera leaf**L. Ior^{1,2,3}, S. Negri², I. Scambi³, O. Sunday¹, F. Guzzo², A. Sagay¹**¹University of Jos, Pharmacology, Jos, Nigeria²University of Verona, Plant Biotechnology, Verona, Italy³University of Verona, Neuroscience, Biomedicine, and Movement Sciences, Verona, Italy⁴University of Jos, Department of Obstetrics and Gynecology, Jos, Nigeria

Neurodegeneration is a process involved in both neuropathological conditions and brain ageing. It is known that brain pathology in the form of cerebrovascular and neurodegenerative disease is a leading cause of death all over the world. No effective treatment for these neurodegenerative diseases has been developed yet. Oxidative stress-mediated neurodegeneration is one of the key pathophysiological factors involved in these diseases. This study is aimed at investigating the neuroprotective effect of the ethylacetate fraction of *Terminalia macroptera* leaf and its isolates on SH-SY5Y neuronal cells. The ethylacetate fraction of *T. macroptera* leaf was subjected to solid phase extraction and preparative high-performance liquid chromatography (HPLC) to yield several polyphenolic compounds. We examine the neuroprotective effects of the ethylacetate fraction and the polyphenols isolated from it against hydrogen peroxide (H₂O₂)-induced cytotoxicity in SH-SY5Y cells. The results revealed that the ethylacetate fraction of *T. macroptera* showed the most favorable antioxidant activity in scavenging free radicals compared to the isolates such as Gallic acid, chebulagic acid, Quercetin-3-O-glucoside, chebulinic acid, vitexin, and ellagic acid. Component elucidation revealed that the ethylacetate fraction of *T. macroptera* is a rich source of phenolic compounds, especially flavonoids and tannins. The ethylacetate fraction of *T. macroptera* has the potential to be a novel neuroprotective agent to be considered in nutraceutical products for preventing oxidative-related disorders. Further investigation is necessary to verify the neuroprotective efficacy and mechanisms *in vivo*.

MTU09-17

Cnestis ferruginea ameliorates kainic acid-induced status epilepticus in rats: role of neuroinflammation and oxidative stress**I. Ishola, A. James, E. Ojo, O. Afolayan, O. Adeyemi**

University of Lagos, Pharmacology, Therapeutics and Toxicology, Idi-Araba, Nigeria

We have earlier reported anticonvulsant of *Cnestis ferruginea* root extract (CF), hence, this study aimed to investigate the mechanism underlying the protective effects of CF on kainic acid (KA)-induced excitotoxicity in the rat hippocampus. Intraperitoneal injection of KA (10 mg/kg) caused seizures and increased the expression of neurotoxic markers, immediate early genes such as cyclooxygenase 2 (COX-2), *c-fos*, brain-derived neurotrophic factor (BDNF), and heat shock protein 70 (hsp70) and a delayed response gene (inducible nitric oxide synthase (iNOS)), which were measured at 6 and 72 h after KA injection, respectively, in the hippocampus. Pretreatment or post-treatment of mice with CF (400 mg/kg, p.o.) delayed the onset of KA-induced seizure as well as reduction in seizures score. KA increased c-Fos (transynaptic marker for neuronal activity) immunoreactivity in DG, CA1, and

CA3 hippocampal regions which was attenuated by pre- and post-treatment of mice with CF. Moreover, CF treatments reduced KA-induced expression of COX-2, BDNF, and iNOS mRNA. Intraperitoneal injection of KA produced significant deficit in the antioxidant enzyme activities (GSH, superoxide dismutase (SOD) and catalase) when compared with the vehicle. However, pre-treatment and reversal with CF 400 mg/kg produced a significant enhancement of antioxidant enzyme activities suggestive of radical scavenging effect. Findings from this study showed that CF treatment suppresses KA-induced hippocampal injury through attenuation of excitotoxicity, neuroinflammation and enhancement of antioxidant defense mechanisms. Thus, suggest the beneficial effects of CF on the treatment of excitotoxicity-induced status epilepticus.

MTU09-18

Biochemical and behavioral evidence for neuromodulatory properties of ellagic acid against D-galactose neurotoxicity in mice**D. Khatri**

Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Department of Pharmacology, Pune, India

Introduction: Ellagic Acid is a non-flavonoid polyphenolic compound found in different berries, mango, areca nut, walnut, green tea, and other fruits, food, and beverages as well as in wine. It has been reported to show different biological activities such as free radical scavenger, antidiabetic, antiangiogenic and antimelanogenic effects, and reduced heart infarction incidence and oxidative liver and kidney damage.

Objective: The aim of the present study was to investigate the protective effects of oral Ellagic acid against motor dysfunction, striatal oxidative stress and mitochondrial deficit induced by **D-galactose** in mice.

Methods: Male mice were divided into different treatment groups. D-galactose (100 mg/kg *s.c.*) was given for 42 days to induced neurodegeneration. Chronic (6 weeks) oral administration of Ellagic acid was given at three different doses (50, 100, 200 mg/kg) to find out neuroprotective effect. The neuroprotective effect was evaluated in term of behavioral (learning, memory and motor coordination) and biochemical (Lipid peroxidation, Nitrite (NO) level, AChE activity, Advanced glycation end products). Antioxidant enzyme estimations were also performed on, reduced glutathione (GSH), Superoxide dismutase (SOD), Catalase (CAT) activity. Mitochondrial Complex activity was performed on mice brain mitochondria for Complex-I (NADH dehydrogenase), Complex-II (succinate dehydrogenase activity) and Complex-III (MTT ability) activity.

Results: Chronic (6 weeks) oral administration of Ellagic acid at three different doses (50, 100, 200 mg/kg) was found to provide a significant neuroprotective effect in a dose-dependent manner in terms of reversing behavioral, biochemical, anti-oxidant and mitochondrial damage induced by D-galactose.

Conclusion: The results suggest that Ellagic acid has neuroprotective activity against D-galactose induced neurodegeneration. The present findings provide a rationale for the uses of Ellagic acid in neurodegenerative disorders.

MTU09-19

Neuroprotective effects of kynurenic acid analogue against secondary cascades of traumatic brain injury in mice**N. Khatri¹, S. Kumar², A. Datusalia^{2,3}, S. Sharma¹**¹Guru Jambheshwar University of Science & Technology, Pharmaceutical Sciences, Hisar, India²National Brain Research Centre, -, Gurugram, India³University of Milan, Pharmacology & Biomolecular Sciences, Milan, Italy

Traumatic brain injury (TBI) is a major cause of fatality and disability across worldwide. Apart from, acute injury, secondary injury cascades such as oxidative stress, mitochondrial dysfunction, excitotoxicity, neuronal cell death etc. are detrimental factors for the life-long disability. Despite many efforts, limited progress has been made towards the development of pharmacological interventions to reduce the effects of TBI. The neuroprotective effects of Kynurenic acid are counterbalanced by 3-hydroxyanthranilic acid which is another neurotoxic metabolite of indoleamine-2,3-dioxygenase pathway. Here, we hypothesize that secondary injury cascade can be rescued by elevating neuroprotection *via* kynurenic acid. In current study, we had investigated the effect of kynurenic acid amide analogue (KAA; BBB permeable) against the secondary cascade after TBI in mice. Our data showed significant increase in mitochondrial dysfunction at 6 h and which persisted even after 72 h of injury. However, cell death of cortical neurons was evident after 24 hours post-injury. Animals (Swiss albino mice, 25-30 g) administered with Kynurenic acid amide analog (KAA, blood brain barrier permeable, NMDA receptor antagonist) (100, 200 and 400 mg/kg, i.p) 30 minutes after injury showed a dose dependent neuroprotective effect on mitochondrial dysfunction (complex-I, II & IV activities), oxidative stress. KAA also improved neuronal survival and neurological function significantly. KAA (100 mg/kg) were found effective at on long-term treatment (i.e. 72 hr to 21 days' time point). Overall, our data shows that KAA promotes neuroprotection against TBI-induced secondary cascade and improve neurological functions in mice model of TBI.

MTU09-20

Neuregulin 1 deficiency in dorsal root ganglia and dorsal roots in friedreich ataxia**A. Koeppen^{1,2}, A. Travis¹, J. Qian², J. Mazurkiewicz³**¹VA Medical Center, Research Service, Albany, USA²Albany Medical College, Pathology, Albany, USA³Albany Medical College, Neuroscience and Experimental Therapeutics, Albany, USA

Friedreich ataxia (FA) is an autosomal recessive ataxia that in the vast majority of cases is due a homozygous guanine-adenine-adenine (GAA) trinucleotide repeat expansion in intron 1 of the frataxin gene. The complex pathological phenotype includes atrophy of the dentate nucleus, hypoplasia and inflammatory destruction of dorsal root ganglia (DRG), spinal cord hypoplasia and degeneration of dorsal spinal columns and dorsal spinocerebellar tracts, myelin deficiency in dorsal roots (DR) and sensory peripheral nerves, concentric cardiac hypertrophy or dilated cardiomyopathy, and destruction of pancreatic beta cells. Neuregulin 1 type III (NRG1 [III]) is a critical signaling protein for the myelination of dorsal roots

and sensory peripheral nerves. Systematic immunohistochemical visualization of NRG1 confirmed paucity and smallness of NRG1-reactive DR axons, and a severe lack of myelination. NRG1 (III) signaling to Schwann cells occurs by binding to ErbB2-ErbB3 heteromers. An antibody to ErbB2 revealed abundant reaction product in DR of FA. Ventral spinal roots in FA displayed normal large NRG1 (III)-reactive axons, myelinated fibers, and abundant ErbB2 in Schwann cells. We conclude that lack of myelination in DR in FA is due to insufficient NRG1 (III) in DRG neurons and downstream fibers. The role of frataxin deficiency in this mechanism is unknown.

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MTU09-21

Lead aggravates the diabetic-induced neurodegeneration and neuro-protecting effect of C. carandas**A. Kumar, R. Singh, B. Sharma**

University of Allahabad, Department of Biochemistry, Allahabad, India

Diabetes, an unresolved metabolic disorder and lead contamination are prevalent problems in contemporary society. Previously, reported suggest that either diabetes or lead exposure resulted in neurodegeneration in male rats. The aim of this study was to evaluate whether diabetic rats exposed to lead demonstrate a higher degree of neurotoxicity, inflammation and neurodegeneration when compared with lead-exposed control rats. And the neuroprotective, anti-inflammatory and antioxidant activity of *C. carandas* constituents were also evaluated. Diabetes was induced by injecting a single dose of streptozotocin (40 mg/kg body weight). Control and diabetic rats were exposed to lead through oral gavage for a period of 21 days and assessed for neuro-inflammatory markers and oxidative endpoints. Significant reduction in brain antioxidant enzyme activity, membrane proteins and ion channels including Acetylcholinesterase, Na⁺-K⁺ ATPase levels and glutathione levels were observed in diabetic rats with an elevation in levels of superoxide anions, hydrogen peroxides, lipid peroxidation. Mild histopathological malformations were observed in the brain of the diabetic rats. TNF- α , IL-6 transcripts and nuclear factor- κ B expression were increased in the diabetic rat brain. Similar oxidative and neurotoxicity was observed in lead-exposed control rats. Further, lead-exposed diabetic rats showed additional deterioration in hippocampal and inflammation end points and noteworthy elevation in oxidative toxicity suggesting that treatment with lead exacerbates neurotoxicity in streptozotocin-induced diabetic rats. The ameliorative efficacy of the aqueous extract of *C. Carandas* was analysed in diabetic rat exposed to lead. The study shows the significant neuroprotective and anti-inflammatory activity of the extract.

MTU09-22

HSP90 co-chaperone stress inducible phosphoprotein-1 is necessary for chaperone activity and neuronal resilience during agingR. Lackie^{1,3}, F. Beraldo^{2,3}, R. Gros^{2,3}, J. Fan³, V. Martins⁴, V. Prado^{2,3}, M. Prado^{2,3}¹University of Western Ontario, Neuroscience, London, Canada²University of Western Ontario, Phys/Pharm, London, Canada³Robarts Research Institute, Molecular Medicine, London, Canada⁴A.C. Camargo Hospital, Molecular & Cell Biology, São Paulo, Brazil

Stress inducible phosphoprotein 1 (STI1) is a co-chaperone of the Hsp70-Hsp90 machinery and can be secreted by cells such as astrocytes. In the extracellular space, STI1 interaction with prion protein (PrP^C) results in pro-survival signaling and neurotrophic effects. Deletion of STI1 in mice is lethal and STI1 haplo-sufficient neurons are less resilient to stress. Recent *in vitro* work has implicated Hsp90 in regulating cellular senescence, a phenotype seen in aging. It remains unknown how reduced STI1 levels affects chaperone machinery function *in vivo* and cellular resilience during aging. To overcome the difficulty of studying STI1 *in vivo* due to embryonic lethality of STI1 KO mice, we generated a mouse line with a hypomorphic *Stip1* allele that produces a partially functional protein (reduced by 80%). These mutant mice have a significant decrease in Hsp90 client proteins and a subset of Hsp90 co-chaperones, suggesting that STI1 acts as a major regulatory node. Moreover, they present age-dependent hippocampal neuronal loss, and consequent memory deficits in the Morris water maze. To determine whether STI1 is required for normal aging in a cell autonomous or non-cell autonomous way we are currently generating neuronal and astrocyte selective STI1 mutant mice. Preliminary results from aged mice with STI1 knocked down in astrocytes revealed no memory impairment or hippocampal neuronal loss. Overall, our work will test whether STI1 is important for maintaining brain cell viability during aging and how its function in the chaperone machinery may maintain proteostasis.

MTU09-23

Physical exercise during pregnancy prevents cognitive impairment induced by amyloid β in adult offspring rats

C. Matte, C. P. Klein, J. B. Hoppe, A. B. Saccomori, J. P. Sagini, M. S. Crestani, P. M. August, R. M. Hozer, M. Grings, B. Parmeggiani, G. Leipnitz, P. Navas, C. G. Salbego

UFRGS, Biochemistry, Porto Alegre, Brazil

Alzheimer's disease (AD) is the main aging-associated neurodegenerative disorder and is characterized by mitochondrial dysfunction, oxidative stress, synaptic failure, and cognitive decline. It has been a challenge to find disease course-modifying treatments. However, several studies demonstrated that regular physical activity and exercise are capable of promoting brain health by improving the cognitive function. Maternal lifestyle, including regular exercise during pregnancy, has also been shown to influence fetal development and disease susceptibility in adulthood through fetal metabolism programming. Here, we investigated the potential neuroprotective role of regular maternal swimming, before and during pregnancy, against amyloid- β neurotoxicity in the adult offspring. Behavioral and neurochemical analyses were performed 14 days after male offspring received a single, bilateral,

intracerebroventricular (icv) injection of amyloid- β oligomers (A β Os). A β Os-injected rats of the sedentary maternal group exhibited learning and memory deficits, along with reduced synaptophysin, brain-derived neurotrophic factor (BDNF) levels, and alterations of mitochondrial function. Strikingly, the offspring of the sedentary maternal group had A β Os-induced behavioral alterations that were prevented by maternal exercise. This effect was accompanied by preventing the alteration of synaptophysin levels in the offspring of exercised dams. Additionally, offspring of the maternal exercise group exhibited an augmentation of functional mitochondria, as indicated by increases in mitochondrial mass and membrane potential, α -ketoglutarate dehydrogenase, and cytochrome c oxidase activities. Moreover, maternal exercise during pregnancy induced long-lasting modulation of fusion and fission proteins, Mfn1 and Drp1, respectively. Overall, our data demonstrates a potential protective effect of exercise during pregnancy against A β Os-induced neurotoxicity in the adult offspring brain, by mitigating the neurodegenerative process triggered by Alzheimer-associated A β Os through programming the brain metabolism.

MTU09-24

Effect of melatonin on methamphetamine (meth)induced alteration of app cleaving enzymes related to Alzheimer's disease

C. Nopparat

Mahidol University, Research center for Neuroscience, Nakhonpathom, Thailand

Methamphetamine (METH) is an addictive drug, which has been found to cause neurotoxicity to central nervous system through multiple mechanisms. METH abusers are likely to develop Alzheimer's disease (AD) and suffer cognitive disabilities as well as alterations in brain chemistry as the pathological cascade observed in AD brains, however, the underlying mechanism of METH-induced Alzheimer's disease remains unknown. Melatonin, hormone is mainly produced by pineal gland plays critical rule in physiological function and brain protective effect. In this study we aimed to investigate the effect of METH on amyloid precursor protein (APP) cleaving enzymes and its upstream pathway in Alzheimer's disease pathway and to determine the protective effect of melatonin on METH-induced Alzheimer's disease. SH-SY5Y cell lines and Male Wistar rats are used to investigate the effect of METH on APP cleaving enzymes and upstream pathway likes GSK3- β . Our results showed that METH significantly increased the β - and γ -secretase, amyloidogenic protein marker. On the contrary METH decreased the α -secretase, non-amyloidogenic biomarker protein. These effects of METH are prevented by pretreatment with melatonin. The result in this study suggested that METH induced the production of amyloid peptide and melatonin exerted it protective effect on METH-induced Alzheimer's disease pathway.

MTU09-25

Assessment of the mechanism of actions of bacopa floribunda on amyloid beta 1-42-induced Alzheimer's disease in male wistar rats**M. Omotola¹, H. Oni¹, B. Owoyele²**¹Afe Babalola University, Ado-Ekiti, Physiology/Neuroscience, Ado-Ekiti, Nigeria²University of Ilorin, Kwara State, Physiology, Ilorin, Nigeria

This study aimed at assessing the effects of *Bacopa Floribunda* (BF) leaves' extracts on hippocampal changes Amyloid beta 1-42 (A β)-induced Alzheimer's disease. A single bilateral dose (4 μ g/ μ l/site) of A β (1-42) was injected into the lateral ventricles using a stereotaxic apparatus while BF (200 mg/kg) was given orally for 21 days. Forty eight (48) adult male Wistar rats (170-220 g) were randomly divided into eight groups (n = 6). Group A received intracerebroventricular (ICV) injection of normal saline, Group B received ICV injection of A β alone, Groups C and D received only Ethanolic and Aqueous extracts of BF respectively, Groups E and F were post-treated with Ethanolic and Aqueous extracts of BF respectively after receiving ICV injection of A β , Groups G and H were pretreated with Ethanolic and Aqueous extracts of BF before the A β ICV injection. Rats were subjected to Y-maze and Novel Object Recognition tests and sacrificed by cervical dislocation. Twenty-four hours after the last administration, rats were sacrificed and brain tissues excised. The hippocampus was removed and some were assayed for the levels of glutamate, acetylcholinesterase, Na⁺ - k⁺ ATPase activities and Amyloid beta deposition using ELISA kits, while the rest were processed for histology using Hematoxylin & Eosin (H&E) and nissl body stain. Data were analyzed using One-way ANOVA followed by a post-hoc test and expressed as Mean $\pm$ SEM. Results showed that BF was able to reverse some perturbations caused by A β (1-42)-induced Alzheimer's disease as evident in changes in the hippocampal levels of acetylcholinesterase, Na⁺ - k⁺ ATPase and glutamate in the different treatment groups.

MTU09-27

Ameliorative potentials of bryophyllum pinnatum on kianic acid induced temporal lobe epilepsy in models
J. Owolabi, O. Fabiyi, J. Olanrewaju, S. Olatunji, A. Odubanjo*Babcock University, Nigeria, Department of Anatomy, Ilesha-Remo, Nigeria*

Bryophyllum pinnatum is reportedly used in managing epilepsy in African folk medicine, albeit without significant empirical or experimental evidences. Effects of aqueous leaf extract of *bryophyllum pinnatum* (AEBP) on the kainic acid induced epileptic activities in the temporal lobes was studied using Wistar rats. Twenty-five (n = 25) adult male Wistars (average weight = 135 g) which were randomly divided into 5 groups. Group A was the control group, and only fed *ad libitum*; Group B was administered Kainic acid only to induce epilepsy; Group C was first administered kainic acid to induce epilepsy and thereafter *bryophyllum pinnatum* (300 mg/kg/day) to observe its ameliorative properties; Group D was administered ketogenic diet (1 kcal/ml/day) after inducing epilepsy with kainic acid and Group E was administered the anti-epileptic carbamazepine (100 mg/kg/day) following initial administration of kainic acid to induce epilepsy. Epilepsy was induced with 10 mg/kg of kainic acid in every instance and confirmed and observed using the Racine scale. After treatment for 21 days, behavioural

observations on memory and cognition were carried out using the Barnes mazes. Animals were sacrificed, and the temporal lobe cortex and hippocampus tissues were processed for histological and immunohistochemistry studies using the hematoxylin and eosin, Nissl stain and glia acidic fibrillary acid proteins techniques. Neurotransmitters- glutamate, serotonin and dopamine activities were assayed in brain homogenates. Histological and histochemical evidences on neurons and astrocyte morphologies and special distribution, astrocyte reactions, cortical histological integrity, neurotransmitters activities showed that *bryophyllum pinnatum* had potentials to ameliorate epilepsy and the effects with significant relative to carbamazepine and ketogenic diet. It also significantly ameliorated behavioural aberrations attributable to epilepsy [p $\leq$ 0.05]. This plant's anti-epilepsy potentials should be explored further.

MTU09-28

Clofibrate, A PPAR- α agonist mitigated sodium fluoride-induced neuro-inflammation, oxidative stress and motor incoordination**A. Oyagbemi, O. Adebisi, B. Ogunpolu, O. Falayi, K. Adigun***University of Ibadan, Department of Veterinary Physiology and Biochemistry, University of Ibadan, Ibadan, Nigeria*

Fluoride is an environmental contaminant that is present in air, water and soil. It is commonly added in minute quantity to drinking water, toothpaste, and mouth rinses to prevent tooth decay. Epidemiological findings have demonstrated that exposure to fluoride induced neurodevelopmental toxicity, developmental neurotoxicity and motor disorders. The neuroprotective effect of clofibrate, a Peroxisome Proliferator-Activated Receptor alpha (PPAR- α) agonist was investigated in the present study. Forty-male Wistar rats were used for this study and were randomly grouped into ten rats per group as control, sodium fluoride (NaF) alone (300 ppm), NaF plus Clofibrate (250 mg/kg) and sodium fluoride plus Lisinopril (10 mg/kg), respectively, for seven days. Sodium fluoride was administered in drinking water while Clofibrate and Lisinopril was administered by oral gavage. Markers of neuronal inflammation and oxidative stress, acetylcholinesterase (AChE) activity and neurobehavioral (Hanging wire and Open Field) tests were performed. Immunohistochemistry was performed on brain tissues and were probed with Glial fibrillary acidic protein (GFAP), Ionized calcium binding adaptor molecule 1 (Iba1) and cerebellar Ca²⁺ binding protein calbindin D-28k (CB). The results showed that NaF significantly increased makers of oxidative stress, neuro-inflammation and inhibited AChE activity. Immuno-staining revealed reactive astrocytes, microgliosis, loss of dendritic spines and arborisation in Purkinje cells in rats administered only NaF. Neurobehavioral results showed that co-treatment of NaF with Clofibrate improved muscular strength, locomotion, reduced anxiety as well as significant reduction in astrocytic count. Altogether, co-treatment of NaF with either Clofibrate or Lisinopril demonstrated neuroprotective effect by mitigating neuronal inflammation, oxidative and motor incoordination. Hence, Clofibrate is a novel drug candidate against neurodegeneration and motor disorders.

MTU09-29

Norvaline, a novel Alzheimer's disease-modifying agent
B. Polis, A. Samson*Bar-Ilan University, Medical School, Safed, Israel*

Alzheimer's disease (AD) is an irredeemable chronic neurodegenerative disorder and the predominant cause of dementia. The disease progression is associated with amyloid plaques' deposition and neurofibrillary tangles' formation in the brain, yet clinical dementia is the end stage of the enduring pathology. Recent evidence points to severe characteristic metabolic dysfunction as a leading cause and hallmark of AD that is apparent decades prior to the disease manifestation. State-of-the-art metabolomics studies prove that complex arginine and branched-chain amino acids (BCAAs) metabolism disturbances accompany AD. Lower plasma valine levels are associated with accelerated cognitive decline, and, conversely, an increase in valine concentration is associated with reduced risk of AD.

We administered an arginase inhibitor norvaline, which is an uncommon non-proteinogenic BCAA and a valine's isoform, chronically to a mouse model of AD. A set of immunohistochemistry, proteomics, and transcriptomics assays was applied to evaluate the neuroprotective effect of the substance and identify the biological pathways activated by the treatment.

The results verify that norvaline reverses the cognitive decline in the AD mice. The neuroprotective effect is associated with significantly reduced hippocampal arginase levels and diminished amyloidosis. Moreover, the treatment moderates the rate of Tau protein phosphorylation, alleviates microgliosis and apoptosis. Additionally, we disclose the treatment-associated increase in the hippocampal expression levels of synaptic plasticity-related proteins, expression levels of cytosolic branched-chain amino acid aminotransferase, and an activation of several, involved in cell survival and neuroplasticity, biological pathways.

The data suggest that norvaline is a potent arginase inhibitor and modulator of glutamate metabolism. The substance possesses various modes of action, which improve the symptoms of AD and even interfere with its pathogenesis. Therefore, norvaline presents a promising neuroprotective molecule with manifold biological potentials that might be tailored for the treatment of a range of neurodegenerative disorders.

MTU09-30

Antibody-based therapeutic approach to target TDP-43 proteinopathy**S. Pozzi¹, S. S. Thamisetty¹, P. Codron², R. Rahimian¹, K. V. Plourde¹, J. Kriz^{1,3}, C. Gravel^{1,3}, J.-P. Julien^{1,3}**¹*CERVO Brain Research Centre, Axis integrative neuroscience and experimental therapies, Quebec city, Canada*²*MITOVASC Institute, MitoLab Unit, Angers, France*³*Laval University, Psychiatry and Neuroscience, Quebec city, Canada*

TAR DNA-binding protein 43 (TDP-43) is a DNA/RNA binding protein mainly localized in the nucleus of cells. In pathological conditions, TDP-43 mislocalizes and aggregates in neuronal cytoplasm forming hyperphosphorylated, fragmented and ubiquitinated inclusions which impair its physiological functions. This condition is called TDP-43 proteinopathy and can be primarily observed in amyotrophic lateral sclerosis (ALS) and frontotemporal degeneration (FTD) cases. TDP-43 has two RNA-recognition domains

(RRM1 and RRM2) both involved in relevant aspects of the protein function. Different studies had highlighted the involvement of the RRM1 domain in TDP-43 proteinopathy. Through the RRM1 domain, indeed, TDP-43 can interact with p65, the main subunit of NF-KB, inducing a hyperactivation of the factor which ultimately lead to increased neuroinflammation and neuron toxicity. Moreover, the RRM1 domain is a sensitive target for detrimental effects, such as oxidation and misfolding, that eventually induce TDP-43 proteinopathy. We generated a monoclonal antibody against the RRM1 domain of TDP-43 with the aim of reducing pathological events mediated by this protein portion. Viral-mediated delivery into the CNS of a single chain antibody, derived from the antigen binding fragment of the monoclonal antibody, in mutant TDP-43 mice was found to reduce cognitive and motor impairments as well as to decrease TDP-43 cytoplasmic mislocalization, aggregation and neuroinflammation. These observations support the feasibility of an immunotherapeutic approach to mitigate TDP-43 pathology in ALS and FTD.

MTU09-31

Pioglitazone reversed hippocampal insulin resistance in an amyloid-beta fibrils induced animal model of Alzheimer's disease**S. O. Rahman¹, S. Parvez², B. P. Panda³, A. K. Najmi¹**¹*Jamia Hamdard, Pharmacology, New Delhi, India*²*Jamia Hamdard, Toxicology, New Delhi, India*³*Jamia Hamdard, Biotechnology, New Delhi, India*

Background: Complications of Alzheimer's disease (AD) have made the development of its therapeutic intervention quite a challenging task. Numerous studies have supported the hypothesis that central insulin resistance plays a significant role in AD. Serine phosphorylation of Insulin Receptor Substrate-1 (IRS-1) has been found to be a contributing factor in neuronal insulin resistance. Pioglitazone (PIO) is a peroxisome proliferator-activated receptor gamma (PPAR- γ) activator, known for its significant antidiabetic functions, has also demonstrated neuroprotective actions.

Methods: In the present study, AD was induced by i.c.v administration of Amyloid- β (1-42) fibrils in Wistar rats. After 7 days of recovery, rats were treated with 10 mg/kg and 20 mg/kg of PIO orally for 28 days. Behavioral analysis was done in the last week of our experimental study. On the 36th day, rats were sacrificed and their hippocampus was separated from the whole brain, then homogenized and stored for biochemical estimations.

Results: PIO significantly reversed the cognitive and memory impairment, as assessed by the Morris water maze test, in A β (1-42) fibrils infused Wistar rats. PIO also significantly attenuated A β (1-42) level, IRS-S307 activity, GSK-3 β activity, TNF- α level, AChE level, nitrite level and oxidative stress in the hippocampus. Histopathological evaluation, done through H&E and Congo red staining, also demonstrated neuroprotective and anti-amyloidogenic effects of PIO in the hippocampus.

Discussions: Our study concludes the protective action of pioglitazone against hippocampal insulin resistance and Alzheimer's disease complications, supporting the potential role of hippocampal insulin resistance targeting against the AD.

MTU09-32

Stress-induced inhibition of 82-KDA choline acetyltransferase nuclear translocation**A. Rai, O. Ojiakor, H. Arora, J. Rylett***Western University, Robarts Research Institute, London, Canada*

Decreased function of basal forebrain cholinergic neurons (BFCNs) in Alzheimer's disease (AD) causes cognitive deficits. Choline acetyltransferase (ChAT) is a key enzyme in cholinergic neurons that synthesizes acetylcholine (ACh). The M-ChAT transcript of human ChAT gene encodes both cytoplasmic 69- and nuclear 82-kDa isoforms of ChAT. We reported that expression of 82-kDa ChAT in neural cell nuclei alters expression of several genes, including some involved in regulation of APP metabolism. Necropsy human brain shows nuclear localization of 82-kDa ChAT in cholinergic neurons, with subcellular distribution changing in aging and mild cognitive impairment and AD with reduced levels of 82-kDa ChAT in nucleus and increased levels in cytoplasm. Being a cysteine-rich protein, 82-kDa ChAT is susceptible to cellular and oxidative stress. Our studies reveal that exposure of SH-SY5Y neural cells to beta-amyloid or oxidative stress reduces 82-kDa ChAT levels in nucleus. We have also characterized homodimerization of 82-kDa ChAT through bimolecular fluorescence complementation assay. Our results show nuclear localization of 82-kDa ChAT homodimers in control neural cells, whereas stressed cells exhibit perinuclear aggregate formation, which may lead to the observed decrease in nuclear localization. Due to primate-specific expression of 82-kDa ChAT and inaccessibility of human brain neurons, we produced BFCNs from human induced pluripotent stem cells (hiPSCs) as a model. We verified differentiation of iPSCs to BFCNs by monitoring expression of cholinergic phenotypic markers and functional parameters, such as ChAT, choline uptake and ACh synthesis. Importantly, 82-kDa ChAT is expressed in these BFCNs and located in nuclei. Taken together, we have demonstrated a possible mechanism for modulation of nuclear translocation of 82-kDa ChAT in stressed cells and developed a model for study of 82-kDa ChAT in human neurons.

MTU09-33

Cassia tora reverses A β 1-42 aggregation *in vitro* and conveys multiple neuroprotective effects in aluminium-induced ad rats**S. K. Ravi¹, R. B. Narasingappa², C. G. Joshi¹**¹*Mangalore University, Biochemistry, Mangalore, India*²*University of Agriculture Sciences, Biotechnology, Bangalore, India*

Alzheimer's disease is a progressive neurodegenerative disorder characterized by the presence of neuritic plaques and neurofibrillary tangles and its multifactorial nature calls for multi-target-directed approaches for therapeutic treatment. This study is firstly aimed at determining the effects of Cassia tora methanolic fraction (MECT) on A β 1-42 aggregation *in vitro*. Secondly, it evaluates the effects on aluminium-induced neurobehavioral and neuropathological changes *in vivo* in rats. MECT was prepared and tested for its ability to prevent and/or reverse A β 1-42 aggregation by measuring thioflavin-T fluorescence and by transmission electron microscopy. For *in vivo* experiments, AIC13 was administered orally for 60 consecutive days in the absence or in the presence of MECT. Behavioural were employed for neurobehavioral assessment. Then, biochemical

assays measuring acetylcholinesterase activity and oxidative stress as well as examination of the expression levels of pro-inflammatory cytokines and BDNF in the hippocampus and frontal cortex were performed. Finally, histopathological assessment of neuronal health in the CA1 and CA3 regions of the hippocampus by cresyl violet staining was carried out. MECT inhibits A β 1-42 aggregation from monomers and oligomers and disintegrates pre-formed A β 1-42 fibrils. Moreover, MECT dose-dependently improves the cognitive and behavioural impairments observed in aluminium-treated rats. Furthermore, the extract alleviates AChE hyperactivity as well as oxidative stress and inflammation observed in the hippocampus and the cerebral cortex of aluminium-treated animals. Finally, MECT precludes aluminium-induced neuronal collapse. Our study reveals that the methanolic extract of Cassia tora is able to prevent most of the AD-related events and therefore stands as a promising mild and natural anti-AD multi-target compound.

MTU09-34

Rage inhibition reduced neuroinflammation and dopaminergic neurodegeneration in a long-term response to LPS systemic inflammation**C. Ribeiro, J. Gasparotto, C. Girardi, P. Brum, D. Peixoto, J. C. Moreira, D. Gelain***Universidade Federal do Rio Grande do Sul, Departamento de Bioquímica, Porto Alegre, Brazil*

Neuroinflammation is one of the major contributors to the progressive loss of dopaminergic (DA) neurons in Parkinson's disease (PD). The receptor for advanced glycation endproducts (RAGE) has been demonstrated as an important mediator of neurodegeneration triggered by inflammation. In this study, we investigated the effect of RAGE inhibition in a long-term response to systemic inflammation in Wistar rats induced by a single dose of lipopolysaccharide (LPS, 5 mg/kg, i.p.). The multimodal RAGE blocker, FPS-ZM1, was administered to selectively inhibit RAGE either intraperitoneally (1 mg/kg, i.p.) one hour before LPS injection or intracranially (40 μ g per rat, i.n.) two months after LPS injection. Immunostaining of Iba-1 and GFAP demonstrated that LPS modulates microglia and astrocyte in the substantia nigra (SN) 15 days, 30 days, 6 months and 10 months after injection. By contrast, 10 months after LPS injection both FPS-ZM1 administrations reduced glial activation, suggesting that RAGE mediates the neuroinflammation resulted from systemic stimulus. In addition, immunostaining of RAGE and TH demonstrated a progressive increase of RAGE in the SN, which is accompanied by DA neurons loss. FPS-ZM1-induced RAGE inhibition also reduced DA neurons loss, showing a neuroprotective effect of FPS-ZM1 against LPS insult. Overall, our results indicate RAGE as mediator of neuroinflammation and DA neurodegeneration triggered by LPS systemic inflammation. Therefore, RAGE inhibition has a potential application in neuroprotective therapies for PD and associated disorders.

MTU09-35

Anthranilate sulfonamides attenuate oxidative stress in human neuronal cells**W. Ruankham¹, W. Suwanjang², V. Prachayasittikul¹, S. Prachayasittikul³, K. Phopin^{1,2}**¹*Mahidol University, Clinical Microbiology and Applied Technology, Bangkok, Thailand*²*Mahidol University, Center for Research and Innovation, Bangkok, Thailand*³*Mahidol University, Center of Data Mining and Biomedical Informatics, Bangkok, Thailand*

Oxidative stress is associated with neuronal damage which is considered to be a risk factor for pathogenesis and development of neurodegenerative diseases (NDs) including Alzheimer's disease (AD), Parkinson's disease (PD), Amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), and others. Thus, antioxidant therapy is a plausible strategy to delay NDs progression. Recently, anthranilic acid and sulfonamides have been reported to possess anti-inflammatory and antimicrobial activities. However, the underlying molecular mechanisms of anthranilic sulfonamide hybrids against oxidative damage have not been fully elucidated. In this study, hybrids of anthranilic sulfonamide incorporating benzenesulfonyl chlorides and anthranilic acid were synthesized, and neuroprotection properties of the compounds against H₂O₂-induced oxidative stress in neuronal cells were also investigated by using MTT assay, carboxy-H₂DCFDA assay, flow cytometry, and western blotting. Both cell viability and reactive oxygen species (ROS) assays showed that pretreatment with anthranilate sulfonamides effectively attenuated H₂O₂-stimulated cytotoxicity and ROS production. Surprisingly, these synthesized compounds promoted antiapoptotic protein (BCL-2) and triggered Sirtuin (SIRT1) signaling pathways in human neuronal cells. Moreover, the binding interaction of anthranilate sulfonamides to SIRT1 protein targets is elucidated and characterized by an *in silico* molecular docking. Taken together, anthranilate sulfonamides might act as sirtuin-activating compounds which are novel therapeutic candidates for NDs.

MTU09-36

Chlorogenic acid protects against MPTP induced neurotoxicity in parkinsonian mice model via its anti-apoptotic activity**S. Singh, S. Rai, H. Birla, W. Zahra, A. Rathore, H. Dilnashin, S. Singh***Banaras Hindu University, Varanasi, India, Biochemistry, Varanasi, India*

Parkinson's disease (PD) being one of the most common neurodegenerative disease is primarily caused by the degeneration of dopaminergic (DA) neurons in the substantia nigra pars compacta of the midbrain. Studies have been conducted in various models including, cell cultures, animal models along with post-mortem brain studies on human bodies to reveal the role of apoptosis in the neuronal cell death in PD. The studies have proven to determine treatment for symptomatic relief but till date, no cure has been identified for PD. Although in the past few years, studies have been correlating neuroprotective effects of chlorogenic acid (CGA) to neurodegenerative diseases and thus, various PD models have been established to study its treatment therapy. With a similar objective,

we aimed to study the effect of CGA, a natural polyphenolic compound, in MPTP-induced mice model of PD. On CGA supplementation, significant motor coordination and the antioxidant defense has been observed in contrast with MPTP-injected mice. Furthermore, the improved tyrosine hydroxylase expression inside the nigrostriatal region in CGA-treated mice supports the neuroprotective effect of CGA. Here, 1-methyl-4-phenylpyridinium (MPP⁺) ion insult in the DA neurons were protected by CGA by reversing aberrant expression of apoptotic markers (Bcl-2, Bax, and Caspase-3). After CGA supplementation, the activity of pAkt1 was promoted, which has further inhibited the apoptosis of DA neurons. In Real-time PCR analysis, CGA treatment after MPTP intoxication showed reduced expression of IL-1 β , IL-2, and IL-6; the pro-inflammatory cytokines. These studies have concluded that against MPTP-intoxication, CGA has offered the neuroprotective effect through its anti-apoptotic activity.

MTU09-37

Role of glutamate dependent signalling pathways during Alzheimer disease and diabetes**N. Singla, A. Shukla, R. Sandhir***Panjab University, Department of Biophysics, Chandigarh, India*

Worldwide, epidemiological findings have revealed that the prevalence of Alzheimer Disease (AD) is more prominent in Diabetes Mellitus (DM) affected individuals. The relationship between neurodegeneration and metabolic disorder is still unclear, which makes them a public health concern. The present study was designed to investigate the role of glutamate dependent signaling pathways during AD and DM. Female Wistar rats weighing 180-200 g were divided into three groups viz: Normal control, A β (1-42) treated (AD model) and Streptozotocin treated (DM model). All the treatments were continued for a total duration of one month. A significant decline in the learning and memory was observed in AD and DM animals when compared to the normal control animals. The levels of neurotransmitters γ -aminobutyric acid and glutamate were also found to be significantly increased in the cerebrum and cerebellum of AD and DM animals in comparison to controls. On the contrary, activities of proteins regulating glutamate metabolism viz: glutamate synthetase, glutamate alpha decarboxylase and glutamate dehydrogenase were found to be significantly decreased in brain of AD and DM animals as compared to controls. The protein expression of glycogen synthase kinase-3, glial fibrillary acidic protein and amyloid precursor protein were also elevated in brain samples of AD and DM animals in comparison to control group. The brain sections of diabetic animals showed similar alterations in the neurohistoarchitecture as that of AD brain sections. Hence, the present study reveals that glutamate dependent signalling pathways plays a vital role in diabetes induced Alzheimer Disease.

MTU09-38

Neurotoxic implications of rotenone induced alpha-synuclein conformers**T. Srivastava^{1,2}, S. Devi^{1,2}, M. Chaturvedi^{1,2}, S. Priya¹**¹Indian Institute of Toxicology Research, Lucknow, System toxicology and health risk assessment group, Lucknow, India²Academy of Scientific and Innovative Research ^{AcSIR}, CSIR-IITR Campus, Lucknow, India

Genetic mutations and environmental factors are believed to initiate the pathogenesis and aggregation of α -synuclein (α -syn), linked to Parkinson's disease (PD) and other synucleinopathies. However, initiation of α -syn unfolding, structural alterations and aggregation upon environment exposure events are still obscure. Here, we studied rotenone- α -syn interactions as rotenone; a pesticide is known to induce the α -syn aggregation. We also characterized the initial events in α -syn misfolding. The study of aggregation kinetics was done with Thioflavin T assay, Circular dichroism and Transmission electron microscopy suggested that rotenone increased the rates of aggregation with initial structural loss and fluctuations at amino acid level in α -syn and form seeds with distinct structural morphology. For rotenone induced α -syn conformers we further performed cytotoxicity studies using MTT assay, AnnexinV apoptosis assay and found them cytotoxic for neuronal cells. The mechanistic study through DCFDA Assay and JC1 assay has been shown that these rotenone exposed α -syn seeds did not cause the oxidative stress but depolarized the mitochondrial membrane potential which results in cell death. In brief we found that rotenone induced α -syn aggregation by affecting the initiation of misfolding events and these aggregated species cause cellular toxicity by altering the mitochondrial membrane potential. Implication of these findings suggests that may be environmental exposure also leads to seeds formation which are cytotoxic and remain dormant till further exposure or favourable conditions and have capability to induce the disease at later stages.

MTU09-39

Nickel-induced neurodegeneration in the hippocampus, striatum and cortex; an ultrastructural insight, and the role of caspase-3 an**O. Sunday¹, I. Omamuyowbi², O. Joshua¹, A. Michael⁴, N. Thajasvarie⁵**¹Babcock University, Neuroscience Unit, Department of Anatomy Babcock University, Ilisan-remo, Nigeria²Federal University of Technology Akure, Department of Anatomical Sciences, Akure, Nigeria³Babcock university, Neuroscience Unit, Department of Anatomy Babcock University, Ilisan-remo, Nigeria⁴Albert Einstein College of Medicine, Department of Molecular Pharmacology, New York, USA⁵University of KwaZulu-Natal, Optics and Imaging Centre, Durban, Durban, South Africa

Human overexposure to nickel (Ni) emanating from the increasing application of Ni compounds in modern technology is a major public health concern. Nickel has been shown to be teratogenic, immunotoxic, genotoxic and carcinogenic. The current knowledge on Ni neurotoxicity is still relatively limited. We have previously demonstrated that Ni treatment alters cognitive and locomotor behaviors, induces oxidative stress and neurodegeneration in brains

of rats. In this study, we examine the ultrastructural changes to neurons in the hippocampus, striatum and cortex of the brain following Ni treatment, as well as attempt to delineate the roles for caspase-3 and α -synuclein in Ni-induced neurodegeneration. Rats were treated with either saline, 10 or 20 mg/kg of nickel chloride for 4 weeks via oral gavage. Electron microscopy analysis revealed ultrastructural alterations in neurons of the hippocampus, striatum and cortex following Ni treatment. Mitochondria structural integrity within neurons were markedly compromised. We also detected elevated caspase-3 activity in hippocampus and striatum, as well as overexpression of α -synuclein in the cortex following Ni treatment. Our study demonstrates that mitochondria are a key target in Ni-induced neurodegeneration. Additionally, we implicate apoptotic pathway via caspase-3 action as the executioner and perturbation of α -synuclein expression in Ni-induced neurodegeneration.

MTU09-40

The role of tau phosphorylation at the AT8 pathological site in brain development**D. Tuerde^{1,2}, K. Furusawa¹, T. Takasugi¹, T. Kimura¹, S. Ishigaki², K. Ando¹, S.-i. Hisanaga¹, G. Sobue²**¹Tokyo metropolitan university, Department of Biological Sciences, Hachioji, Japan²Nagoya University Graduate School of Medicine, Department of Neurology, Nagoya, Japan

Tau is a microtubule (MT)-associated protein, which stabilizes MTs in axons of neurons, contributing the structural support of neuronal network in healthy brains. In contrast, in Alzheimer's disease (AD) brains, tau is phosphorylated around 40 sites and forms aggregates called neurofibrillary tangles, which are supposed to cause neurodegeneration. Among many pathological phosphorylation sites, the AT8 site is particularly interesting because the site used for diagnosis of AD but also is phosphorylated during early brain development. However, it is not completely understood yet how the AT8 reactivity is generated in AD brain and contributes to AD development. We reported that the AT8 site was highly phosphorylated in fetal mice brains, and dephosphorylated at around postnatal day 14 when the neuronal circuit is established. We found hypothyroidism delayed not only brain development but also dephosphorylation at the AT8 site, indicated their direct relationship. Here, we examined localization of phosphorylated tau and non-phosphorylated tau at AT8 site in neurons and found that AT8 phospho-tau was present highly in the cell body and AIS region and less in distal axons. In contrast, non-phosphorylated tau at AT8 site was absent in AIS region and found highly in distal axon. We introduced wild-type (WT) human tau or AT8 site mutants, either Ala or Glu, in tau-knockdown neurons and found that 2E and WT, but not 2A human tau induced axon extension. Thus, axon development requires tau phosphorylated at AT8 site. This is the physiological role of the AT8 phosphorylation in tau, but would also provide important information on AD development.

MTU09-41

Neuroecotoxicology: effects of environmental heavy metal exposure on the brain of african giant rats**I. Usende¹, J. Olopade¹, M. Bentivoglio²**¹University of Ibadan, Veterinary Anatomy, Ibadan, Nigeria²University of Verona, Neuroscience, Biomedicine and Movement Sciences, Verona, Italy

Introduction: Increased exploitation of minerals has led to pollution of environments. Information on brain effects of such exposure is limited. Due to its exploratory activities, the African giant rat (*Cricetomys gambianus*) provides a unique model for ecotoxicological research to determine levels of animal and human exposure to different environmental pollutants. The aim of the present study is to unravel neuropathological features of this animal sampled from agro-ecological zones of Nigeria.

Materials and methods: With ethical approval, the animals were collected in the field in three Nigerian regions according to previously determined data on heavy metal exposure: mangrove forest (high vanadium, selenium); woodland savanna (high lead, selenium, zinc); rain forest (low levels of heavy metals). Immunofluorescence and Immunohistochemical analyses were conducted, focusing on different neuronal cell types sensitive to oxidative stress.

Results: Interesting results were obtained concerning orexin-A and melanin concentrating neurons of lateral hypothalamus and dopaminergic neurons of substantia nigra pars compacta (SNc). Stereological cell counts of tyrosine hydroxylase cells showed a significant loss (-41.8%) of SNc dopaminergic neurons in the animals exposed to vanadium (mangrove), and (-50.7%) in those exposed to lead (woodland savanna), compared to those from rain forest zone. Similarly, a significant loss (-39.9% and -40.8% respectively) of parvalbumin-containing interneurons in the cingulate cortex has been documented in same animal groups compared to those of rain forest.

Conclusion: These perhaps are the first “neuroecotoxicological” findings in distinct neuronal cell groups. The implications of these findings are highly relevant for human population living in these areas, not only in Nigeria but also in similarly polluted areas elsewhere in the world.

MTU09-42

Muscarinic acetylcholine receptors in alcohol use disorder**L. Walker¹, C. Niki¹, A. Lawrence¹, V. Perreau¹, B. Alice², P. Rueda², C. Langmeed², C. Lindsley³, C. Jones³**¹Florey Institute of Neuroscience and mental health, Behavioural neuroscience, Melbourne, Australia²Monash Institute of Pharmaceutical Sciences, Faculty of Pharmacy and Pharmaceutical Sciences, Melbourne, Australia³Vanderbilt Center for Neuroscience Drug Discovery, Departments of Pharmacology and Chemistry, Nashville, USA

Despite the large socioeconomic burden of alcohol use disorders (AUD), therapeutic treatment options are limited. Basic neuroscience has identified a number of targets that contribute to alcohol reward craving and relapse; however few of these successfully translate to human populations. AUDs are characterised by a

transition to compulsive alcohol seeking, which is hypothesized to involve a shift from ventral to dorsal striatum. In addition, a medial to lateral shift in the dorsal striatum is implicated in the transition from goal-directed to habitual alcohol seeking. Muscarinic acetylcholine receptors (mAChRs) are potential targets for AUD treatment as they are expressed within the mesocorticolimbic reward system, including dense expression in the dorsal striatum. Here they modulate dopamine and glutamate release, which may regulate reward processing. To assess the role of mAChRs in AUD, we first conducted genome-wide RNA sequencing in the caudate/putamen of 10 human alcoholics and 10 healthy controls and concurrently examined mAChR expression in the corresponding regions in rat (dorsolateral and dorsomedial striatum) following chronic alcohol consumption/withdrawal using qPCR. Next we examined the role of select mAChR subtypes in alcohol consumption and seeking using selective allosteric modulators. Finally, we probed the role of specific mAChR subtypes in the dorsal striatum in alcohol consumption and seeking. Collectively, our data show that mAChRs are potential novel target pharmacotherapies for the treatment of AUD.

MTU09-43

Alteration of dopaminergic behaviors in a parkinson's disease model through P2x4R modulation by ivermectin
A. Warnecke¹, Moon. S. Kang¹, L. Halbers¹, Micheal. W. Jakowec², D. L. Davies¹¹University of Southern California, School of Pharmacy, Los Angeles, USA²University of Southern California, Neurology Keck School of Medicine, Los Angeles, USA

Dopamine is a key neurotransmitter within the brain that plays a role in the mesolimbic pathway (associated with reward-based behavior) and the nigrostriatal pathway (which is associated with motor control and reward-based cognition). The ability to modify dopaminergic activities within these pathways provides a potential target for the treatment of dopaminergic disorders. Ivermectin (IVM), a P2X4 receptor (P2X4R) positive modulator, has been shown to reduce alcohol consumption in mice and alter L-Dopa induced rotational behavior in the 6-hydroxydopamine (6-OHDA) model. P2X4R knockout mice showed reduced rotational behavior changes (+/- IVM) further implicating P2X4Rs and IVM. To further investigate the effects of IVM on L-Dopa enhancement, I tested additional behaviors linked to the striatum of the medial forebrain bundle in unilaterally lesioned mice via 6-OHDA stereotaxic injection. Following lesion confirmation (via amphetamine [5 mg/kg] challenge) mice were subjected to a battery of behavioral tests to evaluate motor coordination, anhedonistic behavior, learning and memory. IVM (5 mg/kg, I.P.) was administered 8 hours prior to L-Dopa injection (5 mg/kg S.C.) and mice were observed while performing on the rotarod, sucrose preference test or novel object recognition test. L-Dopa and IVM+L-Dopa altered performance on rotarod tests and novel object recognition tests. We found IVM + L-Dopa was able to alter significantly motor coordination and produced a trend in altering learning and memory and anhedonistic behavior in a Parkinsonian mouse model. Overall, these initial findings further illustrate IVM's potential importance as a potential adjunct therapy for Parkinson's disease.

MTU10 Intracellular trafficking & proteostasis (Session A)

MTU10-01

Axonal trafficking of L1CAM in cortical neurons

J. Gallardo^{1,2}, A. Luarte^{1,2}, C. González^{1,2}, A. Couve^{1,2},
J. Sierralta^{1,2}

¹Universidad de Chile, Biomedical Neuroscience Institute ^{BNI},
Santiago, Chile

²Universidad de Chile, Neuroscience Department, Santiago, Chile

Introduction: Neurons are highly polarized cells with two distinct compartments, somato-dendritic and axonal. The maintenance of this polarity depends mainly on axonal proteome. There is increasing evidence supporting that axons and dendrites can autonomously synthesize and export transmembrane (TM) proteins through a local secretory pathway. Although in CNS axons it has been well described the presence of endoplasmic reticulum (ER), the presence or functionality of a Golgi-like structure has been poorly described. Recent evidence showing axonal proteins with immature N-glycosylation in the neuronal surface suggests a Golgi-independent route. Here we study L1-cell adhesion molecule (L1CAM), an axonal TM protein that contributes to the outgrowth and pathfinding

of the growth cone, and which delivery is not fully understood. We hypothesize that the trafficking of L1CAM from axonal ER to plasma membrane (PM) is Golgi-independent in cortical neurons axons.

Methods: We isolated the axonal compartment of cultured embryonic cortical neurons (E18) using microfluidic chambers. To synchronize ER export of L1CAM, we used an ER retention/release system based on FM4/DD. Disruption of ER to Golgi trafficking was achieved with Golgicide-A (GCA) and Brefeldin-A (BFA). WB analysis and glycosidases treatment was performed to study L1CAM N-glycosylation.

Results: L1CAM was locally exported from the axonal ER to PM. Axonal trafficking was resistant to GCA, but sensitive to BFA. There is a differential N-glycosylation profile in axonal and somato-dendritic compartments.

Conclusions: L1CAM can be locally exported in cortical axons but a BFA-resistant Golgi trafficking is necessary. Our results suggest that there is a contribution of Golgi-like structures in local delivery of L1CAM to axonal PM.

MTU11 Glial cells (Session A)

MTU11-01

Oligodendrocyte progenitor cell diversity in the healthy brain

R. Beiter, M. Wall, C. Rivet-Noor, D. Rosen, A. Fernandez-Castaneda, E. Schumann, A. Gaultier

University of Virginia, Department of Neuroscience, Charlottesville, USA

Oligodendrocyte Progenitor Cells (OPCs) make up 2-8% of the adult brain and remain proliferative throughout life. Historically, a majority of the OPC field has focused on their role as a progenitor pool for mature oligodendrocytes, but recent literature has begun to explore the idea that the population of OPCs maintained in the adult brain encompasses multiple diverse subpopulations that may have roles beyond that of producing oligodendrocytes. OPCs have been shown to maintain neuronal health in the hypothalamus and play an integral role in both depression and a mouse model of multiple sclerosis. Further elucidation of both the molecular and functional diversity of OPCs will potentially reveal novel functions for this cell type and provide evidence for the production of new therapeutics for diseases in which OPCs may be implicated. To address the question of overall molecular diversity of OPCs in the adult brain, we have performed single-cell sequencing of PDGFR α -reporter positive cells from the brains of adult mice. Based on unbiased clustering, we have preliminarily identified 4 populations of OPCs. Go Term analysis of these clusters reveals significant enrichment of genes related to a variety of functions, including regulation of dendrite development, cytokine-mediated signaling pathways, and regulation of response to oxidative stress. Ongoing work includes validation of OPC clusters using immunofluorescent techniques to detect transcripts of genes specifically enriched in each OPC cluster. This work provides an overview of the transcriptional state of adult OPCs during homeostasis, as well as a foundation for future investigation into novel functions of OPCs. Identification of subpopulations of OPCs may have important implications for diseases in which OPCs may be playing a significant role.

MTU11-02

Lysosomal function and dysfunction in astrocytes

L. Civiero

University of Padova, Department of Biology, Padova, Italy

During neurodegenerative diseases (NDs), astrocytes become reactive and engulf and degrade dead cells as well as protein aggregates via the lysosomal pathway. As a consequence, lysosomal impairment in these cells interferes with astrocyte function and contributes to the onset and the disease progression of NDs. In PD, astrocytes participate to the clearance of neuronal-released α -syn toxic species. LRRK2 is a kinase that impinges on the lysosomal pathway in different tissues (PNAS 2014, BBRC2016) and alteration of LRRK2 kinase activity in neurons is associated with PD (Front Mol Neurosci 2017, J Neurochem 2015). Of note, LRRK2 is highly expressed in glial cells and mutated LRRK2 might impact on astrocyte functionality. My research aims to define pathological implications of mutated LRRK2 in the phagocytosis/lysosomal

pathways in astrocytes and imply whether targeted therapies focused on restoring astrocytes degradative capacity might be a route for drug intervention in PD.

MTU11-03

Myelin breakdown favors mycobacterium leprae survival in schwann cells

B. D. S. Mietto^{1,2}, B. J. Souza², P. Sammarco-Rosa², M. C. V. Pessolani², F. A. Lara², E. N. Sarno²

¹*Institute of Biological Sciences, Biology Department, Juiz de Fora, Brazil*

²*Oswaldo Cruz Institute, Leprosy Department, Rio de Janeiro, Brazil*

Leprosy neuropathy is a chronic disorder caused by the infection of the peripheral nerve by the intracellular pathogen mycobacterium leprae (M leprae). Schwann cells are remarkable in supporting Mycobacterium leprae persistence in the nerve. While M leprae binding to myelinated Schwann cells has been implicated in demyelinating phenotype, what exactly role M leprae plays during myelin breakdown and clearance are not entirely clear. Here, we provided strong evidence of close interaction of M leprae and degenerating myelin profiles *in vitro* and *in vivo*. We also observed accelerated myelin breakdown and clearance in infected Schwann cells that was accompanied by reduced expression of myelin-related genes *in vitro* and *in vivo*. Furthermore, this increased myelin breakdown was associated with the upregulation of autophagic myelin destruction in Schwann cells *in vitro* and in nerve biopsies. Finally, when we blocked myelin degradation by pharmacological inhibition of JNK/c-Jun pathway in Schwann cells, we drastically reduced M leprae viability in the host cell. Overall, these results provided novel evidence of the ability of M leprae in advancing myelin breakdown to benefit its persistence intracellularly in Schwann cells.

MTU11-04

DAAM2 antagonizes VHL to modulate oligodendrocyte differentiation and remyelination after white matter injury

X. Ding^{1,2,4}, J. Jo^{2,4}, C. Cristobal^{2,3,4}, Y. Qi^{2,4}, S.-H. Yoo⁵, H. K. Lee^{1,2,4}

¹*Baylor College of Medicine, Program in Developmental Biology, Houston, USA*

²*Baylor College of Medicine, Department of Pediatrics and Neuroscience, Houston, USA*

³*Baylor College of Medicine, Integrative Molecular and Biomedical Science Program, Houston, USA*

⁴*Texas Children's, Jan and Dan Duncan Neurological Research Institute, Houston, USA*

⁵*The University of Texas Health Science Center at Houston, Biochemistry and Molecular Biology, Houston, USA*

White matter injury (WMI), or loss of myelinating oligodendrocytes (OLs) results in devastating neurological disorders, such as hypoxic ischemic encephalopathy in neonates and multiple

sclerosis in adults. Despite the robust regenerative capacity of OLs, myelin repair under pathological conditions have been unsuccessful. Indeed, lesions of patients are found populated with stalled OL precursors, highlighting the need of deciphering inhibitory cues. Previously, we identified a novel gene Daam2 that suppresses OL differentiation and WMI repair. Meanwhile, Yuen et al. reported that HIF arrests OL maturation. Upon Daam2 overexpression during tumorigenesis, we observed a significant reduction of VHL, a well-known ubiquitin-ligase targeting HIF, leading to the hypothesis that Daam2 suppresses OL differentiation and WMI repair by antagonizing VHL functions. Using genetic mouse models and OL cultures, we discovered a functional antagonizing relationship between Daam2 and VHL during development, which is conserved in lysolcithin-induced demyelination and hypoxia-induced hypomyelination mouse models. Lastly, Daam2 promotes VHL ubiquitin-proteasomal degradation under the regulation of targeting E3 ligase, the direct manipulation of which is sufficient to alter OL differentiation *in vitro*. Importantly, human expression data indicates promising therapeutic prospects. Together, we propose Daam2-VHL as a novel regulatory mechanism for OL differentiation and a potential therapeutic target for WMI.

MTU11-05

NG2 glia are vulnerable at breaches of the blood brain barrier during secondary degeneration following neurotrauma

M. Fitzgerald^{1,3,2}, T. Clarke¹, C. Bartlett², E. Denham¹

¹Curtin University, Curtin Health Innovation Research Institute, Nedlands, Australia

²The University of Western Australia, School of Biological Sciences, Nedlands, Australia

³Perron Institute for Neurological and Translational Science, Sarich Neurosciences Research Institute Building, Nedlands, Australia

Blood brain barrier disruption accompanies secondary degeneration, both adjacent to and remote from a primary injury, and leads to increased neuroinflammation and damage. NG2 + glia are an integral component of the blood brain barrier, and encompass both pericytes and oligodendrocyte precursor cells. However it is not yet known if oxidative damage to NG2 + glia occurs to a greater degree at sites of blood brain barrier breach than sites where the barrier is intact, thereby perhaps contributing to secondary degeneration. Here we use the partial optic nerve transection model of secondary degeneration in adult female rats and semi-quantify 8-hydroxy deoxyguanosine (8OHdG) immunoreactivity as an indicator of oxidative damage to DNA in NG2 + glia and glial fibrillary acidic protein (GFAP) + astrocytes, together with Immunoglobulin G immunoreactivity as an indicator of blood brain barrier breach. 8OHdG immunoreactivity was increased 1 day after injury in both NG2 + glia and GFAP+ astrocytes surrounding blood vessels ($p \leq 0.001$). However, only in NG2 + glia surrounding vessels, was 8OHdG immunoreactivity higher at sites of blood brain barrier breach than where the barrier was intact ($p \leq 0.01$). Ethynyldeoxyuridine labelling of proliferating cells demonstrated that the percentage of proliferating NG2 + cells around RECA+ blood vessels was increased after injury ($p \leq 0.05$), whereas the percentage of proliferating RECA+ endothelial cells did not increase at this time point. Thus, NG2 + glia may be particularly vulnerable to

oxidative damage at sites of blood brain barrier breach, associated with a proliferative response.

MTU11-06

Effects the remyelination-promoting antibody rHIgM22 on sphingolipid metabolism in primary cultured glial cells

S. Grassi, S. Prioni, L. Cabitta, S. Sonnino, A. Prinetti

University of Milan, Department of Medical Biotechnology and Translational Medicine, Segrate ^{Milano}, Italy

Recombinant human IgM22 (rHIgM22) binds to myelin and oligodendrocytes (OLs) and promotes remyelination in mouse models of multiple sclerosis. Literature suggests that rHIgM22 recruits a multimolecular complex formed by Lyn, integrin $\alpha v \beta 3$ and PDGFR α , triggering Lyn activation and promoting oligodendrocyte precursor cells (OPCs) survival and proliferation. However, its exact mechanism of action remains to be elucidated.

We have shown the involvement of different sphingolipids in rHIgM22 binding at the cell surface, suggesting that reorganization of lipid membrane microenvironment might be relevant in its biological activity.

Thus, we assessed the effect of a 24 hours, single dose treatment with rHIgM22 on sphingolipid metabolism in cultured rat mixed glial cells (MGC), OPCs and OLs. The treatment had no significant effects on the lipid pattern of MGC. However, in OPCs and OLs it determined an increase in the levels of gangliosides GD3 and GM3, both known for their ability to interact with and modulate the activity of different growth factor receptors.

In addition, rHIgM22 determined a reduced activity of the acid sphingomyelinase (ASMase), with a consequent reduction of ceramide (Cer) generation. Ceramide generated by the action of ASMase represents an important pro-apoptotic signal, but also potent regulator for the organization of sphingolipid-rich signaling platforms. Remarkably, genetic deficiency or pharmacological inhibition of ASMase effectively protect against demyelination and other detrimental effects in MS models.

Altogether, our results support the notion that rHIgM22 protective effects might be mediated by alterations of lipid-dependent membrane organization and/or signalling in different cell types present in the niche of MS lesions.

MTU11-07

In vivo activation of microglial Gi signalling using chemogenetics

A. Hogan-Cann^{1,2}, D. Sakae², M. Maksoud^{1,2}, V. Ostapchenko², W.-Y. Lu^{1,2}, M. Prado^{1,2}, V. Prado^{1,2}

¹University of Western Ontario, Neuroscience, London, Canada

²Robarts Research Institute, Schulich School of Medicine & Dentistry, London, Canada

Microglia, the immune cells of the central nervous system, survey their surroundings and respond to external stimuli to maintain homeostasis in the brain. To do this, microglia express an array of receptors that allow them to receive and respond to signals from neighboring cells. Many of these receptors are G protein-coupled receptors, which regulate a variety of microglial functions through different signalling pathways. Gi receptors have been shown, for example, to modulate microglial phagocytosis and

chemotaxis. We have generated mice expressing Gi (hM4Di) Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) selectively in microglia. These mutated muscarinic receptors no longer respond to their endogenous ligand acetylcholine, but they can be activated by clozapine-N-oxide (CNO) and other compounds at doses that are inert at other receptors. These mice therefore allow for the selective activation of microglia Gi signalling *in vivo* without simultaneously affecting receptors on neurons or astrocytes. Activation of microglial Gi DREADD by CNO initiates Gi intracellular signalling pathways in DREADD-expressing microglia. Remarkably, activation of Gi signalling, via CNO injection, does not affect baseline behavior. Furthermore, chronic activation of microglial Gi signalling in mice does not appear to alter the expression of pro-inflammatory cytokines in the brains of LPS-injected mice. Lastly, phagocytic activity of primary microglia is not affected by activation of this pathway. Taken together, our results suggest that specific activation of Gi signalling in microglia is possible and appears to have no negative consequences in healthy mice.

MTU11-08

Dissecting the role of DAAM2 during astrocyte development and associated disease

J. Jo¹, K. Ung³, G. Liu³, X. Ding^{1,3}, B. R. Arenkiel^{2,3}, H. K. Lee^{1,2,3}

¹Baylor College of Medicine, Neurological Research Institute at Texas children's hospital, Pediatrics-Neurology, Houston, USA

²Baylor College of Medicine, Neuroscience program, Houston, USA

³Baylor College of Medicine, Developmental Biology program, Houston, USA

Astrocytes play a vital role in CNS physiology including synaptogenesis and formation of functional blood-brain-barrier. Consequently, astrocytes are associated with numerous neurological disorders and malignancies. However, the molecular mechanisms that control astrocyte development, diversity, and dysfunction remain poorly defined. Development of cell lineages follows a sequential series of differentiative steps, culminating with the differentiation of lineage-specific progenitors into mature populations that execute specific physiological functions. While the paradigm of stepwise lineage differentiation is much appreciated in neuronal and oligodendrocyte lineages, the intermediate steps of astrocyte lineage remain unclear. Therefore, unraveling mechanisms regulating astrocyte development and homeostasis will provide critical insight into the pathology and treatment of multiple neurological disorders. While we discovered previously that Daam2 suppresses oligodendrocyte differentiation during development and repair, how Daam2 operates during astrocyte development remains completely unknown. Here, we found that astrocyte-specific loss of Daam2 results in abnormal astrocyte maturation and alterations in synaptogenesis followed by the aberrant neuronal activity. Additionally, loss of Daam2 enhanced inflammatory responses in photothrombotic stroke model, suggesting critical functions of Daam2 in astrocyte development and tissue repair. To decipher how Daam2 suppresses astrocyte maturation, we performed screening and identified NBCe1, Na⁺/HCO₃⁻ cotransporter as an inverse functional regulator of Daam2 in astrocyte development. Together, these studies elucidate the mechanistic link between Daam2 and its associated genes during astrocyte development and may provide new, tractable pathways of undefined intermediated astrocyte lineage in CNS development as well as associated injury repair.

MTU11-09

Acute toxicity after uptake of copper oxide nanoparticles in glial cells

A. Joshi^{1,2}, K. Jog¹, R. Dringen^{1,2}

¹University of Bremen, Center for Biomolecular Interactions, Bremen, Germany

²University of Bremen, Center for Environmental Research and Sustainable Technology, Bremen, Germany

Copper oxide nanoparticles (CuO-NPs) have been known for their high cell toxic potential. However, interferences by copper ions in CuO-NP preparations have been a hurdle in the investigation of specific nanoparticle-mediated toxicity. In order to distinguish between the adverse effects exhibited by CuO-NPs and ionic copper that was present in the CuO-NP preparation, we have applied the membrane-impermeable copper chelator bathocuproine disulfonate (BCS) in a molar ratio of 20% of total copper, in order to chelate the ionic copper extracellularly released from the CuO-NPs. Physicochemical characterization of CuO-NPs revealed that the presence of BCS did not alter their size or surface charge. Application of CuO-NPs induced a time-, concentration- and temperature-dependent copper accumulation and severely compromised cell viability in C6 glioma cells and primary astrocytes. These consequences were not altered in the presence of BCS, while the tremendous copper accumulation and severe toxicity found upon application of ionic copper was prevented. The observed impairment of cell viability correlated well with the increase in the specific cellular copper content for both types of copper species applied and was only observed for conditions where the specific cellular copper contents exceeded 30 nmol copper per mg protein. The copper-induced toxicity to glial cells was accompanied by an increase in the generation of reactive oxygen species, which was partially prevented by BCS in copper ion-treated glial cells. In conclusion, the application of BCS allows to clearly distinguish between adverse effects caused by extracellular CuO-NPs and copper ions, and demonstrates that intact CuO-NPs are taken up and impair the viability of glial cells.

MTU11-10

Potential of adult oligodendrogenesis as a candidate target for ms therapy

J. Mateus^{1,2}, M. Gomes^{1,2}, R. Soares^{1,2}, S. Paulo^{1,2}, A. Chora², A. Sebastião^{1,2}, S. Xapelli^{1,2}

¹Instituto de Farmacologia e Neurociências, FMUL, Lisboa, Portugal

²IMM-JLA, FMUL, Lisboa, Portugal

Oligodendrocytes, the myelinating cells of the Central Nervous System (CNS), are generated upon differentiation of oligodendrocyte precursor cells (OPCs), which possess proliferative and migratory capabilities and are present in the CNS postnatally. Under pathological conditions such as in Multiple Sclerosis (MS), there is a depletion of oligodendrocytes, but OPCs present in the brain parenchyma or derived from subventricular zone (SVZ) neural stem cells (NSCs) can differentiate, migrate and partially remyelinate the lesioned areas. Herein, we aimed at characterizing the MS mouse model, experimental autoimmune encephalomyelitis (EAE), as well as the process of adult oligodendrogenesis. Behavioural tests were performed to evaluate motor function. Cellular differentiation was assessed by immunohistochemistry for bromodeoxyuridine (BrdU) colocalization with oligodendrocytic markers in brain regions of interest. Western blot and ELISA assays were used for myelin

protein levels and inflammatory cytokine quantification. Results for EAE model characterization suggested that motor impairment is proportional to the clinical score. Moreover, an increase in the levels of the pro-inflammatory cytokine TNF α ($n = 5$, $p < 0.01$), and a tendency for increased IL-1 β were observed in EAE mice. Importantly, a tendency for increased BrdU+ cells in the SVZ, corpus callosum (CC) and cerebral cortex (CT) was observed, accompanied by a significant increase in NG2 + BrdU+ cells in the CC of EAE mice ($n = 3$, $p < 0.05$), hinting at the migration of precursor cells from the SVZ to the CC. Altogether, this work allowed the characterization of the oligodendrogenesis process and of the EAE model throughout time, supporting future studies involving the modulation of adult oligodendrogenesis as a putative therapy for MS.

MTU11-11

Age-related changes in astrocytes contribute to synapse loss and dysfunction

I. Matias¹, L. Diniz¹, L. Neves¹, A. P. Araújo¹, D. Vonk², J. Middeldorp², E. Hol², F. Gomes¹

¹Federal University of Rio de Janeiro, Institute of Biomedical Sciences, Rio de Janeiro, Brazil

²University Medical Center Utrecht, Dept Translational Neuroscience, UMC Utrecht Brain Center, Utrecht, Netherlands

Aging is associated with synaptic deficit and cognitive decline. Several evidences have shown the role of astrocytes in synaptic function during brain development and diseases, although there is still a lack of evidence on their involvement in age-related cognitive decline. Here we investigated the phenotype and function of astrocytes in aging. We used hippocampal tissue from young (2-3 months) and aged (~21 months) C57Bl/6 mice for *in vivo* analysis. We also established an *in vitro* model for astrocyte senescence, in which cultures were maintained for 30-35 days *in vitro* (DIV) (senescent astrocytes) or 7-10 DIV (control astrocytes). We observed a ~90% reduction in synaptic density in the hippocampal dentate gyrus of aged mice. The GFAP immunostaining revealed astrocyte hypertrophy and decreased levels of synaptogenic factors in dentate gyrus of aged mice. Senescent astrocytes cultures showed a reactive phenotype, based on LCN2 immunostaining. The ACM from these cells presented a decreased capacity to support neurite outgrowth and synaptogenesis on neuronal cultures, possibly due to a significant reduction in synaptogenic factors expression and secretion. Our results point to a key role of changes in astrocyte phenotype and function to the age-related synaptic loss and dysfunction. The protocols of this study were approved by the Committee for Animal Research of the Federal University of Rio de Janeiro and the University Medical Center Utrecht. **Support:** CNPq, CAPES, FAPERJ, Ministério da Saúde, ZonMW Memorabel.

MTU11-12

Altered myelinic nanochannel integrity modulates ALS disease progression in SOD1 mutant mice

A. Mot, P. Dibaj, U. Gerwig, I. Tzvetanova, M. Denk-Lobnig, W. Möbius, S. Göbbels, K.-A. Nave

Max Planck Institute of Experimental Medicine, Department of Neurogenetics, Göttingen, Germany

Amyotrophic lateral sclerosis (ALS) is a highly debilitating and fatal disease characterized by the progressive loss of motor neurons. The mechanisms leading to disrupted oligodendroglial support of motor neurons in ALS are poorly understood. To investigate this, we first confirmed that selective removal of oligodendroglial mutant SOD1-G37R expression delays disease onset, prolongs survival, and improves motor performance. Given the recently discovered novel role of oligodendrocytes in supplying nutrients to neurons via a system of nanometer-wide cytoplasmic channels, we utilized immuno electron microscopy and found that mutant SOD1 is present within paranodal loops and the inner periaxonal tongue. This raises the intriguing possibility that SOD1 aggregates within these nanochannels could perturb the motor-driven transport of transporter proteins (i.e. MCT1) within oligodendrocytes and disrupt the free diffusion of nutrients from the oligodendrocyte to the motor neuron. To investigate the role of perturbed myelinic nanochannel integrity as a potential mechanism leading to impaired oligodendroglial metabolic support of motor neurons in ALS, we crossed mutant SOD1-G93A mice with mice lacking expression of CNP, a protein that keeps myelinic nanochannels open by preventing excessive myelin membrane compaction. Double SOD1-G93A and CNP^{null} mutants show reduced survival and worsened neurological scores. Decreased frequency of myelinic nanochannels in CNP^{null} mice could accelerate ALS disease progression in double mutants by further limiting the transport of nutrients from the oligodendroglial compartment to the axonal compartment. These data provide novel insights into the mechanisms leading to impaired oligodendroglial support of motor neurons in ALS.

MTU11-13

The innate capacity of MS oligodendrocytes to produce efficient myelinating oligodendrocytes

S. Mozafari¹, L. Starost², B. Manot³, B. Garcia-Diaz¹, D. Roussel¹, L. Ottoboni⁴, M. Ehrlich², M.-C. Angulo³, G. Martino⁴, T. Kuhlmann², A. B.-V. Evercooren¹

¹Sorbonne University, INSERM U1127, CNRS, UMR 7225, Institut du Cerveau et de la Moelle épinière ^{ICM}, Paris, France

²University Hospital Münster, Institute of Neuropathology, Max Planck Institute for Molecular Biomedicine, Münster, Germany

³Université Paris Descartes, Laboratory of Neurophysiology and New Microscopies, INSERM U1128, Paris, France

⁴San Raffaele Scientific Institute, Institute of Experimental Neurology -DIBIT 2, Division of Neuroscience, Milan, Italy

Multiple Sclerosis (MS) a neuroinflammatory demyelinating disease, could result either from extrinsic activation of the immune system or a process initiated intrinsically by primary cytodegeneration of neuroglia. Whether failed remyelination in MS results from the incapacity of the MS oligodendroglia to function properly, or from environmental cues is still at debate. MS oligodendrocytes are not easily accessible. The induced pluripotent technology constitutes a powerful tool to generate MS oligodendroglia. We showed that transplantation of iPS-derived oligodendrocytes in adult

demyelination condition results in excellent integration and functional remyelination of host axons. Here, we ask whether MS oligodendrocytes exhibit a primary blockade or behave as efficiently as the healthy control cells.

We transplanted Human iPS-oligodendroglia from RRMS patients and their siblings in the developing brain and spinal cord of Shiverer:Rag^{2-/-} mice and sacrificed mice at 4, 8, 12, 16 and 20 weeks-post-transplantation to evaluate their fate and functional properties as myelin-forming cells.

Data showed that MS oligodendroglia survive, vastly distribute/migrate over time and differentiate efficiently in myelin-forming cells generating compact myelin within the murine brain and spinal cord as efficiently as healthy controls. Transcallosal conduction velocities were significantly delayed in non-grafted shiverer mice compared to the wild-type mice but rescued in the grafted mice.

Our data suggest the innate capacity of MS cells to produce efficient myelinating oligodendrocytes. The chimeric mouse-human glial network could be a target as a preclinical model for drug screening of promyelinating compounds for personalized therapy in MS. SM is beneficiary of an ECTRIMS fellowship. Support by the International Progressive MS Alliance Grant #: PA-1604-08492.

MTU11-14

Function of microglia and the exosomes content are influenced by the origin of cells

A.-N. Murgoci^{1,2}, K. Mallah², S. Aboulouard², I. Fournier², M. Salzet², D. Cizkova^{1,2,3}

¹Institute of Neuroimmunology SAV, Center of Regenerative Medicine, Bratislava, Slovakia

²Univ. Lille, Inserm, U-1192, Laboratoire PRISM, Lille, France

³University of Veterinary Medicine and Pharmacy in Kosice, Department of Anatomy, Histology and Physiology, Kosice, Slovakia

Microglia cells are key players of the cross-talk between the nervous and immune systems. In present study we used a combination of proteomic and systemic biology analyses to demonstrate that neonatal microglial cells derived from cortex and spinal cord expressed different phenotypes upon the physiological or pathological conditions. Primary cells were isolated from neonatal rat cortex and spinal cord. For proteomic analyses of microglia cells, the proteins were extracted with RIPA buffer and processed using FASP and nanoHPLC-MS/MS analyses. Functional analyses, including neurite outgrowth and glioma proliferation analysis in 3D spheroid cultures, were performed to test biological activity of cortex microglia exosomes compared with spinal cord microglia exosomes. The results highlight variability in protein production on both cellular and exosome levels. Bioinformatics data reveal for proteins extracted from cortex microglia anti-inflammatory and neurogenesis/tumorigenesis characteristics, while for proteins isolated from spinal cord microglia involvement in the inflammatory response. *In vitro* assays indicate that the microglia located at different CNS areas reveal differential biological functions through released vesicles. While exosomes from both microglia sources enhanced growth of DRGs axons, only the spinal microglia vesicles significantly attenuated glioma proliferation. The results show that exosomes produced by two different sources of microglia do not have the same pattern nor the same biological functions. Thus, microglia function is dependent on its cellular microenvironment which conditions its phenotype. Supported by APVV 15-0613, ERANET Axon Repair, INSERM, SIRIC-ONCOLille Grant-DGOS-Inserm 6041aa.

MTU11-16

Role of coronin-1a in human fetal brain derived astrocyte physiology and activation in HIV-1 neuropathogenesis

H. S. Pandey, P. Seth

National Brain Research Centre, Cellular and Molecular Neuroscience, Gurgaon, India

One of the major challenges of neuroAIDS field is lack of apt model to study HIV-1 neuropathogenesis. This prompted us to develop a well-characterized Human Fetal Brain-derived Neural Precursor cell (hNPCs) culture system. We maintain hNPCs as multipotent stem cells and differentiate neurons and astrocytes for our studies. In HIV/AIDS, astrocytes cause indirect damage to neuron that culminates into neurocognitive deficits. Cellular and molecular mechanisms for astrocyte activation are unclear, hence requires extensive explorations. Recent reports using rat model system, suggest coronin-1a is important for cognitive abilities. Coronin-1a, an actin-binding protein, is associated with important cellular processes such as cell migration, phagocytosis, morphogenesis, cellular trafficking, cytokinesis etc. However, role of Coronin-1a in astrocyte physiology is underexplored. Using hNPCs model, we attempted to investigate the role of Coronin-1a in modulation of astrocytic function by neurotoxic HIV-1 protein Tat. Our studies reveal that HIV-1 Tat can modulate Coronin1A expression in astrocytes. Interestingly, knockdown of coronin-1a resulted in altered physiological features, such as decreased calcium flux, altered PLC γ 1, and ERK1/2 phosphorylation patterns in ATP stimulated astrocytes. Having observed its role in Calcium signaling, we were curious if it also contributes to HIV-1 Tat-induced astroglial activation. Knockdown of this protein alleviates the HIV-1 Tat-induced astrocyte activation marked by measuring the levels of Glial fibrillary acidic protein (GFAP), cytokine, and glutamate release. These results provide novel insights into the field of neuroAIDS by identifying important roles of Coronin-1a in modulation of astrocyte physiology and pathophysiology.

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MTU11-17

Treatment of experimental allergic encephalomyelitis (EAE) by the metabotropic receptor agonist chpg, reduces disease progression

T. Planas-Fontanez^{1,2}, A. D. Stefano², T. Sullivan², Y. Huang², C. Dreyfus²

¹Rutgers University, Toxicology, Piscataway, USA

²Rutgers Robert Wood Johnson Medical School, Neuroscience & Cell Biology, Piscataway, USA

Previous studies demonstrated that a metabotropic glutamate receptor (mGluR) agonist, ACPD (1-amino-1,3-dicarboxycyclopentane), is able to reverse deficits in brain-derived neurotrophic factor (BDNF) and myelin protein levels following injury in a cuprizone model of demyelination. Group I mGluRs (mGluR1 and mGluR5) were localized to astrocytes and effects of ACPD were found to be dependent upon the production of BDNF by these cells. Recent work indicates that similar effects are elicited by an intraperitoneal injection of the Group I mGluR agonist, CHPG (2-chloro-5-hydroxyphenylglycine). In this study, we tested whether CHPG could reverse clinical signs in EAE mice immunized with myelin

oligodendrocyte glycoprotein (MOG). CHPG (20 mg/kg, injected every other day) delayed MOG-induced EAE, and ameliorated clinical signs when treatment was initiated after mice developed hindlimb paralysis. This effect was accompanied by reversal in the loss of BDNF and myelin proteins in the lumbar spinal cord. Moreover, preliminary data revealed increased colocalization of mGluR5 with GFAP+ astrocytes within lesioned sites, with rare colocalization with Iba1 + activated microglia, or CD11b+ microglia and macrophages, suggesting that astrocytes or microglia may be targets of CHPG action. In contrast, no colocalization of the receptor with CD4 + T-helper cells, CD45R+ B-cells, or Ly-6 g+ neutrophils was observed, suggesting that peripheral immune cells do not express mGluR5 at early or late stages of disease. Future studies will be performed to define the cellular mechanisms underlying the effects of CHPG on glial cells and continue to explore the potential of metabotropic agonists as targets for treating demyelinating diseases. Supp. NMSS RG4257B4/1 and NIH RO1 NS036647.

MTU11-19

Regulation of microglial activity by Gq-DREADD mediated signalling

D. Sakae^{1,2,3}, A. Hogan-Cann^{1,2,3}, W. Binning^{1,2,3}, V. Ostapchenko^{1,3}, M. Maksoud^{1,3}, W.-Y. Lu^{1,3}, W. Inoue^{1,3}, V. Prado^{1,2}, M. Prado^{1,2}

¹Robarts Research Institute, Neuroscience, London, Canada

²University Of Western Ontario, Schulich School of Medicine & Dentistry, London, Canada

³University of Western Ontario, Pharmacology and Physiology, London, Canada

G protein coupled receptors (GPCR) are widely expressed across different cell types in the brain. They can act through Gq, Gs or Gi signaling which have been shown to regulate several functions in microglial cells. Microglia are the resident immune cells of the central nervous system. They have an important role responding to injury, infections and removing damaged cells and cross talk to neurons via a number of GPCRs. How activation of Gq-mediated signaling in microglia regulate some of its critical functions *in vivo* is poorly understood, because neurotransmitter receptors are located in all different cell types in the brain. To address this question, we generated a microglia Gq-DREADD (Designer Receptors Exclusively Activated by Designer Drugs; hM3Dq) mouse line. hM3Dq is a mutated muscarinic receptors type 3 that no longer responds to acetylcholine, but is activated by clozapine-N-oxide (CNO) and similar compounds. We confirmed in this mouse line that hM3Dq is expressed only in microglia and that CNO increases intracellular calcium concentrations only in Gq DREADD microglia, indicating Gq pathway activation. Treatment with CNO also increased phagocytosis of fluorospheres by Gq DREADD microglia. *In vivo*, chronic activation of hM3Dq by CNO (ip) does not affect baseline behaviour, however it decreased LPS-induced sickness behaviour and the upregulation of inflammatory cytokines mRNA in the brain. Our results show that hM3Dq-specific microglia mice can be a useful tool to understand how microglia GPCR-signaling modulates its activity *in vivo*.

MTU11-20

Deletion of glial ABCA1 causes glaucoma-like optic neuropathy

Y. Shinozaki¹, K. Namekata², K. Kashiwagi³, N. Ohno^{4,5}, T. Segawa⁶, S. Koizumi¹

¹University of Yamanashi, Dept. Neuropharmacol., Grad. Sch. Med., Yamanashi, Japan

²Tokyo Metr. Inst. Med. Sci., Vis. Res. Project, Tokyo, Japan

³University of Yamanashi, Dept. Ophthalmol., Grad. Sch. Med., Yamanashi, Japan

⁴Natl. Inst. Physiol. Sci., Dev. Neurobiol. Bioinfo., Aichi, Japan

⁵Jichi Med. Univ., Div. Anat., Tochigi, Japan

⁶University of Yamanashi, Cent. Life. Sci. Res., Yamanashi, Japan

Glaucoma is second leading cause of blindness worldwide which is characterized by damages or degeneration of retinal ganglion cells (RGCs). Although an elevated intraocular pressure (IOP) is the main risk factor, it has become apparent that many other factors are involved in the etiology of glaucoma. Among genetic risk factors, single nucleotide polymorphism (SNP) of *ABCA1* gene has been identified as the risk for glaucoma from large scale genome wide association studies (GWAS). However, its pathogenic mechanisms are totally unclear. To clarify this, we raised three issues to be revealed. First, it is unclear whether or not *ABCA1* affects IOP. Second, it is undermined which of gain-of-neurotoxicity or loss-of-function of *ABCA1* causes glaucoma. Third, it is unknown which type of cells contributes to glaucoma. We analyzed conventional *ABCA1* knockout (KO) mice and found that IOP was not changed. We also found that *ABCA1* was highly enriched in astrocytes of ocular tissues. To further elucidate the role of astrocytic *ABCA1*, we generated astrocyte-specific *ABCA1* knockout (cKO) mice. The cKO mice showed significant increase in the number of apoptotic RGCs and reduction in visual function at middle-age (12 months old). Taken together, our data showed that (1) *ABCA1* has no impact on IOP; (2) loss-of-function of *ABCA1* is involved in glaucoma; and (3) *ABCA1* in glial cells contributes to pathogenesis of glaucoma.

MTU11-21

Studying the role of dark microglia in early postnatal development in CX3CR1-deficient mice

M.-K. St-Pierre, F. González-Ibáñez, S. Belhocine, M. Carrier, D. Gosselin, M.-È. Tremblay

CRCHU de Québec-Université Laval, Molecular Medicine, Qc, Canada

Dark microglia (DM), a recently discovered microglial phenotype, has been associated with ultrastructural alterations caused by oxidative stress, as well as down-regulation of microglial homeostatic proteins like CX3CR1. These cells were shown to be abundant in mouse models of psychiatric and degenerative diseases, as well as in normal post-natal development. DM were observed in brain regions like the hippocampus, were associated with an upregulation of cd11b and appear to be involved in synaptic modulation. Although DM can be identified by electron microscopy due to their electron dense cyto- and nucleoplasm, the impossibility to study these cells with other techniques has left many questions unanswered. Our goal is to identify a marker that labels these cells selectively, in order to analyze their molecular signature, regional density, localization, morphology and ultrastructure in a myriad of pathologies. The putative DM marker's specificity was assessed using correlative immunocytochemical light and electron

microscopy. Furthermore, series of brain sections providing a non-biased representation of the brain of young male and female CX3CR1-deficient mice (postnatal day 14 and 21), a model where synaptic dysfunction can be seen, were imaged using a slide scanner to reveal their distribution. Our results revealed that DM are found in unexpected regions in the grey (e.g. striatum) and white matters (e.g. *arbor vitae* of the cerebellum). Moreover, we found that only some DM were positive for our marker, suggesting that multiple DM sub-populations co-exist. Their close association with myelinated axons in the white matter suggest a new potential role for these cells. Further studies will be carried out to investigate DM's role in the white matter of young mice.

MTU11-22

Menadione induces rapid radical formation and MRP1-mediated gssg export in rat astrocytes **J. Steinmeier, R. Dringen**

University of Bremen, Biology/Chemistry, Bremen, Germany

Menadione (2-methyl-1,4-naphthoquinone) is a derivative of vitamin K and takes part in redox cycling, thereby generating reactive oxygen species (ROS) in cells. In brain, astrocytes defend themselves and also neighbouring cells against xenobiotics and toxins using an efficient antioxidant defence system. To test for adverse consequences of menadione on brain cells, primary astrocyte cultures were treated with menadione for up to 6 h. Concentrations of up to 30 μM menadione did not affect viability or cellular glutathione redox state. In contrast, 100 μM menadione caused a quick impairment in lactate release and a delayed increase in extracellular lactate dehydrogenase activity, demonstrating metabolic impairment and loss in membrane integrity, respectively. Already within 5 min after exposure, 100 μM menadione caused formation and cellular accumulation of glutathione disulfide (GSSG) which was accompanied by an increased ROS-staining, clearly indicating oxidative stress. The intracellular GSSG accumulation was followed by an export of GSSG that was prevented by MK571, an inhibitor of the multidrug resistance protein 1 (Mrp1). In glucose-deprived cells glutathione oxidation and ROS formation were already observed for lower concentrations of menadione compared to glucose-fed cells, most likely due to a lack in NADPH regeneration by the pentose phosphate pathway. Co-incubation of astrocytes with dicoumarol, an inhibitor of the menadione-reducing enzyme NAD(P)H: quinone acceptor oxidoreductase 1 (NQO1), did not prevent menadione-induced ROS formation nor GSSG accumulation. These data demonstrate that in primary astrocytes menadione rapidly induced NQO1-independent ROS production and GSH oxidation to GSSG which is followed by a Mrp1-mediated export of GSSG.

MTU11-23

Autotaxin, a regulator of oligodendrocyte differentiation during remyelination

E. Suarez¹, Fatemah. S. Afshar¹, Karen. M. Gorse¹, Wouter. H. Moolenaar², J. L. Dupree¹, B. Fuss¹

¹*Virginia Commonwealth university, Anatomy, Richmond, USA*

²*Netherlands Cancer Institute, Division of Cell Biology, Amsterdam, The Netherlands*

Autotaxin (ATX), also known as ectonucleotide pyrophosphatase/phosphodiesterase 2 (ENPP2) or phosphodiesterase-1 α (PD-1 α)/ATX, is a secreted glycoprotein primarily known for its enzymatic lysophospholipase D (lysoPLD) activity, which generates the lipid signaling molecule lysophosphatidic acid (LPA). LPA, in turn, exerts its functions through activation of a family of G protein-coupled receptors (GPCRs), the so-called LPA receptors. In our own studies, we identified ATX as a protein that is released by cells of the oligodendrocyte (OLG) lineage and functions via two mechanisms to drive OLG differentiation. Initially, we uncovered that ATX, via its C-terminally located modulator of oligodendrocyte remodeling and focal adhesion organization (MORFO) domain, promotes the establishment of a complex and expanded process network by post-migratory, premyelinating OLGs. More recently, we focused on ATX's lysoPLD activity and found that the ATX-LPA axis promotes the expression of genes well-known to be associated with the earlier stages of OLG differentiation via, at least in part, the modulation of histone deacetylation. Studies undertaken in the developing zebrafish substantiated a critical role of ATX in regulating OLG differentiation during development. Interestingly, there is evidence for reduced levels of ATX in the central nervous system (CNS) parenchyma in the major demyelinating disease in humans, Multiple Sclerosis. Here, we show that, similarly, ATX levels are reduced during toxin-induced demyelination. In addition, we present, data that support a role of OLG-derived ATX in regulating OLG differentiation not only during development but also after toxin-induced demyelination.

MTU11-24

Comparison of molecular signatures of OLIG2-lineage astrocyte and GFAP-positive astrocyte using laser microdissection

K. Tatsumi, A. Isonishi, S. Takemura, T. Tanaka, A. Wanaka

Nara Medical University, Anatomy & Neuroscience, Kashihara, Japan

Our lineage tracing study using Olig2^{CreER}; Rosa-CAG-LSL-eNpHR3.0-EYFP (Olig2^{CreER}; YFP) transgenic mice revealed that a subpopulation of Olig2-lineage mature astrocytes (Olig2-astrocytes) distributed widely but unevenly in the adult brain; regions rich in Olig2-astrocytes tended to lack GFAP-positive astrocytes (GFAP-astrocytes). Even within a single brain nucleus, Olig2-astrocytes and GFAP-astrocytes occupied mutually exclusive territories. External globus pallidus (GPe) is one of the representative nuclei. Interestingly, brain nuclei rich in Olig2-astrocytes tended to strongly express GABA-transporter 3 (GAT-3) in astrocytes and vesicular GABA transporter (vGAT) in neurons, suggesting that Olig2-lineage astrocytes may be involved specifically in inhibitory neuronal transmission by forming tripartite synapses.

To compare molecular signatures of the two kinds of astrocytes, we applied laser microdissection to the GPe in combination with

immunohistochemistry. The method enabled us to differentially collect two types of astrocytes from a single section of the GPe, where the territories of Olig2- and GFAP-astrocytes were intermingled. Feeding tamoxifen-containing chow for 1 week to adult Olig2^{CreER}; YFP mice successfully enhanced recombination and subsequent YFP fluorescence. Brain sections were then labeled with anti-GFAP antibody and Alexa 594-labeled secondary antibody. We dissected out YFP-expressing cells with bushy morphologies (Olig2-astrocyte) and Alexa 594-labeled star-like cells (GFAP-astrocyte) from single sections. mRNAs from each type of astrocytes were isolated and subjected to molecular comparison using qPCR. Consistent with our previous report, Olig2-astrocytes expressed lower GFAP mRNA than GFAP-astrocytes. The RT-qPCR analyses further showed that Olig2-astrocytes expressed higher level of GAT-3 gene than GFAP-astrocytes. These results strongly suggest that Olig2-astrocytes constitute a distinct subpopulation of astrocytes subsidiary to inhibitory GABAergic transmission.

MTU11-25

Parallel S1P receptor signalling synergise to induce neuroprotective signalling

C. Tran^{1,2}, S. Humphrey³, B. Heng⁴, J. Teo², H. Stefan¹, A. Don^{2,5}

¹UNSW, School of Medical Science, Sydney, Australia

²Centenary Institute, Lipid Signalling and Neurochemistry, Sydney, Australia

³University of Sydney, School of Life and Environmental Science, Sydney, Australia

⁴Macquarie University, Faculty of Medicine and Health Sciences, Sydney, Australia

⁵University of Sydney, NHMRC Clinical Trials Centre, Sydney, Australia

Sphingosine 1-phosphate (S1P) is an essential lipid metabolite with potent vasculoprotective and neuroprotective properties. S1P signals through its own family of five G-protein coupled receptors, S1P₁-S1P₅, activating intracellular pathways that regulate proliferation, differentiation, and survival. The multiple sclerosis drug Fingolimod is a potent S1P receptor agonist that causes lymphopenia. However, recent research has also established direct neuroprotective properties of Fingolimod on astrocytes in multiple neurodegenerative paradigms including Alzheimer's and Parkinson's disease. In this study, we show S1P upregulates brain-derived neurotrophic factor (BDNF), leukaemia inhibitory factor (LIF), platelet-derived growth factor B (PDGFB), and heparin-binding EGF-like growth factor (HBEGF) in astrocytes but not neurons, and S1P is a much more potent inducer than Fingolimod. Accordingly, in an *in vitro* model of neuronal excitotoxic cell death, S1P significantly attenuates apoptosis whilst Fingolimod does not. Specific antagonists of S1P₁ and S1P₂ both inhibited neurotrophic gene induction in response to S1P, indicating simultaneous activation of both receptors is required. Phosphoproteomic analysis, siRNA, and Western blotting showed that S1P₂ signals through Gα13, RhoA, Jun and Yap to drive neurotrophic gene expression. Fingolimod does not activate S1P₂, explaining why it does not promote significant neurotrophic gene expression in astrocytes. Supplementing Fingolimod with a constitutively active G13 boosts expression of neurotrophic factors. These results demonstrate that S1P utilises dual signalling pathways from independent receptors to maximise neurotrophic gene expression and protection against excitotoxicity.

MTU11-26

Striatin-3 is a novel glial RAC1 effector

M. Weaver^{1,2}, C. Berti³, L. Frick^{2,4}, M. Palmisano⁵, M. Pellegatta⁶, S. Ferguson⁷, C. Namba⁸, M. Selbach⁹, F. Paul⁹, L. Wrabetz^{2,4}, L. Feltri^{2,4}

¹SUNY Buffalo, Neuroscience, Buffalo, USA

²SUNY Buffalo, Hunter James Kelly Research Institute, Buffalo, USA

³New York University, Pathology, New York, USA

⁴SUNY Buffalo, Biochemistry, Buffalo, USA

⁵AstraZeneca, Medical Affairs, Basiglio, Italy

⁶San Raffaele Scientific Institute, Experimental Neurology, Milan, Italy

⁷SUNY Buffalo, Pharmaceutical Sciences, Buffalo, USA

⁸Meharry Medical College, Medicine, Nashville, USA

⁹Max Delbrück Center, Biochemistry, Berlin, Germany

During development, Schwann cells undergo extensive cytoskeletal reorganization as they insert cytoplasmic extensions into axon bundles to sort, ensheath, and myelinate axons. Similarly, following peripheral nerve injury there is extensive actin polymerization around Schmidt-Lantermann incisures as Schwann cells differentiate into a repair phenotype. Both processes are regulated by Rac1. Our lab previously demonstrated that Rac1 activation during development is driven by engagement of β1 integrin with laminins and is essential for radial sorting. Therefore, we performed a proteomic screen to look for novel Rac1 effectors in peripheral nerves and identified striatin-3 (Strn3) as a candidate. Initial *in vitro* data suggests that Strn3 knockdown in SCs decreases their ability to adhere to various substrates including axons and reduces proliferation. We are developing a mouse model with ablation of Strn3 specifically in Schwann cells along with a Strn1/3 double Schwann cell knockout. Additionally, we have found that Strn3 may be highly expressed in axons at nodes of Ranvier and in oligodendrocytes. Therefore, we are also developing mouse models with specific ablation of Strn3 in neurons or oligodendrocytes to characterize the functional significance of Strn3 in axons and myelinating glia of the central nervous system.

MTU11-27

Presentation of acute motor deficit and subsequent recovery following internal capsule demyelination in mice

R. Yamazaki

Gerogetown University, Department of Biology, Washington DC, USA

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the CNS characterized by progressive remyelination failure and accumulated motor disability. However, whether remyelination promotes motor recovery remains unclear. Here, we compared the effect of experimental demyelination with focal ischemia induction in the internal capsule (IC), a white matter region associated with motor impairment in MS and stroke, on motor behavior in mice. First, to induce the demyelination, we injected lysolecithin (LPC) into right side IC using stereotaxic technique. At 7 days post lesion (dpl), demyelination was observed in the IC by immunofluorescence staining and FluoroMyelin. Next, to examine whether this model affects motor function, we performed adhesive tape removal test, cylinder test, wire hang test and ladder walking test. IC-demyelinated mice reduced motor function in mice at 7 dpl. Further, we demonstrated that demyelination of right IC significantly impaired both left forelimb and left hindlimb motor function.

Moreover, these mice exhibited motor deficit until 14dpl, but regained motor function by 28dpl, corresponding with reduced inflammation, decreased axonal dystrophy, and increased oligodendrocytes in lesions. By contrast, injection of endothelin-1 (ET1) into the IC, which is known to induce white matter infarct, displayed lasting motor deficit, which is accompanied by persistent inflammation and axonal dystrophy, and reduced oligodendrocytes in lesions. These results demonstrate that IC demyelination induces acute motor deficit and subsequent motor recovery through remyelination, and suggest that inflammation resolution and the restoration of axonal integrity may be required for successful remyelination and motor recovery. Therefore, IC demyelination is a tractable model for assessing the influence of remyelination on motor behavior, and may be used to complement future drug screens for the identification of compounds for promoting remyelination.

MTU11-28

Guanosine and guanine can differently modulate sumoylation in rat cortical astrocytes

C. Zanella, G. Poluceno, C. Tasca, H. Cimarosti

UFSC, Pharmacology, Florianópolis, Brazil

SUMOylation is a posttranslational protein modification that can participate in endogenous protective mechanisms, and defective SUMOylation is observed in a diverse array of neurological disorders. Guanosine and its metabolite guanine, two endogenous molecules from the purinergic system, also play physiological and protective roles in different disorders. Here we investigated whether guanosine and/or guanine can modulate SUMOylation. Primary cortical astrocytes (14 days *in vitro*) were treated with guanosine and guanine (1, 10, 100, 300 or 500 μ M) for 1 h and 6 h. Data from Western blotting labelling were obtained from the whole membrane signal always divided by its respective loading control (GAPDH). One-way ANOVA followed by Neuman-Keuls *post hoc* test was used to analyze the data. Global SUMO-2/3-ylation increased two-fold in astrocytes treated for 1 h with guanosine (10 - 500 μ M, $n = 4$). Conversely, guanine (at 500 μ M, $n = 4$) decreased global SUMO-2/3-ylation in astrocytes treated for 1 h. SUMO-2/3 levels go back to control levels in astrocytes treated with guanosine (10 - 500 μ M, $n = 4$) for 6 h, however treatment with guanine (at 500 μ M, $n = 4$) for 6 h caused a significant increase in SUMO-2/3 levels. To our knowledge this is the first report where molecules from the

purinergic system are shown to be modulating SUMOylation. These are promising results that raise several questions concerning the functional consequences of this modulation for cellular protection.

MTU11-29

Ionotropic mechanism of NMDA receptor-mediated calcium fluxes in cultured mouse astrocytes: is it modulated by neurons?

K. Skowrońska, J. Albrecht

Mossakowski Medical Research Centre, Polish Academy of Sciences, Neurotoxicology, Warsaw, Poland

It is well established that activation of NMDA receptors (NMDAR) in cultured rodent astrocytes triggers calcium fluxes. In rat astrocytes the fluxes involve calcium derived from both extracellular and intracellular compartments, reflecting ionotropic or metabotropic mechanisms, respectively. Here we analyzed the two mechanisms in cultured mouse astrocytes and the role of neuron-derived factors in the process. Cultured mouse cortical astrocytes were treated with 100 μ M NMDA and changes in the intracellular calcium concentration ($[Ca^{2+}]_i$) were measured with the fluorescent calcium indicator, Fluo-3-AM. The NMDA-dependent fluxes were absent in the presence of a NMDAR channel blocker MK-801 and in cultures with silenced NMDAR subunit GluN1 (siGluN1), but were not altered by incubation with blockers of: i) IP₃ receptor (xestospongine C), ii) ryanodine receptor (ryanodine) and iii) mGluR5 (MPEP). The results indicate that the mechanism by which NMDAR in mouse astrocytes exclusively mobilize extracellular, but not intracellular calcium, reflecting ionotropic mechanism. The ionotropic nature of the fluxes was not altered by preincubation of astrocytes with a medium derived from cultured neurons, indicating that neuron-derived soluble factors are neutral to the process. Evaluation of the calcium fluxes in astrocytes co-cultured with neurons are under way to assess whether direct cell contact between astrocytes and neurons plays a role in establishing the nature of astrocytic calcium fluxes. Supported by National Science Centre of the Republic of Poland (NCN), grant no 2017/27/N/NZ3/02819.

MTU12 Neuron-glia interactions (Session A)

MTU12-01

DTI found structural alterations induced by long-term optogenetics stimulation of striatal medium spiny neurons

Y. Abe, K. Tanaka

Keio University School of Medicine, Neuropsychiatry, Tokyo, Japan

Striatal medium spiny neurons (MSNs) control motor function. Hyper- or hypo-activity of MSNs coincides with basal ganglia-related movement disorders. Based on the assumption that lasting alterations in neuronal activity lead to structural changes in the brain, understanding these structural alterations may be used to infer MSN functional abnormalities. To infer MSN function from structural data, understanding how long-lasting alterations in MSN activity affect brain morphology is essential. To address this question, we conducted a proof-of-concept study using mice that express channelrhodopsin-2 (ChR2) only in the MSNs. We utilized *ex vivo* diffusion tensor imaging (DTI) for comprehensive visualization of structural alterations. One-week optogenetic stimulation was conducted to the hemispherical dorsal striatum (dStr) of mice which express a ChR2-YFP in MSNs. As a result, a rotation behavior induced by optogenetics stimulation to the MSNs was impaired after one-week stimulation. The stimulation decreased fractional anisotropy (FA), reflecting structural alterations of axons and myelin, in the ipsilateral dStr, motor cortex (M), and substantia nigra reticular (SNr), compared with the contralateral side. Histological approach using a super-resolution microscopy showed the smaller diameters of YFP positive axons and dendrites of MSNs. In addition, the diameters of myelin proteolipid protein (PLP) positive axons were smaller and PLP positive myelination was thinner in dStr, M, and SNr. These structural changes were approved by observation with an electron microscopy and were highly correlated with the DTI-FA change. These results indicated that the long-term activation of dStr MSN, resulting in motor dysfunction, altered structures of MSNs and myelinated neurons of the MSN-related circuit. This combinatorial study provides a useful tool to understand the causal relationship with functional and structural alterations.

MTU12-02

Involvement of the gut microbiome-brain microglia axis in a maternal high-fat diet mouse model of neurodevelopmental disorders

M. Bordeleau^{1,2}, C. Bolduc¹, G. Luheshi², M.-E. Tremblay¹

¹*CRCHU de Québec - Université Laval, Neuroscience, Québec, Canada*

²*Douglas Mental Health University Institute, Psychiatry, Montréal, Canada*

A high-fat diet (HFD) during pregnancy and/or nurturing is associated with an increased risk of neurodevelopmental disorders in the offspring. Previous results in mice revealed that maternal HFD (mHFD) leads to the development of behavioral abnormalities,

including stereotypic and repetitive movements, caused by a gut dysbiosis, which promoted altered neuronal network activation. The gut microbiome may influence brain development by altering microglia, the immune cells that regulate the formation of neuronal circuits. However, these effects on microglia and their consequences on the brain and behavior remain largely undetermined. We hypothesized that a dysbiosis of the gut microbiota caused by a mHFD could alter microglial function leading to an imbalance of excitatory/inhibitory neuronal input relevant to known behavioral deficits. To test this hypothesis, female mice received a HFD (rich in saturated and unsaturated fats) for 4 weeks before breeding until weaning of their litter. Offspring's behavior was assessed during adulthood, which identified increased repetitive movements. Another cohort of animals was sacrificed at 30 days of life to collect brain and gut tissues. The microbiome will be analyzed by sequencing of 16S RNA. To assess changes in neuronal circuits and their interactions with microglia, a triple immunostaining will be performed to label excitatory synapses (Vglut1/Homer1) or inhibitory synapses (vGAT/gephyrin) in addition to microglia (IBA1) among the hippocampus and amygdala, two regions involved in the glutamatergic circuit. This project will help to understand the link between gut dysbiosis caused by mHFD and the pathogenesis involving microglia and excitatory/inhibitory imbalance of neurodevelopmental disorders.

MTU12-03

Microglia contribute to the loss of inhibitory synapses in chronic toxoplasma gondii infection

G. Carrillo¹, T. Glauser², J. Teamer¹, Z. Boone¹, I. Blader¹, M. Fox¹

¹*Virginia Tech, Fralin Biomedical Research Institute, Roanoke, USA*

²*University at Buffalo, Department of Microbiology and Immunology, Buffalo, USA*

Schizophrenia is a complex and heterogeneous neurological disorder associated with debilitating cognitive impairment, acquisition of positive symptoms (hallucination and psychosis), and loss of behaviors that are normally present in healthy individuals (apathy and social withdrawal). Evidence from both human patients and rodent models suggest that schizophrenia-associated behaviors result from alterations in the assembly and function of inhibitory synapses, including inhibitory axo-somatic synapses. In addition to genetic causes (such as those examined with genetic mouse models) environmental factors can both increase the risk of schizophrenia and alter inhibitory circuit function in the brain. One such environmental factor is infection with *Toxoplasma gondii*, an intracellular protozoan parasite that infects over one-third of the human population worldwide. We previously discovered abnormalities in inhibitory synapse organization and function in chronically *Toxoplasma*-infected brains. Here, we sought to test whether chronic infection specifically alters axo-somatic inhibitory synapses. We performed ultrastructural analysis of inhibitory axo-somatic synapses in the CA1 region of mouse hippocampus and in layer

V of cerebral cortex using Serial Block Face Scanning Electron Microscopy. In parasite-infected brains we discovered a significant reduction of inhibitory axo-somatic synapses in CA1 and neocortex. Interestingly, we observed a dramatic ensheathment of neuronal somas in these regions by microglia-like cells in *Toxoplasma*-infected brains. These findings were further corroborated with *in situ* hybridization for *Syt1* (a marker for neuronal somas) coupled with immunohistochemistry to visualize phagocytic microglia. Thus, we not only identified a significant reduction in axo-somatic synapses in parasite-infected brains, but our data suggests a role for microglia in inhibitory synapse loss.

MTU12-04

Quetiapine reverses the malfunctions in the behaviour and the neuron-microglia protein systems of prenatally LPS-treated offspring

K. Chamera, M. Szuster, E. Trojan, A. Basta-Kaim

Institute of Pharmacology Polish Academy of Sciences, Department of Experimental Neuroendocrinology, Kraków, Poland

The risk of developing schizophrenia appears to be a serious threat for an adult offspring of mothers exposed to prenatal insults during pregnancy. The immunological aspect seems to indicate the importance of systems controlling neuron-microglia interactions, including chemokines and clusters of differentiation. The study was designed to examine: 1) an influence of the changes induced by the prenatal treatment with lipopolysaccharide (LPS) on the behaviour and protein levels of CX3CL1, CX3CR1, CD200, CD200R in the hippocampus and the frontal cortex of adult offspring rats; 2) an impact of 14-day-treatment with quetiapine on above-mentioned aspects. Every other day from the 7th day of pregnancy, rats were injected with LPS. 3-month-old male offspring were subjected to the behavioural examination (the PPI test). Afterwards, rats were treated with quetiapine for 14 days. The PPI test was performed again and animals were sacrificed to dissect hippocampi and frontal cortices. CX3CL1, CX3CR1, CD200 and CD200R levels were measured using ELISA assays. The results of the PPI test showed disturbances in the prepulse inhibition in LPS adult offspring. These changes were normalized after 14-day injections of quetiapine. Prenatal administration of LPS disrupted homeostasis of CX3CL1-CX3CR1 and CD200-CD200R systems in the examined brain areas and the treatment with quetiapine had a normalizing effect on part of these aspects. Prenatal exposure to lipopolysaccharide causes schizophrenia-related changes in adult offspring rats. The behavioural alterations are followed by disturbances in the protein systems. The antipsychotic drug – quetiapine exerts a positive effect on examined aspects. Nevertheless, the matter raised in the presented summary requires further research. Funding: grant no. 2015/19/B/NZ7/02394, NCN, Poland.

MTU12-05

Alpha-synuclein oligomers enhance astrocyte-induced synapse formation through TGF- β 1 signaling in parkinson's disease model

L. Diniz, I. Matias, A. P. Bérnago, M. N. Araújo, F. B. Aragão, S. V. Alves-Leon, J. M. de Souza, D. Foguel, C. P. Figueiredo, C. Braga, L. Romão, F. Gomes

UFRI, Institute of biomedical sciences, Rio de Janeiro, Brazil

Parkinson's disease (PD) is characterized by selective death of dopaminergic neurons in the substantia nigra, nigrostriatal pathway degeneration, increase of glutamatergic synapses in the striatum and aggregation of α -Synuclein. Evidence suggests that oligomeric species of α -Synuclein (α SO) are the genuine neurotoxins of PD. Whereas several studies support the direct neurotoxic effects of α SO on neurons, their effects on astrocytes have not been directly addressed. Astrocytes are essential to several steps of synapse formation and function, including secretion of synaptogenic factors, control of synaptic elimination and stabilization, secretion of neurogliomodulators, and modulation of extracellular ions and neurotransmitters levels in the synaptic cleft. Here, we showed that α SO induce astrocyte reactivity and enhanced the synaptogenic capacity of human and murine astrocytes by increasing the levels of the known synaptogenic molecule, transforming growth factor beta 1 (TGF- β 1). Moreover, intracerebroventricular injection of α SO in mice increased the number of astrocytes, the density of excitatory synapses, as well as TGF- β 1 levels in the caudate-putamen of injected animals. Inhibition of TGF- β 1 signaling impaired the effect of the astrocyte conditioned medium on glutamatergic synapses *in vitro* and striatal synapse formation *in vivo*; whereas addition of TGF- β 1 protected dopaminergic neurons against synapse loss triggered by α SO. Together, our data suggest that α SO have important effects on astrocytic functions, and describe TGF- β 1 as a new endogenous astrocyte-derived molecule involved in the increase of striatal glutamatergic synaptic density present in early stages of PD.

MTU12-06

Unraveling the role of SUMOylation in peripheral myelination

L. Frick¹, Y. Poitelon², V. Matafora³, A. Bachi³, L. Wrabetz¹, M. L. Feltri¹

¹*University at Buffalo, HJKRI, Buffalo, USA*

²*Albany Medical College, DNET, Albany, USA*

³*Institute of Molecular Oncology Foundation, FIRC, Milano, Italy*

The mechanisms that govern myelination in the peripheral nervous system are not completely understood. Post-translational modifications of proteins are necessary for peripheral myelination, and malfunction of these pathways leads to neuropathies. Whether SUMOylation, covalent attachment of Small Ubiquitin-like Modifier (SUMO) proteins to the substrate, is involved in the formation of myelin and/or in the pathophysiology of peripheral neuropathies is not known. We have recently identified SUMO2 as a novel protein that may be involved in the early interaction between axons and Schwann cells. In the pseudopod system, Schwann cells are cultured on a porous surface, and stimulated to extend pseudopods towards a neuronal membrane preparation. One of the proteins found in the proteome of Schwann cell pseudopods is SUMO2, along with 10 immediate neighbors in the interactome. Using a prediction

algorithm, we evaluated which of the proteins found in the pseudopods have SUMO interaction motifs (SIMs) and/or SUMOylation sites. Interestingly over 85% of those proteins have either SIMs or SUMOylation sites: 67% of proteins may be SUMOylable, 58% of the proteins may interact with SUMO through SIMs. Proteins involved in cytoskeleton organization represent the major category of enriched proteins bearing SIMs and/or SUMOylation sites, and proteins related to regulation of cellular component organization are the most significantly enriched in pseudopods. Pharmacological modulation of SUMOylation and gene targeting of SUMO proteins affect myelination *in vitro*. Our current research is focused on the role of SUMOylation, its substrates, and SUMO2 in Schwann cell function and peripheral myelination. The discovery of SUMO targets will contribute to understanding the mechanisms of PNS development and demyelinating pathologies.

MTU12-07

Circuit-specialized transcriptional control of astrocytes contributes to learning and memory

A. Y.-S. Huang^{1,2}, J. Woo², D. Sardar², B. Lozzi², C.-C. J. Lin², D. Felice³, A. Paulucci⁴, B. Deneen^{1,2,3}

¹Baylor College of Medicine, Program in Developmental Biology, Houston, USA

²Baylor College of Medicine, Center for Cell and Gene Therapy, Houston, USA

³Baylor College of Medicine, Department of Neuroscience, Houston, USA

⁴MD Anderson Cancer Center, Department of Genetics, Houston, USA

Astrocytes are abundant in the brain with diverse functions that contribute to brain homeostasis. However, whether astrocytes in different regions of the brain exhibit specialized characteristics is largely unknown. Here, in order to understand region-specific transcriptional regulation of astrocytes, we knocked out NFIA, a transcription factor pivotal to inducing gliogenesis, in four different regions of the adult mouse brain. We found that NFIA regulated great amount of genes in the hippocampus but only minimal amount of genes in other brain regions. Loss of NFIA in adult astrocytes resulted in aberrant morphology with fewer, shorter processes in the hippocampus. NFIA-deficient hippocampal astrocytes had reduced proximity to neurons, impaired detection of neurotransmitters, and reduced intracellular calcium activity. These astrocytic defects were associated with impaired long-term potentiation (LTP) and learning/memory deficits in astrocytic NFIA knockout mice. Our findings have identified the first region-specific transcriptional mechanism that regulates adult astrocyte morphology and function, and provide further insight into the contribution of astrocytes to learning and memory.

MTU12-08

New neurons reach and regenerate stroke-injured brain tissue by clearing a path through glia

N. Kaneko¹, V. Herranz-Pérez², T. Otsuka³, H. Sano³, N. Ohno^{4,3}, H. B. Nguyen³, T. Q. Thai³, A. Nambu³, Y. Kawaguchi³, J. M. García-Verdugo², K. Sawamoto^{1,3}

¹Nagoya City University Graduate School of Medical Sciences, Department of Developmental and Regenerative Biology, Nagoya, Japan

²Universidad de Valencia, Laboratory of Comparative Neurobiology, Valencia, Spain

³National Institute for Physiological Sciences, Division of Cerebral Circuitry, System Neurophysiology, Neurobiology and Bioinformatics, and Neural Development and Regeneration, Okazaki, Japan

⁴Jichi Medical University, Department of Anatomy, Shimotsuke, Japan

New neurons are continuously generated by the neural stem cells in the ventricular-subventricular zone (V-SVZ) even in the adult brain. After brain injury, the immature new neurons (neuroblasts) migrate toward the lesion. However, the ability of the mammalian brain to regenerate neuronal circuits for functional recovery is quite limited. By time-lapse imaging and immunohistochemistry using a mouse model for ischemic stroke, we showed that neuroblast migration is restricted by astrocytes, a major population of glial cells, activated in response to tissue damage in and around the lesion. To migrate through the meshwork of the astrocytic processes, the neuroblasts secrete a diffusible protein Slit1 to disrupt the actin cytoskeleton in reactive astrocytes that express its receptor, Robo2. By enhancing the Slit1-Robo2 signaling, V-SVZ-derived neuroblasts transplanted into the post-stroke brain could migrate closer to the lesion. Some of these cells matured into neurons possessing morphological and electrophysiological properties of the striatal projection neurons lost by stroke. They were efficiently integrated into the neuronal circuit, resulting in functional recovery in the post-stroke mice. These results suggest that the positioning of new neurons is critical for functional neuronal regeneration in stem/progenitor cell-based therapies for brain injury.

MTU12-09

The role of astrocytes in memory: focus on pattern separation

C. Kramar, V. Ostapchenko, O. R. Ghosh-Swaby, V. Prado, M. Prado, T. Bussey, L. Saksida

University of Western Ontario, Robarts Research Institute, Department of Physiology and Pharmacology, Schulich School of Medicine & Dentistry, London, Canada

Pattern separation (PS), a cognitive function thought to depend on adult born neurons (ABNs) in the dentate gyrus (DG), involves the transformation of representations of distinct features into unique, less overlapping representations, helping to minimize memory interference. As it has recently been shown that astrocytes play a leading role in helping ABNs to integrate and function in DG networks, we hypothesized that astrocytes themselves could play a role in regulating PS. We manipulate astrocytes by expressing excitatory DREADDs specifically on astrocytes by crossing *Glast^{creERT2}*-mice with floxed hM3-Gq-DREADD-mice. We surgically implant cannulas into the DG of each mouse, allowing us to

directly inject clozapine-n-oxide (CNO), or vehicle. We confirm the expression and co-localization of DREADDs on astrocytes, and a lack of DREADDs expression on ABNs or mature neurons. We also validated the functionality of the DREADDs by performing in vitro experiments with astrocytic cultures from these animals and measuring Ca^{2+} levels after CNO induction. We have two main approaches for the study of PS: Spontaneous Location Recognition (SLR); and the touchscreen-based Location Discrimination (LD) task. These tests are the same ones used to reveal a role for ABNs in PS. We found that activation by CNO of the excitatory Gq-DREADDs on the astrocytes of the DG region improved PS performance in both the SLR and LD tasks.

The finding that selective astrocyte manipulation can robustly and selectively improve memory function provides compelling evidence for the importance of astrocytes in cognition, and introduces astrocytes as a novel target for intervention in neurodegenerative and neuropsychiatric diseases affecting memory.

MTU12-10

Specific deletion of neuronal MCT2 or astrocytic MCT4 disturbs the hippocampus-dependent acquisition of information

C. Netzahualcoyotzi, L. Pellerin

University of Lausanne, Department of Physiology, Lausanne, Switzerland

The astrocyte-neuron lactate shuttle (ANLS) hypothesis proposes that neuronal glutamatergic activity leads in astrocytes to a large increase in the production of lactate, which is released in the extracellular space through the monocarboxylate transporter 4 (MCT4) to be taken by neurons via MCT2 and used as an energy substrate to sustain neurotransmission. Lactate released by astrocytes has been suggested to be necessary for working memory. Further, it was demonstrated that MCT1, 2 and 4 are required for the formation of a non-spatial, long-term (24 h) memory and in the expression of plasticity genes. In the present work, we used for the first time the Cre-lox technology to induce the specific deletion of MCT2 in neurons and MCT4 in astrocytes of the dorsal hippocampus to evaluate their requirement for different behavioral tasks. Our results show that the deletion of either MCT2 or MCT4 does not alter innate behavior, but only the acquisition of new information. The short-term storage of information was normal, but long-term memory was significantly affected. However, if the exposition to the new information (training) is sufficiently repeated, it is possible to finally acquire the data and their retrieval in such case is normal. Our results suggest that lactate transport is a critical step in the acquisition of new information, either in the astrocytes or in the neurons. This could be related to the metabolic coupling proposed by the ANLS. Our data also indicate that intense training sessions can induce compensatory responses to overcome MCT deficiencies. Hence, we propose that the ANLS facilitates the acquisition of new hippocampus-dependent information.

MTU12-11

Volume electron microscopy of the white matter in the hereditary demyelinating disease model

N. Ohno^{1,2}, T. Q. Thai², H. B. Nguyen², Y. Sui², K. Ikenaka²

¹*Jichi Medical University, Department of Anatomy, Shimotsuke, Japan*

²*National Institute for Physiological Sciences, Division of Neurobiology and Bioinformatics, Okazaki, Japan*

The connections of neurons are dependent on axons, long neurites which are enriched in the white matter. Many of the axons are ensheathed in the white matter by myelin, and the myelin ensheathment divides axons into structurally and functionally distinct domains. Myelin supports salutatory conduction and maintenance of axonal integrity. Volume electron microscopic imaging of the white matter has recently started providing structural information for the better understanding of physiology and pathology of the white matter. For example, the distribution and morphology of organelles in axons were changed by demyelination in the white matter, and it was suggested that such alterations are beneficial for axonal functions and survival. Among them, mitochondria associated membranes (MAM), physical connections between mitochondria and endoplasmic reticulum, are critical for cellular functions such as Ca^{2+} signaling, lipid transport. Disruption of the connection has been implicated in mitochondrial dysfunction, which has been proposed as a major contributor of axonal degeneration in diseases of myelin. However, the changes and roles of these juxtapositions are still unclear in demyelinated axons. In this study, we investigated three-dimensional ultrastructural changes of axonal MAM in chronic demyelination, using the serial block-face scanning electron microscopy and a mouse model of chronic demyelination caused by extra-copies of proteolipid protein. The results suggest that modulations of MAM as well as mitochondria are caused by chronic loss of myelin, and that such organelle changes are involved in the pathophysiology of hereditary myelin diseases.

MTU12-12

NG2 GLIA-specific KIR4.1 knockout as a tool to understand the impact of neuron-glia synaptic signaling

G. Seifert¹, A. Timmermann¹, A. Boehlen¹, M. Skubal¹, A. Bilkei-Gorzo², A. Zimmer², R. Jabs¹, F. Kirchhoff³, C. Steinhäuser¹

¹*University of Bonn, Institute of Cellular Neurosciences, Bonn, Germany*

²*University of Bonn, Institute of Molecular Psychiatry, Bonn, Germany*

³*University of Saarland, Molecular Physiology, CIPMM, Homburg, Germany*

NG2 glia in grey matter receives direct synaptic input from glutamatergic and GABAergic neurons. During development, NG2 glia upregulate Kir4.1 channels. To test if Kir currents regulate the efficiency of synaptic activation of NG2 glia, we used NG2-CreERT2 knock-in mice to selectively ablate the Kir4.1 gene upon tamoxifen administration.

Mutant NG2 glia, deficient of Kir currents, displayed a more positive resting potential and increased membrane resistance in comparison to control NG2 glia. Monitoring responses upon Schaffer collateral stimulation revealed similar EPSC amplitudes in Kir-deficient NG2 glia compared to control cells. Moreover,

mEPSP amplitudes were enhanced and the time constant of voltage decay was prolonged in Kir4.1 deficient glial cells. To investigate the impact of Kir4.1 deletion in NG2 glia on neural signaling, field potentials were recorded in the hippocampus after stimulation of Schaffer collaterals. Long term potentiation (LTP), induced by theta-burst stimulation, was significantly impaired in the hippocampal CA1 region of mice with NG2 glia-targeted Kir4.1-deficiency. Despite impaired LTP, Kir4.1-deficient mice showed increased novelty preference in the object location recognition test, and improved new partner preference in the partner recognition test. In the hippocampus, NG2 glia-targeted deletion of the Kir4.1 gene entailed an upregulation of MBP protein 8 weeks after tamoxifen injection. These findings show that Kir4.1 channels in NG2 glial cells regulate their excitability, influence myelination and are important for proper hippocampal synaptic plasticity and behavior.

MTU12-13

Muscarinic acetylcholine receptors regulate the expression of KIR4.1 channels and BDNF in cultured mouse astrocytes

S. Shimizu¹, M. Kinboshi^{1,2}, Y. Nakamura¹, N. Oda¹, A. Ikeda², Y. Ohno¹

¹Osaka University of Pharmaceutical Sciences, Department of Pharmacology, Osaka, Japan

²Kyoto University, Department of Epilepsy, Movement Disorders and Physiology, Graduate School of Medicine, Kyoto, Japan

Astrocytes are the most abundant glial cells and regulate neuronal excitability by maintaining ion homeostasis, metabolizing neurotransmitters and secreting neuroactive substances. Recent evidence illustrates that astrocytic brain-derived neurotrophic factor (BDNF) expression is specifically modulated by inwardly rectifying potassium (Kir) channel subunit Kir4.1 channel, which mediate the spatial potassium buffering function of astrocytes (Front. Mol. Neurosci., 10, 408, 2017; Int. J. Mol. Sci., 19, 3313, 2018). In the present study, we investigated the effects of acetylcholinergic agents on mRNA expression of Kir4.1 and BDNF in primary cultured mouse astrocytes in order to explore the neural factors influencing on the Kir4.1-BDNF system. Treatment of astrocytes with acetylcholine (ACh, 3-30 μ M) significantly inhibited Kir4.1 expression and increased BDNF expression in a concentration-related manner. Both inhibition of Kir4.1 and enhancement of BDNF expression by ACh (10 μ M) were antagonized by muscarinic ACh receptor antagonist atropine (3 μ M), however, the nicotinic ACh receptor antagonist mecamylamine (30 μ M) showed no effects. In addition, inhibition of Kir4.1 expression by ACh was significantly antagonized by the selective muscarinic ACh M₁ antagonist pirenzepine (10 μ M), which also inhibited acetylcholine-enhanced BDNF expression. The present results strongly suggest that ACh inhibits Kir4.1 channel expression and increases BDNF expression via activation of muscarinic ACh M₁ receptor in astrocytes.

MTU12-14

Anti-inflammatory effect of carbon monoxide on the neuron-microglia communication

N. Soares¹, C. Queiroga¹, H. Vieira^{1,2}

¹CEDOC - NOVA Medical School, Universidade NOVA de Lisboa, Cell Death and Disease, Lisbon, Portugal

²Instituto de Biologia Experimental e Tecnológica, iBET, Oeiras, Portugal

Microglia, the 'resident immunocompetent cells' of the central nervous system (CNS), are key players in innate immunity and tissue homeostasis. However, dysfunctional microglia contributes heavily to the creation of a toxic inflammatory milieu, a common driving force for the pathophysiology of several CNS disorders. Thus, strategies have been postulated to tackle exacerbated tissue inflammation by modulation of microglial function. Carbon monoxide (CO) is an endogenous gaseous molecule, produced by the degradation of free haem. Long considered a catabolic waste product, it has now emerged as a player in neurobiology, having shown neuroprotective properties in *in vivo* and *in vitro* models. We aimed at studying CO as a modulator of microglial reactivity, focusing on its communication with neurons by limiting inflammation and consequently providing neuroprotection. For this, we used a BV2 microglia-CAD neuron cell line conditioned media protocol. Treating LPS-activated BV2 microglia with CO limited expression and secretion of inflammatory cytokines (TNF- α , NO). Neurons subsequently challenged with inflammatory media displayed high cell death and dysfunction levels. This is partially reverted whenever microglia is pre-treated with CO, indicating the gas promotes neuroprotection in a non-cell autonomous mode. Likewise, CO stimulated the expression of neuronal glycoprotein CD200 and its microglial receptor CD200R1 via PPAR- γ transcription factor. The CD200-CD200R1 axis is involved in the fine regulation of microglial function, and we are elucidating how CO-driven modulation of this tight contact alters cell function, fate and homeostasis. Altogether, this is a stepping stone to understand CO's impact on novel cell-cell regulatory mechanisms.

MTU12-15

Retinal inputs signal through astrocytes to recruit interneurons into mouse visual thalamus

R. D. Somaiya¹, J. Su¹, N. Charalambakis², A. Monavarfeshani¹, U. Sabbagh¹, W. Guido², M. Fox¹

¹Virginia Tech, Fralin Biomedical Research Institute, Roanoke, USA

²University of Louisville, Anatomical Sciences and Neurobiology, Louisville, USA

The lateral geniculate complex includes a number of retinorecipient thalamic nuclei that play important roles in receiving, processing, and relaying image- and non-image forming visual information. Recent studies have revealed that neonatal innervation of these regions by retinal ganglion cell axons play an instructive role in the postnatal development and maturation of thalamic circuits. For example, surgical or genetic removal of retinal inputs at birth impairs the recruitment of local GABAergic interneurons into visual thalamus. Here we sought to identify the mechanisms that underlie retinal input-dependent interneuron migration into visual thalamus. Focusing on this, we first explored transcriptomic changes in neonatal mouse visual thalamus lacking retinal input. Using

microarray analysis and *in situ* hybridization (ISH), we discovered that the expression of Fibroblast Growth Factor 15 (FGF15) in visual thalamus is dependent upon the retinal inputs. To test whether FGF15 was required for interneuron recruitment into visual thalamus, we examined thalamic development in *Fgf15*^{-/-} mutant mice. In these mutants, we observed a significant reduction in GABAergic interneurons both by ISH and by crossing these mutant mice to *Gad67-GFP* reporter mice. We hypothesized that retinal inputs induced FGF15 expression in retinorecipient neurons. To test this, we performed ISH to detect *Fgf15* mRNA in reporter mice that label distinct populations of cells in visual thalamus. Surprisingly, our data revealed that FGF15 is generated by thalamic astrocytes and not neurons, suggesting a novel role for astrocytes in thalamic development. Taken together, these results suggest the existence of novel axon-glia-neuron signaling pathway that underlies subcortical visual circuit formation.

MTU12-16

High fat diet promotes cognitive impairment, neuroinflammation and decreased hippocampal plasticity: role of microglial exosomes

A. Vinuesa¹, M. Bentivegna¹, G. Calfa², F. Filipello³, C. Pomilio¹, A. Gregosa¹, J. Presa¹, M. Matteoli³, J. Beauquis¹, F. Saravia¹

¹*Institute of Biology and Experimental Medicine ^{IByME}, Biological Chemistry, Buenos Aires, Argentina*

²*IFEC-CONICET, Pharmacology, Cordoba, Argentina*

³*Humanitas Clinical and Research Center, Pharmacology, Milan, Italy*

Western dietary habits including high fat foods are increasingly represented in juvenile populations, and constitute one of the factors that affect brain health, potentially leading to long lasting effects. The aim of this study was to assess the impact of an early exposure to a high fat diet (HFD) on mouse hippocampal plasticity. C57BL/6J male mice were exposed to HFD for 6 weeks since weaning. Glucose and IL1 β levels were found to be higher in serum of HFD mice, without overweight. In the hippocampus, neuroinflammation was evidenced by Iba1+ cells reactivity and increased expression of TNF α and IL1 β in HFD group, which also exhibited a strongly reduced neurogenic capability: decreased Ki67+ cells and immature DCX+ neurons in the SGZ of the dentate gyrus. We also found a reduced proportion of mature Dil-labeled dendritic spines from CA1 neurons and diminished levels of the scaffold protein Shank2, suggesting a defective connectivity. Moreover, HFD mice exhibited spatial memory alterations in the novel object location recognition test. To study whether microglia could be mediating HFD-

associated neuronal changes, primary microglia was incubated with palmitate, a saturated fatty acid present in HFD. Palmitate induced a proinflammatory profile as shown by secreted cytokine levels and exosome-like extracellular vesicles that were able to induce an immature dendritic spine phenotype in primary GFP+ hippocampal neurons, in line with the *in vivo* findings. These results provide novel data concerning microglia-neuron communication and highlight that fat excess during an early period of life could negatively impact on hippocampal plasticity in a neuroinflammatory context, where microglia-derived exosomes could be directly implicated.

MTU12-17

Astrocytic insulin-like growth factor-1 protects neurons against excitotoxicity

P. Zheng, B. He, W. Tong

Shanghai Pudong New area People's Hospital, Neurosurgery, Shanghai, China

Background: Exogenous insulin like growth factor-1 (IGF-I) is known to be neuroprotective in animal models with brain insults, while it can also cause hyperexcitability in rodents. In this regard, the role of endogenous IGF-1 in brain responses to brain insults like excitotoxicity, a common pathology in brain injuries remains elucidated. Here, we investigated the potential role of cell-specific endogenous IGF-I in the kainic acid (KA) -induced degeneration of the neurons.

Methods: KA was given to primary cultured cortical neurons and co-cultured astrocytes were added as a supportive system. We evaluated the cell proliferation rate, IGF-1 level in different groups and applied the PCR-Chip assay to explore the downstream of IGF-1. In addition, we applied the viral transfer of astrocytic IGF-1 to rodents treated with KA and assessed the associated molecular marker and behavioural outcomes in these rodents.

Results: We found KA induced increased cell death and hyperphosphorylated tau in neurons; co-cultured astrocytes could prevent these pathologies, and this rescuing effect was abrogated with blockade of the astrocytic IGF-1 with AG1024 (IGF-1R inhibitor). PCR-Chip assay identified that astrocytic IGF-1 could decrease the p-GSK-3 at Thy 216 in neurons treated with KA and this effect was abrogated with AG1024 as well. In addition, *in vivo* study showed that gene transfer of astrocytic IGF-1 decreased p-tau and cognitive dysfunction in KA mice.

Conclusion: Our results show astrocytic IGF-1 exhibit neuroprotective properties in neurodegenerative processes in the CNS.

MTU13 Lipids (Session A)

MTU13-01

Identification of the antigen recognized *in vitro* by RHIGM22, a remyelination-promoting human monoclonal antibody

L. Cabitta¹, S. Grassi¹, S. Prioni¹, L. Mauri¹, M. G. Ciampa¹, Y. Zorina², S. Sonnino¹, A. Prinetti¹

¹Università di Milano, Dipartimento di biotecnologie mediche e medicina traslazionale, Segrate, Italy

²Acorda Therapeutics, Inc, Ardsley, NY, USA

Recombinant human IgM22 (rHIGM22) binds to myelin and oligodendrocytes (OLs), and promotes remyelination in models of multiple sclerosis. rHIGM22 preferentially reacts with sulfatide-positive (O⁴⁺) OLs, and its binding is abolished in brain slices from Cst (-/-) mice, suggesting its binding requires the presence of a product of cerebroside sulfotransferase. However, literature suggests that cell populations lacking sulfatide expression, such as microglia and oligodendrocyte precursor cells, are responsive to rHIGM22, thus the identity of the antigen recognized by this antibody remains to be elucidated.

We tested the binding of rHIGM22 to purified lipids and lipid extracts from various sources using TLC immunostaining and surface plasmon resonance (SPR) with lipid monolayers. Our results show that IgM22 binds to sulfatide and lysosulfatide *in vitro*, while it does not bind to other myelin sphingolipids. In addition, rHIGM22 also reacts with phosphatidylinositol, phosphatidylserine and phosphatidic acid, present in lipid extracts from various sources, including CST ko mice brains, mixed glial cultures, isolated astrocytes and microglia.

These data suggest that sulfatide at the OLs surface might be important for the binding of rHIGM22 to these cells. On the other hand, its ability to bind some glycerophospholipids could explain the biological responses elicited by rHIGM22 in cells lacking sulfatide expression. The *in vitro* reactivity of rHIGM22 suggests that binding of rHIGM22 to intact cells might require a complex molecular arrangement, and, in particular, sulfatide and other membrane lipids might be part of the functional rHIGM22 antigen localized at the cell surface.

MTU13-02

Gender-specific changes to sphingolipid metabolism may sensitise the aging brain to neurodegeneration and Alzheimer's disease

T. Couttas^{1,2}, N. Kain², C. Tran^{1,2}, Z. Chatterton^{1,2,3}, J. Kwok³, A. Don³

¹The Centenary Institute, Lipid Metabolism and Neurochemistry Laboratory, Sydney, Australia

²UNSW Sydney, Prince of Wales Clinical School, Sydney, Australia

³Sydney University, Sydney Medical School, Sydney, Australia

The major risk factors associated with Alzheimer's disease (AD) are age and inheritance of the $\epsilon 4$ allele of the *APOE* gene, which encodes the lipid transporter protein, Apolipoprotein E (ApoE). This suggests a key involvement of lipid transport and metabolism in AD. Sphingolipids, are a class of lipids that exhibit alterations at the prodromal stages of AD, in both brain tissue and serum. Our study

investigated sphingolipids as a function of age and *APOE* genotype in neurologically normal subjects, aged 65 and over. Lipids were quantified from the hippocampus of post-mortem tissue (n = 80) using mass spectrometry. Significant changes to sphingolipids were observed as a function of age, and were gender-specific. Females had a pronounced decline in the sphingosine-1-phosphate:sphingosine ratio ($p = 0.0020$). In contrast, males exhibited increases in ceramides, sulfatide and sphingomyelin ($p < 0.005$). No association between lipids and *APOE* genotype was identified. Previous literature has demonstrated AD is associated with a decline in cerebral glucose utilisation, potentially caused by a loss of insulin receptors at synaptic membranes of the cerebral cortex and hippocampus. Ceramide is a pro-apoptotic lipid implicated as a driver of insulin resistance in metabolic tissue, whereas SIP is associated with increased glucose-stimulated insulin secretion. In the age of precision medicine, there is a need to discern whether risk factors and etiology of AD differs between genders. Our results establish gender-specific differences in sphingolipid metabolism in the aging human brain, which may contribute to a pro-neurodegenerative phenotype.

MTU13-03

Correlation of plasma omega fatty acid index with alpha power during working memory in acute mild traumatic brain injury

A. Fonteh¹, X. Arakaki¹, K. Csator¹, E. Amini², S. Thomas³, R. Goldweber³, M. Harrington¹

¹Huntington Medical Research Institutes, Neurosciences, Pasadena, USA

²USC, Marshall School of Business, Los Angeles, USA

³HMM, Emergency Department, Pasadena, USA

We explored the relationship between alpha power during working memory (WM) processing and the plasma levels of n-3/n-6 polyunsaturated fatty acids (PUFA) in acute mTBI to understand mechanisms and to discover biomarkers of injury. Brain challenges using 0-back and 2-back WM tests were administered within 5 days post-injury with simultaneous electroencephalography (EEG) from 21-head sensors. Plasma unesterified and esterified PUFAs were quantified using gas chromatography/mass spectrometry. The proportions of n-3, n-6, the n-3/n-6 ratio, and the n-3 index were determined and correlated with alpha power for all six brain regions. Esterified n-6 PUFAs negatively correlated with 0-back alpha wave power in the frontal, left temporal, and occipital brain regions while unesterified n-3 negatively correlated with the central brain region of mTBI participants. Esterified n-3/n-6 ratio positively correlated with 0-back alpha power of all six brain regions while esterified n-6 negatively correlated with 2-back alpha power of all brain regions. In contrast, the esterified n-3/n-6 ratio positively correlated with 2-back alpha power in all six brain regions. N-3 and n-6 fatty acids did not correlate with alpha power in the controls. Our studies reveal brain region-specific correlations of 0-back and 2-back alpha frequency with plasma PUFAs in acute mTBI, but not in controls, suggesting that changes in plasma omega-3/6 profile underlie abnormal brain functions during WM challenge at the early phase of mTBI. Therefore, restoring the n-3 PUFA index may enhance prognosis by normalizing alpha power disturbance in mTBI.

MTU13-04

Neuroprotective sphingosine 1-phosphate is essential for amyloid formation, oligodendrocyte survival and cognitive function in AD**M. Lei¹, J. Teo¹, T. Couttas¹, L. Ittner², A. Ittner², T. Karl³, A. Don¹**¹Centenary Institute, The University of Sydney, ACRF, Camperdown, Australia²UNSW, School of Medical Sciences, Kensington, Australia³Western Sydney University, School of Medicine, Campbelltown, Australia

Sphingosine 1-phosphate (S1P) is a potent vasculo- and neuro-protective signalling lipid that promotes neurotrophic growth factor expression and pre-synaptic acetylcholine and glutamate release. S1P is synthesized primarily by sphingosine kinase 2 (SphK2) in the brain. We recently demonstrated pronounced loss of S1P, and SphK2 activity, early in Alzheimer's disease (AD) pathogenesis. Using human female hippocampal tissue samples from neuropathologically normal donors, we recently showed that S1P levels decline with age ($r = -0.5$, $p = 0.002$), leading us to speculate that loss of S1P sensitizes to AD development. To test whether SphK2 deficiency synergises with amyloid beta ($A\beta$) in promoting AD, SphK2 knockout (SphK2^{-/-}) mice were crossed to the J20 mouse model of familial AD amyloidosis.

Surprisingly, SphK2 deficiency reduced $A\beta$ content, plaque burden and reactive astrocyte immunoreactivity in J20 mice. Reduced $A\beta$ was associated with significant improvements in hypersynchronous activity and cross-frequency coupling measured by hippocampal electroencephalography. Despite reduced amyloid burden, SphK2-deficient J20 mice exhibited severe hypomyelination in the hippocampus and cortex, hippocampal atrophy and significant deficits in the Y-maze and social novelty memory tests, when compared to the J20 or SphK2^{-/-} strains.

In summary, endogenous S1P, synthesized by SphK2, is reduced with ageing and AD pathogenesis, yet required for $A\beta$ formation. However, memory deficits and myelin loss and hippocampal volume in J20 mice were exacerbated on a SphK2^{-/-} background, indicating that age-dependent SphK2 depletion promotes neurodegeneration and urging consideration of oligodendrocyte attenuation as a potential enforcer of neurodegeneration in AD.

MTU13-05

Evidence that human glioma cells form pregnenolone via a CYP11A1-independent pathway**Y. C. Lin, V. Papadopoulos**

University of Southern California, Department of Pharmacology & Pharmaceutical Sciences, Los Angeles, USA

The term "neurosteroids" refers to steroid hormones, such as pregnenolone, synthesized and acting in brain. In peripheral steroidogenic tissues, pregnenolone is formed from cholesterol by the cytochrome P450 11A1 enzyme. This conversion involves two hydroxylations at C22 and C20 of cholesterol followed by cleavage of the side chain between C20-22 to form pregnenolone and isocaproic aldehyde. Although pregnenolone is found in the brain, protein expression of CYP11A1 has been difficult to detect. We found extremely low levels of CYP11A1 mRNA in human glioma cells but no protein expression. Therefore, we investigated the ability of human glioma cells to synthesize pregnenolone in a CYP11A1-independent manner. Unlike testicular and adrenal

cortical cells, treatment of glioma cells with the CYP11A1 inhibitor DL-aminoglutethimide or the non-specific CYP inhibitor ketoconazole did not inhibit pregnenolone production by glioma cells. Pregnenolone synthesis can be increased with addition of the substrates 22R-, 22S-, and 20 α -hydroxycholesterols suggesting the involvement of a desmolase activity although the enhanced pregnenolone formation was not blocked by DL-aminoglutethimide or ketoconazole. These data suggest that glioma cells can produce pregnenolone independently of CYP11A1 activity. CYP enzymes are monooxygenases generating free radicals, so we examined whether this alternative pathway involves reactive oxygen species (ROS). Although high doses of DL-aminoglutethimide and ketoconazole increased pregnenolone production by glioma cells, only ketoconazole increased cellular ROS. Addition of the antioxidant Trolox, an analog of vitamin E, did not change pregnenolone production despite blocking ketoconazole's effect on increasing cellular ROS. Treatment with hydrogen peroxide also did not have a significant effect on pregnenolone production. Taken together these results suggest that human glioma cells form pregnenolone via a CYP11A1- and ROS-independent pathway.

MTU13-06

Hexa-associated GM2 gangliosidosis in a family of wild boars**S. Prioni¹, L. Cabitta¹, S. Grassi¹, S. Sonnino¹, V. Bertani², A. M. Cantoni², A. Corradi², V. Jagannathan³, C. Drögemüller³**¹University of Milan, Dep. of Medical Biotechnology and Translational Medicine, Milano, Italy²University of Parma, Department of Veterinary Science, Parma, Italy³University of Bern, Institute of Genetics, Vetsuisse Faculty, Bern, Switzerland

Gangliosidosis are inherited lysosomal storage disorders caused by defective activity of a lysosomal hydrolase required for ganglioside catabolism, resulting in the intra-lysosomal accumulation of undegraded metabolites. The molecular mechanisms linking the lysosomal accumulation to the pathology are still obscure. We report on a novel form of GM2 gangliosidosis in wild boar (*Sus scrofa*). Three littermate wild boars, from a free ranging farm, presented neurological signs (dysmetria, ataxia, quadriplegia and lateral decubitus) at 6 months of age. Viral, bacterial and toxicological analysis were performed to exclude possible exogenous causes of symptoms. Animals were euthanized at approximately one year of age. Necropsy revealed in all affected animals reduced consistency of cerebral and cerebellar parenchyma. Histology revealed enlarged foamy neurons, with diffusely severely vacuolated cytoplasm in brain, cerebellum, spinal cord, peripheral ganglia and retina. EM revealed the presence in neurons of numerous lysosomes, filled by membranous material. Biochemical studies revealed the presence of an elevated amount of GM2 ganglioside, confirming the diagnosis of GM2 gangliosidosis. In addition, genetic analysis revealed the presence of a recessively inherited missense variant (p.Arg499Cys) in the *hexosaminidase subunit alpha (HEXA)* gene located within the GH20 hexosaminidase superfamily domain of the encoded protein. In man and other species, pathogenic HEXA variants are known to be associated with the disease. In conclusion, this HEXA-associated form of GM2 gangliosidosis, described for the first time in wild boars, is thus very similar to human disease.

MTU14 Other topics (Session A)

MTU14-01

Expression of FMRFamide and GFSKLYFamide peptides in *holothuria scabra*: Implication for the neuroendocrine system

A. Ajayi¹, B. Withyachumnarnkul²

¹Kogi State University, Anatomy, Anyigba, Nigeria

²Mahidol University, Anatomy, Bangkok, Thailand

Neuropeptides are key mediators of physiological processes in animals and a considerable amount of information has been accumulated on their diversity and functions across phyla. FMRFamide-related peptides are a large collection of neuropeptides found in invertebrates and vertebrates. They are identified by the possession of a C-terminal -RFamide amino acid sequence and are often coded for by multiple genes. Echinoderms are of phylogenetic importance to chordates in that they are deuterostomes. The sea cucumber, *Holothuria scabra* is a high-premium tropical echinoderm species that is overexploited globally and may be in imminent danger of extinction in some areas. Conservation of this species and its amenability to culture is however hampered by our limited knowledge of its biology, including the neurohormonal system. In this study, FMRFamide peptide, a cardioactive neuropeptide first isolated in a mollusk and GFSKLYFamide peptide, an Echinoderm SALMFamide were investigated for their presence in *H. scabra*. We used indirect immunofluorescence technique with confocal microscope and dot immunoblot assay utilizing polyclonal antisera raised against FMRFamide and GFSKLYFamide peptides. Both FMRFamide- and GFSKLYFamide-immunoreactivity were demonstrated to be widely distributed in *H. scabra* tissues, such as the radial nerve cord, body wall, intestines and coelomic fluid. This underscores the potential physiological roles of these peptides, which might be working as neurotransmitters and neuromodulators in this species.

MTU14-02

Promoting endogenous photoreceptor regeneration in the mammalian retina

C. Boudreau-Pinonneault¹, M. Fries^{3,2}, A. Javed^{3,2}, M. Cayouette^{2,3,1}

¹McGill University, Department of medicine, Montreal, Canada

²Institut de recherches cliniques de Montreal, Cellular neurobiology, Montreal, Canada

³Université de Montreal, Department of medicine, Montreal, Canada

Regenerating the retina using endogenous stem cells is a promising therapy for vision restoration. While fish Müller glial cells (MG) can regenerate the retina, this natural ability was lost in mammals. Recently, however, some genetic manipulations in mouse MG were found to trigger neuron production, but it remains unknown whether MG can generate cone photoreceptors, which are essential for high acuity vision. Interestingly, MG have a similar gene expression profile to late-stage retinal progenitors, and our previous work identified temporal identity factors that can reprogram late progenitors to produce early-born cones. We hence hypothesized that these factors might reprogram MG into cone-producing progenitors. We co-electroporated Cre-dependent

constructs into GlastCre^{ERT};RosaYFP^{fl/fl} retinal explants, which express Cre^{ERT} specifically in MG, allowing expression of genes of interest and cell lineage tracing with the YFP reporter. Of the 21 combinations tested, one was able to reprogram MG into immature cones. MG-derived cells migrated to where cones normally reside, downregulated glial markers, started expressing the cone marker RxRg and adopted a cone-like morphology. These factors were also sufficient to reprogram MG to immature cones *in vivo* in the adult mouse retina, and into more mature cones under certain culture conditions. It remains to be determined whether these cells are functional, but this work suggests that stimulating cone production from endogenous glia might represent a new therapeutic opportunity for retinal degeneration.

MTU14-03

Inner hair cell and neuron degeneration contribute to hearing loss in a DFNA2-like mouse model

C. Carignano¹, E. Barila¹, E. Rías^{1,2}, L. Dionisio^{1,2}, E. Aztiria^{1,2}, G. Spitzmaul^{1,2}

¹Instituto de Investigaciones Bioquímicas de Bahía Blanca, INIBIB, Bahía Blanca, Argentina

²Universidad Nacional del Sur UNS, Departamento de Biología, Bioquímica y Farmacia (DBByF), Bahía Blanca, Argentina

DFNA2 is a progressive deafness caused by mutations in the voltage-activated potassium channel KCNQ4. Hearing loss develops with age from a mild increase in hearing threshold to profound deafness. The first phase starts around 10-15 years old, progressing to the last phase by the age of 70. Studies using transgenic mice for *Kcnq4* expressed in a mixed background demonstrated the implication of outer hair cells (OHCs) at the initial phase. However, it could not explain the last phase mechanisms of the disease. Genetic backgrounds are known to influence disease expressivity. To unmask the cause of profound deafness phenotype, we backcrossed *Kcnq4* knock-out allele to the inbred strain C3H/HeJ and investigated inner and outer hair cell and spiral ganglion neuron (SGN) degeneration across lifespan. In addition to the already reported OHC death, C3H/HeJ strain also exhibited inner hair cell (IHC) and SGN death. We tracked the spatiotemporal survival of cochlear cells by plotting cytochrome c oxidase and neuronal counts at different ages. Cell loss progressed from basal to apical turns with age for both hair cells. Interestingly, the time-course of cell degeneration was different for each cell-type. While for OHCs it was already present by week 3, IHC and neuronal loss started 30 weeks later. We established that OHC loss kinetics slowed down from basal to apical regions correlating with KCNQ4 expression pattern determined in wild-type mice. Our findings indicate that KCNQ4 plays differential roles in each cochlear cell-type impacting in their survival ability. IHC and SGN neuron death generates severe hearing loss that could be associated to the last phase of DFNA2.

MTU14-04

Copper uptake and toxicity in cerebellar granular neurons after application of copper ions or copper oxide nanoparticles**K. Faber^{1,2}, R. Dringen^{1,2}**¹*University of Bremen, Center for Biomolecular Interactions
Bremen, Bremen, Germany*²*University of Bremen, Center for Environmental Research and
Sustainable Technology, Bremen, Germany*

Copper is essential for brain cells as cofactor of various enzymes. Nonetheless, disturbances of copper homeostasis are known to cause oxidative stress and neurological disorders. However, there is only little known on copper uptake and metabolism in neurons. To investigate copper uptake in neurons we have used cerebellar granule neuron-rich primary cultures as model system. The low basal copper content increased strongly after application of copper chloride in a time and concentration-dependent manner, reaching values of up to 40 nmol copper per mg protein after exposure to 100 μ M copper chloride for 30 min. Exposure of neurons to copper chloride in the presence of ascorbate increased cellular copper contents more than 7 times which was accompanied by severe toxicity. Correlation of cellular copper contents with cell viability revealed that specific copper values of above 30 nmol copper per mg protein were accompanied with a strong loss in cell viability. Copper accumulation and copper-induced toxicity was prevented by the application of the copper chelators bathocuproine disulfonate and tetrathiomolybdate, whereas the application zinc ions, known copper transport competitors, did not lower neuronal copper uptake. Comparison of neuronal copper accumulation after application of copper chloride or copper oxide nanoparticles showed that cellular copper contents after incubation with 100 μ M copper chloride was 33 % lower than after incubation with the same concentration of copper oxide nanoparticles. These results demonstrate that copper uptake in neurons is prevented by copper chelators and accelerated by ascorbate which leads to an imbalance of cellular copper homeostasis that severely damages neurons. Moreover, copper accumulation was also observed after application of copper oxide nanoparticles but the mechanisms involved in the uptake need to be further elucidated.

MTU14-05

Targeting mitochondrial dynamics by environmental toxicant bisphenol-A in the rat hippocampus**S. Goyal^{1,2}, A. Tandon^{1,3}, S. J. Singh^{1,2}, J. Shankar⁴,
R. K. Chaturvedi^{1,2}**¹*CSIR-Indian Institute of Toxicology Research, Systems Toxicology
and Health Risk Assessment Group, Lucknow, India*²*Academy of Scientific and Innovative Research^{AcSIR}, CSIR-Indian
Institute of Toxicology Research Campus, Lucknow, India*³*Babu Banarasi Das University, BBD City, Department of
Biochemistry, Lucknow, India*⁴*CSIR-Indian Institute of Toxicology Research, Advanced Imaging
Facility, Lucknow, India*

This study summarized the neuronal demise following Bisphenol-A (BPA) exposure linked impaired mitochondrial biogenesis and dynamics in rat brain hippocampus. Mitochondria are known for their multiple essential cellular functions beyond ATP production/ energy transduction, impacting most of the areas of cell

biology and related mitochondrial medicines in brain. Most of the proteins that help in mitochondria biogenesis (formation of healthy mitochondria) and regulate their dynamics (fission/fusion) are encoded in nucleus. BPA is a known xenoestrogen, found in consumable plastics and causes neuronal apoptosis after chronic exposure in the rat hippocampus and linked cognitive deficits. Any pathogenesis in mitochondria is responsible for impaired mitochondrial dynamics and biogenesis, which is directly associated with cognitive impairment. In this study, we have investigated the gene expression and protein levels of parental factors of mitochondrial biogenesis (PGC1 α , TFAM etc.) and dynamics (DRP-1, MFN-1/2) after BPA exposure in the adult rat hippocampus and in NSC derived neuronal culture. Our result showed reduced protein levels of PGC1 α , TFAM, while imbalanced dynamics showed through excessive fission protein DRP-1. Beside this, our study also investigates the regulatory circuitry and inter-linked events involved in both mitochondrial dynamics and biogenesis. With this, our work holds promising in understanding the role of BPA induced mitochondrial pathophysiology that may give new therapeutic targets to neurodegenerative disorders.

MTU14-06

Metabolic profiles of the synthetic cannabinoid, athpinaca, in human liver microsomes with isomeric discrimination**K. Kitaichi¹, N. Kadamura¹, T. Matsuhisa¹, T. Kinoshita¹, M. Soda¹,
E. Kohyama², T. Chikumoto², H. Nagai², T. Ito^{1,2}**¹*Gifu Pharmaceutical University, Laboratory of Pharmaceutics,
Department of Biomedical Pharmaceutics, Gifu, Japan*²*Gifu Prefectural Research Institute for Health and Environmental
Sciences, Department of Drug and Housing Hygiene, Gifu, Japan*

The illegal use of synthetic cannabinoids (SCs) has become a serious problem worldwide. To promote further research of SCs, precise discrimination of SCs and their metabolites from their isomers and derivatives in human biological specimens is needed. Here, we aimed to develop a method to specifically detect SCs and their structural isomers and to investigate SC metabolic profiles in human liver microsomes (HLMs). ATHPINACA isomer 1 and 2 were incubated with HLMs at designated time points up to 3 hrs. LCMS-IT-TOF data from resulting samples were analyzed by ESI positive/negative mode. The product ion spectra of parent SCs obtained from the protonated molecules revealed a clear difference between the two isomers, likely due to the stability of the resulting adamantyl cation—although chromatography showed similar retention times for both parent compounds. Both parent SCs were quickly metabolized with half-lives of approximately five min. 14 metabolites in isomer 1 and 12 metabolites in isomer 2 were annotated. The major metabolites were di-hydroxylated isomer 1 and mono-hydroxylated isomer 2, suggesting that hydroxylation of the adamantyl moiety is likely the major metabolic pathway of ATHPINACA isomers. This is the first report to characterize the metabolism of ATHPINACA and its adamantyl positional isomer. The information about differences in the product ions of the parent compounds or their major metabolites is useful for discriminating between the two isomers in forensic cases and pharmacokinetic/pharmacodynamic studies.

MTU14-07

L-theanine inhibits the proliferation of neural cell lines via an L-glutamine transporter SLC38A1

S. Maeda¹, Y. Watanabe¹, S. Araki¹, T. Sudo¹, S. Yoshikawa¹, M. Shibata¹, A. Yamamuro¹, Y. Ishimaru¹, Y. Yoshioka¹, T. Kinjyo², N. Kuramoto², Y. Yoneda³

¹Setsunan Univ., Pharm. Sci./Pharmacotherap., Hirakata, Japan

²Setsunan Univ., Pharm.Sci./Mol. Pharmacol., Hirakata, Japan

³Osaka Univ., Grad. Sch. of Dent./Pharmacol., Suita, Japan

L-Theanine (g-glutamylethylamide) is an amino acid contained in green tea leaves with structural analogy to glutamine and is suggested to be taken up into cells mediated by a glutamine transporter SLC38A1. Recently, the oral intake of L-theanine is expected to suppress anxiety, sleep disturbance, and cognitive impairment. Some reports showed that L-theanine possesses anti-cancer activities against some cancers. Here, it was investigated whether L-theanine inhibits cell proliferation and its mechanism is via SLC38A1. The cell proliferation rate was measured by MTT method, and the cell viability was measured by propidium iodide staining. Expression of the amino acid transporter was carried out by real time RT-PCR method. L-Theanine inhibited cell proliferation in mouse motor neuron cell line (NSC-34), mouse neuroblastoma cell line (Neuro 2A) and human neuroblastoma cell line (SH-SY5Y) in a concentration- and time-dependent manner. However, it had little effect in human brain glioblastoma cell line (U-251 MG), mouse astrocyte cell line (C8-D1A), mouse brain endothelial cell line (bEnd3) and human umbilical vein endothelial cells (HUVEC). There was a positive correlation between the L-theanine-dependent inhibition of cell proliferation and the expression level of SLC38A1 mRNA ($r^2 = \sim 0.66$). Therefore, it was suggested that in these neural cell lines, the suppressive effect on cell proliferation was caused by L-theanine which was taken up into the cells via SLC38A1.

MTU14-08

Agrin as a presynaptic differentiation inducer and its proteolytic regulation

M. Oentaryo, C. W. Lee

The University of Hong Kong, School of Biomedical Sciences, Hong Kong, Hong Kong

Agrin, a heparan sulfate proteoglycan molecule, has been well studied for its critical role in promoting acetylcholine receptor (AChR) clustering during postsynaptic differentiation at the neuromuscular junction (NMJ). A previous study also suggested that agrin regulates growth cone guidance in spinal neuron cultures; however, the functional roles of agrin and its proteolytic regulation in presynaptic differentiation remain unclear. Matrix metalloproteinases (MMPs) play critical functions in the remodeling of extracellular matrix (ECM) proteins to control cell migration and motility. Interestingly, membrane-type 1 MMP (MT1-MMP) and secreted MMP-3 have previously been shown to extracellularly cleave agrin that affect synaptic structures. Using *Xenopus* primary cultures, this study aims to investigate how agrin and its proteolytic regulation by MMP activity spatio-temporally modulate presynaptic differentiation at developing NMJs. Firstly, local application of agrin spatially induces the clustering of mitochondria and synaptic vesicles, two well-known presynaptic markers, along the neurites. The findings indicated that agrin is an effective presynaptic differentiation inducer. Secondly, pharmacological inhibition of

MMP activity or reduced expression of MT1-MMP significantly inhibited agrin-induced presynaptic differentiation. We next observed that agrin is spatially enriched at nerve-muscle contact sites, which is coupled with ECM degradation and nerve-induced AChR clustering in nerve-muscle co-cultures. Since agrin is usually secreted globally along the neurites, localization of agrin at synaptic sites inferred that MMP proteolytic activity cleaves agrin that is present in extra-synaptic regions. Lastly, MMP inhibitors or morpholino-mediated MT1-MMP knockdown also inhibited agrin deposition and the formation of nerve-induced AChR clusters at synaptic sites. Taken together, our results demonstrate a previously unappreciated role of agrin in presynaptic differentiation and the regulatory role of MT1-MMP in this process.

MTU14-09

Estrogen-deficiency induced cognitive impairment: role of HB-EGF/EGFR signaling in autophagy and neuronal apoptosis in hippocampus

R. Pandey^{1,2}, N. Chattopadhyay³, Rohit. A. Sinha⁴, S. Bandyopadhyay^{1,2}

¹CSIR-Indian Institute of Toxicology Research, Developmental Toxicology Laboratory, Systems Toxicology & Health Risk Assessment Group, Lucknow, India

²Academy of Scientific and Innovative Research ^{AcSIR}, CSIR-IITR campus, Lucknow, India

³CSIR-Central Drug Research Institute, Department of Endocrinology, Lucknow, India

⁴Sanjay Gandhi Postgraduate Institute of Medical Sciences, Department of Endocrinology, Lucknow, India

Estrogen deficiency in post-menopausal condition promotes hippocampal neuronal apoptosis and learning-memory impairment. However, the underlying mechanism remains uninvestigated. Since excessive autophagy promotes the self-digestion of cells while growth factor signaling promotes cell survival pathway. So, we hypothesized their participation in estrogen deficiency-induced learning-memory impairment, and for this, the ovariectomized (OVX) rat model was used. We observed that estrogen deficiency induces autophagy in hippocampal neurons marked by increased LC3-II, Beclin-1, ATG7, ATG5/12 and autophagosomes along with decreased p62 levels. In addition, autophagy regulators p-AKT/AKT, p-mTOR/mTOR and p-ULK1/ULK1 were down-regulated in OVX rats. Further, we observed mitochondrial localization within the autophagosomes along with decreased VDAC and COXIV levels in OVX rats indicating mitochondrial loss during estrogen deficiency. On investigating up-stream effectors molecules, we identified down-regulated HB-EGF, along with decreased EGFR activation in OVX rats suggesting its role during estrogen deficiency. We observed that 17 β -estradiol or HB-EGF treatment restores autophagy markers, autophagy regulators as well as mitochondrial loss. We finally correlated our observation with neuronal apoptosis and learning-memory impairment. We observed that HB-EGF or autophagy inhibitor, 3-MA treatment not only inhibited OVX-induced apoptosis of hippocampal neurons but also restored learning-memory performances, assessed through Y-Maze and Passive avoidance tasks. Thus, our study highlighted the involvement of HB-EGF/EGFR signaling in mitochondria-associated autophagy in estrogen deficiency which leads to neuronal apoptosis and learning-memory impairment in estrogen-deficient females.

MTU14-10

Characterization of vascular changes in the regenerating optic nerve**B. Rangel¹, L. Benowitz^{2,3,4}, Sd. Lima^{2,3,4}, V. Ibeiro-Resende¹**¹*Federal University of Rio de Janeiro, Institute of Biophysics Carlos Chagas Filho, Rio de Janeiro, Brazil*²*Harvard University, Department of Neurosurgery, Boston, USA*³*Harvard University, F.M. Kirby Neurobiology Center, Boston, USA*⁴*Harvard University, Department of Ophthalmology at Harvard Medical School, Boston, USA*

The vascular and the nervous systems are highly branched networks that are functionally and physically interdependent during development. Vascular patterning and neural wiring share some guidance cues and receptors. Most recently this relationship has also been investigated in the peripheral nervous system (PNS) regeneration. In the PNS, nerves and blood vessels often run in parallel and endothelial cells play an important role on guiding the formation of the bands of Bügnier and serving as a scaffold for regrowing axons. Here, we used the optic nerve crush (ONC) model with a combinatorial treatment that stimulates retinal ganglion cells (RGCs) growth after injury. Our results show that in control animals that didn't receive treatment for regeneration, there was a 2-fold change in the total number of blood vessels 7 days after crush, compared to a unlesioned nerve. Moreover, there were more blood vessels at 0.5 mm after the crush site than in distal parts of the nerve. The combination of treatments promoted extensive regeneration of RGCs and increase in survival. Interestingly, the increased regeneration throughout the nerve is not followed by an increase in the number of blood vessels. There is no difference in the total number of blood vessels 2 weeks after crush between animals that received the combined treatment for regeneration and control animals. This study provides interesting insights into role of blood vessels in the regeneration of an adult CNS tissue.

MTU14-12

Effects of prenatal ischemic hypoxia on cardiovascular risk and rat behavior**T. Silva***State University of Rio de Janeiro, Pharmacology and psychobiology department, Rio de Janeiro, Brazil*

Perinatal stress has been associated with increased susceptibility to affective disorders. Hypertension is a chronic disease that fundamentally compromises the balance of vasodilatory and vasoconstricting mechanisms, causing damage to the organs irrigated by them. There are enough evidences showing a relation between mental and cardiovascular diseases, suggesting that depression could be a risk factor to acute myocardial infarction and cardiovascular mortality. In the present study, we investigated whether there is an association between prenatal hypoxia ischemia, depression and late cardiovascular disease. Ischemic hypoxia was induced by clamping the uterine arteries of pregnant rats (Wistar) for 45 minutes, on the 18th day of gestation. Same conditions were applied to the pregnant rats to form the SHAM group, only without the uterine arteries clamping. Analyses of serotonin, dopamine and their metabolites contents in basal midbrain were performed by the High-performance liquid chromatography technique. Morphometric

analyses was also studied in aortas and hearts tissues. Oxidative stress was studied by the balance between anti-oxidants enzymes and the damage in proteins by carbonylation and immunohistochemistry for 8-isoprostane. The animals were also submitted to systemic arterial pressure measurement using the noninvasive method of caudal plethysmography. The mesenteric artery was isolated to study the performance of the vascular reactivity induced by vasoconstrictors and vasodilators. Data were statistically analyzed using one-way ANOVA and student t test. These results are consistent with the hypothesis of increased vulnerability of the serotonergic system for perinatal stress and the physiological changes in the cardiovascular system before hypertension is established. Although not hypertensive, hypoxic animals are more likely to develop hypertension at older ages than control groups.

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MTU14-13

Cellular and molecular mechanism of bisphenol-a (BPA) mediated effect(s) on protein quality control in the rat hippocampus**S. J. Singh^{1,2}, A. Tandon^{1,3}, S. Goyal^{1,2}, J. Shankar⁴, N. Arjaria⁴, R. K. Chaturvedi^{1,2}**¹*CSIR-Indian Institute of Toxicology Research, System Toxicology and Health Risk Assessment Group/Developmental Toxicology Division, Lucknow, India*²*Academy of Scientific and Innovative Research (AcSIR), CSIR-Indian Institute of Toxicology Research Campus, Lucknow, India*³*Babu Banarasi Das University, Department of Biochemistry, Lucknow, India*⁴*CSIR-Indian Institute of Toxicology Research, Advanced Imaging Facility, Lucknow, India*

Widespread uses of plastic products containing BPA, well known xenoestrogen, have hazardous health impact. In present study, we have investigated the effect(s) of BPA on neurogenesis and protein quality control in rat brain hippocampus. For experimental examination Wistar rats were orally administered BPA (40 & 400 µg/kg body weight) during gestation and postnatal periods. Expression of genes and protein levels were analysed by qRT-PCR and western blotting, respectively. To study proteins localization, immunohistochemical and ultrastructural studies were performed by immunofluorescence and transmission electron microscopy studies *in vitro* and *in vivo*. Results suggested that exposure of BPA in neural stem cells (NSCs) culture derived from rat brain hippocampus showed reduced proliferation and differential potential. Furthermore, we have observed that BPA exposure induces generation of autophagic flux as a protective response in neuronal cells. Electron microscopy analysis revealed that BPA exposure induced generation of autophagosomes and autolysosomes. Moreover gene and protein expression analysis depicts an altered protein quality control in the BPA exposed rat brain hippocampus. Our finding suggests that BPA exposure not only decreases NSCs proliferation and neuronal differentiation, but also increases neurodegeneration, autophagy & apoptosis both *in-vitro* and *in-vivo*. Therefore altered protein quality control might be responsible for BPA induced defects in cognitive function in the rat brain.

MTU14-14

Quercetin modulates neuronal activity of the RAT arcuate nucleus**R. Srisawat, N. Nontamart***Suranaree University of Technology, Institute of Science, Nakhon Ratchasima, Thailand*

Quercetin, an important flavonoid found in fruits and vegetables, possesses antioxidant, anti-inflammatory, and anti-obesity activities. However, there is no evidence to suggest anti-obesity effects of quercetin through central effects, especially for the hypothalamic brain regions that play an importance role in regulation of energy balance. Hence, This study investigated the effects of quercetin on neuronal activity in the hypothalamic food intake regulating areas including the arcuate nucleus (ARC), the ventromedial hypothalamus (VMH), and the dorsomedial of hypothalamus (DMH) and also investigated the effects of quercetin on activity of neuropeptide (NPY) neuron of the ARC in male Wistar rats. Rats orally received vehicle and quercetin (100, 200, 400 mg/ml/kg) for 30, 60, 90 and 120 min. Brains were fixed and sectioned. Free-floating sections were subjected to Fos, NPY and Fos/NPY immunohistochemical staining. The highest number of Fos+ neurons/area were found in the ARC in the rats treated with 100 mg/ml/kg of quercetin for 120 min. In the ARC, there was significantly more number of Fos+ neurons/area in quercetin treated group than vehicle-treated group. Number of Fos+ neurons/area in the VMH in quercetin treated group was significantly lower than in vehicle-treated group. Number of Fos+ neurons/area in the DMH were not different between quercetin and vehicle treatments. Fos+/NPY+ neurons/area, and the ratio and percentage of Fos+/NPY+ neurons to total number of NPY+ neurons in the ARC induced by quercetin were significantly lower than vehicle-treated group. The results suggested that quercetin may involve in the regulation of food intake and energy homeostasis by activate neurons in the ARC and inactivate neurons in the VMH. Quercetin may exert anti-obesity effect by inactivating NPY/AGRP neurons and activate POMC/CART neurons in the ARC.

MTU14-15

Curcumin inhibits bisphenol-a (BPA) mediated rat hippocampal de-myelination via notch signaling**A. Tandon^{1,2}, S. J. Singh¹, M. Gupta¹, N. Singh², S. Goyal¹, J. Shankar³, N. Arjaria³, R. K. Chaturvedi¹**¹*CSIR-Indian Institute of Toxicology Research, System Toxicology and Health Risk Assessment Group/Developmental Toxicology Laboratory, Lucknow, India*²*Babu Banarasi Das University, Department of Biochemistry, Lucknow, India*³*CSIR-Indian Institute of Toxicology Research, Advanced Imaging Facility, Lucknow, India*

Xenoestrogen BPA is component of plastic materials such as feeding bottles and food cans. Previously, we have shown adverse effects of BPA on myelination. The aim of this study is to understand mechanistic action of curcumin against BPA induced alterations in hippocampal myelination. BPA (40 µg/kg b.w, *peroral*) and curcumin (20 mg/kg b.w, *intraperitoneal*) were administered to rats from postnatal day (PND) 21-90. Curcumin treatment after BPA exposure improved immunoreactivity of

fluoromyelin, Olig2⁺/MBP⁺ and MBP⁺/NF⁺. It also significantly increased number and size of oligospheres, augmented number of A2B5⁺/PCNA⁺ (proliferation), MBP⁺/CNPase⁺ (differentiation), β-III tubulin⁺/MBP⁺ (myelination) cells and up-regulated expression and levels of myelin protein. We studied the effect of curcumin on canonical Notch pathway, which is essential for maintenance of oligodendrocyte progenitors (OPCs). *In-silico* studies predicted interaction of BPA and curcumin with Notch1, Hes1 and Mib1. Curcumin treated BPA exposed groups exhibited significantly enhanced gene expression and protein levels of Notch pathway markers. Notch pathway inhibition *via* Notch1 siRNA and DAPT resulted in further significant decline in number of OPCs in BPA exposed group as compared to BPA alone group. OPCs number was not ameliorated after curcumin treatment in Notch inhibited groups indicating notch mediated neuroprotective action of curcumin. Curcumin treatment in BPA exposed group significantly improved learning and memory, ultrastructural architecture and myelin sheath thickness. Results highlight the significance of curcumin as potential therapeutics against xenobiotics induced neurotoxicity.

MTU14-16

The unfolded protein response (UPR) is upregulated in several important regions of the SIDS brain**S. Thomson¹, K. Waters^{1,2,3}, R. Machaalani^{1,3,2}**¹*University of Sydney, Faculty of Medicine and Health, Sydney, Australia*²*Westmead Children's Hospital, Child and Adolescent Health, Sydney, Australia*³*Bosch Institute, Central Clinical School, Sydney, Australia*

The unfolded protein response (UPR) is linked to multiple neurodegenerative diseases such as Alzheimers' and Parkinsons', and has also been implicated in a subset of SIDS infants. Recent work in our laboratory identified an increase in phosphorylated protein kinase R (PKR)-like ER kinase (p-PERK) - a key component of one arm of the UPR - in the hypothalamus of SIDS infants (p<0.000) (Hunt et al., 2015). We aimed to determine whether p-PERK is similarly increased in other brain regions of SIDS babies including the brainstem and cerebellum, as well as any potential links to SIDS risk factors of 'prone sleeping', 'upper respiratory tract infection (URTI)', 'bed-sharing' and 'parental smoking'. The immunohistochemical expression of p-PERK was studied in the brainstem pons, medulla and cerebellum of SIDS (n=28) compared to non-SIDS (n=12) infants. The p-PERK positive neuron percentage was significantly increased in the cuneate nucleus (p=0.035) in SIDS compared to non-SIDS cases, and the inferior olivary nucleus (ION) and locus coeruleus (LC) showed a trend towards the increase (p=0.057, p=0.084 respectively). Analysis for the risk factors showed changes attributed to bed-sharing only, with a significant increase in the purkinje cell layer of the cerebellum (p=0.025) and the dorsal raphe nuclei of the pons (p=0.048), as well as a trend towards the increase in the LC (p=0.073) and the ION (p=0.066) of bed-sharing infants. These results indicate that a certain subset of SIDS infants are experiencing an upregulation of the UPR via the p-PERK pathway, and that bed-sharing plays a contributing role.

MTU14-17

Dopaminergic neuroregeneration in the diencephalon of 6-OHDA-lesioned adult zebrafish-based parkinson's disease model**Y. Vijayanathan¹, S. M. Lim², M. P. Tan¹, F. T. Lim², A. B. A. Majeed², K. Ramasamy²**¹Universiti Malaya, Faculty of Medicine, Kuala Lumpur, Malaysia²Universiti Teknologi MARA, Faculty of Pharmacy, Puncak Alam, Malaysia

Studies on endogenous neuroregeneration using mammalian-based Parkinson's disease (PD) models are often hampered with futile axogenesis. The emergence of zebrafish with remarkable neuroregeneration capacity may address these limitations. This study established a 6-OHDA-lesioned adult zebrafish-based PD model and investigated the neuroregenerative processes of diencephalic dopaminergic neurons (DpN). 25 mg/kg of 6-OHDA were intracerebroventricularly administered into the diencephalon of adult zebrafish (*Danio rerio*). Immunofluorescence was conducted to quantify tyrosine hydroxylase immunoreactive (TH-ir; indicating DpN) and Bromodeoxyuridine-immunoreactive (BrdU-ir; indicating proliferative cells) at brain regions of interest [olfactory bulb (OB), telencephalon, diencephalon]. To elucidate neurodifferentiation activity, Foxa2 and Nurr1 differential gene expressions at different regions were enumerated using qPCR. At day 3 post-lesion, diencephalic TH-ir cell count revealed >85% DpN significantly ($p < 0.05$) ablated when compared to intact fish. Whereas, at day-30 post-lesion TH-ir cell count increased significantly ($p < 0.05$) than day-3 post-lesion; but exhibited no significant difference with intact. Cellular proliferation demonstrated a transient yet significant ($p < 0.05$) decrease and then increase in OB (-55%; +114%) and telencephalon (-73%; +194%) at day 5 and 7 post-lesion, but BrdU-ir cell count in diencephalon remained unchanged at all time points. Conversely, significant ($p < 0.05$) decline and gradual increase of Foxa2 (-44% at day 3 and 9 post-lesion) and Nurr1 (-46% at day 3, 9 and -65% at day 14 post-lesion) were observed in the diencephalon; whilst no significant changes ($p > 0.05$) of both differential markers were discerned in OB and telencephalon. These findings warrant further investigations to harness these potentials and apply towards future human DpN regenerative studies.

MTU14-18

New modulators of the capsaicin receptor TRPV1 in fermented foods**Y. Yamada, T. Sato, M. Hamano, S. Idemoto**

Kindai University, Faculty of Engineering, Higashi-Hiroshima, Japan

TRPV1 (transient receptor potential vanilloid subfamily, member 1), similar to other TRP channels, is a putative six-transmembrane-spanning protein with a pore region localized between transmembrane segments 5 and 6. TRPV1 is a non-selective cation channel with a preference for calcium that is directly activated by noxious temperature (43 °C) or capsaicin. Generally speaking, capsaicin-sensitive neurons are bipolar neurons with unmyelinated axons (C-fibres) and somata in sensory (dorsal root and trigeminal) ganglia. Of note, a subset of sensory neurons with thin myelinated axons (Aδ fibres) is also capsaicin sensitive. TRPV1 is thought to mediate the phenomenon of peripheral sensitization that involves a reduction in

the threshold of activation and an increase in the responsiveness of the peripheral termini of nociceptors. TRPV1 has been reported to have anti-obesity effect by sweating, neuropathic pain curing effect by desensitization, the protective effect against neural cells, and headache inhibition. It was reported that polyamines, by virtue of their cationic charge, can regulate the activity of TRPV1. To investigate new substances acting on TRPV1, we examined the effect of ingredients in fermented food on it. The fermented food contained various amines. We focused the amines contained in Japanese sake. Agmatine and 2-phenylethylamine showed TRPV1 agonist activity and these EC₅₀ were 250 μM and 14.2 μM, respectively. At that time, the EC₅₀ of capsaicin was 3.7 μM. Tyramine, isoamylamine, and putrescine did not show any activity against TRPV1. Lactic acid, phthalic acid, and crotonic acid potentiated TRPV1. It was indicated that the substances existing in sake modulate TRPV1 activity. To investigate the mechanism of action of these substances, we will examine about electrophysiological activities in detail and perform behavioral experiments by animals.

MTU14-19

Effect of repeated transcranial magnetic stimulation on opioid status in fibromyalgia patients by sucrose induced analgesia**A. H. Ansari¹, R. Mathur², K. Mukherjee²**¹Texila American University, Physiology and Neurosciences, East Bank Demerara, Guyana²All India Institute of Medical Sciences, Physiology, New Delhi, India

Fibromyalgia (FM), is a chronic pain syndrome involving altered pain modulation system and no effective treatment yet. The endogenous opioids are one of the important regulators of Gonadotropin Releasing hormone (GnRH) in the hypothalamus. There was an indirect estimation of opioid levels by estimating plasma LH level. Opioids are known to exert an inhibitory control on gonadotrophic releasing hormone (GnRH) neurons. The GnRH neurons in turn control the release of LH and FSH. Endogenous opioid system was assessed in FM patients before and after treatment with Repeated Transcranial Magnetic Stimulation (rTMS) and compared along with subjective symptoms. Series of blood samples were collected from the FM patients (n = 86) and healthy Controls (n = 90) before (2 ml each 10 min earlier) and after (at 0, 5, 10, 15 and 20-min intervals) the participant was provided 25% freshly prepared sucrose solution to drink. The patients were given rTMS as treatment for 4 weeks (5 days per week/20 days). Again, blood samples were collected after the treatment and pre and post treatment data were compared. LH was estimated in blood by Electrochemiluminescence immunoassay methods. The basal LH concentration (lg/dl) in controls was 6.1 2.85 which decreased to 5 min post-sucrose ingestion. It gradually decreased significantly at 5, 10, 15 and 20 min (5.6 2.58, 5.3 2.52, 5.1 2.39, 4.8 2.34 respectively). LH concentration in FM before rTMS remained unaltered from the basal level (4.5 2.12) through 20 min post sucrose ingestion (4.3 2.13, 4.1 2.52, 4.2 2.06, 4.1 2.11) while after rTMS there was significant decrease in LH level after sucrose ingestion, indicating effects of rTMS in FM patients. rTMS elicited a sustained beneficial effect in FM patients.

Poster Sessions Wednesday/Thursday

WTH01 Gene regulation & genetics (Session B)

WTH01-01

Functional organization of MBP transcriptional enhancers H. Bagheri, H. Friedman, A. Peterson

McGill University, Human Genetics, Oncology, Neurology and Neurosurgery, Montreal, Canada

Large caliber axons are ensheathed by myelin, a late evolutionary invention that accelerates action potential conduction with reduced energy expenditure. Myelin is composed of spirally wrapped plasma membrane and proteins one of which, Myelin Basic Protein (MBP), accumulates in both Schwann cells and oligodendrocytes. *Mbp* expression initiates in perinatal mice, peaks during the third post-natal week and thereafter is maintained at reduced levels. Multiple myelin related transcription factors have been characterized and their binding sites within *Mbp* enhancers located. However, our understanding of the functional capacity of such enhancers in their integrated genomic context remains limited. Moreover, four previously described *Mbp* enhancers are located within a chromatin domain enriched in H3K27ac, one signature of super-enhancers with potential to impact autonomous enhancer activity. Previously, we used single copy *lacZ* reporter constructs and controlled transgenesis to characterize the *in vivo* programming conferred by different *Mbp* enhancers. Lineage specificities and developmental programs were assignable to each enhancer although functional inter-enhancer interactions also were encountered. Here, to establish the function/s of these enhancers in the context of the endogenous *Mbp* locus, we used CRISPR to delete them, either singly or in combination, and *Mbp* mRNA accumulation levels in lines of knock-out mice were compared. Despite being part of a putative super-enhancer, the investigated enhancers contributed to expression of the endogenous locus in a largely additive manner. Two enhancers with different transcription factor binding profiles made equal contributions in oligodendrocytes and a third enhancer was active only in Schwann cells. Finally, as MBP is essential and rate limiting for CNS myelin production these enhancer knock-out mice provide stable models of graded hypomyelination applicable in numerous investigations on axon-myelin biology.

WTH01-02

Neuromolecular and behavioral adaptation associated with alcohol deprivation

N. DSilva¹, K. McCullar¹, P. Iturralde¹, T. Blackwater¹, U. Heberlein², R. Azanchi¹, Karla. K. Kaun¹

¹Brown University, Neuroscience, Providence, USA

²Janelia Farm Research Campus, Howard Hughes Medical Institute, Ashburn, USA

Alcohol is the most used psychoactive substance in the world and abuse can lead to addiction. Despite numerous attempts of abstinence, alcohol use disorder remains a debilitating chronic relapsing disorder. Relapse vulnerability is attributed to dysregulation of multiple neurotransmitter interactions, and subtle changes in neurochemical function in the brain following repeated exposures to alcohol. However, the molecular mechanisms that regulate the shift

from alcohol withdrawal to abuse are essentially unknown. Over the past couple of decades, *Drosophila melanogaster* has proven to be a powerful model in which to elucidate the underpinnings of alcohol sensitivity, tolerance, and addiction. Using a new *Drosophila* alcohol deprivation model, we identify the molecular changes associated with gene dysregulation, and the correlated alterations in behavior associated with alcohol withdrawal. Our data suggests that repeated alcohol experiences induce a negative affect state which primes animals to increase their alcohol preference. Here we describe how transcript expression is modulated to consequently alter behavior. This data will help us identify new target genes for future alcohol use disorder and addiction research.

WTH01-03

New AD risk variants are associated with disease pathology and synaptic remodeling

M. Daur¹, J. Frappier^{2,3}, J. Miron^{1,2,3}, D. Dea^{2,3}, C. Picard^{1,2,3}, J. Poirier^{1,2,3}

¹McGill University, Neuroscience, Montreal, Canada

²Douglas Hospital Research Centre, Aging, Cognition and Alzheimer's Disease, Verdun, Canada

³STOP-AD Centre - Centre for Studies on the Prevention of Alzheimer's Disease, Genetics, Verdun, Canada

Introduction: A GWAS performed in a French-Canadian population identified several genetic variants associated with Alzheimer's disease (AD). Our team chose three of these variants for more detailed analysis: rs10493098 (HIVEP3), rs1461684 (CNTN5) and rs11136000 (CLU). In this study, we assessed gene expression in AD brains and the role of the variants in the degenerative process of AD.

Methods: mRNA levels were measured using real-time PCR. Protein levels were measured using ELISA. Choline acetyltransferase (ChAT) activity was measured using radiolabeled acetyl-CoA. Phospho-Tau and A β 42 levels were assessed using Luminex. Gene expression was measured in an animal model with unilateral electrolytic lesions of the entorhinal cortex.

Results: HIVEP3, CNTN5 and CLU mRNA levels were increased in the frontal cortex of AD patients compared to controls. There was no change in protein expression in the frontal cortex of AD patients. HIVEP3 rs10493098 was associated with ChAT activity and neurofibrillary tangles; CNTN5 rs1461684 was associated with A β 42 levels and senile plaques; CLU rs11136000 was associated with A β 42 levels and ChAT activity. Gene expression measured in mice showed increased levels of HIVEP3 during deafferentation (6 days) and regeneration (days 14 and 25) phases and decreased levels of CNTN5 during regeneration phase (days 14 and 60).

Conclusion: These new genetic risk factors correlate with pathological hallmarks of AD and show alterations in human brains and animal models. Further studies will help clarify their role in the disease.

WTH01-04

Blood total cholesterol polygenic score in people at increased risk of Alzheimer's disease

N. Nilsson^{1,3}, J. Poirier^{2,3}

¹McGill University, Integrated Program in Neuroscience, Montreal, Canada

²McGill University, Department of Psychiatry, Montreal, Canada

³Douglas Hospital Research Center, Alzheimer's Disease Genetics Research Unit, Montreal, Canada

Sporadic Alzheimer's disease (sAD) is a multifactorial disease with many factors linking to processes of lipid metabolism. E.g. high blood cholesterol levels specifically in midlife associates with a higher risk of developing sAD and use of cholesterol lowering drugs has been shown to associate with protection. Both blood cholesterol levels and sAD are influenced by genetics. Indeed, in sAD, genetics explain half of the phenotypic variance. However, most of the genetic variants remain unknown. Considering the genetic background of both conditions and that they are linked in terms of risk, we hypothesized that a higher polygenic score (pgs) based on blood total cholesterol (TC) levels would associate with a worsening of sAD biomarkers. Using summary data from a published GWAS on blood TC levels (Willer et al., 2013), a polygenic score was calculated in a cohort of cognitively healthy individuals at a higher risk of sAD; the PREVENT-AD cohort. We confirmed that a TC-pgs correlated well with TC blood levels and identified a p-cutoff of $p < 1e-4$ as the cutoff best predicting TC levels. Furthermore, this score significantly correlated with reduced CSF A β levels in APOE4 positive subjects but not APOE4 negative subjects, whereas no effect was found on CSF p-tau levels. A trend was found for a positive correlation between TC-pgs and CSF tau levels. These results indicate that there is some genetic overlap between TC blood levels and sAD before disease onset, specifically that a higher TC-pgs associates with a worsening of sAD biomarkers.

WTH01-06

Role of miR-183-5P in integrin B1 decrease and blood-brain barrier alteration in hepatic encephalopathy

K. Orzeł, K. Milewski, M. Zielinska

Mossakowski Medical Research Centre Polish Academy of Science, Department of Neurotoxicology, Warsaw, Poland

Hepatic encephalopathy (HE) is a neuropsychiatric syndrome associated with insufficient clearance of circulating toxins by impaired liver. Ammonia is a key pathogenic factor in HE contributing to blood-brain barrier (BBB) permeability impairment, its toxic effects being potentiated by blood-born inflammatory cytokines. Function of BBB might be modulated by the non-coding single-stranded RNAs – microRNAs (miR). It is not clear how altered in HE miRNAs control the BBB. miR-183 family expression is elevated in neurological and auto-immune disorders. Here we show that an elevated levels of the miR-183 cluster members have been observed in cerebral cortex of rats with thioacetamide (TAA)-induced HE, and in simple hyperammonemia (HA) model, with less pronounced inflammatory component assessed by flow cytometry (IL-10, TNF- α , IL-1 β , IL-1 α , IL-6). In the further experiments, the decreased expression of integrin β 1, the miR-183-5p selected protein target, was observed in TAA brain tissue, but not in HA. Using rat brain endothelial cell line (RBE-4), the effect of i) transfection with mimic miR-183-5p (5 μ M and 20 μ M); ii)

treatment with 5 mM ammonium chloride (NH₄Cl) and/or with 50pM Tumor Necrosis Factor- α (TNF- α) applied separately or in a combination for 24 h, on integrin β 1 expression (real-time qPCR, immunocytochemical staining), was analysed. RBE-4 cell adhesion in treated variants was also quantified. Integrin β 1 was significantly reduced in RBE-4 cells treated with ammonia and TNF- α . Accordingly, decreased adhesion to collagen I of RBE-4 cells was observed. Transfection with mimic miR-183-5p down-regulated integrin β 1 expression. Next steps will be conducted to investigate in detail the miR-183-5p-dependent mechanism of BBB altered integrity in HE. Supported by grant 2015/19/8/NZ/01902 and by Knowledge Education Development Programme.

WTH01-07

Genotype-phenotype correlation of SLC6A4 markers with autism spectrum disorder (ASD): initiative towards pharmacogenomics research

U. Rajamma^{1, 2}, S. Guhathakurta², S. Sinha², K. P. Mohanakumar¹, P. Jaiswal²

¹Inter University Centre for Biomedical Research & Super Speciality Hospital IUCBR & SSH, Centre for Development and Aging Research, Kottayam, India

²Manovikas Kendra, Biomedical research & Diagnostics Unit, Kolkata, India

Reports of platelet hyperserotonemia in autism spectrum disorder (ASD) and effective amelioration of behavioural symptoms by selective serotonin reuptake inhibitors, suggest serotonergic dysfunction in ASD. Serotonin transporter (SERT) encoded by *SLC6A4* is a key determinant of 5-HT levels. Therefore, we hypothesise that *SLC6A4* is an autism susceptible locus and SERT is a potential target for hyperserotonemia condition and altered behavioural response in ASD. We have investigated genetic correlation of nine polymorphisms of *SLC6A4* with ASD phenotype, platelet 5-HT levels, mRNA expression of SERT and symptoms severity of ASD. Results revealed sex-bias and parent-of-origin effect in ASD. Significant genetic influence of polymorphisms on specific ASD behavioural phenotypes by CARS assessment was also observed. When sensory dysfunction and activity level showed improvement with age, severity for body-use increased. Low expressing genotypes, C/C and S/S of rs6354 and 5-HTTLPR respectively and A/A of rs7224199 displayed reduced severity for activity level, adaptation to change, taste touch & smell use, and body use. Haplotypes of high expression L allele of 5-HTTLPR showed correlation with increased severity for fear/nervousness. ASD probands exhibited higher platelet 5-HT levels and blood SERT mRNA expression. High expressing STin2 12-repeat allele was associated with elevated mRNA expression and platelet 5-HT level, whereas low expressing rs6354 C/C genotype was correlated with low platelet 5-HT levels. Overall results suggest that *SLC6A4* markers influence specific symptom severity of ASD, through modulation of 5-HT content and SERT expression, which have implication in pharmacogenomics research.

WTH02 Signal transduction & synaptic transmission (Session B)

WTH02-01

Mechanisms of somatodendritic dopamine release in the mouse mesencephalon

B. Delignat-Lavaud, L.-E. Trudeau

Université de Montreal, Neurosciences/Medecine, Montreal, Canada

Dopamine (DA) neurons of the mesencephalon play a key role in motor control, motivated behaviors and cognition. Dopaminergic neurons can release DA not just from axon terminals by a classical exocytosis mechanism, but also from their somatodendritic (STD) compartment through a mechanism still incompletely understood. Somatodendritic release is calcium dependent, but conflicting results exist regarding the extent to which release depends upon extracellular calcium influx in comparison with axonal release (Chen and Rice, 2001; Ford et al., 2010). In the present study, we evaluated in mouse brain slices the calcium sensitivity of STD DA release compared to axonal release using fast scan cyclic voltammetry (FSCV). We find that in fact, both forms of release have the same calcium dependency, emphasizing similarity in the calcium dependent mechanisms of DA release in the striatum and cell body region. Previous work performed in the lab has highlighted the possible regulatory role of the synaptotagmin (Syt) calcium sensor proteins in STD DA release and showed that down-regulation of the somatodendritically expressed Syt4 and Syt7 severely reduced STD DA release in cultured DA neurons, whereas terminal release required Syt1 (Mendez et al., 2011). We are currently exploring the role of the Syt isoforms in STD DA release, *ex vivo*, by measuring DA release by FSCV in brain slices prepared from Syt 1, Syt4 and Syt7 knock-out mice. This work is expected to shed new light into the mechanisms and roles of this still obscure form of DA signaling.

WTH02-02

Cocaine-induced synaptic redistribution of NMDARs in striatal neurons alters NMDAR-dependent signal transduction

I. D. Ramirez¹, A. Sagev¹, D. W. Self¹, S. Kourrich^{1,2}

¹*UT Southwestern Medical Center, Psychiatry, Dallas, USA*

²*Université du Québec à Montréal, Faculté des sciences, Montreal, Canada*

Pharmacological inhibition or genetic down-regulation of NMDARs during cocaine administration impairs the development of several addiction-relevant behaviors and biochemical adaptations developing after prolonged abstinence. While the effects of cocaine on glutamate receptor functions during late abstinence have been extensively studied, early effects on NMDARs signaling, known to contribute to the initiation of addictive processes, remain unclear. NMDAR signaling depends on subunit composition, subcellular localization and interaction with proteins at the postsynaptic density (PSD), where NMDARs and other proteins form supercomplexes responsible for the transduction of signaling activated by NMDAR-induced calcium influx. Also at the PSD, NMDARs interact with lipid-rafts, membrane microdomains involved in signaling compartmentalization. Here, we studied the interaction of the NMDARs with the PSD and lipid-rafts in striatal structures (nucleus accumbens shell and dorsal striatum) after cocaine administration. 24 hrs

after the last cocaine administration (non-contingent intraperitoneal or contingent intravenous self-administration), we observed a decrease in NMDAR at the PSD and lipid-rafts, while we did not detect a decrease in the synaptosomal fraction, regardless of the contingency of drug exposure. This suggests that NMDARs dissociate from the PSD, likely increasing the perisynaptic/extrasynaptic pool. To test this hypothesis, we measured cocaine-induced ERK phosphorylation (ERK pathway is induced by synaptic but is inhibited by extrasynaptic NMDARs). We found that ERK phosphorylation is blocked in animals with previous exposure to cocaine, supporting the conclusion that the loss of interaction with the PSD increases the extrasynaptic pool of NMDARs, thereby altering intracellular signaling triggered by subsequent cocaine exposure. These data suggest that an animal's recent history of cocaine experience alters the subsequent effect of the drug on NMDARs signaling, an adaptation that may contribute to the development of addiction.

WTH02-03

Trace amine-associated receptors as modulators of brain monoaminergic systems

E. Efimova¹, N. Merkulyeva^{1,2}, A. Veshchitskii², N. Katolikova^{1,3}, E. Rubets⁴, M. Mor¹, E. Leonova¹, R. Gainetdinov^{1,5}

¹*St. Petersburg State University, Institute of Translational biomedicine, St. Petersburg, Russia*

²*I.P. Pavlov Institute of Physiology, Laboratory of Neuromorphology, St. Petersburg, Russia*

³*Skoltech Institute of Science and Technology, Biomed, Moscow, Russia*

⁴*St. Petersburg State University, Department of General Physiology, St. Petersburg, Russia*

⁵*St. Petersburg State University, St. Petersburg University Hospital, St. Petersburg, Russia*

Trace amines are structurally close to classical monoamine neurotransmitters but their role in physiology and pathology of mammals remains largely unknown. In the present study, we investigated various parameters of brain monoamine neurochemistry in mice lacking different trace amine associated receptors – TAAR2, TAAR5 and TAAR6. TAAR5-knockout animals had significantly higher tissue content of dopamine (DA) and its metabolites Homovanillic acid (HVA) and 3,4-Dihydroxyphenylacetic acid (DOPAC) in the striatum. Furthermore, TAAR5-knockout animals had higher number of DA neurons and elevated tyrosine hydroxylase mRNA expression level in the Substantia Nigra. Similarly, in TAAR2-knockout animals an elevated DA level in the striatal tissue was observed. In TAAR6-knockout animals, the level of DA was unaltered in the striatum but dopamine turnover rate measured as HVA/DA and DOPAC/DA ratio was higher in the hypothalamus. Also, an elevated expression of monoamine oxidase-B mRNA in the cortex and striatum indicated an increased level of monoamine metabolism. TAAR6-knockout animals had also an elevated serotonin and 5-Hydroxyindoleacetic acid levels in the prefrontal cortex and hippocampus. These data indicate that trace amines acting via TAARs modulate dopamine and serotonin systems. However, various subtypes of TAARs have their own spectrum of action in the brain

and therefore can influence different functions. The work is supported by the Russian Science Foundation grant 19-75-30008.

WTH02-04

The role of CAS adaptor proteins during cerebellar granule cell migration

J. Estep, M. Riccomagno

University of California Riverside, Molecular, Cell and Systems Biology, Riverside, USA

Proper central nervous system (CNS) function requires individual neurons to form synaptic connections with the appropriate partners, ensuring the formation of precise circuits. Central to circuit establishment and refinement events is the need for neurons to dynamically assemble and disassemble cytoskeletal elements in response to diverse extracellular cues. While many ligand-receptor systems have been demonstrated to modulate circuit assembly events, little is understood regarding how these signaling events are perpetuated intracellularly to converge on regulators of the cytoskeletal system. Cerebellar development, in particular the migration of cerebellar granule cells (GCs), presents a unique opportunity to explore how neural progenitors integrate multiple signal modalities to direct migratory events, thus ensuring proper architectural and circuit assembly. Here, we demonstrate the cell-autonomous and non-autonomous roles for the Cas family of adaptor proteins during cerebellar development and GC migratory events. We provide evidence that Cas adaptor proteins act as upstream regulators of Rac GTPase activity and actin assembly during GC radial migration events.

WTH02-05

PVN oxytocinergic projections to the rostral agranular insular cortex and the possible role in nociception

M. Gamal-Eltrabily, A. E. de los Monteros-Zúñiga, G. Martínez-Lorenzana, M. Condés-Lara, A. González-Hernández

Institute of Neurobiology, UNAM, Neurobiología del Desarrollo y Neurofisiología, Queretaro, Mexico

The PVN of the hypothalamus plays a role modulating nociception. Indeed, electrical PVN stimulation induces antinociception at spinal level by direct oxytocinergic projections. Certainly, PVN has other connections with pain-related areas, such as raphe magnus (RMg) and locus coeruleus (LC) nuclei. In this context, the rostral agranular insular cortex (RAIC) is thought to be an integration site of pain transmission, where GABA has a modulatory effect over nociception by recruitment of descending modulatory mechanisms. In this study, we further explore the presence of PVN oxytocinergic innervations towards RAIC. In male Wistar rats (280-310 g), FluoroGold microinjection into RAIC stained cells bilaterally in PVN, some of them, oxytocinergic. Further immunofluorescence studies demonstrated not only the presence of oxytocin receptors (OTR) in the RAIC, but also that OTR seems to be expressed in GABAergic neurons with a close contact with fibers from PVN neurons (anterograde tracer FluoroRubi was injected into PVN). Interestingly, electrical RAIC stimulation decreases the activity of neuronal nociceptive activity of spinal wide dynamic range cells. Accordingly, using a behavioral pain-like model (5% formalin test), we observe that oxytocin microinjection (40 or 400 pmol/40 nl)

into the RAIC diminishes the number of hind paw shakes. This behavioral antinociceptive effect was reversed by the RAIC microinjection of the OTR antagonist (L-368,899, »400 pmol/40 nl) or bicuculline (GABA_A receptor antagonist, »200 pmol/40 nl). Furthermore, intrathecal administration of BRL44408 (an α_{2A} -adrenoceptor antagonist) partially reversed the oxytocin-induced antinociception. Acknowledgments: This study was supported by grants from PAPIIT-UNAM (Grant No. IN200415 to MCL and IA203117, IA203119 to AGH).

WTH02-06

Synapsins regulate alpha-synuclein function

D. Gitler^{1,2}, M. Atias^{1,2}, Y. Tevet^{1,2}, J. Sun³, A. Stavsky^{1,2}, S. Tal^{1,2}, J. Kahn¹, S. Roy^{3,4}

¹*Ben-Gurion University of the Negev, Department of Physiology and Cell biology, Beer-Sheva, Israel*

²*Ben-Gurion University of the Negev, Zlotowski Center for Neuroscience, Beer-Sheva, Israel*

³*University of Wisconsin-Madison, Department of Pathology and Laboratory Medicine, Madison, WI USA*

⁴*University of Wisconsin-Madison, Department of Neuroscience, Madison, WI USA*

The normal function of alpha-synuclein (α -syn) remains elusive. Though recent studies suggest α -syn as a physiologic attenuator of synaptic vesicle (SV) recycling, mechanisms are unclear. We show that synapsin – a cytosolic protein with known roles in SV mobilization and clustering – binds reversibly to α -syn in the presynaptic terminal and is required for α -syn function. Our data offer a critical missing link and advocates a model where α -syn and synapsin cooperate to cluster SVs and attenuate recycling.

WTH02-07

Peroxiredoxin 6 over-expressed mice show depression-like behavior and deficit in 5-HTergic neuronal function

S. M. Gu¹, S. Lamichance², J. Hong¹, J. Yun¹

¹*Chungbuk National University, College of Pharmacy, Chungju-si, Korea South*

²*Wonkwang University, College of Pharmacy, Iksan-si, Korea South*

Peroxiredoxin 6 (PRDX6) is a bifunctional protein with both calcium-independent phospholipase and glutathione peroxidase. Recently, we observed that PRDX6 has an important role in the neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, multiple sclerosis and neurogenesis of neural precursor cells. However, no study has described the relationship of PRDX6 function and depression-like behavior. In our present study, we observed the depression-like behavior using forced swimming test and tail suspension test in over-expressed PRDX6 transgenic (PRDX6 Tg) mice. To study mechanisms underlying depression in PRDX6 mice, we measured 5-hydroxytryptophan (5-HTP)-induced head-twitch responses and serotonin (5-HT) levels in cortex. PRDX6 Tg mice showed prolonged immobility time in forced swimming test and tail suspension test compared with non-Tg mice. 5-HT levels in cortex of PRDX6 Tg mice were lower than that of non-Tg mice. Therefore, we assume that depression of PRDX6 Tg mice is associated with the decrease in brain 5-HT levels and the 5-HT neuronal dysfunction which is evidenced by inhibition

of 5-HTP-induced head twitch response. Our findings provide that PRDX6 may play a role in the depression.

WTH02-08

Agonists of muscarinic acetylcholine receptors exclusively inhibiting camp synthesis

J. Jakubik¹, A. Randáková¹, D. Nelic¹, P. Nwokoye², Q. Su², V. Doležal¹, E. El-Fakahany³, J. Boulos²

¹Institute of Physiology Academy of Sciences of the Czech Republic, Neurochemistry, Prague, Czech Republic

²Barry University, Physical Sciences, Miami Shores, USA

³University of Minnesota College of Pharmacy, Experimental and Clinical Pharmacology, Minneapolis, USA

Majority of G-protein-coupled receptors (GPCRs) mediate several signalling pathways in the cell. Agonists that activate specific signalling pathways to a greater extent than others at the same receptor are termed “biased agonists”. Residues in the binding site that mediate activation of a specific signalling pathway are termed functional hot-spots. Binding of an agonist to one or a subset of functional hot-spots within the binding site results in activation of a subset of signalling pathways and thus in ligand-mediated signalling bias. We have developed agonists biased towards $G_{i/o}$ signalling that is unprecedented at GPCRs. Two of new compounds exclusively inhibited cAMP synthesis in CHO cells, primary cultures and native tissues thus being functionally selective for M_2 and M_4 muscarinic receptors. Molecular modelling revealed interaction only with the subset of amino acid residues in the binding site followed by agonist specific conformation. Allosteric interaction networks leading from the agonist to the receptor G-protein interface were also agonist specific. These compounds may serve as lead-structures in the search for novel non-steroidal and non-opioid analgesics acting via M_2 and M_4 muscarinic receptors.

WTH02-09

Syringaresinol suppresses excitatory synaptic transmission through the presynaptic modulation

M.-H. Kim

Seoul National University College of Medicine, Department of Physiology and Biomedical Sciences, Seoul, Korea South

Syringaresinol (SYR) is a plant polyphenolic lignan that is found in *Castela emoryi* or *Prunus mume*. Here I show that SYR suppresses excitatory but not inhibitory synaptic transmission through presynaptic mechanisms. Bath application of SYR rapidly reduced the slopes of the field excitatory postsynaptic potentials (fEPSPs) at the hippocampal Schaffer collateral (SC)-CA1 synapse in a dose-dependent manner, while inhibitory synaptic transmission remained unchanged. SYR did not affect AMPAR function, including the conductance and the desensitization of AMPARs, but reduced presynaptic release probability and readily releasable pool size. In addition, SYR application exhibited similar features with activation of $G_{i/o}$ protein-coupled receptors in hippocampal neurons as Ca^{2+} currents were reduced and membrane potentials were hyperpolarized by the increased K^+ currents. Collectively, this study identifies SYR as a potent new neuromodulating compound that can be exploited for the development of drug treating various neurological diseases caused by the imbalance between excitatory

and inhibitory neurotransmission such as epilepsy, neurodegenerative diseases, neuropathic pain, and migraine.

WTH02-10

Role of neuroligin in the regulation of presynaptic function

C. Montenegro¹, M. Briza¹, K. Yamashita¹, C. Seidenbecher^{1,2}, P. Zamorano³, V. Torres⁴, D. Montag¹, E. Gundelfinger^{1,2}, K.-H. Smalla^{1,2}

¹Leibniz Institute for Neurobiology, Dept. of Neurochemistry and Molecular Biology, Magdeburg, Germany

²Center for Behavioral Brain Sciences, CBBS, Magdeburg, Germany

³Universidad de Antofagasta, Departamento Biomédico, Antofagasta, Chile

⁴Pontificia Universidad Católica de Chile, Departamento de Biología Celular y Molecular, Santiago, Chile

The Neuroligins Np65 and Np55 are single-pass transmembrane proteins of the immunoglobulin (Ig) superfamily of cell adhesion molecules derived from a single gene (NPTN) by alternative splicing. They are enriched in synapses and have 3 or 2 extracellular immunoglobulin domains, respectively. The role of Neuroligins (Np) in synaptic function has been investigated in several studies, which showed that Np regulates synaptic plasticity including long-term potentiation, formation and stabilization of excitatory synapses and the balance of excitatory/inhibitory synapses. In addition, Np has been shown to be essential for associative learning and memory, preventing retrograde amnesia and for social behavior in adult mice. Although Np has been reported to be present in presynaptic terminals, little is known about its role in presynaptic processes. To address this issue, we characterized presynaptic protein composition and function in primary hippocampal neurons derived from Np knockout mice. In Synaptotagmin antibody uptake assays we observed reduced synaptic vesicle cycling driven by endogenous network activity and after chemical depolarization, an effect that was rescued by over-expression of recombinant Np65- or Np55-GFP constructs. For a more detailed analysis, we used an eYFP synaptotagmin reporter to determine synaptic vesicle (SV) pools and release characteristics at individual synapses. This revealed that the readily-releasable pool (RRP) and total recycling pool (TRP) were reduced and the kinetics of exo/endocytosis were impaired in the absence of Nps. Employing proteomics analyses and STED/Confocal microscopy we identified several neurotransmitter release and vesicle recycling related proteins co-precipitating/co-localizing with Np. Taken together, our results provide new insights into Neuroligin functions at the presynapse. *This work was supported by the German Federal Ministry for Education and Research (BMBF, Support-No. 01DN17002) and Conicyt (Support-No. ConPCI-BMBF 20150065).*

WTH02-12

CXCR3 mediates hippocampal hyperexcitability and synaptic plasticity alterations induced by peripheral viral challenge**M. Reed¹, V. Suppiramaniam^{1,2}, G. Konat³, S. Srinivas¹, J. Bloemer¹, P. Pinky¹, Y. Du¹, S. Setti¹, R. Heslin¹**¹Auburn University, Drug Discovery & Development, Auburn, USA²Auburn University, Center for Neuroscience Initiative, Auburn, USA³West Virginia University School of Medicine, Departments of Biochemistry & Neuroscience, Morgantown, USA

Objectives: A body of clinical evidence has demonstrated that peripheral viral infections exacerbate neurodegenerative conditions, e.g., seizures. We have previously modeled this comorbidity by demonstrating that the acute phase response (APR) instigated by an intraperitoneal (ip) injection of a viral mimetic, polyinosinic-polycytidylic acid (PIC), induces hypersusceptibility to kainic acid-induced seizures. Furthermore, PIC challenge induces neuronal hyperexcitability and alters synaptic plasticity in the hippocampus. At the molecular level, PIC challenge robustly increases hippocampal expression of CXCL10, a ligand of the CXCR3 receptor. Here, we tested the hypothesis that a blockade of CXCR3 mitigates the hyperexcitability and synaptic plasticity alterations associated with PIC challenge.

Methods: Mice received a CXCR3 antagonist, AMG 487 (10 mg/kg, icv), and two hours later APR was induced with PIC (12 mg/kg, ip). Twenty-four hours later, mice were examined for alterations in synaptic plasticity and susceptibility to seizures induced with kainic acid (12 mg/kg sc).

Results: PIC challenge induced an imbalance in synaptic plasticity, as indicated by an increase in both LTP and LTD, and an increase in presynaptic glutamate release and basal synaptic transmission. CXCR3 blockade abrogated the alterations in synaptic plasticity and transmission. Moreover, CXCR3 blockade prevented the seizure hyper-susceptibility associated with peripheral viral challenge.

Conclusions: Our findings suggest that the comorbid effect of peripheral viral infections on seizure may be mediated by CXCL10/CXCR3 signaling via the induction of hyperexcitability. Consequently, CXCR3 may be an attractive therapeutic target.

WTH02-13

Allosteric modulation of the melanocortin 4 receptors by bivalent ions**A. Rinken, R. Link, M.-J. Tahk, S. Kopanchuk**

University of Tartu, Institute of Chemistry, Tartu, Estonia

Melanocortin 4 receptors (MC₄R) are involved in energy homeostasis, neuroprotection, and neurogenesis and therefore have become as important targets for the regulation of body weight and neurological performance. The signal transduction of MC₄ receptors is initiated by a complex ligand binding process, which involves conformational adjustments, oligomerization, and effects of different modulators [1]. Implementation of fluorescence anisotropy assay has enabled on-line monitoring ligand binding to the receptors and allows to characterize kinetic peculiarities of different modulators [2]. We have designed two novel red-shifted fluorescent ligands for the MC₄ receptors, UTBC101 and UTBC102, which have a high binding affinity and suitable kinetic properties for this kind of

studies [3]. The binding of all studied fluorescent ligands to MC₄R was strongly modulated by different bivalent cations. The presence of at least submillimolar concentration of Ca²⁺ was essential to achieve high-affinity specific binding of ligands to MC₄R. Replacement of Ca²⁺ with ions of the same group, Sr²⁺ or Ba²⁺ was considerably less efficient and in the case of Mg²⁺ no specific ligand binding could be detected. This kind requirement of Ca²⁺ ions was found also for MC₄R agonist-dependent activation of adenylate cyclase pathway. The trace metals Zn²⁺ and Cu²⁺ have been found to be allosteric modulators of the ligand binding and signal transduction of MC₄R. Zinc acted as an activator and copper as an inhibitor of the constitutive activity of MC₄R in the absence of ligands at physiological relevant low micromolar concentrations of these ions.

WTH02-14

Activity regulated MIRNA-186-5P controls homeostatic processes in hippocampal neurons**M. Silva¹, B. Rodrigues^{1,2}, J. Fernandes¹, S. Santos¹, L. Carreto³, M. Santos³, P. Pinheiro¹, A. L. Carvalho^{1, 4}**¹Synapse Biology Group, Center for Neuroscience and Cell Biology CNC, University of Coimbra UC, Coimbra, Portugal²Doctoral Program in Experimental Biology and Biomedicine, III, UC, Coimbra, Portugal³Department of Medical Sciences and Institute of Biomedicine

iBiMED, University of Aveiro, Aveiro, Portugal

⁴Department of Life Sciences, UC, Coimbra, Portugal

Homeostatic processes maintain neuronal and circuit function stable in the brain during development and learning, when synapses undergo constant changes. Synaptic scaling is a form of homeostatic plasticity that acts through the regulation of AMPA receptors at synaptic sites to keep neuronal network activity within a physiological range. Although several components of the synaptic scaling apparatus have been characterized, few microRNAs have been linked to the homeostatic plasticity machinery and their role in synaptic scaling is still vastly undervalued. Thus, the main goal of this work was to unveil and characterize novel activity regulated microRNAs that regulate synaptic scaling in the hippocampus. Here, we report that chronic blockade of AMPA and NMDA receptors in hippocampal neuronal cultures induces changes in the neuronal transcriptome and miRNA profile, leading to synaptic scaling. Specifically, we found that synaptic activity blockade persistently down-regulates miR-186-5p expression and that GluA2 AMPA receptor subunit is a direct target of miR-186-5p. Moreover, we show that expression of endogenous GluA2 and composition of AMPA receptors is regulated by miR-186-5p. Lastly, we report that miR-186-5p has a functional role in synaptic scaling mechanisms since manipulation of miR-186-5p levels blocks upscaling of AMPA receptor-mediated currents triggered by synaptic activity blockade. Hence, our findings elucidate a novel activity-dependent miRNA-mediated mechanism for regulation of AMPA receptor expression. Supported by FCT and Fundos FEDER, Portugal.

WTH02-15

Intact synaptic signaling restrains Wnd/DLK-mediated axonal injury response

L. Smithson, L. Junginger, J. Zang, C. Collins

University of Michigan, Molecular, Cellular, and Developmental Biology, Ann Arbor, USA

Neurons respond to injury by invoking plasticity mechanisms to remodel and/or repair damaged circuits, or by undergoing degeneration and death. For any of these responses, activation of the dileucine zipper kinase DLK, also known as *Wallenda* (Wnd) in *Drosophila*, is a key driver.¹ Wnd/DLK protein is actively transported in axons and becomes acutely activated following axonal injury. Despite the fact that many neurons use branched axons to make multiple synaptic connections, the majority of previously reported injury models remove all presynaptic connections made by neurons via complete axotomy of nerve fibers.

Whether neurons respond similarly to single axon branch injuries as they do to complete axotomy is an underexplored question. To investigate the mechanisms that regulate Wnd/DLK activation and response to axonal damage, we developed a branched axon injury paradigm in *Drosophila* larvae. Our data indicate that trafficking and downstream signaling of Wnd/DLK is strongly influenced by injury location in axonal branches. Specifically, we found that Wnd/DLK signaling is only activated by injuries that completely remove all synaptic connections, and that the presence of a single synaptic branch is sufficient to restrain activation of this injury signaling pathway. This finding now draws our attention to synapses to understand the cellular and molecular mechanisms that regulate DLK-mediated injury signaling. ¹Asghari Adib E., Smithson LJ., Collins CA. 2018. An axonal stress response pathway: degenerative and regenerative signaling by DLK. *Curr Opin Neurobiol*, 53:110-119.

WTH03 Neuroinflammation & neuroimmunology (Session B)

WTH03-01

Characterizing complement signaling-mediated neuroinflammation in the irradiated brain

M. Acharya¹, R. Krattli¹, J. Baddour¹, M. Markarian¹, R. Aranda¹, J. Baulch¹, A. Tenner²

¹University of California Irvine, Radiation Oncology, Irvine, USA

²University of California Irvine, Neurobiology and Behavior, Irvine, USA

The debilitating neurocognitive sequelae resulting from clinical radiation therapy (RT) for CNS malignancies are progressive and long-lasting. Despite recent progress, the mechanisms mediating RT-induced cognitive dysfunction are still poorly understood. We have shown that RT-induced cognitive disruption coincides with astrocytic hypertrophy and persistent microglial activation. Therefore, we hypothesize that detrimental changes in glial function significantly contribute to cognitive impairments. The complement system is a potent mediator of microglial and astroglial activation, but it also has a range of non-immune functions in the CNS, including maintenance of cell viability and synaptic pruning. Our data show RT-induced chronic up-regulation in the gene expression profile of reactive astrocytes, microgliosis and elevated co-labeling of complement proteins (C1q, C3a) which was co-incident with cognitive impairments. Thus, inhibition of specific downstream complement cascade events, such as recruitment and activation of inflammatory glia, would promote the potentially beneficial consequences of complement activation. Using microglia-specific conditional C1q knockout mice *C1qa^{FL/FL}:Cx3cr1^{CreERT2}*, our preliminary data show a significant reduction in the complement cascade, astrogliosis and microglial activation in the irradiated brain pointing to a critical role of complement signaling in triggering the RT-mediated brain injury. Concurrently, we are carrying out experiments using a specific complement receptor antagonist (C5aR) to characterize the radiation-response of downstream complement activation cascade, therefore avoiding interference with upstream neuroprotective complement activation using the mouse model of radiation-induced brain injury. Our studies using transgenic mouse model and pharmacological approaches provide a new therapeutic avenue and a translatable approach to curtail radiation-induced cognitive impairments – a critical unmet medical need.

WTH03-02

A novel nanoparticle drug delivery system to promote functional recovery in spinal cord injury in rats

N. Banik^{1, 2}, M. Capone¹, R. Chandran¹, D. Matzelle¹, A. Cox¹, A. Vertigel³, A. Haque¹

¹Medical University of South Carolina, Neurosurgery, Charleston, USA

²Ralph H. Johnson VA Medical Center, Research Center, Charleston, USA

³Clemson University, Bioengineering, Greenville, USA

Spinal cord injury (SCI) is a complex debilitating condition leading to permanent life-long neurological deficits. Currently, no FDA-approved drug exists for the treatment of acute SCI. E2 has anti-inflammatory, anti-oxidant, anti-apoptotic, and neurotrophic

properties, suggesting that E2 warrants clinical evaluation in neurotrauma. However, translation of E2 from preclinical models into clinical practice with the FDA-approved drug Premarin has significant safety concerns. In order to minimize risk and maximize potency, maintaining low to normal serum/systemic E2 with high E2 concentrations in tissue is imperative. The smart drug delivery techniques via nanoparticles may allow for increased drug safety and improved efficacy. We examined the effects of lower doses of E2 (2.5-5.0 µg) via nanoparticle that avoided the high systemic exposures and allowed for enhanced protective and reparative effects on lesioned spinal cord tissue. Results obtained suggest that a single administration of rapid release formulated PLGA-PEG nanoparticles loaded with E2 (nano-E2) can focally deliver E2 to the contused spinal cord with reduced plasma concentrations when compared with i.v. dosing, and can induce anti-inflammatory responses. Mechanistic studies showed that a single 5.0 µg dose of nano-E2 in a gel patch reduced glial scars and improved locomotor function in chronic SCI. Thus, this novel approach for delivery of E2 via nanoparticle may allow for rapid translation of this known neuroprotectant into clinical trials with the ultimate goal of providing a safe and effective therapeutic to treat SCI. Supported by NIH-NINDS, SCIRF, and VA.

WTH03-03

HIV and morphine dysregulate KCC2 and induce gabaergic deficits in neurons via NMDAR, CCR5, and mor A. Barbour

Virginia Commonwealth University, Anatomy and Neurobiology, Richmond, USA

Despite the introduction of combined antiretroviral therapy, the CNS remains susceptible to insult from HIV-1 and inflammatory factors which cause sublethal damage to bystander neurons, providing the cellular basis of HIV-associated neurocognitive disorders (HAND). Opiate use is often comorbid with HIV infection and these patients show exacerbated HAND symptomatology. Little is known about electrophysiological changes associated with HIV ± morphine co-exposure. We developed a dissociated primary human model derived from differentiating human neural progenitors (hNPC) into a mixed neuron-astrocyte culture containing glutamatergic and GABAergic neurons. Optical techniques were used for electrophysiological experiments, thus circumventing the biohazard of sharp electrodes in the presence of HIV. With genetically encoded voltage/calcium indicators, Archon1 and GCaMP6f, we measured human neuron electrophysiological and calcium activity to elucidate changes in excitatory-inhibitory balance due to HIV ± morphine exposure. We determined that HIV and morphine dysregulate neuronal $[Cl^-]_i$, resulting in hyperexcitability. K-Cl cotransporter 2 (KCC2) maintains low $[Cl^-]_i$ necessary for GABA_AR-induced hyperpolarization. Thus, we hypothesized that HIV and morphine decrease expression/activity of KCC2 leading to dysregulated $[Cl^-]_i$ and reduced GABA_AR hyperpolarization. This was confirmed with immunostaining experiments showing significant loss of KCC2 in neurons exposed to supernatant from HIV-infected monocytes (125-500 pg/mL p24) and 500 nM morphine. Further, we determined that the viral proteins transactivator of transcription (Tat) and glycoprotein 120 (gp120; R5-

tropic) contribute to KCC2 loss. These results correlate with significant defects of GABAergic signaling in human neurons exposed to HIV/HIV proteins $\pm$ morphine. KCC2 expression and response to GABA were rescued by co-exposure with KCC2 activity enhancer, CLP257, or targeting upstream pathways. Our data identify KCC2 and upstream activity as a promising, novel target for therapeutic intervention to alleviate functional changes underlying HAND $\pm$ opiate use.

WTH03-04

Understanding interleukin-1 receptor 1 localization after traumatic brain injury **C. Bodnar, A. Bachstetter**

University of Kentucky, Spinal Cord and Brain Injury Research Center, Lexington, USA

Neuroinflammation is increasingly regarded as a major pathology in various diseases affecting the central nervous system. Trauma to the central nervous system is one such area where neuroinflammation has become a major focus, and with over 10 million traumatic brain injuries (TBI) occurring worldwide, understanding the inflammatory reactions after these injuries may be crucial to the development of effective therapies. Following a TBI, it is known that there is a large increase in cytokine release, followed by persistent cytokine dysregulation. Interleukin-1 (IL-1) is a cytokine that is responsible for coordinating the immune response in the body but also affects a variety of cell types in the brain, ultimately altering CNS function, and the neuroimmune axis. IL-1 signals mainly through the IL-1 receptor 1 (IL-1R1). The localization of IL-1R1 in the CNS has been described in the healthy brain, but little is known about how an injury or disease effects localization of IL-1R1, and if there is a gain or loss of IL-1R1 expression on different cell types in the brain after injury. To answer these questions, we have employed a reporter transgenic mouse in which the IL-1R1 gene and protein are tagged to allow for the neuropathological assessment of IL-1R1 regional and cellular localization after a TBI. Using these approaches, we found robust expression of IL-1R1 on neurons, and blood vessels in the brain, with no apparent microglia expression of IL-1R1 at least at acute time points post-injury. These results suggest critical roles for IL-1/IL-1R1 in neuromodulation and the neuroimmune axis.

WTH03-05

Deep immune profiling of peripheral blood reveals a triphasic response and correlations with cognitive outcomes after stroke

M. Buckwalter¹, A. Tsai², K. Berry¹, M. Beneyto², D. Gaudilliere³, E. Ganio², B. Choisy², K. Djebali², Q. Baca², L. Quach¹, L. Drag¹, M. Lansberg¹, M. Angst², B. Gaudilliere², N. Aghaeepour²

¹Stanford University School of Medicine, Neurology, Stanford, USA

²Stanford University School of Medicine, Anesthesiology, Stanford, USA

³Stanford University School of Medicine, Surgery, Stanford, USA

Stroke produces profound local and systemic immune responses. We aimed to characterize the systemic immune response to stroke and determine if it contributes to long-term cognitive disability. Blood was collected for up to 9 timepoints after stroke (days 1, 2, 3,

5, 7, 14, 30, 90, and 365) from 24 consecutive subjects with ischemic stroke. Cognitive function was assessed using MoCA. We used mass cytometry to acquire 240 immune features; 20 immune cell subtypes, their frequency, cell surface markers, and activation states. Elastic Net regularized regression modeling identified three phases of the systemic immune response to ischemic stroke: The acute phase (day 2) exhibited increased STAT3 signaling in innate immune cells. The intermediate phase (day 5) exhibited increased CREB signaling in adaptive immune cells. The late phase (day 90) exhibited persistent elevation of neutrophils and IgM+ B cells. By day 365 there was a return to an immune response comparable to controls. A decline in MoCA scores between day 90 and day 365 after stroke correlated with a stronger inflammatory response in the acute phase ($r = -0.692$, Bonferroni corrected $p = 0.04$). The results demonstrate the utility of a deep immune profiling approach and support the hypothesis that increased inflammation in the subacute time period after stroke may promote later post-stroke dementia.

WTH03-06

Defective microglia-neuronal communication leads to glial activation governing differential gene expression during demyelination

A. Cardona¹, K. Church¹, A. Mendiola³, S. Cardona¹, D. Vanegas¹, S. Lira², C.-H. A. Lin¹

¹The University of Texas at San Antonio, Biology-STCEID, San Antonio, USA

²Icahn School of Medicine at Mount Sinai, Immunology, New York, USA

³University of California San Francisco, Gladstone Institute, San Francisco, USA

Multiple sclerosis (MS) a disease characterized by the demyelination of axonal nerve fibers in the central nervous system (CNS) causing disruption in nerve impulses leading to motor and cognitive deficits. In the CNS, the CX3CR1 receptor is only found on microglia and signals through neuronal derived Fractalkine (FKN). This binding is known to inhibit microglia mediated neurotoxicity. To test the hypothesis that defective CX3CR1-FKN signaling leads to microglial toxicity and robust demyelination we utilized the cuprizone model of demyelination/remyelination. Our findings show severe demyelination, neuronal damage, and delayed remyelination accompanied by microglia clustering in demyelinated regions of the corpus callosum (CC) in CX3CR1-KO, FKN-KO and mice expressing the human CX3CR1^{I249/M280} polymorphic variant, that has reduced binding affinity to FKN. In order to elucidate the mechanism by which CX3CR1-FKN regulates oligodendrogenesis and neurogenesis, we performed NanoString analysis on RNA extracted from tissues isolated from the SGZ to identify differentially expressed genes involved in neurogenesis, cell maintenance and immune regulation. The data revealed an up-regulation in genes involved in oxidative stress to neurons, notch signaling by microglia, and carbohydrate catalysis and a down-regulation in genes involved in stem cell maintenance in CX3CR1-KO and human CX3CR1^{I249/M280} expressing mice. This study has the potential to provide gene target therapies for the advancement of the MS field by providing evidence of a neuroprotective role of CX3CR1 that can be manipulated to enhance remyelination and repair.

WTH03-07

Intravital two-photon imaging reveals distinct morphology and infiltrative properties of glioblastoma-associated macrophages and M

Z. Chen^{1,2}, J. Ross¹, D. Hambardzumyan^{1,2}

¹Emory University, Pediatrics, Atlanta, USA

²Emory University, Winship Cancer Center, Atlanta, USA

Characterized by dismal survival rates and limited responses to therapy, glioblastoma (GBM) remains one of the most aggressive central nervous system (CNS) malignancies. Recently, the role of tumor-associated macrophages (TAMs) in the progression of these tumors has demonstrated TAMs are significant contributors to tumor growth, invasion, and therapeutic resistance. TAMs, which include brain-resident microglia and circulating bone marrow derived-monocytes, are typically grouped together in histopathological and molecular analyses due to the lack of reliable markers of distinction. To develop more effective therapies aimed at specific TAM populations we must first understand how these cells differ both morphologically and behaviorally. Further, we must develop a deeper understanding of mechanisms encouraging their infiltration and how these mechanisms can be therapeutically exploited. In this study we combine immunocompetent lineage tracing mouse models of GBM with high-resolution open skull two-photon microscopy to investigate the phenotypical and functional characteristics of TAMs. We demonstrate TAMs are composed of two morphologically distinct cell types that have differential migratory propensities. We show that bone marrow derived monocytes are smaller, minimally branched cells and are highly migratory compared to microglia which are larger, highly branched, stationary cells. Additionally, two populations of monocytic macrophages were observed, which differed by CX3CR1 expression and migratory capacity. Lastly, we demonstrate the efficacy of anti-VEGF blockade for prohibiting TAM infiltration, especially against bone marrow-derived macrophages. Together, our data clearly defines characteristics of individual TAM populations and suggests combination therapy with anti-vascular and anti-chemotaxis therapy may be attractive options for the treatment of these tumors.

WTH03-08

Vagal nociceptor neurons sense TH2 cytokines **T. Crosson**

Université de Montréal, Pharmacology, Montréal, Canada

Modalities detected by nociceptors are broader than pressure and temperature and include signals as various as cytokines and pathogens to immunoglobulins and even microRNAs. In different models of type 2 (Th2) immunity responses, dorsal or vagal sensory neurons induce either itch or pain hypersensitivity, and several studies describe a bidirectional communication with immune cells. Previous work by Talbot et al. showed that nociceptors amplify pathological immune responses in allergic airway inflammation. Here, we used live calcium microscopy of cultured Nodose Ganglia neurons to measure the ability of vagal nociceptors to sense TH2 cytokines. IL4, IL5, IL13, IL33 and TSLP all triggered calcium influxes in TRPV1 expressing nociceptors. Interestingly, the response to IL33 was increased in nociceptors from a mouse model of allergic airway inflammation. Those results support a context-dependent neuro-immune interplay

and suggest a heightened importance of pain neurons in driving allergic inflammation.

WTH03-09

Blocking bet proteins is neuroprotective in ischemic stroke

K. DeMars, C. Yang, B. Sanz, E. Candelario-Jalil

University of Florida, Neuroscience, Gainesville, USA

Neuroinflammation after stroke significantly contributes to neuronal cell death. Bromodomain and Extra Terminal Domain (BET) proteins are essential to inflammatory gene transcription. BET proteins (BRD2, BRD3, BRD4, and BRDT) have varied effects including chromatin remodeling, histone acetyltransferase activity, and as scaffolds to recruit transcription factors; they couple chromatin remodeling with transcription. BRD2/4 are of particular interest to stroke-induced neuroinflammation that contributes to delayed cell death as they are required for NF- κ B-dependent gene transcription. We hypothesized that targeting BET proteins for degradation with dBET1, a proteolysis targeting chimera (PRO-TAC) that combines the highly selective BET inhibitor JQ1 and a ligand for cereblon E3 ubiquitin ligase, will reduce brain injury in ischemic stroke. Male mice were subjected to permanent or transient occlusion of the middle cerebral artery and received either vehicle, JQ1, or dBET1 at various times after stroke. Neurobehavioral tests were performed before and after stroke induction. Infarct volume was quantified at 48 h or 72 h. Data showed that BET degradation significantly reduced infarct volume in permanent focal cerebral ischemia in aged mice, and this was associated with reduced brain levels of pro-inflammatory mediators including TNF- α , CXCL1, CXCL10, CCL2, and matrix metalloproteinase-9. Importantly, treatment with the BET degrader dBET1 resulted in a significant improvement in stroke-induced neurological deficits. Collectively, these data indicate that BET proteins are a novel target for neuroprotection in ischemic stroke.

WTH03-10

Hypertension associated neuroinflammation and cognitive decline are attenuated by targeting the sphingosine-1-phosphate pathway

N. Don-Doncow¹, L. Vanherle¹, F. Mathes¹, Y. Zhang¹, A. Messier^{1,2}

¹Lund University, Department of Experimental Medical Sciences, Lund, Sweden

²Lund University, Wallenberg Centre for Molecular Medicine, Lund, Sweden

Background: Hypertension affects more than 30% of people worldwide and is a major modifiable risk factor for the development of cognitive decline. As the relationship between high blood pressure and inflammation has become clearer, increased attention has started to focus on hypertension-associated neuroinflammation and neurodegeneration. Particularly, the hypertension-associated elevation of circulating pro-inflammatory T-lymphocytes has been shown to contribute to target organ damage in the brain. However, precise mechanisms how hypertension leads to neurodegeneration are still elusive and specifically, the current understanding of T-lymphocyte infiltration into the brain is limited. Recent evidence has

implicated the bioactive phospholipid sphingosine-1-phosphate (S1P) in hypertension-associated T-lymphocyte trafficking and thus, evolves as a potent therapeutic target in hypertension-mediated neuroinflammation.

Objective: Our earlier work showed that blocking S1P generation via SphK2 prevents inflammatory responses typical of hypertension. Therefore, we investigated the potential therapeutic effects of pharmacological SphK2 inhibition on cognitive decline and neuroinflammation.

Materials and Methods: In an Angiotensin-II murine model of hypertension, we assessed the potential therapeutic effect of specific SphK2 inhibition on (1) cognitive deficits using novel object recognition tests, (2) neuronal integrity using Golgi-Cox staining, (3) immune cell populations using flow cytometry, and (4) S1P levels using mass spectrometry.

Results: Treatment with an SPHK2 inhibitor significantly reduced systemic inflammation, which was accompanied by an improvement in cognitive function and attenuation of neuroinflammation.

Conclusion: Our results suggest a role for the S1P pathway in hypertension-mediated neurodegenerative processes (i.e., neuroinflammation). Thus, the inhibition of SphK2 might evolve as new therapeutic strategy to efficiently control hypertension-related neuroinflammation and neurodegeneration.

WTH03-11

Role of CD44 reactive astrocytes following traumatic brain injury

A. Early, A. Gorman, J. Morganti

University of Kentucky, Sanders-Brown Center on Aging, Lexington, USA

The current knowledgebase regarding the pathobiological contribution of reactive astrogliosis in the brain following traumatic brain injury is poorly understood. To begin to understand astrocyte-specific response to TBI, we examined a time course spanning acute-subacute intervals following TBI; 1, 3, and 7 days post-surgery using 3-month-old C57BL/6J mice that received either sham or controlled cortical impact (CCI) contusion surgery. Two cohorts were generated; one for histological quantification and a second for gene expression profiling. Reactive astrogliosis was quantified using GFAP, S100beta, and Aqp4 as histological markers. Secondly, at the prescribed interval, ipsilateral neocortex and dorsal hippocampus was used for ACSA-2 magnetic bead astrocyte enrichment. Astrocyte-specific response to TBI was profiled using a focused array of 46 genes. Our time course profiling revealed that the bulk of disparate responses occurred at the 3d interval, notably exacerbated expression of *CD44*, relative to sham. Gene profiling was examined using principle component analysis (PCA) and hierarchical clustering, revealing conserved groupings between each surgical cohort. We next examined the transcriptional profile of CD44⁺ vs. CD44⁻ astrocytes following TBI, using an astrocyte-specific reporter strain. Using these reporter mice, 3 months old, we examined the 3d post-surgery interval (sham and TBI) via FACS of CD44⁺ astrocytes (e.g. CD44⁺tdTomato⁺) versus CD44^{neg}tdTomato⁺ astrocytes. Sorted cells were again processed for RNA isolation and analyzed on the same focused gene array as above. Our current data indicate that TBI-induced CD44⁺ reactive astrocytes acquire a predominantly pro-inflammatory response (e.g. *CCL2*), with a concomitant decrease in genes associated with synaptic support (e.g. *GPC6*),

compared to CD44^{neg} astrocytes. Collectively, our findings identify a novel subset of astrocytes that may play an integral role in the deleterious neurodegenerative sequelae following TBI, highlighting these cells as a potential therapeutic target.

WTH03-12

Selective immunomodulatory and neuroprotective effects of NOD2 receptor agonist on APP/PS1 mouse model of Alzheimer disease

A. F. Maleki, G. Cisbani, M.-M. Plante, N. Laflamme, P. Préfontaine, J. Baillargeon, M. Rangachari, J. Gosselin, S. Rivest

Laval university, CHU de Québec Research Center and Department of Molecular Medicine, Faculty of Medicine, Quebec, Canada

Muramyl dipeptide (MDP) is minimal bacterial peptidoglycan recognized by NOD2 receptor. Peripheral administrations of MDP convert Ly6C^{high} into Ly6C^{low} monocytes. Previously we showed Ly6C^{low} monocytes efficiently eliminate Aβ microaggregates from brain microvasculature. We investigated whether MDP immunomodulatory effects could influence neuropathology of mouse model of AD. 3-months old APP_{sw}/PS1 transgenic male mice and age-matched C57BL/6J mice were used for high frequency (2-times/week) over 6-months and low frequency (once a week) over a 3-months period of intraperitoneally MDP (10 mg/kg) administrations. Flow cytometry analysis of monocyte subsets in blood, behavioral and post mortem analyses were performed. Two-photon microscopy using APP/PS1/CX3CR1^{gfp/+} mice were conducted to study vascular Aβ clearance by Ly6C^{low} monocytes upon MDP administration. FACS results showed MDP in both type of treatments significantly regulated monocyte subsets. Water T maze results showed improvement in memory deficits in the treatment group. PSD95, LRP1 and MCP-1 levels significantly increased, whereas ICAM-1 significantly decreased and Iba1 did not change in treatment group compared to the control. Using two-photon microscopy study, we found that MDP increased surveillance of Ly6C^{low} monocytes to small Aβ aggregates present on APP/PS1/CX3CR1^{gfp/+} cortical blood vessels to potentially mediate vascular Aβ clearance. Our findings demonstrate selective immunomodulatory effects of MDP. Our results suggest that PSD95 and LRP1 may be key players in these neuroprotective properties via enhancement of synapse functions and vascular Aβ clearance. Taken together, our results suggest that MDP is beneficial in early phases and to some extent late phases of AD.

WTH03-13

Persistent neuropathology in a RAT model of acute op intoxication

P. Lein¹, S. Supasai¹, D. Rowland²

¹University of California, Davis, Molecular Biosciences, Davis, USA

²University of California, Davis, Center for Molecular and Genomic Imaging, Davis, USA

Acute intoxication with the organophosphorus (OP) cholinesterase inhibitor, diisopropyl fluorophosphate (DFP), can cause status epilepticus. Aggressive treatment with atropine and benzodiazepines increases survival but does not protect against neurobehavioral deficits. Here, we characterize neuropathology at 3 and 6 months

after acute DFP intoxication. Adult male Sprague-Dawley rats were administered DFP (4 mg/kg, sc), atropine (2 mg/kg, im) and 2-PAM (25 mg/kg, im). Forty min later, animals were administered vehicle or a benzodiazepine [diazepam (DZP), 5 mg/kg, ip or midazolam (MDZ), 0.7 mg/kg, im]. GFAP and S100 β immunostaining of brains from DFP-intoxicated animals not administered benzodiazepine revealed significant reactive astrogliosis in the thalamus, CA1, CA3, amygdala and piriform cortex at 3 and 6 months post-DFP. Dual IBA-1/CD68 immunostaining was significantly increased in the thalamus, CA1, CA3, amygdala, and piriform cortex at 3 months and persisted in the CA1 and piriform cortex at 6 months. DZP did not protect against neuroinflammation, whereas MDZ mitigated astrogliosis in thalamus and CA1 at both time points, and reduced microglial activation in CA1, and piriform cortex at 3 months. Calcium deposits were detected in the brain of > 75% rats, as determined by micro-CT imaging and Alizarin Red S staining. At 3 months post-DFP, calcium deposits were visible in the thalamus of DZP, not MDZ-treated animals. At 6 months, both DZP and MDZ-treated rats exhibited thalamic calcium deposits. These data indicate that current medical countermeasures for acute OP intoxication do not effectively protect against chronic neuropathology, and suggest that persistent neuroinflammation and/or dysregulated calcium homeostasis contribute to long-term morbidity. Supported by NIH CounterACT grant # NS079202.

WTH03-14

Targeting interferon lambda promotes recovery during CNS autoimmunity

S. Manivasagam¹, J. Williams³, L. Vollmer¹, A. Archambault², R. Klein¹

¹Washington University in St. Louis, Medicine, St. Louis, USA

²Washington University in St. Louis, Neurology, St. Louis, USA

³Cleveland Clinic, Neurosciences, Cleveland, USA

Multiple sclerosis (MS) is a chronic autoimmune, demyelinating disease that affects 2.5 million people worldwide. MS is characterized by pathologic infiltration of lymphocytes and macrophages into the central nervous system (CNS) that leads to demyelination and axonal injury. Axonal injury is strongly correlated with disease progression and permanent disability. Available treatments for MS have varying efficacy, do not impact transition to progressive disease, or promote axonal recovery. Here, we show that interferon lambda (IFN λ) may play a role in progression of CNS autoimmune diseases. IFN λ is a new member of the interferon family of proteins and is related to type I interferon. As IFN λ has not been widely studied outside of viral models, it is unknown whether its immunomodulatory properties impact other inflammatory diseases, including MS. In preliminary studies, we found that IFN λ signaling impacts recovery in mice with experimental autoimmune encephalomyelitis (EAE), a well-established model for MS. Mice with targeted deletion of the IFN λ receptor (*Ifnlr1*^{-/-}) demonstrated improved clinical recovery compared to wild-type (WT) animals. This recovery was linked to resolution of inflammation and prevention of axonal injury. *Ifnlr1*^{-/-} mice exhibited decreased activation of T cells and subsequent decreases in inflammatory cytokine production. Targeting IFN λ using neutralizing antibodies resulted in similar improvements in clinical disease and axonal damage. Finally, in human spinal cord tissue, we found increased levels of IFN λ in lesions of secondary progressive MS patients compared to relapsing remitting MS patients. These data suggest

that IFN λ may promote disease progression during autoimmune neuroinflammation and be a novel therapeutic target for MS.

WTH03-15

The role of PTEN on microglia dynamics

S. Marik, K. Oluyemi, A. Milano

Pace University, Biology, Pleasantville, USA

Phosphatase and tensin homolog (PTEN) is best known for its role as a tumor suppressor, yet it also plays an important role in neuronal morphology. Previous studies demonstrate that a reduction of PTEN results in longer dendritic and axonal arbors. However, it is unknown if a reduction of PTEN may also impact neuronal morphology in cell non-autonomous ways. Microglia, the resident macrophages of the central nervous system, also contribute to synapse elimination during development. Here we compare microglia morphology, motility and dynamics in developing zebrafish with reduced *ptenb* with wild type conspecifics to determine if reduced PTEN impacts microglia. Using time-lapsed confocal microscopy we observe that at 2.5 days post-fertilization (dpf) there is no significant difference between nearest neighbor distance (nnd) as microglia are entering the tectum in WT (29.92 $\pm$ 2.35 mm) and *ptenb*^{-/+} (29.16 $\pm$ 2.06 mm). However, at 3 dpf WT microglia were significantly more distributed within the tectum (42.22 $\pm$ 5.93 mm, nnd) than *ptenb*^{-/+} (27.04 $\pm$ 1.32 mm, nnd). Interestingly, *ptenb*^{-/+} fish were also less motile compared to WT at 5 dpf, but not at 3 dpf. Taken together, these findings suggest that PTEN does not alter microglia colonization in the brain, instead a reduction in PTEN impacts microglia distribution and motility at a developmental time point that is crucial for axonal growth and pruning which suggests that PTEN can also impact neurite length in a cell non-autonomous fashion.

WTH03-16

Interleukin-1 alpha contributes to hippocampal neural progenitor cell proliferation and differentiation following injury

C. McPherson, J. Harry

National Institute of Environmental Health Sciences, Neurotoxicology Group, North Carolina, USA

Beneficial and detrimental effects of interleukin-1 (IL-1) signaling are reported in the neural progenitor cell (NPC) response to injury. The localization and source of IL-1 regulates the distinct neuroimmune activities of IL-1 in the brain. To examine the impact of microglia-derived IL-1 on hippocampal NPC proliferation and differentiation we induced selective neuronal death in the dentate granule cell layer (GCL) by trimethyltin (TMT, 2.3 mg/kg; i.p.) in adolescent CD1 mice and examined the subsequent NPC response. Peak neuronal apoptosis occurred at 2d, coinciding with an increase of bromodeoxyuridine (BrdU) + NPCs in the subgranular zone (SGZ) and GCL. IL-1 α protein expression was elevated and localized to microglia and IL-1 receptor 1 (IL-1R1) to Nestin+ NPCs within the GCL. *In vitro*, IL-1 α stimulated NPC proliferation and differentiation from Nestin+/Sox2 + neural precursors to doublecortin+ (DCX) transit amplifying NPCs. *In vivo*, the migratory pattern of Nestin+ cells from the SGZ and DCX+ cells in the GCL

coincided with increased expression of *Il1a*. With resolution of the injury, down-regulation of microglia, and repair of the GCL, NPCs that were generated during the injury response expressed the mature neuronal protein NeuN and successfully integrated into the GCL resulting in a full repopulation of GCL neurons. The newly-generated neurons were functionally integrated into the hippocampal circuitry as demonstrated by cFos expression following Morris Water Maze training and a recovery of maze performance to control levels. These data suggest a role for microglia and IL-1 α in regulation of NPC proliferation and differentiation for self-repair following hippocampal injury. Supported by NIEHS Division of Intramural Research: Z01 ES01623 & ES021164.

WTH03-17

NGR-FC protein delivered by hematopoietic cells enhances neurorepair in a multiple sclerosis model
S. Petratos¹, M. J. Kim¹, J. H. Kang¹, J. Y. Lee^{4, 1}, P. Theotokis³, S. Strittmatter²

¹Monash University, Neuroscience, Melbourne, Australia

²Yale University School of Medicine, Cellular Neuroscience, Neurodegeneration and Repair Program, CT, USA

³AHEPA University Hospital, B', Department of Neurology, Thessaloniki, Greece

⁴ToolGen Inc., R&D Therapeutics, Seoul, Korea

Nogo receptor 1 (NgR1) is the high affinity receptor for the potent myelin-associated inhibitory factors (MAIFs) that make up part of the inflammatory extracellular milieu during experimental autoimmune encephalomyelitis (EAE). Signaling through the NgR1 complex has been shown to be associated with axonal degeneration in an animal model of MS and neuronal deletion of this receptor homologue, in a disease specific manner is associated with preserving axons even in the context of neuroinflammation. The local delivery of NgR(310)ecto-Fc, a therapeutic fusion protein has been successfully applied as a treatment in animal models of spinal cord injury and glaucoma. As multiple sclerosis (MS) and EAE exhibit large numbers of inflammatory cell infiltrates within central nervous system (CNS) lesions, in this study, we utilized transplantable HSCs as a cellular delivery method of the NgR(310)ecto-Fc fusion protein. There were no immunomodulatory effects of the transplanted, modified HSCs with LV-NgR(310)ecto-Fc on immune cell lineages in EAE-induced recipient female mice. We identified CNS infiltrating macrophages as the immune-positive cell type expressing for the myc-tagged NgR(310)ecto-Fc. These differentiated phagocytes predominated the extensive immune cell infiltrated regions at the peak of clinical disease with extensive demyelination with associated engulfment of NgR-Fc protein-MAIF complexes. Importantly, three animals transplanted with LV-NgR(310)ecto-Fc-over-expressing HSCs, recovered from the peak of neurological symptoms associated with EAE, exhibiting axonal regeneration and eventual remyelination in the white matter tracts. These results suggest that HSCs can be utilized as carriers of the therapeutic NgR-Fc protein for specific delivery into multifocal EAE lesions and can potentiate neurological recovery.

WTH03-18

Corpora amylacea in human hippocampal brain tissue exhibit a homogeneous distribution of neo-epitopes
M. Riba^{1, 2}, E. Augé^{1, 2}, I. Bechmann³, N. Llor¹, M. Krueger³, J. Vilaplana^{1, 2}, C. Pelegrí¹

¹Universitat de Barcelona, Facultat de Farmàcia i Ciències de l'Alimentació, Barcelona, Spain

²Universitat de Barcelona, Institut de Neurociències, Barcelona, Spain

³University of Leipzig, Institute for Anatomy, Leipzig, Germany

Corpora amylacea (CA) are spherical or ovoid polyglucosan bodies that accumulate primarily in the periventricular and subpial regions of the human brain during aging process and some neurodegenerative diseases. In recent work, we reported that they contain some neo-epitopes recognized by natural IgMs, revealing a possible link between them and natural immune system. Here, we used transmission electron microscopy techniques to perform an ultrastructural study to precisely localize the neo-epitopes in CA and complemented them with immunofluorescence and confocal microscopy studies. Results indicate that CA in human hippocampal brain tissue exhibit a homogeneous distribution of neo-epitopes and also indicate that, in immunohistochemical studies of CA, some epitopes of the central part are not always accessible to the antibodies and can only be labeled when CA has been sliced up. Thus, previous studies about CA in which immunohistochemical techniques were used might produce erroneous interpretations. These results are consistent with previous results about neo-epitopes in mouse PAS granules, which are structures that share some features with human CA. The post-embedding labeling in immunoelectron microscopy studies of PAS granules showed that the neo-epitopes were located in the whole granule. Finally, the presence of neo-epitopes on the whole CA reinforces the theory of CA as structures involved in the entrapment of damaged and non-degradable products and that have a role in protective or cleaning mechanisms.

WTH03-19

Microglia-mediated inflammation in diabetic retinopathy
B. Sarker¹, S. Cardona¹, D. Vanegas¹, K. Church¹, A. Mendiola², A. Cardona¹

¹The University of Texas at San Antonio, Biology-STCEID, San Antonio, USA

²Gladstone Institute of Neurological Disease, Neurology, San Francisco, USA

Diabetic retinopathy (DR) is a microvascular complication of diabetes and leading cause of blindness. Increasing evidence suggests that microglia-mediated inflammation plays a significant role in neuronal and vascular damage, but mechanisms contributing to microglia activation, proliferation, and pro-inflammatory cytokine release are not well understood. Our studies from diabetic murine models showed that fractalkine (FKN), a neuronal-derived chemokine, and its microglial receptor CX3CR1 exert anti-inflammatory and neuroprotective roles in DR. Also, systemic inflammation accelerated fibrinogen deposition and perivascular microglial clustering. We hypothesize that aberrant CX3CR1 signaling leads to blood-retinal barrier damage, and fibrinogen-mediated microglia activation leads to neuronal loss and inflammation, which can be dampened by reducing fibrinogen levels. We also hypothesize that DR pathology in human retinas mirrors observations from mouse

models. To examine retinal capillary degeneration, acellular capillaries were quantified in non-diabetic and diabetic CX3CR1-WT, CX3CR1-KO, and humanized CX3CR1 mice. Acellular capillaries were increased in diabetic CX3CR1-KO mice compared to non-diabetic mice. To characterize retinal pathology in mice expressing human CX3CR1 variants and in the human retina, microglial densities, astrogliosis, and fibrinogen deposition were evaluated. Histopathological analysis revealed increased gliosis and fibrinogen extravasation in diabetic patients. Our results indicate that pathology observed in diabetic human retinas is reproduced in experimental models and that CX3CR1 signaling plays a key role in mediating neuroprotection. Future studies will determine the effect of anticoagulants or defibrinogenating agents in DR initiation and progression. Determining CX3CR1-mediated microglial regulation mechanisms to dampen inflammation is expected to positively impact implementation of new treatment strategies via stimulation of CX3CR1 by FKN alone or in combination with anti-fibrin therapies.

WTH03-20

Novel process of myelin debris clearance by glovenin[®]-I treatment in the lyssolecithin demyelination of mouse sciatic nerve

Y. Setoguchi, A. Hayashi, N. Suzuki, S. Kaneko, H. Tanaka, D. Yanaoka, H. Baba

Tokyo University of Pharmacy and Life Sciences, Department of Molecular Neurobiology, Hachioji-shi, Japan

The Intravenous Immunoglobulin (IVIg) therapy is first choice of the treatment of immune-mediated neuropathy. Recently we have reported that IgG antibodies against large-myelin protein zero (L-MPZ) are included in human γ -globulin preparation glovenin[®]-I (Nihon Pharmaceutical Co., LTD.). IVIg treatment shows protective effect on chemical demyelinating condition and induction of M2 macrophages in acute phase in mouse experimental model. To clarify the role of glovenin in neuropathy treatment, we examined immunohistological changes of demyelinating regions in glovenin-treated mice. ICR mice were injected with 1% Lyssolecithin directly into bilateral sciatic nerves, and either glovenin or control saline was injected intravenously 24 h later. The sciatic nerves were examined on the day 7 and 14 after Lyssolecithin injection. On the day 7, abundant debris was found in the demyelinated regions and inside the phagocytic macrophages. Most of these debris was stained by anti-myelin basic protein (MBP) antibody. Myelin and myelin debris were detected with anti-human IgG (hIgG) antibody in glovenin injected group, but not in saline group. Some MBP+/hIgG+ and relatively large-sized hIgG+ debris was also observed in the IVIg treated mice. While substantial number of MBP+ debris was still present on the 14th day, hIgG+ myelin debris was significantly decreased. Instead, CD68 + phagocytic macrophages were labeled by anti-hIgG antibody, suggesting that direct binding of hIgG to myelin debris via L-MPZ enhanced its clearance by macrophages. Thus, IVIg treatment of the Lyssolecithin-induced demyelination is effective partly through IgG antibody reaction against L-MPZ in glovenin.

WTH03-21

AKT3-mediated protection against inflammatory demyelinating disease

B. Shafit-Zagardo, J. DuBois, A. Ray, R. Aflakpui, R. Gruber, F. Macian, Y. Zhang

Albert Einstein College of Medicine, Pathology, Bronx, USA

Akt is a serine/threonine protein kinase that plays a major role in regulating multiple cellular processes. While the isoforms Akt1 and Akt2 are involved in apoptosis and insulin signaling respectively, the role for Akt3 remains uncertain. Akt3 is predominantly expressed in the brain, and total deletion of Akt3 in mice results in neurodegeneration and a reduction in brain. Previously, we found that Akt3^{-/-} mice have a significantly worse clinical course during myelin-oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE), an animal model in which autoreactive immune cells enter the CNS, resulting in inflammation, demyelination, and axonal injury. Spinal cords of Akt3^{-/-} mice are severely demyelinated and have increased inflammation compared to WT, suggesting a neuroprotective role for Akt3 during EAE. To specifically address the role of Akt3 in neuroinflammation and maintaining neuronal integrity, we used several mouse strains with different manipulations to Akt3. During EAE, Akt3^{Nmf350} mice (with enhanced Akt3 kinase activity) had lower clinical scores, a lag in disease onset, a delay in the influx of inflammatory cells into the CNS, and less axonal damage compared to WT mice. Mice with a conditional deletion of Akt3 in CD4⁺ T-cells had an earlier onset of EAE symptoms, increased inflammation in the spinal cord and brain, and had fewer FOXP3 + cells and FOXP3 mRNA expression. No difference in EAE outcome was observed when Akt3 expression was deleted in neurons (Syn1-CKO). These results indicate that Akt3 signaling in T-cells and not neurons is necessary for maintaining CNS integrity during an inflammatory demyelinating disease.

WTH03-22

ER stress initiates janus kinase (JAK) 1-dependent gene expression in astrocytes

S. Sims, G. Meares

West Virginia University, Microbiology, Immunology, and Cell Biology, Morgantown, USA

Neurodegenerative diseases are associated with the accumulation of misfolded proteins in the endoplasmic reticulum (ER). ER stress occurs when the protein folding capacity of the ER is overwhelmed, resulting in unfolded protein response (UPR) initiation to restore homeostasis. Unresolved UPR activation leads to cell death and inflammation. Evidence indicates ER stress and inflammation are linked, and we have described a Janus Kinase (JAK) 1- Signal Transducer and Activator of Transcription (STAT) 3-dependent mechanism that promotes expression of inflammatory mediators like Interleukin-6 (IL-6) and chemokine C-C motif ligand 2 (CCL2). Using siRNA knockdown and RNA-seq, we found that JAK1 regulates over 10% of ER stress-induced gene expression in astrocytes. This includes genes not previously associated with JAK1 signaling, such as tribbles (TRIB) 3 and growth arrest and DNA damage inducible (GADD45) α . RNA-seq revealed that JAK1 drives a distinct gene expression program in response to ER stress compared to that induced by cytokines. Less than 10% of the ER stress-induced JAK1-dependent genes are also induced by cytokine

stimulation. GADD45 α and TRIB3 are known ATF4 target genes, therefore we investigated activating transcription factor (ATF) 4, which is established to be induced in a PERK-dependent manner in response to ER stress. We found that TRIB3 and GADD45 α were both JAK1 and ATF4 dependent in response to ER stress and pharmacologically inhibiting the kinase domain of JAK1 fails to abrogate ER stress-induced expression of these genes. These data demonstrate that JAK1 elicits noncanonical signaling during ER stress that we hypothesize is independent of JAK1 kinase activity. These findings suggest JAK1 is a major driver of transcriptional adaptation in response to cellular stress utilizing novel signaling mechanisms to regulate gene expression.

WTH03-23

Dihydromyricetin exerts neuroprotection in ischemia reperfusion induced neuronal damage by inhibiting apoptosis and astrogliosis

D. Singh¹, H. Wasan¹, U. Sharma², Ak. Dinda³, R. KH¹

¹All India Institute of Medical Sciences, Department of Pharmacology, New Delhi, India

²All India Institute of Medical Sciences, Department of NMR, New Delhi, India

³All India Institute of Medical Sciences, Department of Pathology, New Delhi, India

Despite advances in understanding the pathophysiology of cerebral ischemia, tPA remains to be the only treatment option. Increasing number of evidences suggests that apoptosis and astrocytes activation exacerbates damage after stroke. Dihydromyricetin (DHM), a flavonoid, has shown to be neuro-protective by its anti-inflammatory and anti-apoptotic properties. Current study evaluates the hypothesis that sub-acute DHM treatment may exert neuro-protection via inhibiting apoptosis and astrogliosis. In male Wistar rats (270 $\pm$ 20 g), middle cerebral artery was occluded for 90 min using a silicon coated Doccol suture followed by reperfusion for 3 days. DHM (100 mg/kg) was administered immediately and 2 h after reperfusion followed by single dose every 24 h for next 2 days. After 72 h of ischemia, T-2 MR images were acquired for infarct damage. Rats were euthanized, perfusion fixed with paraformaldehyde, cryosections were stained with NeuN and GFAP, counterstained with DAPI. TUNEL assay was used for neuronal apoptosis. MRI data revealed DHM treatment significantly ($p < 0.05$) decreased cerebral infarct, signal intensity and ADC values when compared with MCAo group. TUNEL staining showed significantly ($p < 0.01$) increased number of neuronal apoptotic cells in MCAo. Treatment with DHM significantly ($p < 0.05$) reduced the number of apoptotic neurons. Immunofluorescence study revealed that DHM significantly ($p < 0.05$) reduced neuronal damage and activated astrocytes in peri-infarct region. Our results indicate the neuro-protective effect of DHM in ischemia reperfusion injury by inhibiting apoptosis and astrogliosis indicating protective potential of DHM in experimental stroke.

WTH03-24

An anticancer drug regulating neuroinflammation and pain hypersensitivity: a BCR-ABL inhibitor GNF-2 attenuates glial activation

K. Suk, G. J. Song, M. H. Rahman, D. Gupta, J. Kim, M. Jha

Kyungpook National University School of Medicine, Department of Pharmacology, Daegu, Korea South

GNF-2 is an allosteric inhibitor of Bcr-Abl. It was developed as a new class of anti-cancer drug to treat resistant chronic myelogenous leukemia. Recent studies suggest that c-Abl inhibition would provide a neuroprotective effect in animal models of Parkinson's disease as well as in clinical trials. However, the role of c-Abl and effects of GNF-2 in glia-mediated neuroinflammation or pain hypersensitivity has not been investigated. Thus, in the present study, we tested the hypothesis that c-Abl inhibition by GNF-2 may attenuate the inflammatory activation of glia and the ensuing pain behaviors in animal models. Our results show that GNF-2 reduced lipopolysaccharide (LPS)-induced nitric oxide and pro-inflammatory cytokine production in cultured glial cells in a c-Abl-dependent manner. The small interfering ribonucleic acid (siRNA)-mediated knockdown of c-Abl attenuated LPS-induced nuclear factor kappa light chain enhancer of activated B cell (NF- κ B) activation and the production of pro-inflammatory mediators in glial cell cultures. Moreover, GNF-2 administration significantly attenuated mechanical and thermal hypersensitivities in experimental models of diabetic and inflammatory pain. Together, our findings suggest the involvement of c-Abl in neuroinflammation and pain pathogenesis and that GNF-2 can be used for the management of chronic pain.

WTH03-25

Pro-inflammatory function of SPNS2/S1P in microglia

G. Wang, L. Zhong, Z. Zhu, A. Elsherbini, E. Bieberich, H. Qin

University of Kentucky, Physiology, Lexington, USA

Neuroinflammation contributes to the pathogenesis of neurodegenerative disorders, such as Alzheimer's disease (AD) and Parkinson's diseases. We have found that spinster homolog 2 (Spns2), a bona fide S1P transporter, promotes microglia pro-inflammatory activation *in vitro* and *in vivo*. Spns2 knockout (Spns2KO) led to significantly reduced levels of pro-inflammatory cytokines induced by amyloid-beta peptide 1-42 (A β 42) when compared to littermate controls in primary cultured microglia. S1P supplementation increased, while Fingolimod (FTY720), a S1P receptor 1 (S1PR1) functional antagonist, partially dampened A β 42-induced pro-inflammatory cytokine generation, suggesting that Spns2 promotes microglia pro-inflammatory activation through S1P-signaling. Spns2KO significantly reduced A β 42-induced nuclear factor kappa B (NF κ B) activity. S1P increased, while FTY720 dampened, A β 42-induced NF κ B activity, suggesting that Spns2 activates microglia inflammation through, at least partially, NF κ B pathway. Spns2KO mouse brains showed significantly reduced A β 42-induced microglia activation/accumulation and reduced levels of pro-inflammatory cytokines when compared to age-matched controls. More interestingly, Spns2KO ameliorated A β 42-induced working memory deficit detected by Y-Maze. In summary, these results suggest that Spns2/S1P promotes pro-inflammatory polarization of microglia and may play a crucial role in AD pathogenesis.

WTH03-26

Targeting VCAM1 to reduce post-stroke neuroinflammation**K. Zera¹, T. Peterson¹, H. Yousef^{1, 2}, D. Lee^{1, 2}, T. Wyss-Coray^{1, 2, 3}, M. Buckwalter^{1, 4}**¹Stanford University, Neurology and Neurological Sciences, Stanford, USA²VA Palo Alto, Health Care System, Palo Alto, USA³Palo Alto Veterans Institute, for Research, Palo Alto, USA⁴Stanford University, Wu Tsai Neurosciences Institute, Stanford, USA

Ischemia-induced neuroinflammation is associated with worse outcomes. The innate immune response influences short-term recovery. Later adaptive responses lead to chronic neuroinflammation that causes neurodegeneration remote to the ischemic lesion and consequent vascular dementia. In a mouse model of vascular dementia, B lymphocyte influx causes a progressive cognitive deficit. Vascular cell adhesion molecule 1 (VCAM1) is an endothelial protein that facilitates vascular-immune cell interactions by binding very late antigen-4 (VLA-4), and plasma levels are elevated in stroke patients. Therefore, we hypothesized that acute anti-VCAM1 treatment in mice would reduce microgliosis and astrogliosis, while delayed treatment would reduce B and T lymphocyte infiltration. Adult (3-month-old) C57BL/6 mice (N = 10/group) underwent permanent middle cerebral artery occlusion and were dosed with anti-VCAM1 4 h post-stroke before sacrifice at 72 h. Microgliosis and astrogliosis were quantified as percent area immunostained in the infarct border by Iba1 and GFAP, respectively. Acute treatment reduced microgliosis 32.32% ($p = 0.0476$) and astrogliosis 35.25% ($p < 0.03$). Additionally, adult mice (N = 10/group) were treated with anti-VCAM1 immediately (4 h) or later (4d) post-stroke and euthanized 3 weeks later. B cell infiltration was quantified as percent stroke core immunostained by B220, while T lymphocyte infiltration was quantified as CD3 + cells in the core. Delayed anti-VCAM1 significantly reduced B and T cell infiltration by approximately 25% ($p = 0.0015$) and 50% ($p = 0.0192$), respectively. In contrast, early anti-VCAM1 had no effect on B/T cell infiltration. Together, these findings establish VCAM1 as a possible target to treat stroke-induced neuroinflammation.

WTH03-27

Microglia-specific down-regulation of TGF- β -activated-kinase-1 contributes to neuroprotection after murine cerebral ischemia**T. Zeyen¹, A. Reich¹, Jörg. B. Schulz^{1, 2}, P. Habib¹**¹Medical School, RWTH Aachen University, Department of Neurology, Aachen, Germany²Forschungszentrum Jülich GmbH and RWTH Aachen University, JARA-BRAIN Institute of Molecular Neuroscience and Neuroimaging, Jülich, Germany

Background: Neuroinflammation and apoptosis play a crucial role in the expansion of the infarct core and the adjacent penumbra after cerebral ischemia. The key regulator of gene transcription NF- κ B and the MAP kinases JNK, p38/MAPK and ERK share a common upstream activator, the transforming growth factor- β -activated kinase 1 (TAK1). TAK1 is up-regulated after stroke and seems to have cell specific response pattern. Microglia are a main source of TAK1.

However, little is known about the function and regulation of microglial-specific TAK1 after cerebral ischemia.

Purpose: To gain further information about the biological function and regulation of TAK1 in microglial cells after cerebral ischemia.

Methods: Tamoxifen-dependent conditional down-regulation of TAK1 in microglial cells was induced in CX3CR1-Cre^{ER}-TAK1^{fl/fl} mice. The Cre negative CX3CR1-TAK1^{fl/fl} mice served as a control group. We performed 30 min of transient middle cerebral artery occlusion (tMCAo) followed by 72 h of reperfusion. Laser Doppler flowmetry during surgery confirmed sufficient MCA occlusion. Weight, general status and focal neurologic dysfunction were evaluated at various time-points before and after tMCAo or sham surgery. Lesion sizes were determined via computer-assisted infarct volumetry. Examining cell specific interactions primary microglia and mixed cell cultures were subjected to oxygen-glucose deprivation (OGD). TAK1-deficiency was induced by using tamoxifen and/or the general TAK1-inhibitor 5-7-Oxozeanol.

Results: Infarct volumes, neurological deficits and weight loss after tMCAO were reduced in TAK-1 deficient mice compared to the control group. Microglial specific TAK1-down-regulation as well as a general TAK-1 inhibition significantly increased ischemic tolerance and decreased mRNA- and protein-levels of pro-inflammatory cytokines, such as TNF- α , IL-1 β and IL-6 after OGD.

Conclusion: Microglial-specific down-regulation/inhibition of TAK1 offers a promising strategy in stroke therapy.

WTH03-28

Effects of fatty acids on cell cycle progression in neuroblastoma cells**H. Zhou, C. J. Urso**

Seton Hall University, Biological Sciences/Arts & Sciences, South Orange, USA

Circulating levels of free fatty acids have been reported to be elevated in obese subjects, morbidly obese subjects, overweight/obese subjects with diabetes mellitus, patients with severe non-insulin-dependent diabetes mellitus, and obese patients with non-alcoholic fatty liver diseases. While fatty acids are known to be important for various cellular functions, the effects of elevated levels of free fatty acids on cell cycle progression remain to be defined. This study focused on studying the effects of different fatty acids on the viability and cell cycle progression of neuroblastoma cells. The neuroblastoma cell line, Neuro-2A, was treated with different fatty acids including saturated fatty acids, such as palmitic acid, and unsaturated fatty acids, including oleic acid and linoleic acid, for 24 h, the cell cycle of these cells were examined by flow cytometry. The number of cells in 2N phase was significantly decreased and the number of cells in 4N phase was significantly increased following 24 h treatment with 200 μ M palmitic acid as compared to cells treated with BSA. Both oleic acid and linoleic acid not only protected Neuro-2A cells from palmitic acid-induced cell death, but also restored palmitic acid-induced defects in cell cycle progression. Furthermore, while oleoylethanolamide blocked palmitic acid-induced cell death and cell cycle defects, sulfo-N-succinimidyl oleate (SSO) reduced palmitic acid-induced cell cycle defects without a significant effect on palmitic acid-induced cell death. Delineation of the mechanisms by which fatty acids affect cell cycle progression in neuroblastoma cells will help to understand the mechanisms of obesity-associated pathologies and shed light on potential intervention strategies.

WTH04 Molecular basis of disease (Session B)

WTH04-01

Autocrine/paracrine IGF-1 in DRG neurons drives neurite outgrowth and is suppressed in the diabetic state
R. Aghanoori^{1,2}, D. R. Smith², P. Agarwal^{1,3}, Vernon. W. Dolinsky^{1,3}, V. Yathindranath⁴, D. W. Miller^{1,4}, P. Fernyhough^{1,2}

¹University of Manitoba,, Dept of Pharmacology & Therapeutics,, Winnipeg, Canada

²University of Manitoba, Children's Hospital Research Institute of Manitoba, Winnipeg, Canada

³University of Manitoba, Kleyesen Institute for Advanced Medicine, Winnipeg, Canada

Insulin-like growth factor-1 (IGF-1) declines in serum of diabetic patients and animal models with type 1 and 2 diabetes as disease progresses. We hypothesized that impaired autocrine/paracrine IGF-1 in dorsal root ganglia (DRG) contributed to neurodegeneration in diabetic sensory neuropathy. DRG neuron cultures and tissues from age-matched control or streptozotocin (STZ)-induced type 1 diabetic rats were used for *in vitro* and *in vivo* studies. IGF-1 gene expression in liver and DRG were significantly ($p < 0.05$) lower in diabetic rats vs control. DRG neurons derived from control rats secreted higher IGF-1 levels into the culture media compared to cultures from diabetic rats ($p < 0.05$). IGF-1 mRNA was expressed in neurons of the DRG rather than in glial cells as determined by RNA-FISH and Northern blot analysis. The hyperglycemic state suppressed IGF-1 mRNA in DRG neurons which was relieved by treatment with (10 nM) IGF-1 or an aldose reductase inhibitor, Sorbinil. Bioinformatic screening and chromatin immunoprecipitation revealed NFAT1 and CEBP- β functional binding sites on the IGF-1 promoter in DRG neurons. Either IGF-1 neutralizing antibody or two IGF-1-targeting encapsulated siRNAs (in nanoparticles) downregulated IGF-1 receptor and Akt S473 phosphorylation, and lowered background neurite outgrowth. In conclusion, down-regulation of endogenous IGF-1 in DRG neurons in diabetes may contribute to distal dying-back neurodegeneration and up-regulation of neuronal IGF-1 may be a promising target for therapy. *Funded by CIHR grant # MOP-130282.*

WTH04-02

Lipid dynamics in rat model of vascular dementia by desims imaging
R. C. Parreira¹, D. L. R. Severiano¹, O. C. Oliveira-Lima¹, G. A. Vasconcelos³, B. L. Marques¹, C. H. X. Custodio¹, M. V. Gomez², A. C. O. Pinheiro⁴, R. S. Gomez⁵, B. G. Vaz³, M. C. X. Pinto¹

¹Universidade Federal de Goiás, Instituto de Ciências Biológicas, Goiânia, Brazil

²Santa Casa de Misericórdia, Instituto de Ensino e Pesquisa, Belo Horizonte, Brazil

³Universidade Federal de Goiás, Instituto de Química, Goiânia, Brazil

⁴Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Belo Horizonte, Brazil

⁵Universidade Federal de Minas Gerais, Departamento de Cirurgia, Belo Horizonte, Brazil

Vascular dementia (VD) is a neurodegenerative disease caused by cerebral blood supply problems that profoundly disrupts lipid homeostasis in the brain. The understanding of lipid dynamics in VD could reveal biomarkers and novel molecular targets for pharmacological intervention. In this study, we performed the two-vessel occlusion model (2VO) of Vascular Dementia and use the Desorption Electrospray Ionization - Imaging Mass Spectrometry (DESI-IMS) technique for mapping lipids on rat brain tissues. The brain imaging demonstrated that arachidonic acid, docosahexaenoic acid levels were significantly reduced in the hippocampus and cortex of animals submitted to 2VO model when compared to control animals. Decanoic acid and a diacylglycerophosphate were increased in 2VO model. Partial least squares Discriminant Analysis (PLS-DA) can discriminate between 2VO group and control group, where the ion γ -linolenic acid (m/z 277) and stearic acid (m/z 283) had the highest discrimination potential. Taken together, these findings indicate that lipid dynamics are changed in chronic ischemia induced by 2VO model and γ -linolenic acid is a potential biomarker for Vascular Dementia

WTH04-03

Altered abundance of the epitranscriptomic mark M6A following focal ischemia

A. K. Chokkalla^{1,2}, S. L. Mehta¹, T. Kim¹, B. Chelluboina¹, R. Vemuganti^{1,2,3}

¹University of Wisconsin-Madison, Neurological Surgery, Madison, USA

²University of Wisconsin-Madison, Cell and Molecular Pathology Graduate Program, Madison, USA

³William S. Middleton Veterans Administration Hospital, -, Madison, USA

RNAs can undergo > 100 chemical modifications collectively termed as epitranscriptomic modifications. A most abundant RNA modification in the adult brain is methylation of adenosine at N6 position (N6-methyladenosine; m⁶A) which is shown to modulate synaptic plasticity, cognition, stress response and glioma growth. We currently evaluated the effect of focal cerebral ischemia (stroke) on m⁶A epitranscriptome in adult mouse brain. Transient middle cerebral artery occlusion (MCAO) significantly increased the global m⁶A levels in the peri-infarct area of the ipsilateral cerebral cortex of both male and female mice compared to sex-matched sham controls (by 3 to 4-fold; $n = 5/\text{group}$; $p < 0.05$). In addition, mRNA and protein expression of m⁶A demethylase FTO decreased significantly in the peri-infarct cortex compared to sham (by 50 to 60%; $n = 4/\text{group}$; $p < 0.05$). FTO down-regulation following focal ischemia was observed to be mainly in the NeuN+ neurons than GFAP+ astrocytes and IBA1 + microglia. Whereas, expression of the m⁶A methylases (METTL3, METTL14 and WTAP) remained unaltered following focal ischemia. Microarray profiling of the immunoprecipitated methylated RNA showed that 584 mRNAs and 131 lncRNAs were differentially hypermethylated in the ischemic brain compared to sham control (fold change > 2; $n = 5/\text{group}$; $p < 0.05$). This is a first study that showed that stroke significantly alters cerebral m⁶A epitranscriptome which might have functional implications for the post-stroke pathophysiology.

WTH04-04

Adenosine A_{2A}-dopamine D₂ receptor heteromers in schizophrenia

F. Ciruela¹, M. López-Cano¹, L. Callado², E. Aso¹, F. Contreras³, X. Altafaj¹, M. Masahiko⁴, V. Fernández-Dueñas¹, José. M. Menchón³, M. Valle-León¹

¹*Universitat de Barcelona-IDIBELL, Departament de Patologia i Terapèutica Experimental, Hospitalet de Llobregat, Spain*

²*University of the Basque Country, Department of Pharmacology, Leioa, Spain*

³*Hospital Universitari de Bellvitge-IDIBEL, Servei de Psiquiatria, Hospitalet de Llobregat, Spain*

⁴*Hokkaido University School of Medicine, Department of Anatomy, Sapporo, Japan*

Schizophrenia is a chronic and severe neuropsychiatric disorder with an unknown aetiology. Current treatments are often insufficient to coat all the symptomatology, thus the search for alternative and/or complementary therapies constitutes a big contest in psychiatry. Indeed, adenosine, a well-known neuromodulator in the central nervous system, has been highlighted because of its ability to modulate both dopaminergic and glutamatergic neurotransmission. Here, we studied the relationship between adenosine A_{2A} receptor (A_{2A}R) and dopamine D₂ receptor (D₂R) expression and their impact on psychotic-like behavior in mice. Thus, using a classical pharmacological animal model of schizophrenia, namely the chronic administration of phencyclidine (PCP), we correlated PCP-induced psychotic-like behavior with a significant increase of striatal D₂R expression. Interestingly, A_{2A}R deletion (i.e. A_{2A}R^{-/-} mouse), also associated to psychotic-like behavior, was concomitant with striatal D₂R overexpression. Finally, when the expression of A_{2A}R and D₂R in human caudate necropsies from schizophrenic patients was assessed a significant increase in both receptors was found. Nevertheless, we evaluated the A_{2A}R/D₂R heteromer content in these human necropsies through a new AlphaLISA-based assay and a significant reduction in the A_{2A}R/D₂R heteromer amount was detected in caudate membranes from schizophrenic patients. Overall, it could be postulated that promoting A_{2A}R/D₂R heteromer formation in schizophrenia might constitute an alternative non-dopaminergic strategy for the management of such devastating disease.

WTH04-05

Sex differences in obesity-mediated neuroinflammation and impairment of hypothalamic function

D. Coss

University of California, Riverside, Biomedical Sciences, Riverside, USA

Increase in the prevalence of obesity has coincided with an elevated risk of type II diabetes, cardiovascular disease, cognitive disorders, and reproductive problems. Metabolism and energy balance, and other critical functions such as reproduction, thirst, thermoregulation, and circadian rhythms, are controlled by hypothalamic neurons that secrete neuroendocrine peptides. To analyze mechanisms of obesity-mediated impairment of hypothalamic function, we fed male and female mice high fat diet (HFD) or control diet with equal sucrose levels. Male mice fed HFD exhibit diminished neuroendocrine peptide expression, decreased testosterone and reduced sperm count. Female mice on the other hand are resistant to endocrine changes. Male mice display high levels of

several inflammatory cytokines in the hypothalamus, as well as in circulation. Microglia become reactive following exposure to the HFD, also specifically in the hypothalamus of male mice, but not in the female. Increased numbers were detected juxtaposed to the circumventricular areas of the hypothalamus that contain fenestrated capillaries with a leaky blood-brain barrier. Elevated cytokines affect the expression of hypothalamic neuropeptides, either directly or alternatively via changes in synaptic molecules that regulate activity-dependent gene expression. To determine if ovarian estrogens are important for protection of female mice, we ovariectomized the females and placed them on the HFD. Female mice are protected from inflammatory and neuroendocrine changes regardless of the presence of ovarian hormones. Ovariectomized females gained weight at the same rate as male mice, but failed to show a decrease in neuropeptide expression or an increase in inflammatory markers that we found in obese males. Therefore, contrary to our hypothesis, ovarian estrogens are not necessary for protection in females. Delineating the mechanisms whereby inflammatory and metabolic signals influence hypothalamic function will provide insight into etiology of obesity-mediated disorders.

WTH04-06

Lipid dynamics in LPS-induced neuroinflammation by desi-MS imaging

M. C. X. Pinto¹, O. C. Oliveira-Lima¹, J. Carvalho-Tavares², M. F. Rodrigues¹, M. V. Gomez^{3,2}, A. C. P. Oliveira¹, R. R. Resende², R. S. Gomez², B. G. Vaz¹

¹*Universidade Federal de Goiás, Departamento de Farmacologia, Goiania, Brazil*

²*Universidade Federal de Minas Gerais, Departamento de Fisiologia e Biofísica, Belo Horizonte, Brazil*

³*Instituto de Ensino e Pesquisa Santa Casa de Misericórdia, Departamento de Pesquisa, Belo Horizonte, Brazil*

It is well-established that bacterial lipopolysaccharides (LPS) can promote neuroinflammation through receptor Toll-like 4 activation and induces sickness behavior in mice. This phenomenon triggers changes in membranes lipid dynamics to promote the intracellular cell signaling. Desorption electrospray ionization mass spectrometry (DESI-MS) is a powerful technique that can be used to image the distribution of lipids in the brain tissue directly. In this work, we characterize the LPS-induced neuroinflammation and the lipid dynamics in C57BL/6 mice at 3 and 24 h after LPS injection. We have observed that intraperitoneal administration of LPS (5 mg/kg body weight) induces sickness behavior and triggers a peripheral and cerebral increase of pro- and anti-inflammatory cytokine levels after 3 h, but only IL-10 was up-regulated after 24 h. Morphological analysis of hypothalamus, cortex and hippocampus demonstrated that microglial activation was present after 24 h of LPS injection, but not at 3 h. DESI-MS revealed a total of 14 lipids significantly altered after 3 and 24 h and as well as their neuroanatomical distribution. Multivariate statistical analyzes have shown that ions associated with phosphatidylethanolamine [PE(38:4)] and docosatetraenoic acid [FA (22:4)] could be used as biomarkers to distinguish samples from the control or LPS treated groups. Finally, our data demonstrated that monitoring cerebral lipids dynamics and its neuroanatomical distribution can be helpful to understand sickness behavior and microglial activation after LPS administration.

WTH04-08

ATF4 regulates neuronal death in Parkinson's disease models**M. Demmings, S. Cregan***University of Western Ontario/Robarts Research Institution, Schulich School of Medicine, Neuroscience, London, Canada*

Parkinson's Disease (PD) is characterized by the loss of dopaminergic neurons in the substantia nigra. However, mechanisms underlying this neuronal loss remain largely unknown. ATF4, a key mediator of the Integrated Stress Response (ISR), is a transcription factor that during prolonged activation can induce the expression of several downstream pro-apoptotic target genes. Both oxidative stress and mitochondrial dysfunction are associated with PD and these factors are known to activate the ISR. In this study, we have determined, that both PD neurotoxins (MPP⁺ and 6-OHDA) and pre-formed alpha-synuclein fibrils (PFFs) elicit the ISR and cause sustained upregulation of ATF4 protein in mouse primary cortical and mesencephalic neurons. Furthermore, we have identified that this increase in protein leads to ATF4-dependent transcriptional activation of known pro-apoptotic genes. Importantly, using neurons derived from ATF4 +/+ and ATF4 -/- mice, we have shown ATF4 to be necessary for neuronal apoptosis and that ATF4-deficient dopaminergic neurons display attenuated cell loss following exposure to PD neurotoxins or PFFs. These novel molecular findings highlight ATF4 and the ISR as a potential therapeutic target in PD.

WTH04-09

Relative roles of neurons and astrocytes in glycogen-induced neurodegeneration**J. Duran^{1, 2}, A. Gruart³, José. M. Delgado-García³, Joan. J. Guinovart^{1, 2}**¹*IRB Barcelona, Molecular Medicine Programme, Barcelona, Spain*²*Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas, CIBERDEM, Madrid, Spain*³*Pablo de Olavide University, Division of Neurosciences, Seville, Spain*

Glycogen, a branched polymer of glucose, is the sole carbohydrate reserve in mammals. Glycogen plays key roles in the brain, although it is present at much lower concentrations than in muscle and liver. By characterizing several animal models depleted of brain glycogen, we have shown that this polysaccharide contributes to learning capacity, activity-dependent changes in synapse strength, and susceptibility to epilepsy. However, in some conditions, glycogen is abnormally accumulated in the brain. The most striking example of this is Lafora disease, a fatal neurodegenerative condition that starts as myoclonus epilepsy and proceeds to rapid cognitive deterioration and death. The hallmark of the disease is the presence of high numbers of insoluble glycogen aggregates, known as Lafora bodies (LBs), in the brain. We have demonstrated that the accumulation of this aberrant glycogen accounts for the susceptibility to epilepsy, electrophysiological changes and neurodegeneration observed in models of the disease. Furthermore, and against the general belief that LBs accumulate exclusively in neurons, we have demonstrated that most LBs are deposited in astrocytes. This accumulation of astrocytic glycogen induces neurodegeneration and is involved in the pathology of Lafora disease. Our findings change

the current view of the role of glycogen in the brain, revealing that the accumulation of this polysaccharide in neurons and astrocytes contributes to neurodegenerative diseases.

WTH04-10

Early pathologies in the 3XTG-ad mouse model of Alzheimer's disease**M. Fahnstock¹, A. Shekari¹, R. Hatkar¹, J. Teeling⁴, P. Forsythe², D. Ma³, T. Florica¹, M. Kapadia¹, B. Sakic¹**¹*McMaster University, Dept. of Psychiatry & Behavioural Neurosciences, Hamilton, Canada*²*McMaster University, Dept. of Pathology & Molecular Medicine, Hamilton, Canada*³*McMaster University, Dept. of Medicine, Hamilton, Canada*⁴*University of Southampton, Centre for Biological Sciences, Southampton, UK*

Triple-transgenic (3xTg-AD) mice are known for learning/memory deficits and AD-like pathology. Recent cohorts of one-year-old 3xTg-AD males no longer show plaque/tangle deposition, yet they exhibit autoimmune manifestations and low brain weight of unknown etiology. In this study, we investigated the time course of neuromorphological changes and the development of systemic autoimmunity. Planimetric brain analysis, microglial activation and immunoglobulin deposition were measured in brain slices from one-year-old and two-month-old male 3xTg-AD mice and wild-type controls. Immunological changes were measured in mice at 2 weeks, 2 months and 1 year of age. Embryonic neurons were grown in microfluidic chambers for 7 days before measuring axon length. Total brain area was smaller in 3xTg-AD mice than in controls. Even after differences in total brain area were accounted for, 3xTg-AD mice exhibited smaller hippocampal dentate gyrus area and thinner piriform cortex. Enhanced microglial activation was accompanied by reduced hippocampal immunoglobulin deposition in 1-year-old 3xTg-AD mice. Immunological changes were apparent even in the second week of postnatal life. Surprisingly, embryonic 3xTg-AD neurons exhibited decreased axon length compared to controls. Immunological changes suggesting neuroprotective autoimmunity occur in 3xTg-AD mice as early as two weeks after birth. However, impaired axon outgrowth in embryonic neurons suggests the influence of either the maternal immune system and/or genetic background. These results may provide a valuable preparation for understanding the role of the immune system in curbing AD-like disease.

WTH04-11

Exploring trafficking mechanisms promoting inhibitory synapse down-regulation during cerebral ischemia**J. Garcia, S. Gookin, N. Quillinan, K. Smith***The University of Colorado - Anschutz Medical Campus, Pharmacology, Denver, USA*

GABAergic synaptic transmission is essential to maintain proper neuronal excitability. GABA_A receptors (GABA_ARs) are the major mediators of synaptic inhibition in the brain and are clustered by the scaffold, gephyrin, opposite GABAergic terminals. Inhibitory synapses are extremely plastic and can modify their shape, size and strength in response to multiple forms of stimulation, thereby shifting neuronal excitatory/inhibitory balance. These shifts can alter

long-term neuronal excitability and function, a common feature in numerous brain disorders including ischemia. During cerebral ischemia, inadequate blood flow to the brain leads to oxygen and glucose deprivation (OGD), which contributes to (i) delayed cell death of vulnerable neuronal populations and (ii) alterations to the long-term excitability and function of surviving populations. Previous work reports down-regulation of GABA_ARs and other inhibitory synaptic proteins following OGD, but the mechanisms responsible for GABAergic synapse down-regulation remains undefined. Here we investigate whether signaling from over excitable excitatory synapses leads to the depression of GABAergic synapses during OGD. Stimulation of NMDA receptors throughout long-term potentiation (LTP) is known to cause depression of nearby inhibitory synapses in a calcineurin (CaN) dependent manner. We show glutamatergic receptor activation and calcium influx is important for down-regulating GABAergic synapses following OGD. Specifically, CaN activation plays a role in synaptic GABA_AR down-regulation by receptor declustering but has no role in gephyrin down-regulation during OGD. Further, depression of GABA_ARs is mediated by receptor endocytosis, which is regulated by receptor phosphorylation. This data suggests that GABA_AR down-regulation is mediated by receptor declustering and endocytosis during cerebral ischemia.

WTH04-12

The ADNP fragment NAP (CP201) corrects synapse density/brain cognitive plasticity in the autism ADNP deficient mouse

I. Gozes

Tel Aviv University, Dept Human Molecular Genetics and Biochemistry, Tel Aviv, Israel

Activity-dependent neuroprotective protein (ADNP) was cloned and characterized twenty years ago as a secreted glial protein providing potent neuroprotection. A small 8-amino-acid ADNP fragment, NAP, was then discovered, as mimicking and enhancing the endogenous ADNP neuroprotective activity. Our mouse studies revealed that ADNP is essential for brain formation and cognitive functions. Now, *de novo* mutations in *ADNP* were identified as causing an autism-like syndrome, namely the *ADNP* syndrome, making ADNP as one of the leading autism/intellectual disability causing genes. Furthermore, original *in vitro* studies from our lab showed that the ADNP drug candidate NAP binds to microtubule end binding proteins (EBs) through its SxIP motif, to regulate dendritic spine formation. These results now extend to an *in vivo* model, the *Adnp*-deficient (heterozygous) mouse. This mouse exhibits altered synapse density (hippocampus and cerebral cortex, encompassing mushroom, stubby and thin dendritic spines) paralleled by developmental, motor, and cognitive deficits, which were essentially corrected by NAP treatment. Strikingly, the observed impairments in the *Adnp*-deficient mouse correspond to the human *ADNP* syndrome, characterized by global developmental delays, intellectual disabilities, speech impediments and motor dysfunctions. Taken together, a better understanding of the *ADNP* syndrome is provided paving the suggesting NAP (CP201) for further clinical development. Activity-dependent neuroprotective protein deficiency models synaptic and developmental phenotypes of autism-like syndrome.

WTH04-13

Erythropoietin regulates lifeguard protein family members grina and faim2 after transient cerebral ischemia

P. Habib¹, A. Reich¹, J. B. Schulz^{1,2}

¹Medical School, RWTH Aachen University, Department of Neurology, Aachen, Germany

²JARA-BRAIN Institute of Molecular Neuroscience and Neuroimaging, Forschungszentrum Jülich GmbH and RWTH Aachen University, Aachen, Germany

Background and Purpose: Erythropoietin (EPO) confers potent neuroprotection against cerebral ischemia through a variety of biochemical mechanisms. Lifeguard (LFG) protein family members exert inhibitory activities in apoptosis and necroptosis. FAIM2 (LFG2) is neuroprotective against murine focal ischemia and is regulated by EPO. Similar to FAIM2, GRINA (LFG-1) is predominantly expressed in the brain. The role of GRINA transient brain ischemia, its potential synergistic effects with **FAIM2** and its regulation by EPO treatment were assessed.

Methods: We performed middle cerebral artery occlusion (MCAo) for 30 min followed by 72 h of reperfusion in GRINA-deficient (GRINA^{-/-}), FAIM2-deficient (FAIM2^{-/-}), double-deficient (GRINA^{-/-}FAIM2^{-/-}) and wild-type littermates (WT) mice. We administered EPO or saline 0, 24 and 48 h after MCAo. We subjected primary murine cortical neurons (pMCN) of all mouse strains to oxygen-glucose deprivation (OGD) after GRINA and/or FAIM2 gene transfection.

Results: Compared to wild-type controls GRINA^{-/-} had a similar increase in infarct volumes as FAIM2^{-/-} ($p < 0.01$). We observed the highest neurological deficits and largest infarct sizes in double-deficient mice. EPO administration up-regulated GRINA and FAIM2 mRNA levels in wild-type littermates. EPO decreased infarct sizes and abrogated neurological impairments significantly in wild-type controls. GRINA and/or FAIM2 deficient mice showed increased expression levels of cleaved-caspase 3 and of pro-apoptotic BAX. Further, caspase 8 was up-regulated in FAIM2^{-/-} and caspase 9 in GRINA^{-/-} mice. Overexpression of GRINA and FAIM2 in wild-type and in double deficient pMCN significantly decreased cell death rate after OGD.

Conclusions: GRINA and FAIM2 are highly expressed in the brain and convey EPO-mediated neuroprotection after ischemic stroke involving different caspases.

WTH04-14

The role of methylglyoxal, a metabolite accumulating in type II. diabetes, in the central component of chronic diabetic pain

Z. Hegyi, K. Docs, Z. Meszar, M. Sivado, P. Szucs

University of Debrecen, Department of Anatomy, Histology and Embryology, Debrecen, Hungary

The pathomechanism of chronic pain in diabetes mellitus can easily be explained by the development of peripheral polyneuropathy. However, disturbances of glucose metabolism in type II diabetes leads to the accumulation of various bioactive metabolites, such as methylglyoxal (MGO). As MGO may induce centrally-restricted effects, here we investigated how MGO influences the normal function of glial cells and neurons in

the spinal dorsal horn, where primary pain processing takes place.

MGO increases calcium signaling in, and induces the release of TNF α and IL-1b from, primary cultured spinal astrocytes and microglial cells. Intrathecal administration of MGO not only decreases the paw withdrawal threshold in rats, but also increases the number of reactive astrocytes and microglia, and the amount of TNF α and IL-1b in the spinal dorsal horn. Bath application of MGO evokes calcium transients in astrocytes and simultaneously, slow inward currents in interneurons in the spinal dorsal horn.

Our results indicate that MGO induces reactive spinal astrocytes and microglial cells which release inflammatory cytokines and lead to the development of neuroinflammation. In addition, MGO indirectly induces a slow depolarization in spinal dorsal horn neurons, an effect most probably dependent on gliotransmission. These mechanisms may be sufficient to induce a peripheral neuropathy-independent central sensitization, leading to allodynia and hyperalgesia in type II. diabetes.

WTH04-15

TAU in marmoset brains, its isoform expression and phosphorylation

S. Hisanaga¹, G. Sharma¹, A. Huo¹, T. Kimura², S. Shiozawa³, R. Kobayashi³, N. Sahara², M. Ishibashi⁴, S. Ishigaki⁴, T. Saito¹, K. Ando¹, S. Murayama⁵, M. Hasegawa⁶, G. Sobue⁴, H. Okano³

¹Tokyo Metropolitan University, Biological Sciences, Hachioji, Japan

²National Institute of Radiological Sciences, Functional Brain Imaging Research, Chiba, Japan

³Keio University School of Medicine, Physiology, Tokyo, Japan

⁴Nagoya University Graduate School of Medicine, Dementia and Neurodegenerative Disease, Nagoya, Japan

⁵Tokyo Metropolitan Institute of Gerontology, Brain bank, Tokyo, Japan

⁶Tokyo Metropolitan Institute of Medical Science, Dementia and Higher Brain Function, Tokyo, Japan

Tau is a microtubule-associated protein, expressed predominantly in axons of neurons. Hyperphosphorylated tau is a major component of neurofibrillary tangles (NFTs), a pathological hallmark in Alzheimer's disease (AD). Aggregates of hyperphosphorylated tau are also detected in many neurodegenerative diseases, those are collectively called "tauopathy". Mutations of tau are a cause of frontotemporal dementia and Parkinsonism linked to chromosome 17 (FTDP-17). Transgenic mice with mutant tau were generated as a model of tauopathy but primate animals are expected to be a preferred model close to human. Common marmoset is poised as a non-human primate model for age-dependent neurodegenerative diseases. However, there are no biochemical studies on tau in marmoset brains. Here, we investigated the expression of tau isoforms and phosphorylation, two important properties of the tau protein. Unexpectedly marmoset tau did not have the "primate-unique motif" in the N-terminal region. We found that marmoset tau resembled to mouse tau in isoform expression rather than human tau; while three-repeats tau was expressed in neonatal marmoset as well as human and mouse, four-repeats tau alone was detected in adult marmoset different from human tau. Tau in newborn marmoset brains was phosphorylated at AT8, AT180 and PHF-1 AD pathological sites but the reactivities were lost with tau in adult marmoset brains whereas Ser202 in AT8 site and Ser404 in PHF-1

site were phosphorylated. The present results provide useful information on tau expression and phosphorylation in brain of marmoset, which would be used as a primate model of neurodegenerative diseases.

WTH04-16

Expression analysis of astrocyte-related receptors in epilepsy lesions

M. Itoh

National Center of Neurology and Psychiatry, Mental Retardation and Birth Defect Research, Kodaira, Japan

Epilepsy is one of the major neurologic diseases, and astrocytes play important roles in epileptogenesis. To investigate possible roles of astrocyte-related receptors in patients with intractable epilepsy associated with focal cortical dysplasia (FCD), hippocampal sclerosis (HS) and other conditions, we examined resected epileptic foci from 51 patients, including 23 FCD (including type I, IIa, and IIb), 20 HS, 5 tuberous sclerosis complex, and 3 low-grade astrocytoma. Control samples were from 21 autopsied brains of patients without epilepsy or neurologic deficits and 5 patients with pathologic gliosis without epilepsy. Immunohistochemical and immunoblot analyses with antibodies against purinergic receptor subtypes P2RY1, P2RY2, P2RY4, potassium channels of Kv4.2 and Kir4.1, and metabotropic receptors of mGluR1 and mGluR5 were performed. Anti-gial fibrillary acidic protein (GFAP), anti-NeuN, and anti-CD68 immunostaining was used to identify astrocytes, neurons, and microglia, respectively. Most GFAP-immunopositive astrocyte cells in the brain samples from patients with epilepsy were P2RY1-, P2RY2-, P2RY4-, Kv4.2-, Kir4.1-, mGluR1-, and mGluR5-positive, whereas samples from controls and pathologic gliosis showed lower expression levels of these astrocyte-related receptors. The findings suggest that, although these receptors are necessary for astrocyte transmission, formation of the neuron-glia network, and other physiologic functions, overexpression in the brains of patients with intractable epilepsy may be associated with activation of intracellular and glio-neuronal signaling pathways that contribute to epileptogenesis.

WTH04-17

Ouabain protects cortical neurons from hyperhomocysteinemia induced neurotoxicity

M. Ivanova, P. Abushik, D. Sibarov, A. Kokorina, S. Antonov

Sechenov Institute of Evolutionary Physiology and Biochemistry Russian Academy of Sciences, Comparative Neuroscience, St. Petersburg, Russia

Ouabain is a cardiac glycoside, which was previously shown to induce neuroprotection in excitotoxic stress caused by ionotropic glutamate receptor agonists. Homocysteine (HCY) is an inherent agonist of ionotropic NMDA receptors in the brain. Excessive HCY accumulation in CSF (hyperhomocysteinemia) results in neurotoxic effects. Here we study the mechanisms of ouabain induced neuroprotection in cultured cortical neurons treated with high 100 μ M L-HCY (corresponds to severe hyperhomocysteinemia). HCY treatment of neurons for 4 h decreased neuronal survival to $56 \pm 3\%$ with $19 \pm 2\%$ apoptotic and $25 \pm 4\%$ necrotic cells. When ouabain at 0.1-1 nM was co-applied, the fraction of live cells did not

significantly decrease during HCY treatment. Incubation in HCY for 24 h resulted in $49 \pm 3\%$ of apoptotic neurons and did not increase necrosis. Ouabain at 0.1–1 nM prevented neuronal death during HCY treatment so that the ratio of live, necrotic and apoptotic neurons was similar to untreated cultures or control values. In addition, under these particular conditions the number of neurons that are immunopositive for antiapoptotic protein Bcl-2 remained similar to the control value. In further experiments we studied intracellular signaling pathways that are triggered by Na(+)/K(+)-ATPase to induce ouabain neuroprotective effects. With this aim the inhibitors of protein kinase-A (PKA), CaM-kinase-II (CaMKII) and protein kinase-C (PKC) were tested as possible inhibitors of the ouabain effects. An inhibition of PKA, CaMKII or PKC did not change the ouabain induced neuroprotection during 4 h incubation with HCY. However PKC, but not PKA or CaMKII inhibition during 24 h HCY treatment prevented ouabain effect on neuronal survival. Thus, protein kinase-C mediated signaling pathway is involved in ouabain induced neuroprotection. Supported with Russian Science Foundation grant #16-15-10192.

WTH04-18

Memory deficits and plasticity genes in a rat model of Alzheimer disease

D. Jerusalinsky¹, S. DoCarmo², V. Baez¹, V. Bercowicz¹, C. Sister¹, C. Cuello²

¹School Medicine, Univ. Bs as, IBCN, LaN&N, Buenos Aires, Argentina

²McGill University, Dept Pharmacology and Therapeutics, Montreal, Canada

McGill-R-Thy1-APP Wistar-transgenic (Tg) rats, bearing human amyloid precursor protein with Swedish and Indiana mutations of familial Alzheimer's disease (AD), are suitable for testing learning and memory at AD onset. Three month old (mo) Homozygous Tg rats show cognition deficits; human amyloid- β (A β) accumulates from first week, developing extracellular amyloid pathology in 6–9 mo animals. Hemizygous Tg (He) does not develop extracellular plaques even at 20 months. Three, 4 and 6 mo He male rats and their wild-type litter-mates (WT) explored an open field (OF) for 5 min. When tested 24 h later (long-term-memory, LTM), denoted habituation to the environment. Same He and WT rats were trained for object recognition (OR) and inhibitory avoidance (IA) of a mild foot-shock. All groups discriminated new versus known object 1 h later (short-term-memory), but 4 and 6 mo He did not show OR-LTM neither IA-LTM. Then, some plasticity genes were investigated in 4mo He rats. There were no significant differences for PSD95, Arc, GluR1 AMPA receptor or NR1- and NR2A-NMDA receptor subunits; though NR2B and CaMKIIb mRNAs levels were significantly higher at the hippocampus, suggesting an expression increase. These results strongly suggest that deficits in certain LTM develops from 4 months. Further investigation is necessary to interpret hippocampal CaMKIIb and NR2B mRNAs increase, which could be due to intracellular Ca²⁺ rise following overstimulation by A β /A β oligomers.

WTH04-19

Increased seizure sensitivity in PHD finger protein 24 (Phf24)-knockout rats

M. Kato¹, N. Kunisawa¹, S. Shimizu¹, K. Kawakita¹, S. Kawaji¹, Higor. A. Iha¹, T. Kuramoto^{2, 3}, T. Mashimo^{2, 4}, T. Serikawa^{1, 2}, Y. Ohno¹

¹Osaka Univ. Pharm. Sci., Dept. Pharmacol., Osaka, Japan

²Kyoto Univ., Inst. Lab. Anim., Kyoto, Japan

³Tokyo Univ. Agric., Dept. Anim. Sci., Kanagawa, Japan

⁴Osaka University, Inst. Exp. Anim. Sci., Osaka, Japan

PHD finger protein 24 (Phf24), also known as G α_i -interacting protein (GINIP), interacts with G α_i subunit and facilitates GABA_B receptor-mediated functions (e.g., analgesia). We previously demonstrated that the expression of Phf24 was markedly down-regulated in Noda epileptic rat (NER), a genetic rat model of epilepsy (Behav. Genet., 47, 609, 2017). However, the role of Phf24 protein in the central nervous system (CNS), especially its function in modulating epileptogenesis, has not been studied in detail. Here, to clarify the function of Phf24 in regulating seizure susceptibility, we analyzed behavioral phenotypes of the newly developed Phf24-knockout (KO) rats. Seizure susceptibility of Phf24-KO rats was assessed using chemically- and electrically-induced seizure tests. Behavioral score and incidence of seizures induced by pentylenetetrazole (PTZ, 30–40 mg/kg) were significantly increased in Phf24-KO rats than in control (F344) rats. Phf24-KO rats also showed higher sensitivity to electrical shock-induced seizures. In addition, kindling development with repeated PTZ treatments (30 mg/kg/day, 10 days) was significantly facilitated by the Phf24-KO. Furthermore, immunohistochemical analysis of c-Fos expression, a biological marker of neural excitation, revealed that Phf24-KO rats showed a significantly higher Fos expression than control animals in the cerebral cortex, amygdala, hippocampus and thalamus. These results suggest that Phf24 play an important role in controlling the susceptibility to epileptic seizures, which is probably involved in epileptogenicity of NER.

WTH04-20

Exploring the role of post translational modifications for APLNR in the mouse central nervous system

T. Kinjo¹, S. Ebisawa¹, T. Nokubo¹, H. Higashi¹, K. Uno¹, S. Maeda², N. Kuramoto¹

¹Setsunan University, Molecular pharmacology, Pharmaceutical Sciences, Hirakata, Japan

²Setsunan University, Pharmacotherapeutics, Pharmaceutical Sciences, Hirakata, Japan

Apelin receptor, APLNR, is a member of G protein coupled receptor related to angiogenesis, blood pressure and even neuroprotection. The amino acid sequence suggests that APLNR has two N-glycosylation sites. Here, we have detected the APLNR proteins which was overexpressed in HEK293TN (HEK-APLNR) cells, or in the central nervous system of the mouse. APLNR in the HEK-APLNR cells was quite heat labile and disappeared even at boiling. APLNR-like immunoreactivities were localized not in the plasma membrane but broadly in the cytosol. Western blot analysis revealed that, there were two bands around the desired molecular weight of APLNR. Among of the two, the band with slow mobility, was disappeared by the sample incubation with Peptide-N-Glycosidase F (PNGase F) for cleaving N-glycosylation sites. On the other hand,

when attempting to detect APLNR in the mouse spinal cord, multiple bands at approximately 10 kDa greater than the desired molecular weights were detected. Among of them, the band with a slow mobility, was disappeared by PNGase F, while it still had higher molecular weight. These findings suggest that multi-post translational modifications, not only N-glycosylation, but isopeptidation etc. might be required to form the functional APLNR in the central nervous system.

WTH04-21

Beneficial effects of sound exposure on auditory cortex development in a mouse model of fragile x syndrome

A. Kulinich¹, S. Reinhard², M. Rais¹, J. Lovelace², D. Binder¹, K. Razak², I. Ethell¹

¹University of California, Riverside, Biomedical Sciences, Riverside, USA

²University of California, Riverside, Psychology, Riverside, USA

Fragile X syndrome (FXS) is the most common genetic cause of autism and intellectual disability. *Fragile X mental retardation (Fmr1)* gene knock out (KO) male mice display core deficits of FXS, including abnormally increased sound-evoked responses, and show delayed development of parvalbumin (PV) cells in the auditory cortex. However, it remains unknown how sound deprivation or enrichment during development may affect auditory hypersensitivity. Here, we report that *Fmr1* KO male mice raised under a sound-attenuated environment showed impaired PV cell development in the auditory cortex similar to mice raised in the regular vivarium. In addition, increased dendritic spine density in L2/3 and L5/6 excitatory neurons and enhanced N1 amplitude of event related potentials (ERPs) were observed in the auditory cortex of sound-attenuated *Fmr1* KO compared to WT. In contrast, developmental exposure of mice to 14 kHz tones (5 Hz repetition rate) increased density of PV cells and reduced sound-evoked ERP amplitude in *Fmr1* KO to WT levels. Finally, TrkB phosphorylation was reduced in the auditory cortex of sound-attenuated *Fmr1* KO but was enhanced in sound-exposed *Fmr1* KO, suggesting that BDNF-TrkB signaling may be regulated by sound exposure to influence PV cell development. Interestingly, while hyperactivity was observed in *Fmr1* KO male mice raised in the regular vivarium, both sound-attenuated and sound-exposed *Fmr1* KO mice showed WT-like activity levels. Together, our results demonstrate that acoustic exposure, but not attenuation, during early developmental window restores molecular, cellular and functional alterations in the auditory cortex of *Fmr1* KO mice to WT levels.

WTH04-22

Effects of neonatal hypoxia on the development of serotonergic innervation and cognitive functions

K. Lee^{1, 2}, B. Chattopadhyaya^{1, 2}, G. D. Cristo^{1, 2}

¹Université de Montréal, Neuroscience, Montréal, Canada

²CHU Ste. Justine Research Center, Neuroscience, Montréal, Canada

Perinatal hypoxia is caused by prolonged oxygen deprivation to a newborn infant during birth. Although many studies have focussed on understanding the mechanisms underlying the neurological outcome following severe hypoxia, the specific cellular and network

changes caused by mild perinatal hypoxia (MPH) are still elusive. However, this is an important issue since recent studies suggest children who experience MPH show subtle cognitive deficits and behavioral problems such as impaired working memory and episodic long-term memory capacity, and social and attentional deficits. Serotonin (5-HT) has long been known to be important for both behavioural and cognitive functions such as learning, memory and social preference. Whether the dysregulations of the 5-HT system contribute to MPH-induced neurological problems has been left largely uncharted. To date, the only studies that investigated the association between 5-HT system and neonatal hypoxia have only used severe hypoxia-ischemic models and have completely ignored the sex variable. We have recently established a mouse model of MPH, which show long-term deficits in social behaviour, working memory and episodic memory. To investigate whether MPH has any effects on the development of the 5-HT system, we characterized 5-HT expression levels and 5-HT innervation in prefrontal (PFC) and auditory cortices of both female and male MPH mice and control littermates. Preliminary data suggest that both 5-HT expression and 5-HT innervation complexity are reduced in adult MPH mice specifically in the PFC. Identifying the effects of MPH on 5-HT system development and function will lead towards a better understanding of how cognitive impairments associated with MPH occur and may pave a path toward the development of pharmacological strategies for the treatment of the cognitive abnormalities observed in children that experienced MPH.

WTH04-23

A bioid approach to identify the interactome of the orphan nuclear receptor NUR77 (NR4A1)

D. Levesque¹, F. Dodat¹, D. Cotoir-White^{1, 2}, S. Mader²

¹University of Montreal, Faculty of pharmacy, Montreal, Canada

²University of Montreal, IRIC, Montreal, Canada

Involvement of Nur77 (NR4A1), a member of the nuclear receptor family, in dopaminergic neurotransmission and Parkinson Disease is well documented. We showed that ectopic Nur77 expression in the *substantia nigra* is involved in dopaminergic cell loss in rat models. However, the putative mechanism of action of Nur77-dependent dopamine cell loss remains unexplored. We propose to identify Nur77 interactome using a proximity-dependent biotin identification (BioID) approach. Our BioID utilizes a new generation of ascorbate peroxidase (APEX2) that is fused in N- and C-terminal to Nur77, and a fluorescent protein (ametrine) in self-cleavable bicistronic constructs. When expressed in living cells and stimulated with biotin-phenol, these BioID-fusion constructs biotinylate interacting endogenous proteins. Following biotin-affinity capture, biotinylated proteins can be identified using mass spectrometry. First, we generated the constructs and confirmed, in HEK293 cells, that Nur77-APEX2 fusion protein did not alter the transcriptional activity of Nur77 or that APEX2 is still active. Finally, we performed proteomic analysis of Nur77 interactors in HEK293 cells with both constructions. We identified a total 162 biotinylated proteins with high probability scores (> 95%). Interestingly, 126 biotinylated proteins were present with both APEX2-Nur77 and Nur77-APEX2 constructs. Moreover, most abundant and significant pathways enriched included nuclear, gene expression and DNA binding related proteins (String program). Finally, we also identified some proteins already known to interact with Nur77 (PARP1, TRIM28). This newly developed assay will be used to

identify the interactome of Nur77 in different cellular contexts (e.g., after exposure to a neurotoxin) to better understand the mechanism of action of Nur77 in dopamine cell loss in Parkinson's disease models.

WTH04-24

SHRNA-dependent generation of single NF1 transcript glioma cell lines reveals isoform-specific functions

D. Mangoura, C. Peta, E. Tsirimonaki, S. Mylonas, D. Samouil

Biomedical Research Foundation of the Academy of Athens, Neurosciences Center, Athens, Greece

Neurofibromatosis-1 (NF-1), caused by mutations in the *NF1* gene, is the most commonly inherited cancer predisposition syndrome, presenting as low grade gliomas in children, while adults have a 5-fold risk for glioblastoma. The weak phenotype-genotype association plus lack of therapies emphasize the need to understand the function of the NF1 gene product, the RasGAP neurofibromin, and in particular of its isoforms. These derive from alternative splicing of exon31 in the corresponding RasGAP activity domain, GRD, or of exon51 that bears a functional NLS. These editings may produce as many as 4-transcripts [GRDI/II x NLS/ΔNLS] per cell and thus as many as 4-neurofibromins. Using SF268 glioblastoma cells as a model and employing shRNA approaches (pINDUCER tet-inducible lentiviruses), we have now generated stable cell lines that express specified *NF1* transcripts. Having estimated (qPCR) percentages of all 4-*NF1* transcripts, we currently study cell lines that have lost both GRDII transcripts (NLS or ΔNLS), thus expressing only NLS or ΔNLS GRDI transcripts, and both NLS transcripts (GRDI and II), now expressing only GRDI or GRDII-ΔNLS transcripts. Each isoform or combination of isoforms imposed distinct morphologies, as established with confocal imaging of F-actin showing extensive remodeling of stress fibers and focal adhesions, with concurrent changes in F-actin binding proteins expression, most notable paxillin, as well as gains in vimentin and in proliferation rates. More importantly, after we verified the previously shown higher RasGAP activity of GRDI over GRDII transcripts, we now establish for the first time that NLS transcripts confer differential regulation on Ras activation temporal and amplitude profiles, thus impacting the biological outcome of growth factors.

WTH04-25

Beta-hydroxybutyrate attenuates the unfolded protein response and stimulates the autophagic flux after cerebral ischemia

L. Massieu¹, T. Montiel¹, C. Geronimo^{1, 2}, S. Flores¹, B. Bernal¹, Y. Heras¹, L. Tovar¹

¹*Universidad Nacional Autonoma de Mexico, División de Neurociencias. Neuropatología Molecular, México, México*

²*Center for Integrative Biology, Faculty of Sciences. Universidad Mayor., Santiago, Chile*

Ischemic stroke is a leading cause of disability worldwide and among the third and the fifth cause of death. To date there is no effective treatment against ischemic brain injury. Treatment with the ketogenic diet or the ketone body beta-hydroxybutyrate (BHB), has shown protection against ischemic brain damage. However, the

mechanisms involved are still not clear. The accumulation of unfolded proteins can contribute to ischemic injury due to prolonged activation of the unfolded protein response (UPR). Defective autophagy also contributes to ischemic damage. We have investigated whether protection by D-BHB infusion correlates with diminished UPR activation and stimulation of autophagy. Results show that 24 h after the occlusion of the medial cerebral artery, the RNA-like ER kinase (PERK) pathway of the UPR is activated, as eif2a phosphorylation and the abundance of ATF4 and CHOP transcription factors increase in the ischemic core and the penumbra. Increased transformation of LC3-I to LC3-II but no reduction in p62, was observed indicative of impaired autophagic degradation. In D-BHB-treated animals the activation of the PERK pathway was inhibited, which correlated with decreased LC3-II and p62 content suggesting the stimulation of the autophagic flux. D-BHB-treated rats showed a reduction of the lesion size, the number of degenerating cells and the activation of caspase-12. Results suggest that D-BHB post-treatment attenuates the activation of the UPR and sustains protein homeostasis during ischemic reperfusion, due to the stimulation of the autophagic flux. This work was supported by IN205416 PAPIIT-UNAM and CONACYT grants to LM.

WTH04-26

Targeting sphingosine-1-phosphate signaling to treat heart failure-induced memory deficits

A. Meissner^{1, 3}, F. Uhl¹, D. Lidington², F. Matthes¹, L. Vanherle¹, S.-S. Bolz²

¹*Lund University, Department of Experimental Medical Science, Lund, Sweden*

²*University of Toronto, Department of Physiology, Toronto, Canada*

³*Lund University, Wallenberg Center for Molecular Medicine, Lund, Sweden*

Medical advancements significantly improved survival rates for heart attacks. Yet, this extension in longevity comes at a cost as heart failure (HF) chronically reduces cerebral perfusion. This is frequently accompanied by cognitive decline. Statistically, cognitive impairment is 5× more prevalent in HF patients relative to the age-matched general population. Due to lacking knowledge regarding underlying mechanisms, no curative treatments or strategies for HF-associated cognitive decline exist. We discovered that HF associates to augmented signaling of sphingosine-1-phosphate (S1P) in several tissues including the brain. During HF, such enhanced S1P signaling roots from dysfunctional cystic fibrosis transmembrane regulator (CFTR) expression and activity, which we identified as critical regulator of S1P degradation. Imbalances in S1P homeostasis were linked to several processes relevant for normal brain functioning, including vascular and immune responses and synaptic function. We set out to investigate the therapeutic potential of a novel molecular target linking alterations in cerebral sphingosine-1-phosphate (S1P) signaling to neurological deficits during experimental HF. In a mouse model of chronic HF, we tested if correcting the HF-induced reduction of CFTR expression improves neuronal integrity by normalizing specific S1P signaling in the brain (i.e., regulation of cerebral blood flow). Our data demonstrate the therapeutic potential of CFTR corrector compounds to improve HF-associated memory deficits by normalizing cerebral blood flow and recovering neuronal integrity (i.e., dendrite morphology and dendritic spine density).

Our results put forward a new mechanistic understanding of HF-associated brain complications and leave ground to consider CFTR correctors as potential treatment options for HF-associated cognitive decline.

WTH04-27

The cholesterol ester transfer protein (CETP) in cholesterol homeostasis in the brain

L. Münter¹, F. Oestereich¹, S. Efre¹, E. Yang², P. Chaurand²

¹McGill University, Department of Pharmacology and Therapeutics, Montreal, Canada

²Université de Montréal, Department of Chemistry, Montreal, Canada

Objectives: CETP has been linked to Alzheimer's disease through epidemiological studies, but has never been investigated on the molecular level in the context of Alzheimer's disease. Interestingly, the major difference in the cholesterol metabolism between humans and mice is the lack of CETP in the latter. Our objective was to determine if a humanized cholesterol metabolism in CETP transgenic mice affects cerebral cholesterol levels and the transcriptional profile.

Methods: CETP transgenic mice received either a standard or a 1% (w/w) high cholesterol diet *ad libitum* between 3 to 6 months of age. Brain cholesterol levels were determined using matrix-assisted laser desorption/ionization imaging mass spectrometry. Transcriptional profile was determined by microarray. Protein and mRNA expression levels were determined by standard biochemical analyses.

Results: CETP transgenic mice possessed up to 30% more cholesterol in the brain than wild-type mice. The blood-brain-barrier in those mice is, however, intact. To investigate if such cholesterol derives from synthesis, we purified astrocytes from adult CETP transgenic mice versus controls and analyzed the mRNA expression profile. In contrast to our expectations, genes involved in cholesterol synthesis were down regulated. Instead, we observed an activation of EP4 and gamma-secretase signaling pathways.

Conclusion: We conclude that CETP expression elevates cerebral cholesterol levels and as a consequence stimulates gamma-secretase activity. We hypothesize that CETP activity will stimulate amyloid-beta production.

WTH04-28

Pharmacological regulation of the amyloid-degrading enzyme neprilysin after prenatal hypoxia

N. Nalivaeva^{1, 2}, D. Vasilev^{2, 3}, N. Dubrovskaya^{2, 3}, O. Alekseeva², D. Kozlova², E. Kochkina², N. Tumanova², A. Turner¹, I. Zhuravin^{2, 3}

¹University of Leeds, School of Biomedical Sciences, Leeds, United Kingdom

²Institute of Evolutionary Physiology and Biochemistry, RAS, Physiology and Pathology of CNS, Saint Petersburg, Russia

³Saint-Petersburg State Pediatric Medical University, Research Institute, Saint Petersburg, Russia

The neuropeptidase neprilysin (NEP) is the major amyloid-degrading enzyme in the brain and periphery and a therapeutic target in Alzheimer's disease. As we have shown NEP expression and activity in rat cortex (Cx) and hippocampus (Hip) decrease

with age and are reduced in rat offspring subjected to prenatal hypoxia (PH). Following our cell culture studies, we analyzed the effect of various compounds on NEP expression and activity in the brain and blood of male Wistar rats subjected to PH (E14, 7% O₂, 3 h). Administration (*i.c.* or *p.o.*) of a caspase inhibitor Ac-DEVD-CHO (Ac-D), a histone deacetylase inhibitor valproic acid (VA) or an antioxidant epigallocatechin gallate (EGCG) increased NEP mRNA, protein levels and activity in the Cx, Hip or blood serum which were reduced after PH. Ac-D administration was accompanied by restoration of the APP C-terminal fragment, AICD, levels while VA injections correlated with reduced HDAC binding and increased AICD occupancy at the NEP gene promoter. In all paradigms, increased NEP expression and activity were accompanied by improved performance in memory tests, which correlated with an increased number of dendritic spines in the Cx or Hip. Further studies of NEP and other amyloid-degrading enzymes regulation will help to design possible strategies for prevention of neurodegeneration and cognitive deficits caused by various insults and A β accumulation. Supported: RFBR (19-015-00232), Russian state budget (AAAA-A18-118012290373-7).

WTH04-29

Pretreatment with MSO attenuates behavioral but not electrographic seizures elicited by pilocarpine in juvenile rats

M. Pawlik¹, M. Obara-Michlewska¹, S. J. Czuczwar², J. Łuszczki², A. Acewicz³, T. Wierzb-Bobrowicz³, M. Kołodziej⁴, M. Popek¹, J. Albrecht¹

¹Mossakowski Medical Research Centre Polish Academy of Sciences, Department of Neurotoxicology, Warsaw, Poland

²Medical University of Lublin, Department of Pathophysiology, Lublin, Poland

³Institute of Psychiatry and Neurology, 1st Neurology Department, Warsaw, Poland

⁴Warsaw University of Technology, Institute of Theory of Electrical Engineering, Measurement and Information Systems, Warsaw, Poland

24-day rats received pilocarpine (Pilo) subsequent to methylscopolamine and lithium (van der Hel et al. Eur J Neurosci, 2014). Some "Pilo" animals received 2.5 h before Pilo, 75 mg/kg bw. of a glutamine synthetase (GS) inhibitor, methionine sulfoximine ("Pilo+MSO"). Behavioral seizures were assessed by Racine points, EEG by a subcranially mounted electrode (8401 Data Conditioning & Acquisition System, Pinnacle Technology), GS activity in hippocampal and cerebral cortical homogenates (hh, cch) by colorimetry (Shapiro&Stadtman, Methods Enzymol., 1970). In "Pilo" rats, behavioral and EEG seizures which commenced ~15' post-Pilo, were recorded during 1 h. The mean Rp of 2.5 $\pm$ 0.5 and sum of Rp of 32.5 $\pm$ 5.9 measured in "Pilo" rats, decreased in "MSO+Pilo" rats to 1.6 $\pm$ 0.1 and 21.2 $\pm$ 4.9, respectively. EEG power was not altered by MSO. GS activity increased above control in cch of "Pilo" rats, was down to ~17% of control in cch and hh of rats treated with MSO only, and equaled control in cch and hh of "Pilo + MSO" rats. The results suggest that either i) MSO targeted brain areas indirectly involved in the response to Pilo, and/or ii) MSO or its metabolites interacted with Pilo and/or muscarinic receptors prior to glutamatergic activation.

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WTH04-30

Behavioral, circuitry and molecular aberrations by region-specific deficiency of the high-risk autism gene CUL3

M. Rapanelli¹, T. Tan¹, W. Wang¹, X. Wang², P. Zhong¹, Z.-J. Wang¹, L. Frick³, L. Qin¹, K. Ma¹, J. Qu², Z. Yan¹

¹SUNY-University at Buffalo, Department of Physiology and Biophysics, Buffalo, USA

²SUNY-University at Buffalo, New York State Center of Excellence in Bioinformatics and Life Sciences, Buffalo, USA

³SUNY-University at Buffalo, Hunter James Kelly Research Institute, Buffalo, USA

Cullin 3 (Cul3) gene, which encodes a core component of the E3 ubiquitin ligase complex that mediates proteasomal degradation, has been identified as a true high-risk factor for autism. Here, by combining behavioral, electrophysiological and proteomic approaches, we have examined how Cul3 deficiency contributes to the etiology of different aspects of autism. Heterozygous mice with forebrain Cul3 deletion displayed autism-like social interaction impairments and sensory-gating deficits. Region-specific deletion of Cul3 leads to distinct phenotypes, with social deficits linked to the loss of Cul3 in prefrontal cortex (PFC), and stereotypic behaviors linked to the loss of Cul3 in striatum. Correlated with these behavioral alterations, Cul3 deficiency in forebrain or PFC induces NMDA receptor hypofunction, while Cul3 loss in striatum causes a cell type-specific alteration of neuronal excitability in striatal circuits. Large-scale profiling has identified sets of misregulated proteins resulting from Cul3 deficiency in different regions, including Smyd3, a histone methyltransferase involved in gene transcription. Inhibition of the up-regulated Smyd3 in forebrain Cul3-deficient mice ameliorates social deficits and restores NMDAR function in PFC. These results have revealed for the first time a potential molecular mechanism underlying the manifestation of different autism-like behavioral deficits by Cul3 deletion in cortico-striatal circuits.

WTH04-32

Hyperphenylalaninemia causes cholinergic alterations in brain of young rats

P. Schuck¹, M. Oliveira², L. Kist¹, R. Martins³, E. Streck², M. Bogo¹, R. Kubrusly³, A. Zugno², G. Ferreira⁴

¹Pontificia Universidade Católica do Rio Grande do Sul, Escola de Ciências, Porto Alegre, Brazil

²Universidade do Extremo Sul Catarinense, Programa de Pós-graduação em Ciências da Saúde, Criciúma, Brazil

³Universidade Federal Fluminense, Departamento de Fisiologia e Farmacologia, Niterói, Brazil

⁴Universidade Federal do Rio de Janeiro, Instituto de Bioquímica Médica, Rio de Janeiro, Brazil

Phenylalanine (Phe) accumulates in tissue and body fluids of phenylketonuria (PKU) patients. Brain injury is a clinical characteristic of PKU patients, although the pathophysiology of this damage is poorly understood. The aim of the present work was to investigate the *in vivo* effects of phenylalanine (Phe) on choline acetyltransferase (ChAT) and acetylcholinesterase (AChE)

activities. For the *in vivo* experiments, animals received a single subcutaneous administration of saline (control group) or 5.2 $\mu\text{mol/g}$ Phe plus 0.9 $\mu\text{mol/g}$ p-chlorophenylalanine (HPA group). One hour after the administration, animals were euthanized by decapitation; brain structures were isolated and homogenized. ChAT and AChE activities and its relative RNA expression and phospho-PKA content were determined. Animals subjected to acute HPA had higher AChE activity in cerebral cortex and striatum. On the other hand, AChE mRNA expression was not altered by HPA. In order to verify the mechanisms by which AChE activity is affected, the content of phospho-PKA was assayed. Phospho-PKA content was higher in cerebral cortex, but not in striatum of animals submitted to HPA. Our results suggest that HPA induces cholinergic alterations. Since cholinergic imbalance is associated to failure and progressive neurologic decline in learning and memory functions, it is tempting to speculate that AChE alterations might contribute to the intellectual deficiency observed in HPA patients.

WTH04-33

TDP-43 stabilizes transcripts encoding the core stress granule protein G3BP1

H. Sidibé^{1, 3}, Y. Khalfallah^{2, 3}, G. DiTomasso², A. Aulas^{2, 3}, L. Destroimaisons³, J.-E. Deshaies³, J. Alex Parker^{1, 3}, P. Legault², M. Tétreault^{1, 3}, C. V. Velde^{1, 3}

¹Université de Montréal, Neurosciences, Montréal, Canada

²Université de Montréal, Biochimie, Montréal, Canada

³CRCHUM, Neurosciences, Montréal, Canada

Amotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the selective loss of motoneurons. The reasons underlying this selective death remain incompletely understood. Motoneurons are non-renewing cells subjected to numerous stresses throughout life that can be deleterious for their survival. One mechanism used by neurons to counter stressful events is the formation of stress granules (SG). These granules allow for a translational shift towards synthesis of pro-survival factors and storage of non-translating mRNAs. Defects in their formation negatively affect cellular survival. SG assembly requires the SG core protein G3BP1 (GTPase-Activating-Protein (SH3-Domain)-Binding-Protein1). G3BP1 and SG are suggested to be critical to (moto) neurons as the genetic deletion of *G3BP1* in rodents is either embryonic-lethal with neuronal loss or results in locomotor deficits and paralysis, depending on the genetic background. Moreover, poliovirus, an enterovirus that replicates in motoneurons and causes their selective death, inactivates G3BP1, leading to the blockage of SG assembly. 97% of ALS cases feature the mislocalization of the RNA-binding-protein TDP-43 (TAR-DNA-binding-protein-43) from the nucleus to the cytoplasm. We previously demonstrated that TDP-43 regulates SG assembly via G3BP1. Thus, we hypothesize that TDP-43-dependent regulation of G3BP1 is central to selective motoneuron vulnerability in ALS. Here, we characterized the TDP-43-dependent regulation of G3BP1 and investigated the relevance of this regulation in the motor cortex of ALS cases. Specifically, we show that TDP-43 binds, with high affinity, an evolutionary-conserved *trans*-element within the G3BP1 3'UTR. Additionally, we demonstrate that TDP-43 cytoplasmic accumulation compromises G3BP1 levels *in vivo* and *in vitro*.

WTH04-35

A novel approach for region-specific mouse brain dissociation and microchip-based cell sorting of neurons & neural stem cells

M. Sturges¹, S. Reiß², C. Wittwer², J. Gaiser², A. Bosio², M. Jungblut²

¹Miltenyi Biotec Inc, San Diego, USA

²Miltenyi Biotec GmbH, Bergisch Gladbach, Germany

Tissue dissociation and the preparation of single-cell suspensions with high cell viability and minimal cell debris are prerequisites for reliable cellular analysis and cell sorting. In the case of the adult rodent brain, sophisticated mechanical and enzymatic treatments are required to successfully disaggregate the tightly connected neural cells. When only small brain regions are available, the process becomes even more challenging. Especially when high viability, recovery, and functionality are crucial for successful downstream processing. Previously, we developed sophisticated technologies for the automated dissociation of whole adult rodent brain. This process is based on mechanical dissociation using the gentleMACS™ Octo Dissociator with Heaters and an optimized enzymatic treatment, followed by an effective clearing procedure to eliminate cell debris and erythrocytes. To extend this work, we further refined our approach to generate single-cell suspensions from small brain regions. This process results in high cellular viability and neural cell yield with a minimum of contaminating debris. From these high-quality single-cell suspensions, we were able to isolate neurons or neural stem cells (NSCs) using the MACSQuant® Tyto®, a novel multiparameter cell sorting device that uses a microchip-based technology for sterile and gentle cell isolation. This approach resulted in highly pure (80-99%) and viable (> 90%) neurons from cerebellum, cortex, and olfactory bulb. Furthermore, NSCs from the subventricular zone (SVZ) were identified with a new marker combination and successfully sorted at high purity (> 95%) and viability (> 90%). Isolated NSCs formed a large number of neurospheres, which gave rise to secondary neurospheres and differentiated into different neural cell types.

WTH04-36

Establishment of a new animal model for hereditary sensory autonomic neuropathy VI by conditional deletion of dystonin

H. Takebayashi¹, N. Yoshioka¹, H. Sano², S. Chiken², A. Nambu²

¹Niigata University, Division of Neurobiology and Anatomy, Niigata, Japan

²National Institute for Physiological Sciences, Division of System Neurophysiology, Okazaki, Japan

DYSTONIN (DST) is the causative gene for the hereditary sensory autonomic neuropathy VI (HSAN6) which encodes cytoskeletal linker protein. *Dystonia musculorum (dt)* mice carrying mutation in the *Dst* locus show sensory neuropathy and progressive motor symptoms. Although *dt* mice are similar to HSAN6 patients, the pathophysiological analysis has been hampered due to their early postnatal death. We had generated conditional *Dst* gene trap mice. The *Dst* gene trap allele can be switched from the mutant (*Dst^{Gt}*) to functional (*Dst^{Gt-inv}*) allele or from functional (*Dst^{Gt-inv}*) to mutant (*Dst^{Gt-DO}*) allele by Cre-mediated recombination. Homozygous mice carrying *Dst^{Gt}* or *Dst^{Gt-DO}* allele showed motor symptoms. To

generate *Dst* conditional KO mice mainly in the PNS and hindbrain, *Dst* gene trap mice were crossed with *Wnt1-Cre* transgenic mice in which Cre recombinase is expressed in the neural crest cells, cerebellum and midbrain in the embryos. As expected, Cre-mediated switch from *Dst^{Gt-inv}* to *Dst^{Gt-DO}* allele occurred in DRG and sympathetic ganglion neurons. In *Wnt1-Cre; Dst* cKO mice, ATF3, a stress-induced transcription factor, was up-regulated in both DRG and sympathetic postganglionic neurons. Accumulation of neurofilament in DRG neurons, denervation of sensory nerve in skin, and axonal degeneration in the spinal cord were also observed. These pathological remarks suggest that *Wnt1-Cre; Dst* cKO mice suffer from sensory and autonomic disturbance. Since *Wnt1-Cre; Dst* cKO mice showed increased life span compared to *dt* mice, detailed behavioral and physiological analyses become possible. Therefore, *Wnt1-Cre; Dst* cKO mouse is a new animal model for late-onset HSAN6.

WTH04-37

ATP-independent opening of LRRC8-containing volume-regulated anion channels and swelling-activated glutamate release in astrocytes

C. Wilson, Alexander. A. Mongin

Albany Medical College, Department of Neuroscience and Experimental Therapeutics, Albany, USA

The glutamate-permeable Volume-Regulated Anion Channel (VRAC) is thought to be a major pathway for ischemic stroke damage in the brain. This notion is based on observations that the VRAC blockers tamoxifen and DCPIB potently protect rodents against experimental stroke and reduce intra-ischemic glutamate release. The limitation of this theory is that VRAC activity has been shown to require intracellular ATP, which is expected to be depleted in the ischemic tissue. In the present study, we explored the metabolic constraints of the swelling-activated release of glutamate in rat brain astrocytes, the cell type that shows prominent swelling in stroke. To model stroke conditions *in vitro*, cultured astrocytes were exposed to chemical ischemia with sodium cyanide (NaCN, to inhibit mitochondrial respiration) and 2-deoxy-D-glucose (DDG, to block glycolytic flux). Intracellular ATP levels ([ATP]_i) were measured using a luciferin/luciferase assay, and glutamate release was quantified with the non-metabolizable glutamate analog, D-[³H] aspartate. VRAC activity was stimulated by exposure to hypoosmotic media (30% reduction in osmolarity), and its specific contribution confirmed with siRNA targeting the essential VRAC subunit, LRRC8A. With this approach, we found that complete metabolic inhibition with DDG+NaCN lowered [ATP]_i by ~90% and completely blocked swelling-activated glutamate release from swollen astrocytes. However, when astrocytes were exposed to more dramatic degrees of cell swelling (-50% and -70% hypoosmotic media), the metabolic block of VRAC was partially or nearly completely overridden. Since the astrocytic processes in the infarction core can swell as much as ten-fold, our findings suggest that VRAC can be active in the ischemic tissue, even upon complete metabolic inhibition.

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WTH04-38

DJ-1 regulates the integrity and function of ER-mitochondrial association through interaction with IP3R3-GRP75-VDAC1**X. Zhu¹, Y. Liu^{1,2}, X. Ma¹, H. Fujioka³, J. Liu², S. Chen²**¹Case Western Reserve University, Dept of Pathology, Cleveland, USA²Shanghai Jiao Tong University School of Medicine, Department of Neurology, Shanghai, China³Case Western Reserve University, Electron Microscopy Core Facility, Cleveland, USA

ER and mitochondria form close associations that serve as critical signaling platform to regulate many key cellular processes including calcium signaling, phospholipids synthesis, mitochondrial biogenesis and dynamics, autophagy and cell death, disturbance in many of which were involved in Parkinson's disease (PD). Loss-of-function mutations in DJ-1 are associated with autosomal recessive early-onset PD but the underlying mechanism is not clear. In this study,

we aimed to determine whether DJ-1 deficiency cause deficits in the integrity and function of ER-mitochondria association and explore the underlying mechanism. We found that DJ-1 knockout caused significant impairments in the structure of ER-mitochondria association and disturbed mitochondrial calcium uptake and mitochondrial function in neuronal cells. Mechanistically, we demonstrated that DJ-1 localizes to mitochondrial-associated membrane (MAMs) where it forms a macrocomplex with IP3R3-Grp75-VDAC1, the well-known complex responsible for the association and calcium crosstalk between the two organelles, through direct interaction both *in vitro* and *in vivo*. Such an interaction is critical for the stabilization and function of IP3R3-Grp75-VDAC1 complex. We further demonstrated that DJ-1 is essential in the regulation of IP3R3 homeostasis since DJ-1 knockout led to the loss of IP3- and Ca²⁺-induced IP3R3 degradation and accumulation of IP3R3 at MAMs. Together, these results have revealed a novel mechanism critical for ER-mitochondria association and suggested the involvement of DJ-1 in the regulation of IP3R3 homeostasis in general, and identified a novel pathogenic mechanism for DJ-1 related PD in specific.

WTH05 Brain development & cell differentiation (Session B)

WTH05-01

MTOR inhibition restricted to a postnatal sensitive period rescues the deficits in PV cell connectivity caused by loss of TSC1

C. Amegandjin^{1,2}, M. Choudhury^{1,2}, V. Jadhav^{1,2}, J. Carriço², A. Quintal¹, M. Berryer^{1,2}, B. Chattopadhyaya², G. D. Cristo^{1,2}

¹Université de Montréal, Département de Neurosciences, Montréal, Canada

²CHU Sainte-Justine Research Center, CHU Sainte-Justine, Montréal, Canada

Properly functional cortical circuits depend on the correct development of inhibitory interneurons. In particular, the axonal arborisation and synapse density of parvalbumin (PV)-positive GABAergic interneurons undergo striking changes in the young brain. The mechanisms controlling the development of PV cell connectivity are still not well understood. The mTORC1 pathway, which is regulated by Tuberous Sclerosis (TSC) 1 and 2 proteins, has been implicated in controlling several aspects of neuronal development. How and whether mTORC1 signaling affects PV cell development is unknown. Here, we showed that Tsc1 knockout (KO) in single PV interneurons in cortical organotypic cultures caused a premature increase in terminal axonal branching and bouton density formed by mutant PV cells, followed by a striking loss of perisomatic innervation after the 4th postnatal week. To investigate the role of mTORC1 in PV cells *in vivo*, we bred Tsc1^{lox} with *Nkx2.1*-Cre and PV-Cre mice to knockout Tsc1 before and after birth, respectively. Both conditional KO mice showed mTORC1 hyperactivation in PV cells. Consistently to what observed following Tsc1 KO in single PV cells, PV cell perisomatic innervations were increased at P18, but decreased at P45 in *Nkx2.1*-Cre;Tsc1^{lox/lox} mice compared to controls. Further, both conditional KO mice showed alterations in social behavior. Finally, treatment with the mTOR inhibitor Rapamycin restricted to the third postnatal week was sufficient to rescue deficits in PV cell innervation in organotypic cultures and social behavior in heterozygous but not homozygous mice. All together, these results suggest that mTORC1 signaling regulates both the developmental time course and the maintenance of PV cell innervations. Further, altered PV cell connectivity may be one of the pathological mechanisms contributing to social behavioral deficits in diseases characterized by mTOR dysregulation. Treatment restricted to specific sensitive periods may ameliorate PV cell synapse loss and social behavior deficits.

WTH05-02

Effects of Val66Met BDNF polymorphism on cortical gabaergic circuit refinement

P. Chehraz

University of montreal, Neuroscience, Montreal, Canada

Parvalbumin (PV)-expressing GABAergic interneurons constitute the majority of interneurons in the cortex and strongly regulate principal cell output and plasticity. One of the strongest modulators of PV network development and experience-dependent plasticity is Brain-Derived Neurotrophic-Factor (BDNF). BDNF is first synthesized as a precursor (proBDNF) which is cleaved to generate mature

BDNF (mBDNF) and the prodomain (pBDNF). We have previously shown that proBDNF induces PV cell synapse pruning. Emerging data suggest that a common single-nucleotide polymorphism (SNP) in pBDNF, i.e. methionine (Met) substituting for valine (Val) at codon 66 (Val66Met), is associated with genetic predisposition to anxiety and depression. Here, we investigated whether and how different pBDNF SNPs affect cortical PV interneuron axon morphology and synapse development. We labeled isolated PV interneurons and their axons, by driving GFP expression with a previously characterized promoter, in cortical organotypic cultures treated with either pBDNF-Met66 or pBDNF-Val66 and quantified two aspects of PV cell axonal innervation: 1) perisomatic innervation around individual pyramidal cell somata visualized by NeuN immunostaining, and 2) the extent of pyramidal neurons innervated by a single PV cell. Our preliminary data show that pBDNF Met66 has a more severe effect on the maturation of PV cell innervations than pBDNF Val66. Excessive PV synapse pruning might contribute to the higher risk of developing psychiatric disorders associated with the presence of Val66Met.

WTH05-03

The adhesion-GPCR BAI1 shapes dendritic arbors through contact-dependent, BCR-mediated RhoA activation causing growth arrest

J. Duman

Baylor College of Medicine, Dept. of Neuroscience, Houston, USA

A central neurodevelopmental challenge is establishing circuits linking 10¹⁰ CNS neurons. This process yields 10¹³ synapses in human adults that drive all cognition. Synapse and circuit defects precipitate cognitive and mood disorders. Axons and dendrites must develop properly to enable functional synaptogenesis. Dendritic arbors exhibit a striking diversity of neuronal type-specific forms that not only facilitate circuit connectivity but also determine neuronal computational properties. These complex forms arise from an intricate interplay between gene expression programs and signaling pathways that integrate environmental information; the molecules and interactions that mediate this interplay remain largely unknown. The adhesion-GPCR brain-specific angiogenesis inhibitor (BAI1) is highly expressed in hippocampus, cortex, and striatum. We showed that BAI1 is required for excitatory synaptogenesis through activation of the small GTPase Rac1 and interactions with the synaptic organizer neuroligin-1. We report that BAI1 also plays a critical role in the dendritic arborization of hippocampal pyramidal neurons by triggering late developmental dendritic growth arrest through localized activation of the small GTPase RhoA, a frequent functional antagonist of Rac1. Surprisingly, BAI1 does not activate RhoA through Gα_{12/13}, but via the breakpoint cluster region (Bcr) protein. Bcr mediates growth arrest through its GAP (inhibitory) function against Rac1, but we show that BAI1 activates its cryptic RhoA-GEF (activator) function. Moreover, both Bcr Rac1-GAP and RhoA-GEF activities are required for dendritic growth arrest. These results indicate that the signals terminating net dendrite growth are complex and multilayered. We are ascertaining the relationship between the BAI1-instigated synaptogenic and anti-dendritogenic signals and the nature of BAI1-mediated contact-dependence.

WTH05-04

Cannabinoid-inhibited proliferation in the embryonic retina: an investigation on the role of purinergic receptors
H. Freitas¹, R. Reis¹, A. Ventura², G. França²¹*Universidade Federal do Rio de Janeiro, Carlos Chagas Filho
Institute of Biophysics, Rio de Janeiro, Brazil*²*Universidade Federal Fluminense, Institute of Biology, Niterói,
Brazil*

Retinal development is accomplished in the vertebrate through crosstalk between different steps, such as genesis, proliferation, differentiation, and death, all of which involve signals between progenitor cells in diverse morphofunctional stages. Previous investigations shed light on ATP and the P2Y1 receptor as positive switches for proliferation, while P2Y12 receptor activation prevented retinal population growth. Cannabinoid receptors were shown to be expressed very early in the embryonic retina, and evidence revealed that these receptors modulate death, but not proliferation, through the P2X7 receptor in glial progenitors. To further explore the effects of cannabinoid receptors in the inhibition of proliferative behavior, avian retinal embryonic cells were cultivated *in vitro* and assayed for viability, proliferation, morphology, and intracellular calcium. Our data reveal that the effect exerted by the cannabinoid agonist WIN-55,212-2 on proliferation and on the loss of cell viability (up to 50%) could only be observed in the early stages of development (7 days embryonic and 2 days *in vitro*, E7C2), while well-differentiated cultures (E7C8) were unaffected by the cannabinoid. When E7C1 cultures were treated for 24 h, an extensive loss of morphologically identifiable glial cells was observed. Cell death, however, did not affect ATP responsiveness in these cultures when examined for calcium transients at E7C10. The cannabinoid effect on viability was absent in enriched Müller glia cultures (E9C21). Finally, a P2Y12 receptor antagonist, PSB 0739, prevented the loss of cell proliferation in E7C2 cultures, indicating that it's partially responsible for the effects of cannabinoid receptor activation on population growth in the retina.

WTH05-05

Loss of PCDH12 causes cell migration and differentiation defects in human embryonic stem cell-derived neuroprogenitors**A. G. Gamboa, J. Rakotomamonjy, L. Rylaarsdaam, D. Thomas***Northwestern University, Physiology, Chicago, USA*

Protocadherin (PCDH)12 is a cell adhesion molecule, member of the cadherin superfamily, that mediates homophilic cell-cell interactions. Loss of PCDH12 was previously shown to result in microcephaly, facial dysmorphism, epilepsy, and developmental disability. How PCDH12 loss of function leads to malformations of cortical development such as microcephaly, and disorders associated with disrupted neuronal circuitry such as epilepsy is unknown. Here, we investigated the effects of PCDH12 deletion in neuroprogenitors derived from human embryonic stem cells. We showed that PCDH12 absence affects cell migration. Neuroprogenitors lacking the cadherin failed at migrating as far as wild-type (WT) cells due to disrupted directional persistence. PCDH12 knock-out cells did not show any proliferative defect or change in cell death rate. However, we observed a decrease in cell cycle re-entry when compared to WT neuroprogenitors, suggesting a premature exit from the progenitor state and early neuronal differentiation. Our data suggest that abnormal cell

migration and fate determination could be responsible for the disrupted cortical development and neuronal circuitry observed in patients carrying homozygous PCDH12 variants. These results provide insight into the cellular mechanisms regulated by PCDH12 during brain development.

WTH05-06

The effect of prenatal exposure to methadone and morphine on the cerebellum using the developing chicken embryo**M. G. Hadera¹, J. M. Andersen^{1, 2}, S. Steinsland², R. E. Paulsen¹**¹*University of Oslo, School of Pharmacy, Oslo, Norway*²*Oslo University Hospital, Department of Forensic science, Oslo, Norway*

Introduction: Methadone is used in opioid maintenance therapy during pregnancy, though reports indicate potential for long-term neurodevelopmental deficits. The mechanisms by which it influences neurodevelopment is not yet known. The developing chicken embryo is an attractive model with potential for nonclinical safety studies of pharmaceuticals.

Methods: On embryonic day (ED) 13 (single exposure) or ED13 and 16 (repeated exposure), methadone and morphine at 20 mg/kg were injected onto the chorioallantoic membrane of chicken (*Gallus gallus*) embryos. On ED17 the cerebellum was harvested and homogenized. Western blotting, rt-PCR and DAMGO binding analysis was performed on the cerebellar tissue homogenate. We also studied the effect of the opioids on neurite outgrowth of primary granule neurones culture derived from E17 chicken cerebellum using a timelapse live-cell imaging performed with an Essence Bioscience IncuCyte Zoom running NeuroTrack module.

Results: We observed reduced levels of PAX6 (30%; One-way ANOVA, $p = 0.005$) and MMP9 (40%; $p = 0.04$) in the ED13 single methadone exposure. Binding to DAMGO was also reduced in this group (35%; $p = 0.05$). rt-PCR analysis indicated reduction in the expression of MOR, POMC, PDYN and an increase in GluN2B in this group. We did not observe changes in both exposure protocols in the morphine and the repeated E13 and 16 methadone exposure groups. Methadone reduced neurite length in primary granule neuron culture in an NMDA dependent manner.

Conclusion: The developing chicken embryo is a promising alternative animal model for testing of effects by medications on neurodevelopment. Methadone appears to affect molecular markers of brain development and the opioid system.

WTH05-07

Evolutional analysis of the protein phosphorylation sites in the growth cone**M. Igarashi¹, S. Okuda¹**¹*Niigata University Graduate School of Medical & Dental Science,
Department of Neurochemistry and Molecular Cell Biology,
Niigata, Japan*²*Niigata University Graduate School of Medical & Dental Science,
Lab of Bioinformatics, Niigata, Japan*

The growth cone is an essential structure formed in the developing axons for accurate neuronal network formation. To know the molecular mechanisms of the growth cone activity and its regulation

should greatly contribute to the profound understanding the whole basis of neuronal network rearrangement, in response to the environment. However, in the mammalian growth cone behavior, these problems are poorly approached so far. We focused the phosphorylation which is the most important protein modification in the cellular signaling pathways, and performed the phosphoproteomic analysis of the growth cone membrane (GCM) prepared from the developing rodent brain, to comprehensively know the phosphorylation sites and their frequency for neuronal development. We identified 30,000 phosphopeptides derived from the 5,000 phosphorylation sites of 1,200 proteins. A considerably large amounts of these sites were phosphorylated by the proline-directed protein kinases such as JNK, which is known to be involved in axon growth within a wide range of animals from *C. elegans* or *Drosophila* to mammals. Bioinformatic analysis revealed that most of these sites were conserved within the vertebrates, however, were not conserved in more simpler model organisms such *C. elegans* or *Drosophila*. In particular, most of the highly frequent phosphorylation sites (more than 20 times) in GCM were not conserved in *C. elegans* or *Drosophila*. We concluded that the mammalian signaling pathways in GCM are totally distinct from the model organisms. (Ref.) Kawasaki A et al.: iScience 4; 190 [18].

WTH05-08

Region-specific expression of pcna and dcx in adult brain of pre-pubertal male Japanese quail exposed to Di(N-butyl) phthalate

A. Ihunwo¹, U. M. Bello², G. F. Dlamini¹

¹University of the Witwatersrand, Anatomical Sciences, Johannesburg, South Africa

²Ahmadu Bello University, Veterinary Anatomy, Zaria, Nigeria

Phthalates esters, including di(n-butyl) phthalates, DBP, are endocrine-disrupting chemicals which can adversely affect neuronal differentiation. Although few studies investigated biological impact of phthalates on brain development, early exposure to phthalates can disrupt normal neurodevelopment, particularly in the hippocampus. This study investigated neuronal cell proliferation and changes in specific regions: hippocampus, mesopallium, nidopallium, medial pre-optical nucleus and ventricles in 6-weeks old quail (*Coturnix japonica*) brains exposed to 10, 50 and 400 mg/kg of di(n-butyl) phthalate for 30 days. Perfusion-fixed brains, post-fixed in 4% PFA and 50 µm frozen sections were processed using cresyl violet staining for brain cyto-architecture, proliferative cell nuclear antigen (PCNA) for neuronal cellular differentiation and Doublecortin (DCX) for immature neurons respectively. Intense staining of PCNA and DCX immunolabelled cells was observed along the ventricles while some moderate PCNA-ir stained cells were present along the medial preoptic nucleus. In addition, positive DCX-ir cells were predominant in the basal regions of pallial areas in both DBP-treated and control groups. On quantification, no significant differences between control and DBP-treated groups, although, two to three-fold increase in cell numbers, with no significant differences, existed between the low and high doses DBP-treated groups. Results suggest that DBP does not seem to have significant effect on cell numbers but affect cellular arborization. Our data suggests that ongoing neurogenesis provides a sensitive indicator of early or delay environmental stress such as exposure to DBP.

WTH05-09

Understanding the role of SYNGAP1 in GABAergic circuit development and function

V. Jadhav^{1, 2}, T. Badra^{1, 2}, G. D. Cristo^{1, 2}, B. Chattopadhyaya¹, J. Michaud^{2, 3}

¹CHU Ste-Justine Research Center, Brain & Child Development, Montreal, Canada

²University of Montreal, Department of Neurosciences, Montreal, Canada

³University of Montreal, Department of Pediatrics, Montreal, Canada

Haploinsufficiency of Syngap1 gene encoding the Synaptic Ras-GTPase Activating protein is associated with intellectual disability, autism spectrum disorder and epilepsy. Syngap1 is a negative regulator of Ras and of AMPA receptor trafficking to the postsynaptic membrane, thereby regulating the process of synaptic plasticity and neuronal homeostasis. Haploinsufficiency of Syngap1 leads to synaptic plasticity alterations, behavioral abnormalities and cognitive deficits in mouse models. In particular, several studies have shown that Syngap1 regulates the time course of the maturation of dendritic spines and glutamatergic synapses in excitatory neurons; in contrast, the role of Syngap1 in inhibitory, GABAergic neurons is relatively uncharted. GABAergic neurons are a diverse class of neurons with different morphology, connectivity and physiological properties. They play an important role in neural circuit development and plasticity. Parvalbumin (PV)-expressing interneurons, one of the major classes of cortical GABAergic interneurons, form synapses onto the soma and proximal dendrites of pyramidal cells and are involved in the synchronization of the firing rate of pyramidal cell populations. We showed that haploinsufficiency of Syngap1 specifically in GABAergic cells derived from the medial ganglionic eminence (MGE), which include Parvalbumin and Somatostatin-expressing interneurons, decreases PV cell numbers and connectivity. In addition, these mutant mice show impaired context-dependent fear memory. We are currently investigating the molecular mechanisms underlying these phenotypes. A better understanding of the role of Syngap1 in GABAergic cell development may shed light on the involvement of GABAergic circuit alterations in the cognitive deficits caused by Syngap1 haploinsufficiency in humans.

WTH05-10

Mood stabilizing drugs activate adult neural stem cells-neurogenesis system

K. Nakaji

Shiga University of Medical Science, Medical Science, Otsu, Japan

Neural stem cells (NSCs) not only produce all neurons and glia in the developing brain but also reside in the adult brain and supply new neurons to the olfactory bulb and hippocampus, which play significant roles in the olfaction and some types of memory. NSCs attract much attention as a resource of cell replacement therapy for impaired central nervous system. However, efficient and clinically feasible strategy to activate endogenous NSCs is not currently available. We have previously demonstrated that mood stabilizing drugs, which are used to treat patients with bipolar disorder, enhance the self-renewal capability of mouse NSCs *in vitro* at therapeutically relevant concentrations in the cerebrospinal fluid. In this study, we examined the effect of a novel type of mood stabilizing drugs,

lamotrigine, on the self-renewal of NSCs *in vitro*. Also, we chronically administrated lamotrigine to mice and evaluated the effects on the proliferation of glial cells and neurogenesis in the subgranular layer of dentate gyrus, subependymal zone, and cortex *in vivo*. In addition, we conducted behavioral battery after administration of lamotrigine. Here, we found that lamotrigine possesses similar pharmacological function to classical mood stabilizers, such as valproate, carbamazepine and lithium. These results provide insight into the mechanism of psychiatric diseases such as bipolar disorder as well as the development of novel therapies.

WTH05-11

Role of post-translational arginylation in glial cells during CNS myelination

A. Palandri, L. Bonnet, J. F. Martin, M. Hallak, M. Galiano

National University of Cordoba, Biology Chemistry, Cordoba, Argentina

Post-translational arginylation of proteins is mediated by Arginyltransferase (Ate1), which modifies multiple substrates, such components of actin cytoskeleton, and modulates biological processes during embryogenesis, cell migration, proliferation and neuronal growth; however, its role in glia cells is poorly understood. Oligodendrocytes (OLs) are responsible of myelin formation in the central nervous system (CNS), essential for the rapid propagation of neuronal action potential as well as for metabolic support of axons. OLs are generated from OL precursor cells (OPC) that undergo morphological changes through differentiation and myelination. The aim of this study was to analyze the relevance of Ate1 in OLs during CNS myelination. Conditional deletion (cKO) of Ate1 from OLs with the *Cnp*-cre promoter resulted in differential changes on OL differentiation throughout CNS development. Ate1 cKO animals had reduced OLs (Sox10 +) number in the corpus callosum (CC) at P14. But at P21, OLs number was unchanged and its maturation, defined by CC1 expression, appeared normal in the CC. However, in the spinal cord of cKO, the number of mature (CC1 +) OLs was reduced, showing that deletion of Ate1 in OLs resulted in developmental delay that varies between different regions of the CNS. Additionally, local OPC proliferation was increased in the CC at P21 in Ate1 cKO mice, relative to control mice, whereas at the same time the astrocytes population was increased. These results suggest that deletion of Ate1 in OLs caused a localized increase of OPC proliferation and a concomitant astrogliosis response. Future studies would address which proteins are modified by Ate1 during OLs maturation. Studies supported by FONCYT 2015.

WTH05-12

NEGR1 and FGFR2 cooperatively regulate cortical development and core behaviors related to autism spectrum disorders in mice

F. Pischedda¹, J. Szczurkowska², B. Pinto², F. Managò², Carola. A. Haas³, M. Summa⁴, R. Bertorelli⁴, F. Papaleo², Michael. K. Shafer⁵, L. Cancedda², G. Piccoli¹

¹University of Trento, Center for Integrative Biology ^{CIBIO}, Trento, Italy

²IIT, Department of Neuroscience and Brain Technologies, Genova, Italy

³University of Freiburg, Department of Neurosurgery, Freiburg, Germany

⁴IIT, Department of Drug Discovery and Development, Genova, Italy

⁵Johannes Gutenberg-University, Department of Anesthesiology and Focus Program Translational Neurosciences, Mainz, Germany

Autism spectrum disorders (ASD) are neurodevelopmental conditions with diverse etiologies, all characterized by common core symptoms such as impaired social skills and communication, as well as repetitive behavior. Cell-adhesion molecules (CAMs), receptor tyrosine kinase (RTK) and associated downstream signaling have been strongly implicated in both neurodevelopment and ASD. We found that down-regulation of the CAM *Negr1* or RTK *FGFR2* similarly affect neuronal migration and spine density during mouse cortical development *in vivo* and results in impaired core behaviors related to ASD. Mechanistically, *Negr1* physically interacts with *FGFR2* and modulates *FGFR2*-dependent ERK and AKT signaling by decreasing *FGFR2* degradation from the plasma membrane. Accordingly, *FGFR2* over-expression rescues all defects by *Negr1* knock-down *in vivo*. *Negr1* KO mice presented phenotypes similar to *Negr1*-down-regulated animals. These data indicate that *Negr1* and *FGFR2* cooperatively regulate cortical development and suggest a role for defective *Negr1*-*FGFR2* complex and converging downstream ERK and AKT signaling in ASD.

WTH05-13

The contribution of oxytocin to the regulation of actin cytoskeleton and scaffolding proteins in early mice development

A. Reichova¹, S. Bukatova¹, Z. Bacova¹, F. Muscatelli-Bossy², J. Bakos^{1,3}

¹Biomedical Research Center, Slovak Academy of Sciences, Department of Neurobiology, Bratislava, Slovakia

²Institut de Neurobiologie de la Méditerranée, INSERM, Perinatal Imprintings and Neurodevelopmental Disorders, Marseille, France

³Faculty of Medicine, Comenius University, Institute of Physiology, Bratislava, Slovakia

The aim of the present study was to investigate the contribution of oxytocin to neurogenesis, neuronal differentiation, neuritogenesis and regulation of the expression of small GTPases. Incubation in the presence of oxytocin (1 μM, 8 days) increased the proportion of neurons (67%) compared to control group (42%). Incubation in the presence of oxytocin (1 μM, 48 h) significantly increased neurite length in the primary hippocampal cells isolated from the neonatal C57BL/6 mice. The number of neurite branches measured by Sholl analysis increased in the presence of oxytocin as well. Oxytocin administered to the mice (P2, P3, 2 μg/pup) significantly enhanced

mRNA and protein levels of GTPase RhoB without any effect on RhoA expression in the hippocampus. These changes were accompanied by increased levels of mRNA for TIAM1, PAK2 and N-WASP as effectors which convey signals to the regulation of actin cytoskeleton. Oxytocin treatment resulted in a significant increase in the gene and protein expression of the microtubule associated protein 2, as well as in the significant increase of mRNA levels of PSD95, SHANK2 and major scaffolding proteins in the excitatory postsynaptic density. Overall, it can be suggested that the activation of oxytocin signaling pathway contributes to the neurogenesis and to the regulation of actin cytoskeleton and scaffolding proteins allowing the neurite growth. The mechanism of the oxytocin effect could be mediated by small GTPases and their pathways. *Supported by VEGA 2/0116/16, APVV-15-205, SK-FR-2017-0012.*

WTH05-14

Vitamin D dietary supplementation rescues rett syndrome phenotypes of MECP2 mutant mice

M. Ribeiro, J. MacDonald

Syracuse University, Biology, Syracuse, USA

Rett syndrome (RTT) is an X-linked neurodevelopmental disorder that affects females almost exclusively. RTT is caused by mutations in the *MECP2* gene, which encodes a transcriptional regulator that is essential for normal CNS function. Among the numerous symptoms of the disorder, the cortical neurons of RTT patients show reduced soma area, dendritic complexity and spine density. A previous study in *Mecp2*-null RTT model mice demonstrated that these phenotypes are, at least in part, caused by increased activation of the NF- κ B pathway. Interestingly, genetically attenuating NF- κ B signaling rescues dendritic complexity of cortical layer II/III callosal projection neurons (CPN) in *Mecp2*-null mice. Moreover, we have found that vitamin D, a known inhibitor of NF- κ B signaling, reduces the pathway activation and rescues neurite outgrowth of cortical neurons *in vitro*. Strikingly, our data also demonstrate that treating 4-week-old *Mecp2*-null mice with custom chow containing increased concentrations of vitamin D rescues soma area, dendritic complexity and dendritic spine density of CPN *in vivo*. A similar result was found in 5-month-old heterozygous female mice, which better recapitulate the disorder in humans. To evaluate if the neuronal morphological rescue could ameliorate heterozygous female mice motor and sensory behavior, we are currently investigating their behavior under our vitamin D dietary supplementation paradigm. Altogether, these data suggest that vitamin D dietary supplementation could be a simple, cost-effective new therapeutic avenue for RTT, via NF- κ B signaling attenuation.

WTH05-15

Long-term decreases in N-acetylaspartate after perinatal brain injury results from perturbed de novo synthesis

J. Scafidi¹, T. Johnson¹, B. Talbot¹, S. Lin², P. Wang², S. Scafidi³

¹Children's National Medical Center, Neuroscience, DC, USA

²Howard University College of Medicine, Molecular Imaging, DC, USA

³Johns Hopkins School of Medicine, ACCM, Baltimore, USA

Children born extremely premature have long-term neurodevelopmental delays and decreased N-acetylaspartate (NAA) - a

neurometabolite specifically synthesized in mitochondria of neurons. We aimed to determine the mechanisms that cause decreased NAA using different rodent models of perinatal brain injury (chronic hypoxia and moderate systemic inflammation). At one month of age, mice were subjected to hippocampal-dependent behavioral tests and ¹H-MRS of the hippocampus. Using immunohistochemistry, we compared cell density of post-mitotic neurons. Mitochondrial copy number was quantified by qRT-PCR. Protein expression of the enzymes responsible for NAA synthesis and degradation were analyzed. The precursors of NAA were measured using mass spectroscopy and synthesis of NAA was assessed using ¹³C-nuclear (N)MR. Our results indicate that mice that underwent perinatal injury had significantly decreased NAA on ¹H-MRS and performed significantly worse on behavioral tests. No differences were detected in: i) neuronal cell density; ii) mitochondrial DNA number; iii) expression of NAA synthesis or degradation enzymes; and iv) measurements of acetyl coA and aspartate. Using ¹³C-NMR, we determined that *de novo* NAA synthesis was decreased. Since acetyl-coA is an important precursor for fatty acid (FA) synthesis in the developing brain, we evaluated lipid composition and found no differences in medium and long-chain FAs, however, very long chain FA were significantly decreased. The percent enrichment of ¹³C-containing FAs was increased in the injured group. Our data strongly suggest that the decrease in NAA after perinatal brain injury is due to diminished *de novo* synthesis and NAA precursors being diverted to fuel up-regulated FA synthesis during the recovery period.

WTH05-16

Cas adaptor proteins regulate cortical migration and lamination

W. Wong¹, Y. C. Wong¹, B. Zareian², E. Zagha², Martin. M. Riccomagno¹

¹University of California, Riverside, Molecular, Cell, & Systems Biology, Riverside, USA

²University of California, Riverside, Psychology, Riverside, USA

Accurate cell migration and stratification are critical for the formation of functional neural circuits. The mammalian cortex is a highly stratified structure and provides an excellent model for understanding how neuronal laminar organization is established. Migrating neurons encounter multiple guidance cues as they migrate radially to the appropriate layer. How multiple cues are integrated within the migrating cell is not well understood. To uncover the signaling events essential for radial migration, we studied how Cas signaling adaptor proteins function during cortical development. The three embryonically expressed Cas proteins show strong and specific expression in the cortex, in a manner consistent with playing a role in radial migration and stratification. They have been hypothesized to mediate integrin and guidance cue signal transduction during neural development. Here, we provide *in vivo* genetic evidence that Cas proteins serve a functional and redundant role during cortical lamination. At the anatomical level, *Cas* triple conditional knockout (*CasTcKO*) mice display severe cortical phenotypes that resemble the human neurodevelopmental disorder, cobblestone lissencephaly. Electrophysiological and behavioral studies revealed distinct functional deficits in *CasTcKO* mice, providing insights into the consequences of altered lamination on cortical processing. Overall, our data support an essential role of Cas adaptor proteins for the formation of cortical circuits and their function.

WTH05-17

Regulation of proliferative activity by peroxynitrite in the newly generated cells after neuronal degeneration in the hippocampus**M. Yoneyama, Y. Ikeda, T. Yamaguchi, Y. Onaka, K. Ogita***Setsunan University, Faculty of Pharmaceutical Science, Hirakata, Japan*

It is now clear that there is a continual turnover of the mammalian hippocampal dentate gyrus (DG) neurons throughout life even in adult brain. Various neurological injuries are widely recognized as promoting endogenous neurogenesis in DG. Reactive oxygen species (ROS) modulate various cellular functions including cell proliferation, differentiation, survival, and death. Post-injury production of ROS may be one of the regulators for neurogenesis after neurodegeneration. Our previous studies demonstrated that the systemic treatment with trimethyltin chloride (TMT) causes the granule cell loss in the DG of adult mouse, with being regenerated in the dentate granule cell after the neuronal loss. The goal of the present study was to elucidate the involvement of peroxynitrite in proliferation of neural stem/progenitor cells (NPCs) after neuronal degeneration. In vivo experiments, mice were given TMT to prepare slices for immunostaining using antibody against nestin (NPCs marker) and 3-nitrotyrosine (3-NT, a product of tyrosine nitration by peroxynitrite). Cells positive for nestin and 3-NT markedly increased in the DG on day 3 after TMT treatment. In vitro experiments using the NPCs derived from the DG on day 3 post-TMT, the exposure to apocynin (NADPH oxidase inhibitor) or L-NAME (nitric oxide synthase inhibitor) significantly decreased the cell proliferation. Conversely, KT5823 (G kinase inhibitor) significantly increased the cell proliferation. Our results suggest that peroxynitrite has a critical role in proliferative activity in the NPCs generated following neuronal degeneration in the DG.

WTH05-18

Phosphorylation of cytoskeletal proteins in axon initial segments**T. Yoshimura¹, M. Rasband², T. Katayama¹**¹*United Graduate School of Child Development, Osaka University, Department of Child Development and Molecular Brain Science,, Osaka, Japan*²*Baylor College of Medicine, Department of Neuroscience, Houston, USA*

The axon initial segment (AIS) is a structurally and molecularly unique neuronal compartment of the proximal axon that functions as both a physiological and physical bridge between the somatodendritic and axonal domains. The AIS has two main functions: to

initiate action potentials and to maintain neuronal polarity. The AIS has ion channels, cell adhesion molecules, extracellular matrix molecules and cytoskeletal scaffolds. It has been reported that mutations in AIS proteins cause neurodevelopmental and psychiatric disorders. Super resolution microscopy techniques have provided new insights into the structure of this AIS cytoskeleton. The actin/spectrin/ankyrin-based cytoskeleton forms a periodic structure with actin filaments in the AIS. The molecular mechanism by which AIS cytoskeleton is regulated remains unclear. Here, we report that AIS cytoskeletal proteins are phosphorylated. This phosphorylation is required for AIS formation.

WTH05-19

Intracerebral infusion of ganglioside GD3 augments the adult neural stem cell pool in mouse brain**R. Yu, D. Li, F.-L. Tang, Y. Itokazu***Medical College of Georgia, Augusta University, Neuroscience and Regenerative Medicine, Augusta, USA*

Adult neural stem cells (NSCs) are located primarily in the subgranular layer of the dentate gyrus (DG) in the hippocampus and the subventricular zone (SVZ) of the lateral ventricles of the brain. NSCs are fundamental cells that can differentiate into various types of cells in the developing nervous system, and, in mature brain, for adult neurogenesis. It has been suggested that NSCs contribute to repairing damaged or degenerated CNS. Throughout neural development, dynamic changes are observed in the composition of carbohydrate-rich molecules, including gangliosides. We previously showed that ganglioside GD3 is the predominant species in NSCs; reduced postnatal NSC pools are observed in both the SVZ and DG of GD3-synthase knockout mouse brains. Specifically, deficiency of GD3 in GD3S-KO animals revealed a dramatic reduction in cellularity in the DG of hippocampus of the developing mouse brain, resulting in severe behavioral deficits in these animals. To further evaluate the functional role of GD3 in postnatal brain, we performed rescue experiments by intracerebroventricular (icv) infusion of ganglioside GD3 in adult GD3S-KO animals and found that it could restore the NSC pools and enhance the self-renewal capability of NSCs. Our results thus demonstrate that exogenously administered ganglioside GD3 is capable of restoring the biological function of postnatal NSCs. Since ganglioside expression profiles are associated not only with normal brain development, but also with pathogenic mechanisms of diseases, we anticipate that administration of exogenous gangliosides, such as GD3, could represent a novel strategy for promoting adult neurogenesis in damaged brain for disease treatment. (Supported by USPHS NIH RO1 NS094161-02).

WTH06 Bioenergetics & metabolism (Session B)

WTH06-01

Lipids as metabolic energy reserves in white matter tracts
**E. Asadollahi¹, A. Trevisiol¹, A. Saab², E. Dale Abel³, T. Ruhwedel¹,
O. Jahn⁴, P. Dibaj¹, K. Kusch¹, J. Hirrlinger^{1, 5}, G. Saher¹,
W. Möbius¹, C. M. Kassmann¹, K.-A. Nave¹**

¹Max Planck Institute of Experimental Medicine, Neurogenetics, Göttingen, Germany

²University of Zürich, Institute of Pharmacology and Toxicology, Zürich, Switzerland

³University of Iowa, Department of Internal Medicine, Iowa, USA

⁴Max Planck Institute of Experimental Medicine, Proteomics Group, Göttingen, Germany

⁵University of Leipzig, Carl-Ludwig-Institute for Physiology, Leipzig, Germany

In the vertebrate central nervous system, oligodendrocytes synthesize myelin as a lipid-rich multilayered membrane sheath that electrically insulates axons for fast impulse propagation. Myelinating glial cells can provide spiking axons with lactate, a metabolic support function of glia that precedes the evolution of myelin. In these non-myelinating species, glial cells harbor lipid droplets, a cellular energy source that may have evolved into the lipid-rich myelin compartment of vertebrates. We therefore wondered whether myelin lipid metabolism in mammals can be a source of metabolic energy that is utilized during a metabolic crisis. Using the myelinated mouse optic nerve as a model system, we found that glucose deprived oligodendrocytes readily metabolize myelin lipids and survive, unlike astrocytes, for 24 h by relying on fatty acid beta-oxidation. Catabolism of myelin lipids even supports basic ATP levels of the myelinating axon. Our data suggest a revised model of myelin as a lipid based metabolic reserve that can prolong oligodendrocyte survival and axonal integrity upon transient energy deprivation, a finding relevant for neurodegenerative diseases.

WTH06-02

FRET-based real time ATP measurements in sensory neurons of normal or diabetic rats

**P. Fernyhough¹, M.-R. Aghanoori¹, V. Margulets²,
L. Kirshenbaum², D. Gitler³**

¹University of Manitoba, Pharmacology & Therapeutics, Winnipeg, Canada

²University of Manitoba, Physiology & Pathophysiology, Winnipeg, Canada

³Ben-Gurion University of the Negev, Department of Physiology and Cell Biology and Zlotowski Center for Neuroscience, Beer-Sheva, Israel

The distal dying-back of nerve fibers is a hallmark of diabetic neuropathy. The provision of energy in the form of ATP is challenging for neurons with long axons. We hypothesized that energy supplementation via glycolysis and/or mitochondrial oxidative phosphorylation is compromised in nerve endings thus contributing to axonal degeneration in diabetic conditions. DRG neuron cultures from age-matched control or streptozotocin (STZ)-induced type 1 diabetic rats were used for *in vitro* studies. Three plasmids containing ATP sensors of varying affinities (detectable by

FRET technology and live cell confocal imaging) were transfected into neurons to study endogenous ATP levels in real time. FRET efficiency (YFP/CFP ratio) of the ATP sensors AT1.03 (low affinity) and AT1.03 YEMK (medium affinity) were significantly higher than the mutant (AT1.03 R122/6K) in DRG neurons in both cell bodies and neurites ($p < 0.0001$). Using the AT1.03 YEMK construct, treatment with oligomycin (an ATP synthase inhibitor in mitochondria) decreased the ATP levels in neurites and cell bodies of DRG neurons ($p < 0.05$). Blockade of glycolysis using 2-deoxy-D-glucose (2-DG: a glucose analog) also lowered ATP levels ($p < 0.001$). Both neurites and cell bodies of DRGs from diabetic rats showed a diminishment of ATP levels when compared to neurons from control rats ($p < 0.01$). In conclusion, low ATP levels in cell bodies and distal axons may contribute to the energy deficit in nerve in diabetes and could trigger distal dying-back nerve degeneration. Funded by St Boniface Research.

WTH06-03

ARALAR/AGC1: therapeutic approaches
**I. Liébana^{1,2,3}, M. J. Casarejos^{4,5}, M. Caamano^{1,2,3},
A. Alcaide^{1,2,3}, J. Satrustegui^{1,2,3}, B. Pardo^{1,2,3}**

¹Centro de Biología Molecular Severo Ochoa, Universidad Autónoma de Madrid, Molecular Neuropathology, Madrid, Spain

²Centro de Investigación Biomédica en Red de Enfermedades Raras, CIBERER, Madrid, Spain

³Instituto de Investigación Sanitaria Fundación Jiménez Díaz, IIS-FJD, Madrid, Spain

⁴Instituto Ramón y Cajal de Investigación Sanitaria, Hospital Ramón y Cajal, IRYCIS-HRC, Madrid, Spain

⁵Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas, CIBERNED, Madrid, Spain

Aralar/AGC1/Slc25a12, the aspartate-glutamate carrier from brain mitochondria, is the regulatory step in the malate-aspartate NADH shuttle. Aralar deficiency in humans and mice causes severe hypotonia, seizures and secondary postnatal hypomyelination possibly related to the lack of neuronal N-acetylaspartate (NAA). In mice, Aralar deficiency in striatum causes a decrease in dopamine (DA) and an increase in DOPAC/DA ratio. Intraperitoneal injections of β -hydroxybutyrate (bHB) increase the expression of proteins essential for myelin in Aralar-KO brain, such as myelin associated glycoprotein (MAG) and myelin basic protein (MBP). The bHB impact on brain NAA content and whether NAA represents a mandatory precursor of postnatal myelin lipid synthesis remains to be elucidated. HPLC analysis reveals that bHB also improve DA metabolism, increasing DA content, and reducing DOPAC/DA ratio in striatum. On the other hand, bHB exerts a beneficial role in Aralar-KO primary cortical neurons *in vitro*. It improves basal and glutamate-stimulated respiration, which are impaired in Aralar-KO neurons. In addition, this ketone body protects against glutamate-induced neuronal excitotoxicity. In this regard, bHB might work as an alternative substrate to glucose bypassing Aralar deficiency, improving neuronal metabolism, myelination and nigrostriatal DA system. But whether bHB effects are only mediated by its metabolic use in Aralar-KO neurons still needs to be determined.

WTH06-04

Metabolism of [1,6-¹³C]glucose in the cerebellum of 18 day old male and female rats: comparison with cerebral metabolism**M. McKenna¹, G. Ferreira¹, A. Karimi¹, J. Waddell¹**¹Univ. Maryland School of Medicine, Dept of Pediatrics, Baltimore, MD, USA²Federal Univ. of Rio de Janeiro, Institute of Medical Biochem., Rio de Janeiro, Brazil

The cerebellum plays an integrative role in brain, however, metabolism in developing cerebellum has not been studied. We performed ex vivo studies of cerebellar and cortical metabolism in brains from 18 day old rats 30 min after i.p. injection of [1,6-¹³C] glucose (543 mg/kg). High resolution ¹H and ¹³C spectra were obtained at 25°C on a Bruker Avance III 950 MHz NMR spectrometer. The concentration of metabolites in cerebellum was distinctly different than cerebrum. Incorporation of label from metabolism of [1,6-¹³C]glucose into the neurotransmitters glutamate and GABA, and also into glutamine and aspartate were lower in cerebellum than cerebrum. Labeling of glutamine relative to glutamate was higher in cerebellum than in the cerebrum 0.37 ± 0.01 vs 0.26 ± 0.01 ($p < 0.001$ by Mann-Whitney U test). The cycling ratio for glutamate (GLU C3/GLU C4) was lower in cerebellum than cerebrum. In contrast, the cycling ratio for glutamine (GLN C3/GLN C4) was higher in cerebellum than in the cerebrum (0.59 ± 0.04 and 0.45 ± 0.04 , respectively; $p < 0.05$) indicating active metabolism in astrocytes. There was relatively more labeling of GABA from glial precursors in cerebellum than cerebrum. The relative labeling of GABA C2 from glutamate synthesized in neurons was not different. Ex vivo ¹³C-NMR studies provide insight into the relative amount of metabolism via neuron and astrocyte specific pathways in cerebellum compared to cerebrum in developing brain. Supported in part by NIH grants 5-P01 HD016596 and P01 HD085928.

WTH06-05

Herbal molecule corrects nigral neuronal mitochondrial dynamics and bioenergetics to protect against experimental PD**K. Mohanakumar^{1,2}, R. Singh^{1,2,3}, T. Sengupta², J. Vinayagam³, D. Dutta^{1,2}, N. Ali^{1,2}, D. N. Nthenge-Ngumbau^{1,2}, J. Chakraborty², R. K. Paidi^{1,2,3}, P. Jaisankar³**¹Inter University Centre for Biomedical Research & Super Speciality Hospital, CDAR, Kottayam, India²CSIR-Indian Institute of Chemical Biology, Cell Biology & Physiology, Kolkata, India³CSIR-Indian Institute of Chemical Biology, Medicinal Chemistry, Kolkata, India

Parkinson's disease (PD) is the most common neurodegenerative movement disorder, resulting from the loss of dopaminergic neurons in the nigrostriatal system. Mitochondrial dysfunction is known to be an integral component of PD pathogenesis, and the present study investigated a novel herbal moiety, tetrahydroisoquinoline (TIQ) for its potential to correct mitochondrial dynamics and electron transport chain functional recovery. TIQ, isolated and characterized from an herb that is traditionally used in treating PD in Ayurveda system of medicine has been investigated in MPTP-, or MPP+-mediated nigral dopaminergic lesion, in mice or rats respectively.

TIQ administration per-orally in parkinsonian mice or in hemiparkinsonian rats, i.p. provided significant improvements in akinesia, catalepsy and swimming ability in mice, and reduced severity of apomorphine- or amphetamine-mediated rotational bias in rats. TIQ treatment helped to recover striatal dopamine to normal levels, and corrected the impaired mitochondrial state-3 respiration in striatal mitochondria, as well as in PD cybrids. Over-expression of the mitochondrial fission protein, Drp-1 was effectively contained by TIQ treatment in parkinsonian mice. On the other hand, the loss in the levels of mitochondrial fusion protein Mfn-2 in substantia nigra was improved by TIQ treatment. These results suggested the strength of this novel molecule as a drug contender for diseases that have mitochondrial aberration as the basis.

WTH06-06

The interplay between O-GlcNAc and phosphorylation on tyrosine hydroxylase activity and catecholamines synthesis**B. Rodrigues, M. Clodmiro, A. Rego, I. Oliveira, D. Beckman, S. Ferreira, F. Mello, A. Todeschini, R. Reis, W. Dias**

Federal University of Rio de Janeiro, Biophysics Institute, Rio de Janeiro, Brazil

The Hexosamine biosynthetic pathway (HBP) uses about 2% to 5% of all glucose that enters the cell, and has UDP-GlcNAc as final product. This is used as substrate for intracellular O-GlcNAc; a post-translational modification (PTM) resulting from the addition of GlcNAc on serine and/or threonine in proteins. This reaction is catalyzed by OGT, and the removal reaction is made by OGA. The balance of such enzymes will regulate the levels of O-GlcNAcylated proteins, which similarly to phosphorylation, are active in many cellular processes. Tyrosine hydroxylase (TH) is the rate-limiting step enzyme in catecholamine synthesis, hydroxylating L-tyrosine to obtain L-DOPA. Our aim is to investigate the role of O-GlcNAc on the tyrosine hydroxylase activity and catecholamines synthesis. Using PC12 cells, we show that O-GlcNAc is modulated during neuritogenesis. Also, O-GlcNAcylation acts on the control of the phosphorylation levels on serine 40 in TH, where stimulation by 28% on the increase in phosphorylation at serine 40 decreases O-GlcNAc in 26%; while increase of intracellular O-GlcNAc in 19% reduces serine 40 phosphorylation by 16%. In addition, increasing the intracellular O-GlcNAcylation significantly reduces the levels of L-DOPA. Enzyme activity confirms that down-regulation on TH's O-GlcNAc increases its activity by the increase on serine 40 phosphorylation. These data implies on a mechanism that integrates carbohydrate metabolism with the catecholamines pathway; where competition between O-GlcNAc and phosphate at tyrosine hydroxylase serine 40 site modulates its activity, controlling the synthesis of catecholamine levels.

WTH06-07

The anti-obesity treatment efficacy of SSRI preparations can be dependant by adapted eating habits

S. S. Yi¹, K. Lim¹, J. Lee¹, Y. Kim¹, S. Yoon¹, I. K. Hwang², Y. S. Yoon²

¹*Soonchunhyang University, Biomedical Laboratory Science, Asan, Korea South*

²*Seoul National University, Anatomy and Cell Biology, Seoul, Korea South*

Over the several decades, increasing attention has been paid to the roles and regulatory mechanisms of pharmaceutical agents in treatment of obesity. Many number of anorectic agents have been released such as specific-serotonin reuptake inhibitor (SSRI) in the market for several decades. SSRI is well known to have an anti-appetite and anti-depressant effect, however, it has a great disadvantage that it is found the body weight is rebounded finally in clinic. In addition, several reports suggest SSRI's anti-appetite

efficacy may be due to the presence of certain nutrients or palatable food, which indicate a wide variety of patients' responses. Therefore, we compared the concurrent animal preference of two foods with vehicle group while repeatedly dosing Fluoxetine, a well known SSRI, to highly adapted animals on either high-fat diet or normal-chow for ten days prior to injection of the agent. Before this study, we examined the effects of fenfluramine on food preference under almost the same conditions with this study, however, the results of Fluoxetine were somewhat different from those of Fenfluramine. Although the tolerance effects were not as rapid as those of Fenfluramine by repeated administration of Fluoxetine, the results of weight-gain were evident, and the response to Fluoxetine tended to be different depending on the type of pre-adapted foods. It is concluded treatment of the agent for anti-obesity can be altered by the adapted food types of obese patients. (This work has supported by the National Research foundation of Korea (NRF) grant funded by the Korea government(MSIT)(No.2018R1D1A3B07047960).

WTH07 Neuronal plasticity & behavior (Session B)

WTH07-01

BETA2* nicotinic acetylcholine receptors expressed by striatal neurons control behaviour in mice in a sex-dependent manner

A. Abbondanza¹, J. Höfflin², H. Janickova¹

¹*FGU, Neurochemistry, Prague, Czech Republic*

²*Rheinische Friedrich-Wilhelms Universität Bonn, Bonn, Germany*

While the function of nicotinic acetylcholine receptors (nAChRs) expressed by nerve terminals projecting to the striatum have been extensively studied, less is known about the nAChRs expressed by striatal neurons. Striatal projecting neurons (SPNs) express very low levels of nAChRs as they are mainly expressed by striatal interneurons. Electrophysiological studies have shown that specific types of GABAergic interneurons (GABA_{IN}s) are activated by nicotine and they inhibit SPNs as a result of this activation. Hence, nAChRs expressed by GABA_{IN}s may be important in modulation of striatal output and behavioral control. To determine how $\beta 2^*$ nAChRs expressed by GABA_{IN}s contribute to the control of striatal-based behavior, we selectively deleted $\beta 2$ subunit in the dorsal striatum by injecting $\beta 2$ -floxed mice with AAV-Cre vector. After confirming that the deletion only occurs in the injected area and the vector is not retrogradely transported, we sought to characterize the mice using behavioral tests and histological and biochemical measurements. First data obtained with one cohort of mice consisting of both males and females showed the effect of deletion might differ between sexes. In tasks testing depressive-like behaviour, sociability and motivation, behaviour was altered in male mutants while females did not differ from the non-deleted controls. Therefore, we conclude the $\beta 2$ deletion in the GABA_{IN}s in the dorsal striatum may have stronger effect in males than in females and we decided to focus predominantly on the male sex in our future experiments. Support: This work was supported by the Grant Agency of the Czech Republic grant [19-07983Y]. J.H. was supported by DAAD RISE program during her internship.

WTH07-02

GluN2A KD alters neuronal synaptic plasticity in neuronal mature cultures

M. Acutain¹, C. Vazquez¹, D. Jerusalinsky¹, A. Salvetti², V. Baez¹

¹*Institute of Cellular Biology and Neurosciences^{IBCN}, School of Medicine, University of Buenos Aires^{UBA}, CABA, Argentina*

²*Cancer Research Center of Lyon^{CRCL}, Inserm, Lyon, France*

Synaptic plasticity refers to long lasting changes in synapses that have been related to the structural bases of memory and learning processes. For several years, NMDA receptors (NMDAR) have been involved in those processes as well as in several neuropathologies. NMDAR are composed by two GluN1 obligatory subunits and two regulatory subunits: GluN2 (A-D) or GluN3 (A-B). In cognitive related brain structures GluN2A and GluN2B are the most expressed regulatory subunits, that undergo a tightly regulation. Whereas GluN2B expression is characteristic of immature synapses, GluN2A is present in mature synapses. In order to better understand the role

of GluN2A in synapses, we transduced mature neuronal cultures with AAV-eGFP vectors: one codifying a specific shRNA anti GluN2A, AAV-sh2A, and the other carrying a shRNA scramble as control, AAV-shSc. We verified that AAV-sh2A knocks down GluN2A mRNA and protein levels (GluN2A KD). Moreover we evaluated neuronal morphology and two synaptic proteins: Syn-1 and PSD95. In GluN2A KD cultures we confirmed the increase in dendritic arbor complexity observed previously. Interestingly, in those cultures we found a significant decrease in GluN1 protein, while GluN2B protein levels did not change. Furthermore, in those cultures the expression of Syn-1 and PSD95 was increased, which suggest that synaptic function was altered. For these reason we analyzed GluN2A KD cultures by Ca^{+2} imaging and observed changes in the excitability of neurons after chemical LTP. These results suggest that GluN2A KD induce a rearrangement of NMDAR expression that modifies synaptic physiology.

WTH07-03

HDACS class IIa: distinct effects among psychostimulant drugs on the mesocorticolimbic system

M. Bernardi¹, O. Torres², M. Sosa¹, B. Gonzalez¹, J. Muñiz¹, F. Urbano³, E. Garcia-Rill⁴, J.-L. Cadet⁵, V. Bisagno¹

¹*ININFA, Laboratory of Stimulant Drugs, CABA, Argentina*

²*San Diego Mesa College, Department of Behavioral Sciences, SD, USA*

³*IFIBYNE, Laboratory of Physiology and Molecular Biology, CABA, Argentina*

⁴*CTN, Department of Neurobiology and Developmental Sciences, LR, USA*

⁵*NIH/NIDA, Molecular Neuropsychiatry Research Branch, Baltimore, USA*

Psychostimulants produce different profiles: while some drugs cause neuroplastic changes that leads to cognitive deficits and addiction, others are considered cognitive enhancers. It is known that epigenetic mechanisms are contributory factors in drug-induced neuroadaptations, specifically, dysregulation of histone deacetylases (HDACs) was proposed as a contributor to establish and maintain aberrant transcriptional programs associated with changes in cognitive functions and behaviors. HDACs are divided into zinc-dependent (class I (HDAC1,2,3,8), class IIa (HDAC4,5,7,9), class IIb (HDAC6,10), or class IV (HDAC11)) or NAD-dependent (class III (sirtuins1-7)) enzymes. Class IIa are phosphorylated and exported from nucleus to cytoplasm and can be regulated by calcium and neuronal activity. In this study we tested acute administration of methamphetamine (1 mg/kg), modafinil (90 mg/kg), caffeine (10 mg/kg) or methylphenidate (10 mg/kg) in mice to investigate potential changes on gene expression of HDACs4,5,7 measuring mRNA and protein levels in medial prefrontal cortex (mPFC) and dorsal striatum (DS). After a single injection, all psychostimulants increased locomotion. At mRNA level, modafinil decreased HDAC7 and HDAC5 in DS but increases their levels in mPFC. Methylphenidate increased HDAC5 in mPFC and decreased in DS. Methamphetamine increased HDAC4 and HDAC5, while decreased HDAC7 in mPFC. Our results show that the effects of different psychostimulant on class IIa HDACs are brain area-

specific. Experiments are underway to explore correlations between gene expression and protein levels.

WTH07-04

Hippocampal subregions express distinct dendritic transcriptomes that reveal differences in mitochondrial function in CA2

S. Farris^{1,2,3}, James. M. Ward³, Kelly. E. Carstens³, M. Samadi³, Y. Wang³, Serena. M. Dudek³

¹Virginia Tech Carilion Research Institute, Developmental & Translational Neurobiology Center, Roanoke, VA, USA

²Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Department of Biomedical Sciences & Pathobiology, Blacksburg, VA, USA

³National Institute of Environmental Health Sciences, National Institutes of Health, Neurobiology Laboratory, Research Triangle Park, NC, USA

RNA localization is one mechanism that neurons use to spatially and temporally regulate gene expression at synapses. The repertoire of RNA transcripts in adult dendrites *in vivo* and their role(s) during learning and memory are only beginning to be explored. Advancements in RNAseq have led to the identification of thousands of RNA transcripts in hippocampal CA1 dendrites. However, it is unknown whether different hippocampal cell types express distinct dendritic transcriptomes. Here, we tested the hypothesis that cells exhibiting distinct forms of synaptic plasticity will have differences in dendritically localized RNAs. In particular, we were interested in area CA2, a small subregion of the hippocampus that is resistant to long-term potentiation and important for encoding social experience. Using laser capture microdissection and RNAseq, we discovered that each hippocampal subregion expresses a unique complement of dendritic RNAs. Specifically, we identified over 1,000 differentially expressed dendritic RNAs in the hippocampus, suggesting that local translation plays an important and overlooked layer of cell type-specific regulation. Further, by focusing gene ontology analyses on plasticity-resistant CA2, we identified an enrichment of mitochondria-associated pathways and provide functional evidence that these pathways influence CA2 plasticity and mitochondrial respiration. In sum, our results support accumulating evidence that thousands of RNAs are present in adult dendrites and likely function to regulate cell type-specific processes important for learning and memory.

WTH07-05

CGRP revealed fear memory retention disorder via NPAS4 expression in mice

N. Hashikawa-Hobara, S. Mishima, A. Otsuka, N. Hashikawa

Okayama University of Science, Life Science, Okayama, Japan

Calcitonin gene-related peptide (CGRP), which is produced in both peripheral and central nervous system, is well known as a potent vasodilator. Although CGRP plays an important role in central nervous system, its effect on hippocampus-dependent fear memory is still not clear. 8-week-old male C57BL6J mice were examined to passive avoidance test or contextual fear learning test. Mice were given a 0.2 mA foot shock when entered black compartment. In the contextual fear learning test, mice were given a 0.3 mA foot shock. After fear conditioning, mice were given

saline or CGRP (0.5 nmol) by intracerebroventricular administration. CGRP injections shortened the avoidance latency in passive avoidance test, and also reduced freezing time in contextual fear learning test. To examine which gene is involved in CGRP-mediated extinction of fear memory, microarray assay was performed. CGRP injections significantly increased *Npas4* gene rather than saline treatment. *Npas4* knockdown inhibited CGRP-mediated memory retention disorder with increase freezing time in mice. We also found that CGRP increased dephosphorylation of nuclear histone deacetylase 5 (HDAC5), which is known to be involved in epigenetic regulation of NPAS4, in the mouse hippocampus. MC1568, HDAC class IIa inhibitor, also recovered CGRP-mediated memory retention disorder in mice. Furthermore, the amount of HDAC5 bound to the *Npas4* promoter site was significantly suppressed by CGRP in the Chromatin immunoprecipitation assay. These results suggest that HDAC5 and NPAS4 might be involved in CGRP-mediated fear memory retention disorder.

WTH07-06

Enriched environment ameliorates synaptic and behavioral impairments in mouse models of schizophrenia

Y. Huang, Q. Zheng, X. Li, A. H. K. Fok, C. S. W. Lai

The University of Hong Kong, LKS Faculty of Medicine, Hong Kong, Hong Kong

Schizophrenia is a neurodevelopmental psychiatric disorder with an age of onset in late adolescence and early adulthood. Although the precise pathogenesis of the disease is remaining elusive, synaptic and behavioral alternations have been identified in human and animal studies. Recent evidence implicates that synaptic dysfunction plays an important role in schizophrenia pathogenesis. Structural imaging of postmortem studies shows a decrease of dendritic spine density on pyramidal neurons (PNs) in the prefrontal cortex (PFC) of schizophrenia patients. Spines undergo experience-dependent formation and elimination during development, allowing the establishment and remodeling of connectivity within neuronal circuits. Longitudinal studies in wild-type rodents indicate that exposure to enriched environments (EE) during early life increases brain-wide spine density. In animal schizophrenic models, EE has been demonstrated to reverse key schizophrenia-like behaviors, such as hyperactivity and sensorimotor gating deficits, but the effect of EE on dendritic spine plasticity is still unclear. Here, we studied two mouse models of schizophrenia-relevant disease processes: a chronic MK801 administration pharmacological model and a PV-*ErbB4*^{-/-} conditional knockout model, where *ErbB4* was selectively ablated in parvalbumin-positive GABAergic interneurons. We used *in vivo* two-photon transcranial imaging of adolescence fluorescent mice to investigate the structural plasticity of PNs' dendritic spine in the frontal cortex. We found that long-term exposure to EE ameliorates synaptic and schizophrenia-like behavioral impairments in these two mouse models of schizophrenia. Further studies will focus on better understanding how EE induces its beneficial effects on synaptic and behavioral levels, which might provide a new direction for future development of therapeutics in clinical practice.

WTH07-07

Leaf extracts from *dendropanax morbifera* léveillé ameliorate mercury-induced reduction of hippocampal function

I. K. Hwang¹, W. Kim¹, H. Y. Jung¹, H. J. Kwon², D. W. Kim², Y. S. Yoon¹, S. S. Yi³

¹College of Veterinary Medicine, and Research Institute for Veterinary Science, Seoul National University, Department of Anatomy and Cell Biology, Seoul, South Korea

²Research Institute of Oral Sciences, College of Dentistry, Gangneung-Wonju National University, Department of Biochemistry and Molecular Biology, Gangneung, South Korea

³College of Medical Sciences, Soonchunhyang University, Department of Biomedical Laboratory Science, Asan, South Korea

Dimethylmercury-treated rats showed reduced discrimination index in novel object recognition test and took longer to find the platform than did control group. Compared with dimethylmercury treatment alone, supplementation with DML or galantamine significantly ameliorated the reduction of discrimination index and reduced the time spent to find the platform. In addition, the number of platform crossings was lower in the dimethylmercury-treated group than in controls, while the administration of DML or galantamine significantly increased the number of crossings than did dimethylmercury treatment alone. Cell proliferation and neuroblast differentiation, assessed by Ki67 and doublecortin immunohistochemical staining was significantly decreased in the dimethylmercury treated group versus controls. Supplementation with DML or galantamine significantly promoted cell proliferation and neuroblast differentiation in the dentate gyrus. In addition, treatment with dimethylmercury significantly increased AChE activity in hippocampal homogenates, while treatment with dimethylmercury+DML or dimethylmercury+galantamine significantly ameliorated this increase. These results suggest that DML may be a functional food that improves dimethylmercury-induced memory impairment and ameliorates dimethylmercury-induced reduction in cell proliferation and neuroblast differentiation, and demonstrates corresponding activation of AChE activity in the dentate gyrus.

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WTH07-08

Analysis of transcriptome profile in the rat prefrontal cortex with risk-averse and risk-seeking preferences in a gambling task

J.-H. Kim¹, M. J. Kwak², S.-H. Jung⁴, W. Y. Kim¹, M. J. Ku², Y.-J. Chung^{3,4}

¹Yonsei University College of Medicine, Dept of Physiology, Seoul, Korea South

²Yonsei University College of Medicine, Brain Korea 21 Plus Project for Medical Sciences, Seoul, Korea South

³The Catholic University of Korea College of Medicine, Dept of Microbiology, Seoul, Korea South

⁴The Catholic University of Korea College of Medicine, Integrated Research Center for Genome Polymorphism, Seoul, Korea South

Proper measurement of risk is important for making rational decisions, and maladaptive decision making may underlie various psychiatric disorders. However, differentially expressed genetic profiling involved in this process is still largely unknown. In the

present study, we examined using next-generation sequencing (NGS) technique whether there are differences in gene expression profiles in the prefrontal cortex (PFC) when rats make different choices toward risk in rat gambling task (rGT). Rats were trained to learn the relationships between 4 different light signals on the screen and accompanied reward outcomes or punishments set up with different magnitudes and probabilities. Once they showed a stabilized pattern of preference upon free choice, rats were classified into risk-averse or risk-seeking group. After accomplishment of rGT, rats were decapitated, the PFC was dissected out from their brains, and NGS was performed with total RNA extracted. We found that there were significant differences in 498 genes (fold change > 1.3, $p < 0.05$) up or down-regulated in risk-seeking compared to risk-averse group. Among those, with a few top ranked genes, post analysis for validation is currently under the investigation. These results suggest that differential gene expression profile appearing in the PFC may importantly contribute to the preference toward risk choice in rGT.

WTH07-09

Fibroblast growth factor and ARA290 fused with elastin-like polypeptides for neuroregeneration after spinal cord injury

S. Kumar¹, S. Rastogi², T. Theis³, M. Schachner³, M. Yarmush¹, F. Berthiaume¹

¹Rutgers, The State University of New Jersey, Biomedical Engineering, Piscataway, USA

²Rutgers, The State University of New Jersey, Department of Cell Biology and Neurosciences, Piscataway, USA

³Rutgers, The State University of New Jersey, W.M. Keck Center for Collaborative Neuroscience, Piscataway, USA

Traumatic spinal cord injury (SCI) is one of the most devastating traumas that lead to sudden loss of sensorimotor and autonomic functions below the level of injury besides chronic pain, pressure ulcers, and respiratory problems. Primary mechanical insult to the spinal cord triggers a cascade of molecular and cellular events, which tend to cause severe secondary damage (e.g. local edema, ischemia, inflammatory response etc.). There is a limited neurological recovery with extensive neurological dysfunction with lifelong disability and poor quality of life for SCI patients and their families. We have recently developed a fusion proteins namely fibroblast growth factor (FGF-2) and ARA290 fused with elastin-like polypeptides (ELPs). The expression vector containing FGF2-ELP and ARA290-ELP were transformed into *E. coli*. The bacteria were grown overnight and then lysed by sonication. The fusion proteins were purified by inverse transition cycling at 4°C and 40°C. Fusion proteins were characterized and their biological activity was tested using cerebellar granule cells (e.g. live/dead survival assay and neurite outgrowth) and human endothelial cells (e.g. tube formation assay) *in vitro*. Our results suggested a better neurite outgrowth with FGF2-ELP in a dose-dependent (1-1000 nM) manner as compared to FGF-2 and ELP. Similarly, ARA290-ELP treatment (1-3 µM) suggested better survival *in vitro* and *in vivo* (30 mg/kg). The efficacy of this modality for SCI recovery is unknown but if successful, it may provide a low-cost biologic approach to improve SCI patient outcomes.

WTH07-10

Cholinergic processing in the medial prefrontal cortex of a mouse model for neuropathic pain**K. Kummer¹, M. Mitrić¹, T. Kalpachidou¹, M. Zeidler¹, A. Seewald², F. Ferraguti², M. Kress¹**¹Medical University of Innsbruck, Division of Physiology, Innsbruck, Austria²Medical University of Innsbruck, Department of Pharmacology, Innsbruck, Austria

Chronic neuropathic pain constitutes a major public health issue, but the underlying disease mechanisms are only partially understood. The involvement of the medial prefrontal cortex (mPFC) in pain chronification has already been established and disruption of cholinergic transmission to mPFC has been associated with the impairment of cognitive functions, a frequent comorbidity of chronic pain. Nevertheless, the role of cholinergic projections to the mPFC in the processing of painful stimuli has so far been widely neglected. Therefore, we investigated cholinergic processing in acute mPFC slices from spared nerve injury (SNI) and sham-operated mice by either pharmacological stimulation in multielectrode array (MEA) recordings or endogenous acetylcholine release induced by optogenetic stimulation during patch-clamp recordings. Also, functional and morphological investigation of basal forebrain (BF) cholinergic neurons was performed. MEA recordings demonstrated that SNI reduced the mPFC network activity in response to muscarinic receptor activation, which was revealed to be M1 receptor dependent. This reduction was also observed in patch-clamp recordings of prelimbic (PrL) but not infralimbic (IL) layer 5 pyramidal neurons. Finally, patch-clamp recordings of BF cholinergic neurons revealed SNI-associated hyperexcitability, disinhibition and morphological changes in SNI compared to sham treated controls. These findings show that chronic pain is associated with alterations in cholinergic synaptic transmission at PrL pyramidal neurons that are suggested to be based on SNI induced disinhibition and subsequent hyperexcitability of BF cholinergic neurons.

WTH07-11

Motor impairment in mice with a gain-of-function mutation in retinoic acid receptor beta (RARB)**N. Lemmetti^{1,2}, C. Nassif¹, Jacques. L. Michaud^{1,2}**¹CHU Ste-Justine Research Center, Brain and Child Development, Montreal, Canada²Université de Montréal, Neurosciences, Montreal, Canada

Retinoic acid (RA) plays a critical role during brain development by binding to receptors that function as ligand-activated transcription factors. We previously described mutations in the retinoic acid receptor beta gene (*RARB*) in patients with dystonia. We found that these mutations enhance RA-induced transcriptional activity 2- to 3-fold over the WT receptor, suggesting a gain-of-function (GOF) mechanism. Loss of *Rarb* in mice cause motor impairment and a reduction of striatonigral neurons due to premature differentiation of their progenitors. We hypothesize that the motor impairment of patients with *RARB* GOF mutations is caused by increased *RARB* signaling in the striatum, possibly disrupting homeostatic control of the same pathways as those affected by decreased *Rarb* signaling. In order to investigate this hypothesis, we introduced p.R394C, the equivalent of the recurrent p.R387C GOF mutation found in some patients, at the *Rarb* genomic locus in mice. Behavioral assessment

of *Rarb*^{R394C/+} mice showed a short stride and dramatically reduced motor coordination in the rotarod paradigm. Moreover, these mice show a decreased number of D2- but not D1-expressing neurons in the striatum. *Rarb*^{R394C/R394C} mice are born at the expected mendelian ratio but they show a waddling gait, their growth is compromised and they die perinatally. In order to understand the cellular basis of the motor impairment associated with p.R394C, we are currently characterizing the striatum of *Rarb*^{R394C/+} and *Rarb*^{R394C/R394C} mice using additional molecular markers and transcriptomic studies.

WTH07-12

Hippocampal activity induced by increasing memory demand in an olfactory recognition task is altered in aged rats**P. Moreno-Castilla, Lynde. M. Wrangler, Edward. L. Rivera, Sharyn. L. Rossi, J. Long, P. Rapp**

National Institute on Aging, Laboratory of Behavioral Neurosciences, Baltimore, USA

Recognizing a stimulus as previously encountered is crucial in everyday life and a capacity that frequently declines in older adults. Specifically, aged humans incorrectly identify a novel stimulus as familiar, but the neurobiological basis of these deficits remains controversial. In order to investigate the function of brain circuits that may underly such errors, we took advantage of rodents' exceptional olfactory abilities and their natural preference for novelty to develop an olfactory recognition task that assessed accuracy as a function of memory demand (short- and long-term retention) in young and aged Long-Evans rats. We found that aging did not affect either the total time rats explored presented odors or recognition of a novel odorant after a short delay (0.5 h). In contrast, olfactory recognition after a long delay (24 h) was sensitive to aging and mirrored individual differences in water maze performance tested in the same animals. Neuronal activity was assessed by quantifying the immediate-early gene products *Arc* and *c-Fos* after the olfactory memory tests. In addition, we performed a total examination of the brain regions involved with optical clearing and fluorescence deep-tissue imaging techniques by using light-sheet microscopy. Aged rats that fail to recognize the novel odor after the long delay showed fewer active granule cells in the dentate gyrus and hippocampal CA3 region relative to young and aged rats that performed successfully. Interestingly, aged impaired rats displayed increased hippocampal activation in comparison with young and aged unimpaired animals after testing on short-term odor recognition. These findings suggest that memory demand-dependent hippocampal activation is altered in normal aging.

WTH07-13

Unique contributions of amyloid and tau to the entorhinal cortical-hippocampal axis dictate behavioural deficits in TGF344-ad rats**C. Morrone^{1,2}, P. Bazzigaluppi³, T. Beckett¹, M. Hill¹, M. Koletar³, B. Stefanovic^{3,4}, J. McLaurin^{1,2}**¹Sunnybrook Research Institute, Biological Sciences, Toronto, Canada²University of Toronto, Laboratory Medicine and Pathobiology, Toronto, Canada³Sunnybrook Research Institute, Physical Sciences, Toronto, Canada⁴University of Toronto, Medical Biophysics, Toronto, Canada

Alzheimer's disease is the leading cause of dementia, and poses a major health burden on the elderly population. Current disease models describe a long prodromal phase beginning in early adulthood, with progressive accumulation of Ab pathology first, which is followed by neurofibrillary tangles of hyperphosphorylated tau and neuronal loss. Multiple Ab-targeted therapies have demonstrated promising results in preclinical models, however all have failed to meet cognitive endpoints in the clinic. The regional distribution of neurodegeneration and tau pathology better predict cognitive outcomes than Ab pathology, and these pathologies are absent in commonly used preclinical models. Therefore, we investigated the successes and failures of Ab attenuation in disease bearing TgF344-AD rats, which better represent a complete Alzheimer's disease-like phenotype, with the goal of understanding the connection between pathologies and behaviour. Ab attenuation exerted variable effects on behaviour, with rescue in pattern separation and executive function deficits, but no effect on activities of daily living and spatial memory. Hippocampal reduction in Ab promoted the clearance of tau pathology, and resilience in hippocampal neurogenesis and neuronal function, underlying behavioural rescue. In the entorhinal cortex, reduction in Ab did not promote the clearance of tau, and dysfunction in the entorhinal-hippocampal network persisted, underlying spatial memory deficits. Therefore, Alzheimer's disease pathology can progress independent of Ab in the symptomatic stage, and therapeutic design should be focused on combinatorial approaches.

WTH07-14

Alpha7 nicotinic agonist reverse bla hyperactivity and attenuation of dopaminergic activity induced by chronic mild stress in rats**G. Neves^{1,2}, A. Grace¹**¹University of Pittsburgh, Department of Neuroscience, Pittsburgh, USA²Federal University of Rio de Janeiro, Institute of Biomedical Sciences, Rio de Janeiro, Brazil

Studies showing that nicotinic receptors (nAChR) may play a role in mood regulation increased the interest in targeting the cholinergic system for major depressive disorder treatment. Modulation of nAChRs in the basolateral amygdala (BLA) is sufficient to produce an anti-immobility effect in the mouse tail suspension test, however, how nAChR impact BLA neuronal activity in relation to mood is not well-characterized. In this work, we used the chronic unpredictable mild stress (CMS) model to investigate the potential antidepressant-like effect of a $\alpha 7$ nAChR full agonist and the

involvement of BLA-induced changes in dopaminergic neurotransmission in its mechanism of action. All experimental procedures were conducted according to NIH guidelines and approved by University of Pittsburgh Institutional Animal Care and Use Committee. Male adult Sprague-Dawley rats were exposed to four weeks of CMS. Behavioral and electrophysiological experiments were performed within one week following stress. A single administration of 7 nAChR full agonist PNU282987 reversed the stress-induced increase in forced swim immobility time. CMS exposure decreased the number of spontaneously active dopamine neurons in the ventral tegmental area and increased the firing rate of putative projection neurons in the BLA. Both stress-induced electrophysiological changes were reversed by a single administration of PNU282987. In summary, our findings indicate a potential antidepressant effect for $\alpha 7$ nAChR full agonist that involves a mechanism distinct from those of classic antidepressants: normalization of BLA hyperactivity and, consequently, of DA hypofunction. These observations corroborate the role of $\alpha 7$ nAChR as a potential important target for novel antidepressant drug development. **Support:** NIH (MH191180).

WTH07-15

C-Fos reactivity and functional alterations of cortical neural structures of early undernourished lactating wistar rats**M. Ortiz, M. Regalado, C. Torrero, M. Salas**National Autonomous University of Mexico ^{UNAM}, Institute of Neurobiology, Department of Developmental Neurobiology and Neurophysiology, Querétaro, Mexico

Early undernutrition and environmental deficiencies are epigenetic conditions that severely interfere with the development and functions of the young brain with long-term consequences on the maternal behavior, learning, and electrical activities. These pups' alterations disrupt the transmission, encoding and integration of the messages ascending from subcortical levels to the cerebral cortex. The late-emerging effects of pre- and neonatal undernutrition on the pup retrieval by early underfed lactating Wistar dams were correlated with reduced immunoreactivity of C-Fos protein at the anterior cingulate, medial prefrontal cortex and basolateral amygdala examined on lactation days 4 and 12. The F0 dams and the F1 control subjects received a balanced diet during the gestation and the lactating periods. In the underfed group, pregnant F0 dams received different percentages of a balanced diet, after birth, prenatally underfed (F1) pups continued their undernutrition by remaining with a nipple-ligated mother for 12 h. Weaning occurred at 25 days of age, and pups were subsequently provided with an ad lib diet. At 90 days of age, F1 dams were maternally tested (10 min), and her pups were separated; 90 min later dams were sacrificed, and their brain removed for immunohistochemistry. Early underfed F1 dams showed prolonged retrieval latencies by grasping pups by inappropriate body areas; this behavioral alteration was correlated with significant reduction in immunoreactivity of C-Fos protein on neural cortical structures. The findings suggest that early undernutrition can have deficiencies in the mechanisms underlying the maternal behavior that correlated with decreased activity on the maternal circuit.

WTH07-16

Effect of lactobacillus gasseri OLL2809 on mouse depressive-like behavior

A. Otsuka, T. Fukunaga, R. Yamamoto, N. Hashikawa, N. Hashikawa

Okayama University of Science, Life Science, Okayama, Japan

Introduction: Probiotics have been reported they have a beneficial effects on depressive symptoms in humans. In this study, we used social defeat stress model to investigate the effect of lactobacillus gasseri OLL2809 on the depressive-like behavior in mice.

Methods: Male C57BL6J mice were used for 8 weeks of age. Social defeat stress were applied for 4 weeks, followed by receiving OLL2809 was mixed in normal diet to 2×10^9 cfu, for 2 weeks. After administration, behavioral tests were conducted on open field test, forced swimming test, tail suspension test and sucrose preference test.

Results: In the open field test, locomotor activity, rearing activity and time spent in the center area by stressed and stressed with OLL2809 were not significantly different. Administration of OLL2809 improved depression-like behavior in forced swim test and sucrose preference test. These results suggest that OLL2809 may have an antidepressant effect.

WTH07-17

A high caloric diet induces memory impairment and disrupted synaptic plasticity in aged rats

S. Paulo^{1,2}, C. Miranda-Lourenço^{1,2}, R. Belo^{1,2}, R. Rodrigues^{1,2}, S. Tanqueiro^{1,2}, J. Fonseca-Gomes^{1,2}, A. Sebastião^{1,2}, S. Xapelli^{1,2}, M. Diógenes^{1,2}

¹*Instituto de Farmacologia e Neurociências, FMUL, Lisbon, Portugal*

²*iMM-JLA, FMUL, Lisbon, Portugal*

The alarming increase in sugar/fat consumption over the last decades, leading to overweight/obesity, is a major health concern nowadays, being associated with a higher risk of metabolic and cardiovascular diseases. Recent evidence suggests a possible deleterious effect of this type of diet on cognition, which may exacerbate natural age-related cognitive decline. Thus, we aimed at unravelling the effects of a high caloric diet (HCD) in aged rats upon synaptic plasticity and memory performance.

Male rats were fed with a high-sucrose/fat diet from 1 to 24-months-old and analysed at 24 months-old. Weight and glycemia were measured and behaviour tests were performed. Field excitatory postsynaptic potentials (fEPSPs) were recorded upon a long-term potentiation (LTP) protocol. Adult hippocampal neurogenesis was evaluated and the levels of brain-derived neurotrophic factor TrkB receptors were appraised.

HCD animals were obese ($p < 0.001$), while glycemia levels were decreased ($p < 0.05$). These animals presented greater anxious-related behaviour (open-field test) and significant episodic long-term memory impairment (novel-object recognition test) (novel vs familiar object: CTL, $p < 0.05$, HCD, $p > 0.05$), accompanied by a reduction in LTP ($p < 0.05$). TrkB full-length receptor levels were decreased in the hippocampus of HCD animals ($p < 0.05$). However, no changes were observed concerning the number of immature neurons in the dentate gyrus of the hippocampus. These results

suggest that a HCD leading to obesity impairs memory performance and synaptic plasticity at old-age, possibly due to changes in TrkB receptors.

WTH07-18

TGF-beta1 in cognitive functions in a healthy brain

E. Pershina^{1,2}, E. Kamaltdinova^{1,2}, S. Levin^{1,2}, V. Arkhipov^{1,2}

¹*Pushchino State Inst. of Natural Sci., -, Pushchino, Russia*

²*Inst. of Theoretical and Exptl. Biophysics, -, Pushchino, Russia*

The non-canonical function of transforming growth factor $\beta 1$ (TGF- $\beta 1$) was investigated: its participation in the mechanisms of learning and memory in the healthy brain. Male Wistar rats were trained to perform the task with food reinforcement. Endogenous level of TGF- $\beta 1$ were measured in the using ELISA at different periods after training. The mRNA level of both TGF- $\beta 1$ itself and its receptors was determined by the method of qPCR. The results showed that the training led to a decrease in TGF- $\beta 1$ protein level in the hippocampus, however, the gene expression of this cytokine and its receptors didn't change. In the prefrontal cortex, an increase in TGF- $\beta 1$ protein level was observed after training, which remained elevated after 1 h and 24 h after learning and returned to normal level after 72 h. Gene expression of TGF- $\beta 1$ in the prefrontal cortex decreased immediately after training, recovering to the control level 1 h after training. In the prefrontal cortex, training has led to a slight decrease in gene expression for the TGFBR1 receptor, which mean a compensatory change in response to an elevated level of the TGF- $\beta 1$ protein. Considering the special role of the prefrontal cortex in memory consolidation and the revealed changes in the expression of TGF- $\beta 1$ and its receptors in this structure, it can be assumed that TGF- $\beta 1$ is involved in the consolidation of memory traces. This assumption is supported by experiments in which the pharmacological inhibition of the TGF- $\beta 1$ signaling pathway with SB431542 led to memory impairment in rats in the passive avoidance test. This study was funded by a grant of the Russian Science Foundation (project No.16-15-10356).

WTH07-19

Early postnatal FMR1 loss from cortical excitatory neurons elicits auditory processing deficits in a mouse model of FXS

M. Rais¹, J. Lovelace², X. Shuai¹, S. Bishay¹, A. Palacios¹, D. Binder¹, K. Razak², I. Ethell¹

¹*University of California, Riverside, Division of Biomedical Sciences, Riverside, USA*

²*University of California, Riverside, Psychology Department, Riverside, USA*

Fragile X Syndrome (FXS) is a common monogenic form of autism spectrum disorder (ASD). Symptoms of FXS include anxiety, intellectual disability, and abnormal sensory processing. Altered auditory responses in humans with FXS and *Fmr1* KO animals suggest that abnormal development of the auditory circuits may underlie the deficits. Hypersensitive neuronal circuits develop during third postnatal week (P14-P21 period). This period coincides with the critical period plasticity (CPP) window in the rodent auditory cortex, a postnatal window of structural and functional circuit development driven by sensory input. Since the development

of acoustic representations in primary auditory cortex is profoundly influenced by early experience, here we examined how deletion of *Fmr1* from cortical excitatory neurons during this period affects auditory processing phenotypes in the auditory cortex. Mice were developed to remove FMRP specifically from the cortical excitatory neurons during the P14-P21 window through Cre-mediated deletion of floxed *Fmr1* gene in excitatory neurons using CaMK2a promoter. We found that similar to global *Fmr1* KO the density of PV/PNN cells was reduced in Cre^{CaMK2a}/*Fmr1*^{Flox/y} cKO mice, whereas cortical MMP-9 gelatinase activity and resting EEG gamma power were enhanced. In addition, TrkB phosphorylation was increased in Cre^{CaMK2a}/*Fmr1*^{Flox/y} cKO mice, suggesting dysregulation of BDNF-TrkB signaling in the auditory cortex of these mice. The Cre^{CaMK2a}/*Fmr1*^{Flox/y} cKO mice also show increased locomotor activity and anxiety-like behaviors. These results indicate that FMRP loss in cortical excitatory neurons during P14-P21 period is sufficient to elicit abnormal cellular, electrophysiological and behavioral phenotypes in mice.

WTH07-20

Relaxin-3 receptor (RXFP3) expression by gaba neurons in hippocampus and amygdala and effects of RXFP3 activation on behaviour

V. Rytova¹, M. Nategh^{1,2}, T. D. Aumann¹, H. Albert-Gasco³, C. A. Reid^{1,2}, R. A. D. Bathgate^{1,2}, F. E. Oloucha-Bordonau³, S. Ma^{1,2}, A. L. Gundlach^{1,2}

¹The Florey Institute of Neuroscience and Mental Health, Parkville, Victoria, Australia

²The University of Melbourne, Florey Department of Neuroscience and Mental Health, Parkville, Australia

³Universitat Jaume I, Unitat Predepartamental de Medicina, Castellon, Spain

The neuropeptide, relaxin-3, preferentially activates the G_{i/o}-protein-coupled receptor, RXFP3 in brain. Relaxin-3/GABA projection neurons constitute an ascending network enriched in limbic areas such as amygdala, ventral hippocampus (vHip), septum and prefrontal cortex, involved in stress, arousal and emotional behaviours. Here, we characterized the effects of chronic RXFP3 activation in vHip or medial amygdala (MeA) on anxiety and social avoidance. In vHip, RXFP3 mRNA was detected in somatostatin (SOM)/GABA neurons. AAV vectors driving local secretion of RXFP3 agonist, R3/I5, were bilaterally injected into vHip or MeA of adult Sprague-Dawley rats (7-10 per group). Chronic vHip RXFP3 activation *decreased* time and distance in the open arms of an elevated plus maze (EPM) and the light zone of a light-dark box (LDB); and decreased social interaction with a conspecific stranger, compared to control (all $p < 0.05$). Conversely, chronic RXFP3 activation in the MeA *increased* time and distance in EPM open arms and the centre of a large open field (LOF). In MeA, RXFP3 mRNA was detected in GABA neurons, and co-expressed with oxytocin receptor mRNA. We are currently testing the effect of RXFP3 agonist (0.5-1 μ M RXFP3-A2) application on interneuron activity in hippocampal brain slices from wildtype and transgenic mice. These studies should provide a better understanding of the neurochemical/neurophysiological basis of anxiety behaviour, with potential novel therapeutic targets for anxiety disorders.

WTH07-21

The sigma-1 receptor: a molecular hub for psychostimulant drugs in the nucleus accumbens A. Segev¹, I. Delint-Ramirez¹, F. Garcia-Oscos¹, A. Pavuluri¹, S. Kourrich^{2,1}

¹University of Texas Southwestern Medical Center in Dallas, Department of Psychiatry, Dallas, USA

²Université du Québec à Montréal, Département des Sciences Biologiques - CERMO-FC, Montreal, Canada

The psychoactive properties of psychostimulant drugs such as cocaine (COC) and methamphetamine (METH) have long been considered a consequence of their ability to enhance dopamine (DA) signaling. Recently, we found that COC-induced firing rate depression (FRD) in nucleus accumbens shell (NAcSh) medium spiny neurons (MSNs), a physiological adaptation that contributes to behavioral sensitization to cocaine, is independent of DA signaling, but dependent on the endoplasmic chaperon protein sigma-1 receptor (σ 1R). Here we found that METH (2 mg/kg i.p for 5 days.), a psychostimulant drug with pharmacological properties distinct from COC, also induces a FRD in NAcSh DA D1 receptor-containing MSNs (D1R-MSNs). Similar to cocaine, METH-induced neuronal intrinsic plasticity is independent of DA signaling, but dependent on σ 1R binding to Kv1.2 potassium channels, a mechanism that leads to enhanced Kv1.2 levels at the plasma membrane. Using site-directed mutagenesis of σ 1R binding site for METH in HEK293T cells, we found that METH binding to σ 1R is necessary for METH-induced enhanced surface Kv1.2. Interestingly, Methylphenidate (Ritalin[®]), a psychostimulant drug used for the treatment of Attention Deficit Hyperactivity Disorder and that exhibits addiction liability, engages the same mechanism. Our study provides direct evidence that besides actions mediated through conventionally studied mechanisms, psychostimulant drugs also engage a common DA-independent, but σ 1 binding-dependent mechanism that contributes to behavioral response to cocaine.

WTH07-22

Gene expression regulation in dentate gyrus during memory formation

A. Shvadchenko^{1,2}, O. Erlychenkova¹, A. Vinarskaya¹, M. Roshchina¹, M. Volobueva¹, N. Bal¹, P. Balaban¹

¹Institute of Higher Nervous Activity and Neurophysiology of RAS, Cellular Neurobiology of Learning Lab, Moscow, Russia

²I.M. Sechenov First Moscow State Medical University, Center of Master's Programs, Moscow, Russia

It was shown that nitric oxide can regulate gene expression in neurons. To investigate genes which are controlled by nitric oxide, we analyzed differential expression of transcripts in hippocampal cultured neurons during NOS blockade by L-NAME. We revealed that *Igf2* gene expression can be regulated by nitric oxide. In present experiments we have studied the relationships between nitric oxide production and *Igf2* and *c-fos* expression in hippocampus during memory formation. Rats were trained according to contextual fear conditioning paradigm followed by hippocampus or dentate gyrus isolation 1 h after training. Trained groups were intraperitoneally injected with saline solution or L-NAME (30 mg/kg) 30 min before training session. Gene expression analysis was performed using real-time PCR. We revealed that *c-fos* expression in trained animals was significantly increased as compared to naïve control ($p < 0.05$).

L-NAME exposure significantly lowered *c-fos* expression as compared to the trained group ($p < 0.05$). We didn't observe any differences in *Igf2* expression between groups in whole hippocampi. We observed a tendency of *c-fos* and *Igf2* expression increase in isolated dentate gyrus ($p < 0.1$) during memory formation in vehicle-injected trained group. Expression of both genes did not change in the L-NAME-injected group after training as compared to naïve control. These data suggest that nitric oxide may regulate *c-fos* and *Igf2* gene expression in dentate gyrus during memory formation. This study is supported by RSF grant № 18-75-10112.

WTH07-23

Allosteric neurotensin receptor 1 modulator confers beta-arrestin bias and selectively attenuates addiction-associated behaviors

L. Slosky, L. Barak, M. Caron

Duke University, Cell Biology, Durham, USA

Psychostimulant and opioid addictions are growing international public health concerns that are inadequately managed by available therapeutics. A shared feature of psychostimulants and opioids is their ability to incite aberrant activation of the mesolimbic dopamine system. Restoration of dopamine signaling homeostasis may be achieved by targeting the G protein-coupled receptor (GPCR) neurotensin receptor 1 (NTR1). Characteristic of GPCRs, NTR1 signals not only through the canonical activation of G proteins, but also through β -arrestins to mediate distinct cellular and physiological effects. Drug-like, small molecule NTR1 agonists have remained elusive, despite decades of screening using G protein-based approaches. Employing a β -arrestin-based screening platform, we have identified a promising preclinical lead: compound SBI-553. Here, we report that SBI-553 exhibits novel pharmacodynamic properties, acting as both a β -arrestin biased activator and a positive allosteric modulator that biases neurotensin-NTR1 signaling towards the β -arrestin pathway. Critically, this β -arrestin-biased modulator attenuated behavioral evidence of methamphetamine, cocaine and remifentanyl exposure in mouse models of drug use without the side effects characteristics of unbiased NTR1 agonism. In line with SBI-553's biochemical mechanism of action, studies in neuron-subtype-specific β -arrestin2 knockout mice revealed that SBI-553 behavior modulation requires β -arrestin2 expression in striatal dopamine D2 receptor-expressing neurons. These data reveal central roles for NTR1 and β -arrestin in substance use disorders and identify a novel agent whose development may lead to improved clinical outcomes for patients.

WTH07-24

Characterization of a novel animal model of episodic hepatic encephalopathy

F. Tamnanloo^{1,2}, R. Ochoa-Sanchez^{1,2}, M. Tremblay¹, Christopher F. Rose^{1, 2}

¹Centre de recherche du CHUM ^{crCHUM}, -, Montréal, Canada

²Université de Montréal, Médecine, Montréal, Canada

Introduction: Hepatic encephalopathy (HE) is a neuropsychiatric syndrome, a major complication of chronic liver disease (CLD/cirrhosis). The primary cause of hospital admissions for cirrhotic patients is an overt episode of HE. Precipitating factors of HE

frequently lead to an increase in blood ammonia. Patients who have experienced multiple episodes of HE are associated with persisting neurological complications post-liver transplantation. Currently, the impact of HE episodes on neurological integrity is unknown. We hypothesize that multiple episodes of HE will accelerate and/or intensify neurological deterioration. To date, an animal model of episodic HE is lacking. Therefore, our goal was to characterize an animal model of episodic HE and to evaluate the impact of cumulative episodes on neurological status in cirrhotic rats. **M&M:** Animal model of CLD and HE: 6-week bile-duct ligation (BDL) rats, and Sham-operated controls were used. Ammonium acetate was used to induce HE episodes, every 4 days starting at 3-weeks post-surgery (total 5 episodes). After the last episode, we assessed motor-coordination (RotaRod), anxiety (elevated plus maze), as well as, short-term and long-term memory (novel object recognition). Rats were then sacrificed, and plasma ammonia measured.

Results: Short-term memory ($p < 0.05$) and motor-coordination ($p < 0.05$) were reduced in both non-episodic and episodic BDL groups vs Sham-operated controls. Long-term memory impairment ($p = 0.06$) and increased anxiety (+60.0%, $p < 0.05$) were found only in episodic vs non-episodic BDL rats. Moreover, there was an increase in blood ammonia (+30.4%, $p = 0.06$) in episodic vs non-episodic BDL rats.

Conclusion: The induction of HE episodes escalates neurological impairments in cirrhotic rats. Thus, this new episodic HE model represents a good approach to explore the pathological mechanism arising from multiple episodes, as well as further investigate brain sensitivity to ammonia. Moreover, this model is an excellent platform to investigate novel therapies to prevent or treat episodic HE.

WTH07-25

Autism-related deficits via dysregulated NSF-dependent membrane protein trafficking

M. J. Xie^{1,2,3}, K. Iwata^{1,2,3}, Y. Ishikawa⁴, Y. Fukazawa⁵, H. Matsuzaki^{1,2,3}

¹University of Fukui, RCCMD, Yoshida-gun, Japan

²University of Fukui, LSIC, Yoshida-gun, Japan

³Osaka University, UGSCD, Osaka, Japan

⁴Maebashi Institute of Technology, Department of Systems Life Engineering, Gunma, Japan

⁵University of Fukui, Department of Morphological and Physiological Sciences, Yoshida-gun, Japan

Background: Abnormal serotonin transporter (SERT) function has been implicated in autism. New serotonin transporter binding protein, *N*-ethylmaleimide-sensitive factor (NSF), found to be reduced in postmortem brains and lymphocytes of autistic subjects. Thus, we investigated whether the NSF knockout mice showed autistic behaviors and their molecular mechanism.

Methods: To assess autistic like behavior, we used three-chambered task, ultrasonic vocalizations analysis and open field test. Alteration of endogenous SERT membrane expression was evaluated in the raphe. To examine AMPA receptors location in the synaptic membrane, we used freeze-fractured replica-immunolabeling study. Furthermore, to examine synaptic functions, we measured evoked field EPSPs in CA1 of the hippocampal slice. The LTD and LTP were induced by low-frequency stimulation (LFS) and high-frequency stimulation (HFS) of the Schaffer collaterals, respectively.

Results: We found social interaction abnormalities and communication deficits in the *NSF*^{+/-} mice, compared with the *NSF*^{+/+} mice. Anxiety was detected in the *NSF*^{+/-} mice. At the postsynaptic level, the AMPA receptor expression revealed a significant decrease in synaptic membrane of the *NSF*^{+/-} mice. Furthermore, the LTD was impaired in the CA1 of the *NSF*^{+/-} mice, but normal LTP was

found in the both mice.

Conclusions: NSF is associated with autism and has an important role in the regulation of behavior. NSF is involved in the synaptic plasticity through membrane protein trafficking, and it might be related to the pathophysiology of behavioral impairments.

WTH08 Clinical studies, biomarkers & imaging (Session B)

WTH08-01

Sensory perception and processing alterations in SYNGAP1, a mouse model of SYNGAP1 haploinsufficiency
M. I. Carreno-Munoz^{1,2}, B. Chattopadhyaya¹, K. Agbogba^{1,2}, S. Lippe^{1,2}, J. L. Michaud^{1,2}, G. D. Cristo^{1,2}

¹CHU Sainte Justine, Brain Development, Montreal, Canada

²University of Montreal, Neurosciences, Montreal, Canada

Autism-spectrum disorder (ASD) is a range of neurodevelopmental disorders characterized by impaired social interaction, stereotyped behaviours, cognitive impairments and sensory deficits. While there are hundreds of genes involved in ASD, SYNGAP1 gene is particularly gaining attention because of the frequency and penetrance of loss-of-function variants found in patients as well as the wide range of comorbid brain disorders associated with SYNGAP1 pathogenicity. In both human patients and animal models, Syngap1 haploinsufficiency leads to cognitive and social problems, altered neural circuit excitability, spontaneous seizure and unadaptative behaviours. Until now, most of the research on the deficits caused by SYNGAP1 haploinsufficiency has focused on cognitive problems but recent works point to the possibility that altered sensory processing may strongly contribute to cognitive problems and other altered behaviours associated with ASD. Indeed, sensory symptoms are becoming an important early biomarker, since they are often documented as early as 6 months of age in infants later diagnosed with ASD (considerably earlier than development of other higher cognitive functions as attention). Although sensory problems have been largely reported in ASD, whether auditory and visual perception and processing are altered in Syngap1 haploinsufficient mice is not known. Here we study different aspects of auditory and visual perception in a mouse model of SYNGAP1 haploinsufficiency. Our preliminary results reveal altered electrophysiological activity underlying both auditory and visual perception.

WTH08-02

Potential biomarkers in human serum for severity of acute ischemic stroke: mouse to man via analyses of mouse brain proteome

Z. Gu¹, J. Cui¹, H. Song¹, H. Zhou¹, J. Hou¹, J. Cheng¹, Y. Lu², S. Li³, W. Chen⁴, W. Cai⁴, G. Sun¹, C. Wu⁴

¹University of Missouri, Pathology&Anatomical Sciences; Computer Science, Columbia, USA

²Texas State University, Genetic Center, San Marcos, USA

³University of Maryland, Chemistry, College Park, USA

⁴Second Affiliated Clinical College of Fujian University of Traditional Chinese Medicine, Neurology, Fuzhou, China

Acute ischemic stroke (AIS) is a devastating neurological disease. Biomarkers may assist identification of stroke mechanisms and predict the risk of complications in stroke patients. We examined ischemic mouse brain proteome with bioinformatic analysis and investigated expressed proteins at different cerebral sub-regions in a mouse model of ischemia and identified differentially expressed proteins in mouse cortex, hippocampus, and striatum. Ingenuity Pathway Analysis suggested involvement of inflammatory response, Nrf2-mediated antioxidant responses, and neurodegenerative signaling pathways. From this initial analysis, two candidate biomarkers, clusterin (CLU) and cystatin C (CST3), were further examined in serum samples of

patients with AIS within 24 h of stroke onset and together with matched health controls. CLU levels in AIS were significantly higher within 24 h of stroke onset as compared to controls, and the values showed positive correlation with both NIHSS scores and time after stroke onset. CST3 levels also showed significant increase in stroke patients as compared with controls. These data demonstrate that elevated levels of serum CLU and CST3 may serve as peripheral biomarkers for assessing AIS severity. Furthermore, quantitative proteomics together with bioinformatic analyses can provide a novel translational strategy for identifying proteins/pathways that aid in the discovery of novel biomarkers associated with ischemic stroke.

WTH08-03

Quantification of posterior cingulate cortex glucose hypometabolism in patients with mild cognitive impairment using F-18 FDG pet

F. Peng

University of Texas Southwestern Medical Center, Radiology, Dallas, USA

Objectives: Patients with mild cognitive impairment (MCI) at early stage of Alzheimer's disease were found to have glucose hypometabolism in posterior cingulate cortex (PCC). However, it is sometimes challenging to recognize mild decrease of FDG uptake in PCC on PET images by visual assessment. The objective of this retrospective study was to conduct a quantitative PET analysis of cerebral FDG uptake in the brains of MCI patients when no abnormal FDG uptake was detected on prior F-18 FDG PET by visual assessment.

Methods: MCI Patients with prior F-18 FDG brain PET were searched in a data base established in our PET center. A quantitative PET analysis was conducted to assess cerebral FDG uptake using a data of normal cerebral FDG uptake installed in our facility, in correlation with the prior results of clinical neuropsychological tests.

Results: Five MCI patients with previous normal F-18 FDG brain PET by visual assessment were identified and subjected to further quantitative analysis of cerebral FDG uptake. Three patients were found to have low FDG uptake in PCC and one patient was found to have low FDG uptake in bilateral parietal lobe by quantification, which were not detected by previous visual assessment. No significant abnormal FDG uptake was detected in one of five MCI patients.

Conclusion: Both visual assessment and quantitative analysis are essential for detection of posterior cingulate cortex glucose hypometabolism in MCI patients using F-18 FDG PET.

WTH08-04

Molecular and functional characterization of early-stage Parkinson's disease

A. Rajan¹, D. LaRocca², Q. Du¹, C. Neville², S. DeVita¹, A. Montefusco¹, R. Ericson¹, A. Brindle¹, D. Mihaila³, F. Middleton²

¹Quadrant Biosciences, Research, Syracuse, USA

²SUNY Upstate Medical University, Neuroscience & Physiology, Syracuse, USA

³SUNY Upstate Medical University, Neurology, Syracuse, USA

Parkinson's disease (PD) is a progressive debilitating neurodegenerative disorder, affecting 2% of the population over the age of 65, that exerts a major burden on patients, families, and society.

Because most cases are idiopathic, there is considerable interest in identifying biomarkers that might indicate the earliest signs of PD to facilitate interventions to delay or modify disease course. We sought to identify such biomarkers through comprehensive characterization of the salivary microtranscriptome along with detailed functional assessments of subjects with early stage PD. We recruited 58 PD subjects and 38 age- and gender-matched controls. Subjects completed detailed assessments of motor, cognitive, balance, autonomic, and chemosensory/smell functions. RNA was extracted from saliva, stranded RNA-sequenced, and reads aligned to human non-coding RNA and oral microbiome databases. Sequencing data were then investigated for robust correlations with phenotypic data. Human ncRNAs identified included microRNAs, PIWI-interacting RNAs, and small-nucleolar RNAs.

We identified distinct subsets of each class of ncRNAs that appeared highly-changed in early-stage PD, and were reasonably accurate in classifying PD. Interestingly, among the miRNA biomarkers, several of the predicted gene targets are known PD susceptibility genes (SNCA, PARK2, ATG12, HTRA2, and LRRK2). Several ncRNAs correlated with standard scores for PD progression, cognition, daily living, motor function, and quality of life measures. Unexpectedly, some identified microbial strains correlated with these standard scores. These results suggest the salivary microtranscriptome represents a highly-accessible and informative microenvironment offering new insights into the

pathophysiology of early-stage PD, with promising utility for discovering biomarkers.

WTH08-05

Comparison of oxidative stress parameters between healthy control and idiopathic chronic fatigue

C.-G. Son

Daejeon University, Institute of Traditional Medicine and Bioscience, Daejeon, Korea South

The linkage between oxidative stress and idiopathic chronic fatigue (ICF) has not been explored in detail. This study thoroughly compared the serum levels of biomarkers for oxidative stress and antioxidants from 103 subjects with ICF (20 men and 83 women) to those of 82 healthy volunteers (27 men and 55 women). Oxidative parameters, which included reactive oxygen species (ROS), malondialdehyde (MDA) and F2-isopropanol, and tumor necrosis factor- α (TNF- α) were significantly elevated, while antioxidant parameters, which included total antioxidant activity (TAC), catalase, superoxide dismutase, SOD and GSH activity, were decreased compared to those of healthy subjects (by approximately 1.2- to 2.3-fold, $p < 0.05$ or 0.01). Our results confirmed that oxidative stress is a key contributor in the pathophysiology of ICF, and firstly explored the features of oxidative stress parameters in ICF subjects compared to a healthy population.

WTH09 Neurodegeneration and mental health (Session B)

WTH09-01

Glutamate transporter *eaat1* (GLAST) in human prefrontal cortex; interactome and expression in brains of chronic alcoholics

V. Balcar¹, M. A. Kashem¹, N. Sultana¹, D. Pow²

¹The University of Sydney, School of Medical Sciences, Sydney, Australia

²The University of Queensland, Centre for Clinical Research, Brisbane, Australia

Our previous studies using post-mortem samples of alcoholic brain tissue have identified changes in expression and abundance of many proteins, neurotransmitters and their metabolites. The focus of our current studies has been on glutamate transporter EAAT1/GLAST that normally resides in plasma membranes of glial cells and has previously been reported as significantly increased in human alcoholic prefrontal cortex. We estimated EAAT1/GLAST expression in human alcoholic brains and then used healthy brain tissue to reveal any proteins specifically interacting with EAAT1/GLAST thus being potentially impacted by changes in EAAT1/GLAST expression *in vivo*. Post-mortem samples were analysed by Western blotting to compare the expressions of EAAT1/GLAST in healthy and alcoholic brains. Using the non-alcoholic prefrontal brain cortex we determined EAAT1/GLAST “interactome” i.e. the set of proteins specifically interacting with EAAT1/GLAST. EAAT1/GLAST was significantly more abundant (1.6-fold in total; 2.2-fold in the fraction containing plasma membranes) in the prefrontal cortical tissue from alcoholic brains compared to that from healthy controls. In the healthy brains, 38 proteins specifically interacted with EAAT1/GLAST. These proteins can be classified as contributing to the cell structure (6 proteins; 16%), energy and general metabolism (18 proteins; 47%), neurotransmitter metabolism (three proteins; 8%), signalling (6 proteins; 16%), neurotransmitter release at synapses (three proteins; 8%) and Ca²⁺ buffering (two proteins; 5%). The findings represent an extension of our previous proteomic and metabolomic studies of human alcoholism revealing another aspect of the complexity of changes imposed on brain by chronic long-term consumption of ethanol.

WTH09-02

Reduced glucose transporter-1 trafficking to the plasma membrane impairs brain glucose utilization in Alzheimer's disease

S. Barger^{1,3}, Y. Ou¹, R. Hendrix², J. Davis¹

¹University of Arkansas for Medical Sciences, Department of Geriatrics, Little Rock AR, USA

²Washington University, Department of Neurology, St. Louis MO, USA

³Central Arkansas Veterans Healthcare System, GRECC, Little Rock AR, USA

Alzheimer's disease (AD) manifests lower rates of cerebral glucose consumption. We find that accumulation of amyloid β -peptide (A β) in the CNS also perturbs peripheral glucose handling in the BRI-A β 42 transgenic (A β -tg) mouse line, utilized because it obviates APP over-expression, itself a modulator of insulin levels.

A β -tg mice had elevated blood glucose levels, and glucose tolerance was impaired, as seen in 80% of AD patients. Comprehensive laboratory animal monitoring system (CLAMS) cages were employed to test for differences in food consumption, physical activity, or respiration; no differences were found between A β -tg and wild type (WT). Likewise, insulin was unaltered, and no systemic insulin resistance was detected. Gluconeogenesis was not altered; A β -tg were not significantly overweight; we detected no differences in blood lipids, inflammatory factors, relevant endocrine factors, nor hypothalamic neuropeptides. A β -tg mice did, however, show reduced cerebral consumption of glucose. To explore this phenomenon we analyzed the levels and cellular fractionation of glucose transporter-1 (GLUT1) and other glucose transporters. Alone among these, parenchymal GLUT1—discernible from endothelial GLUT1 by gel mobility—showed less translocation to the plasma membrane. In cerebral tissue from AD patients, parenchymal GLUT1 evinced a similar blockage in translocation. Finally, primary cultures of astrocytes mirrored this phenomenon after exposure to A β ₁₋₄₂. These findings indicate that impaired peripheral glucose regulation in AD may result from lower cerebral consumption, itself caused by the direct effect of A β on astrocytes, which are critical for shuttling glucose from the vasculature to neurons. Supported by U.S. National Institute on Aging P01AG012411-19.

WTH09-03

Comparing the morphology and physiology of PD-vulnerable neuronal populations

S. B. Nanni¹, M.-J. Bourque², L.-E. Trudeau^{1,2}

¹Université de Montréal, Neuroscience, Montreal, Canada

²Université de Montréal, Pharmacology and Physiology, Montreal, Canada

There is a growing body of evidence exploring the causative factors rendering neurons vulnerable in Parkinson's disease (PD). Much of this work posits that chronic mitochondrial oxidative stress and impaired proteostasis are driving the cellular pathological mechanisms. One key distinguishing characteristic of PD-vulnerable neurons is their vast projecting axonal arborizations, a factor thought to contribute significantly to their elevated bioenergetic needs, increased basal oxidative stress and ensuing vulnerability. Recent work in our group has provided evidence for a tight link between neuronal vulnerability, basal bioenergetics and axonal arbor size, when comparing dopaminergic (DA) neurons of the substantia nigra, ventral tegmental area and olfactory bulb. To further strengthen and extend this hypothesis, we now aim to determine if a similar link between axonal arbor size and vulnerability can be found in other long-range projection neurons. We used a mouse primary culture system to compare noradrenergic neurons of the locus coeruleus, serotonergic neurons of the raphe nuclei and cholinergic neurons of the dorsal motor nucleus of the vagus and pedunculopontine nucleus. We find that these neurons possess an intrinsic capacity to establish a long and complex axonal arbor that is larger than that of VTA neurons but comparable to that of SNc DA neurons. We are presently extending this comparison by evaluating mitochondrial density and polarity as well as oxidative

stress in the axonal domain of these same neuronal populations. Finally, we are comparing and contrasting the relative vulnerability of these PD-vulnerable nuclei to PD-relevant *in vitro* stress assays including oxidative stress (H_2O_2) and proteosomal stress. Preliminary results suggest that LC neurons are surprisingly less vulnerable to H_2O_2 than SNc DA neurons.

WTH09-04

Serotonergic system is affected in a drosophila model of Parkinson's disease

J. Campusano, R. V. Zárate, N. Navarro, S. Hldalgo, D. Molina-Mateo, M. E. Andrés, N. Fuenzalida-Urbe

Pontificia Universidad Catolica de Chile, Biología Celular y Molecular, Santiago, Chile

Parkinson's disease (PD) is a degenerative disorder characterized by several motor symptoms (MS) including shaking, rigidity, slow movement and difficult walking. In addition, no motor symptoms (NMS) which include olfactory dysfunction, depression, anxiety and sleep disorders have been also described. Although PD is described by a progressive loss of dopaminergic neurons, there are other neural systems affected including the serotonergic system. Abnormalities in this system in patients with PD have been associated with NMS both at the pre-symptomatic and symptomatic stages of the disease, and more recently with MS as well. Nevertheless, little is known on how the serotonergic system contributes to the onset of MS or how it is involved in pre-symptomatic stages of the disease. In this work, we used a *Drosophila* model of PD generated by a deletion in the fly *PINK1* gene (*PINK1*^{B9}). These mutants exhibit a pre-symptomatic phase with olfactory impairment and a symptomatic phase with locomotor defects. Here, we characterized the serotonergic system in *PINK1*^{B9} flies. During the pre-symptomatic phase in the PD fly model, a lower serotonin content and defects in transporter (SERT) activity were detected. In order to increase the serotonergic signaling in young *PINK1*^{B9} flies before the onset of locomotor defects we fed flies with a selective SERT blocker. This treatment partially prevented the locomotor defects observed in old *PINK1*^{B9} flies, but it negatively affects the locomotion in older w¹¹¹⁸ control flies. Thus, we propose that a possible dysfunction in the serotonergic system during the pre-symptomatic phase could contribute to the Parkinsonian phenotype in older *PINK1*^{B9} flies. Fondecyt 1161375:

WTH09-05

Determination of M6A mRNA methylation in Parkinson's disease model

X. Chen, C. Yu, M. Guo, X. Zheng

Beijing University of Technology, College of Life Science and Bioengineering, Beijing, China

Parkinson's disease (PD) is a common age-related neurological disorder with its pathogenesis still not fully elucidated. Reports have shown that epigenetic mechanisms including DNA methylation are associated with PD. However, the role of mRNA methylation in PD still remains unclear. N6-methyladenosine (m6A) is the most prevalent occurs in the mRNA of eukaryotes and plays a vital role in the post-transcriptional regulation. In our study, we investigated the amount of m6A mRNA methylation in both cell and rat model of PD induced by 6-hydroxydopamine (6-OHDA). We found that the

global m6A modification of mRNAs is down-regulated in PC12 cell model and in the striatum of PD rat brain. To test whether the decreasing m6A modification of mRNA is interrelated with its demethylases (FTO and ALKBH5), we then identified their distribution. In cell model, the results showed that FTO was up-regulated, with no difference of ALKBH5, indicating down-regulation of m6A in cell model was due to FTO. In rat model, the expression of FTO was enhanced in midbrain, with the elevation of ALKBH5 in striatum. As the onset of PD is mainly in the SNpc of midbrain, we found no significant difference in the m6A content in midbrain; however, the expression of demethylase FTO was increased significantly. We speculated that high expression of FTO in midbrain could transmit to striatum by the axons of dopaminergic neurons and decrease m6A level together with ALKBH5. These results suggest that m6A modification of mRNAs is down-regulated in PD models, which may be due to the over-expression of its demethylases. Collectively, our results provide a novel view of mRNA methylation to understand the epigenetic regulation of Parkinson's disease.

WTH09-06

CDNF enhances development and survival of stem cell derived dopaminergic neurons

R. Cohen¹, N. Francis^{1,2}, N. Zhao³, H. Calvelli¹, A. Saini¹, J. Gifford⁴, G. Wagner⁴, Z. Pang², P. Moghe^{1,3}

¹Rutgers, Biomedical Engineering, Piscataway, USA

²Rutgers RWJMS, Neuroscience and Cell Biology, New Brunswick, USA

³Rutgers, Chemical and Biochemical Engineering, Piscataway, USA

⁴Rutgers, Psychology, New Brunswick, USA

Cell transplantation studies offer a way to treat neurological disorders such as Parkinson's Disease, but require robust dopaminergic neuronal cells. One way to produce such material would be from genetically unmodified patient specific or immunologically neutral Induced Pluripotent Stem Cells (iPSCs). Using a single Episomal plasmid with a cocktail of small molecules, a stable iPSC line was generated. A 3 step protocol was used to generate DOPA2 cells, a neuronal precursor line; (1) differentiation into neural lineages as spheres with high dose Retinoic Acid, Y27632 Rock Inhibitor, and an increasing gradient of sonic hedgehog signaling for 7 days; (2) amplification and selection for 3-4 passages/weeks as adherent cultures with CDFN and a defined cytokine cocktail described to be secreted from PA6 cells, a dopaminergic inducing mouse mesenchymal line (3) differentiation into mature neurons using CDFN and canonical dopaminergic factors, such as GDNF, BDNF, Activin A, TGF- β 3, Notch inhibition, and elevation of cAMP. In the second step, the combination of CDFN, cytokines and small molecules allowed for the rapid expansion and enrichment of the DOPA2 dopaminergic precursor cell line, while in the third phase CDFN allowed for the robust survival of dopamine secreting neurons developed in 2D culture and was necessary when cells were seeded, grown and then transplanted in a novel 3D matrix. Currently, we are evaluating the biological activity of a CDFN nano particle that may be useful in non-cell-based approach to treat neurological disorders.

WTH09-07

Cellular prion protein is required for toxicity mediated by soluble aggregates of neurodegeneration-causing proteins

G. Corbett¹, W. Hong¹, M. Lao¹, A. Asfaw¹, T. Hall¹, L. Ding¹, A. DeSousa¹, S. Linse², M. Frosch³, J. Collinge⁴, D. Harris⁵, M. Perkinson⁶, A. Billinton⁶, T. Young-Pearse¹, D. Walsh¹

¹Brigham & Women's Hospital and Harvard Medical School, Neurology, Boston, USA

²Lund University, Biochemistry, Lund, Sweden

³Massachusetts General Hospital and Harvard Medical School, Neurology, Charlestown, USA

⁴University College London, Prion Unit, London, England

⁵Boston University School of Medicine, Biochemistry, Boston, USA

⁶AstraZeneca, Neuroscience, Cambridge, England

Neurodegenerative diseases affect tens of millions of people worldwide. Most of these disorders are associated with the presence of macroscopic aggregates formed from neuronal proteins of unrelated sequences. While substantial efforts have focused on these tell-tale histological lesions, emerging data indicates that soluble aggregates of the same proteins are the true drivers of pathogenesis. Here, we report that soluble, prefibrillar aggregates of amyloid- β protein (A β), α -synuclein (α Syn) and tau bind with high affinity to the N-terminus of the cellular prion protein (PrP^C) and that deletion of specific PrP sequences prevents these interactions. Furthermore, soluble aggregates of all three proteins bind to PrP^C on the surfaces of neurons, causing dose- and time-dependent loss of neuritic integrity that can be precluded by deleting or immunotargeting PrP^C. Importantly, soluble extracts of Alzheimer's disease, dementia with Lewy body and frontotemporal dementia brains also induce PrP^C-dependent neurotoxicity in a manner that can be prevented by removal of the disease-relevant proteins (i.e. A β , α Syn or tau). Collectively, these results indicate that PrP^C plays a central role in a variety of late-life neurodegenerative diseases and that therapeutic targeting of PrP^C may offer a means to treat conditions which often involve multiple proteinopathies.

WTH09-08

Mitochondrial PKA is neuroprotective in a cell culture model of Alzheimer's disease

R. Dagda, T. D. Banerjee, K. Reihl

University of Nevada, Reno, Pharmacology, Reno, USA

Alzheimer's disease (AD) is a neurodegenerative disease characterized by progressive memory loss and cognitive decline. The pathological features of AD include the accumulation of extracellular amyloid beta peptide (A β ₄₂) accompanied by oxidative stress, mitochondrial fragmentation and dysfunction, and neuron loss. Neurodegeneration and mitochondrial fragmentation in AD is believed to be partially attributed to a decrease in neuroprotective signaling mediated by PKA, a serine/threonine kinase that promotes neuronal survival and mitochondrial fusion by associating with its mitochondrial-targeted protein scaffold A-kinase anchoring protein 121 (AKAP121). We hypothesized that: 1) a decrease in PKA signaling at the mitochondrion contributes to neurodegeneration in AD, and 2) restoring PKA signaling at the mitochondrion can reverse mitochondrial fragmentation and neurodegeneration in neurons in AD models. By immunohistochemistry, we showed that the level of AKAP121, but not of other mitochondrial proteins, is

significantly reduced in primary neurons treated with A β 1-42 peptide (10 mM, 24 hrs.), and in hippocampal and cortical brain slices from 6-month-old 5XFAD mice. We surmised that restoring the level of AKAP121 might reverse neurodegeneration and mitochondrial pathology induced by A β 1-42 in cortical neurons. Transiently expressing wild-type, but not a PKA-binding deficient mutant of AKAP121, was able to reverse mitochondrial fission, loss of dendritic mitochondria, dendrite retraction and apoptosis in primary neurons treated with A β 1-42. Mechanistically, the protective effects of AKAP121/PKA is mediated through PKA-mediated phosphorylation of the fission modulator Drp1, as transiently expressing a PKA phosphomimetic mutant of Drp1 (Drp1-S656D) phenocopies AKAP121's ability to reduce neurodegeneration induced by A β 1-42. Overall, our data suggests that augmenting PKA signaling at the mitochondrion via expression of AKAP121 is neuroprotective in *in vitro* models of AD, and AKAP121 is a potential anti-AD therapeutic target.

WTH09-09

Global RNA-SEQ reveals the timeline of transcriptomic changes associated with neuroinflammation in a mouse model for cINCL

M. Domowicz¹, W.-C. Chan², J. Andrade², N. Schwartz¹

¹The University of Chicago, Department of Pediatrics, Chicago, USA

²The University of Chicago, Center for Research Informatics, Chicago, USA

Neuronal Ceroid Lipofuscinoses (NCLs), are a group of inherited, early onset, fatal neurodegenerative diseases associated with mutations in 13 genes. All forms of the disease are characterized by lysosomal accumulation of fluorescent storage material, as well as profound neurodegeneration. In this study we used a well characterized model of classical late infantile NCL (cLINCL) in which the tripeptidyl peptidase 1 (*Tpp1*) gene is disrupted by gene targeting, resulting in loss of detectable TPPI activity and leading to progressive neurological phenotypes including ataxia and increased motor deficiency. We have analyzed forebrain/midbrain and cerebellar transcriptional differences at 2, 3 and 4 months of age in control and TPPI-deficient mice by global RNA-sequencing to identify genes and pathways that may contribute to progression of the neurodegenerative process. Progressive neurodegenerative inflammatory responses involving microglia and astrocytes and endothelial cell were observed, accompanied by activation of leukocyte extravasation signals and up-regulation of NO and ROS production. Several astrocytic (i.e., *Gfap*, *C4b*, *Osmr*, *Serpina3n*) and microglial (i.e., *Ctss*, *Itgb2*, *Itga*, *Lyz2*) genes were identified as good markers for assessing disease progression as they showed increased expression levels *in vivo* over time. Based on these gene expression changes we concluded that neuroinflammation starts, for the most part, after 2 months in the *Tpp1*^{-/-} brain and that activation of microglia and astrocytes occur more rapidly in cerebellum than in the rest of the brain; confirming increased severity in this region. These findings have led to a better understanding of cLINCL pathological onset and progression, which may aid development of future therapeutic treatments for this disease.

WTH09-10

Pathological roles of neurexins in α -synuclein pathology in Parkinson's disease and Lewy body dementias**A. Fallon^{1,2}, A. Lee^{1,3}, Y. Naito^{1,3}, H. Takahashi^{1,2,3}**¹*Institut de recherches cliniques de Montréal, Synapse development and plasticity, Montreal, Canada*²*Université de Montréal, Neuroscience, Montreal, Canada*³*McGill University, Integrated Program in Neuroscience, Montreal, Canada*

Parkinson's disease (PD) is a neurodegenerative disease associated with an α -synuclein (α -syn) pathology, but its underlying molecular mechanisms remain unknown. α -syn is the primary component of Lewy bodies and Lewy neurites, both present in PD and Lewy Body Dementias (LBD). α -syn can form oligomers and fibrils, which are toxic for neurons and synapses. In addition, α -syn could be released and uptaken from neuron to neuron, resulting in spreading of α -syn pathology. Yet, little is known about how α -syn leads to synaptic pathology and what mechanism underlies α -syn spreading. We have recently found that α -syn interacts with the β -isoform of the neurexin (NRX) family members (β -NRXs). NRXs are presynaptic cell adhesion molecules that regulate synapse formation, plasticity, neurotransmitter release and cognitive function. My project is to evaluate how the α -syn- β -NRX interaction contributes to α -syn pathology and spreading. We hypothesize that this interaction affects synaptic function and composition of NRXs and mediates neuron-to-neuron transmission of α -syn. To test our hypothesis, the α -syn-NRX interaction was characterized by performing cell surface binding assays and pull-down assays. Second, the effects of α -syn on NRX trafficking and functions was tested through internalization assays and an artificial synapse formation assays, respectively. Finally, I will elucidate if NRXs mediate the uptake and the propagation of α -syn by performing respectively pH-Rodo uptake assays and microfluid three chamber assays. This work will contribute to our better understanding of molecular mechanisms of α -syn pathology and spreading, providing new molecular insight into PD and LBD.

WTH09-11

Delayed onset and progression of amyotrophic lateral sclerosis in SOD1-G37R/THY1-YFP16 mouse model**F. Fiore***Université de Montréal, Neurosciences, Montréal, Canada*

Amyotrophic Lateral Sclerosis (ALS) is a fatal and rapidly progressing neurodegenerative disease characterized by neuromuscular junction (NMJ) denervation and motor neuron (MN) death. In order to study denervation mechanisms *in vivo*, we crossed SOD1^{G37R} mice with thy1-YFP16 mice. Surprisingly, ALS onset occurred much later in SOD1-YFP mice compared to SOD1 mice. We therefore investigated SOD1-YFP disease progression and motor phenotype. We observed a delayed onset and slower disease progression in SOD1-YFP mice along with the preservation of their motor function for a longer period of time. We then looked at NMJ denervation and motor neuron survival. SOD1-YFP mice showed less denervation and postsynaptic disorganization, suggesting a better overall preservation of their NMJs. They also displayed increased motor neuron survival in lumbar spinal chord slices. Finally, we analyzed synaptic transmission at the NMJ. Preliminary results show no difference in synaptic activity between SOD1-YFP

and SOD1 mice, suggesting early synaptic transmission is unaffected. In this study, we characterized ALS evolution in SOD1-YFP mice and found a significant delay in disease onset and slower progression. The exact cause of this delay remains to be determined.

WTH09-12

Changes in dorsal hippocampal calcium levels and behavior before, during, and after ad pathology in the 5XFAD and hne mouse models**A. Ghoweri¹, L. Ouillette², H. Frazier¹, K. Anderson¹, C. Gant¹, R. Parent², G. Murphy², O. Thibault¹**¹*University of Kentucky, Pharmacology and Nutritional Sciences, Lexington, USA*²*University of Michigan, Department of Molecular and Integrative Physiology, Ann Arbor, USA*

Highlighted in the calcium hypothesis of brain aging and dementia, an altered state of calcium handling in neurons has an impact on several physiological parameters, including the Ca²⁺-dependent potassium potential, the afterhyperpolarization (AHP). One hallmark of field CA1 neuronal aging in the hippocampus is an increased AHP, accompanied with elevated levels of intracellular calcium. Though a robust association between calcium and the AHP has been illustrated in normal aging, how the two phenomena contribute to disease-state aging remains largely unknown. Recent work has reported reduced levels of L-type voltage sensitive calcium channels (L-VSCCs) in older APP and PS-1 transgenic mice, suggesting calcium dysregulation in AD mouse models may vary from that seen in aging. In this study, we are identifying the effects of aging on the calcium-dependent AHP and intracellular calcium levels in the 5xFAD and HNE models. Using sharp electrode electrophysiology and calcium imaging (OGB-1), we are beginning to observe an attenuated AHP in the 4 month 5xFAD animals compared to 1.5 months. Analyses of behavior data (MWM) does not show deficit until later time points (6-7 months). These data support the notion that reduced neuronal calcium signaling could be a precipitating factor in the manifestation of behavioral deficits, rather than an increase in neuronal calcium seen in normal aging.

WTH09-13

Alteration of brain energy induced by psychological stress affects motor function in a rat model of Parkinson's disease**M. Grigoruta^{1,2}, R. Dagda², A. Martinez-Martinez¹**¹*Universidad Autonoma de Ciudad Juarez, Chemical Biology, Juarez, Mexico*²*University of Nevada Reno, Pharmacology, Reno, USA*

Parkinson's disease (PD) is a multifactorial progressive neurodegenerative disorder. A loss of *substantia nigra* (SN) neurons leads to clinical symptoms including rigid tremors and bradykinesia. In the brain, psychological stress can increase oxidative damage, neurodegeneration, and thereby contribute to neuropathologies. While the link between psychological stress and brain health has received high level of attention, the implication of psychological stress in the onset and progression of PD is not known.

Hypothesis: We hypothesized that distress alters energy production in the brain, elicits oxidative stress and affects

motor function in wild-type (WT) and in Parkinsonian rats (PINK1-KO).

Results: By using an XF24^e Extracellular Analyzer, we observed that psychological distress diminished up to 50% of mitochondrial respiration and glycolysis in the prefrontal cortex (PC) and concomitantly elicited an increase in mitochondrial respiration in SN from brains derived both WT and PINK1-KO rats. By performing immunohistochemical analysis of tyrosine hydroxylase neurons, we observed intrinsic neurodegeneration in PINK1-KO rats at two and a half months of age, neuropathology that was not enhanced by psychological distress. In addition, psychological distress induced motor deficits and significantly reduced hind leg strength in WT rats in a similar manner as PINK1-KO rats. Finally, Western blot analysis of brain lysates showed that the protein content of several mitochondrial antioxidants proteins, mitochondrial markers and of brain-derived neurotrophic factor were significantly reduced because of psychological distress.

Conclusion: Our data suggest that psychological distress can elicit alterations in brain bioenergetics, induce fatigue and motor deficits in a similar manner as in PD without inducing SN degeneration.

WTH09-14

Functional recovery of Alzheimer disease mice by functional gene-expressing neural stem cells and microglial cells

H. Gwak¹, H.-R. Seong^{1,2}, D. W. Seo^{1,2}, E. S. An², E.-K. Choi^{1,2}, S. S. Joo³, D. Park⁴, Y.-B. Kim^{1, 2}

¹Chungbuk national university, College of Veterinary Medicine, Cheongju, Republic of Korea

²Designed Cells Co., Ltd., Cheongju, Republic of Korea

³Gangneung-Wonju National University, Department of Marine Molecular Biotechnology, Gangneung, Republic of Korea

⁴Korea National University of Education, Department of Biology Education, Cheongju, Republic of Korea

In Alzheimer disease (AD) patients, amyloid β (A β) peptide-mediated degeneration of cholinergic system utilizing acetylcholine (ACh) for memory acquisition is observed. Since AD therapy using acetylcholinesterase (AChE) inhibitors are only palliative for memory deficits without reversing disease progress, cell-based therapeutic approaches are attracting attention. To recover cognitive function and to eliminate the causative A β peptides, we established F3.ChAT human neural stem cells (NSCs) encoding choline acetyltransferase (ChAT) gene (an ACh-synthesizing enzyme) as well as HMO6.NEP human microglial cells encoding neprilysin (NEP) gene (an A β -degrading enzyme) and HMO6.SRA cells encoding scavenger receptor (SRA) gene (an A β -uptaking enzyme). The cells (2×10^5 cells) were transplanted intracerebroventricularly to mice showing memory loss induced by cholinotoxin AF64A, and brain A β accumulation, ACh concentration, and cognitive function were analyzed. Transplanted NSCs and microglial cells were found to express their functional genes in the mouse brain and restored the learning and memory function of AF64A-challenged mice by eliminating A β deposit and recovering ACh level, in which the effects were further enhanced by combinational treatments with F3.ChAT and HMO6.NEP or HMO6.SRA cells. The cell therapy also attenuated inflammatory astrocytic response by reducing A β accumulation. Taken together, it is expected that NSCs and

microglial cells over-expressing ChAT, NEP or SRA genes could be candidates for replacement therapy of AD.

WTH09-15

NMDA-induced mitochondrial depolarization and subsequent neurodegeneration regulated by intracellular potassium levels

H. Higashi, T. Kinjo, K. Uno, N. Kuramoto

Setsunan University, Laboratory of Molecular Pharmacology, Faculty of Pharmaceutical Sciences, Hirakata-shi, Japan

Intracellular potassium ion level is higher than extracellular space, which is regulated by basically sodium pump. Potassium ion generates a resting potential on the cell membrane by passing through the leak channels. Openings of the other potassium channels on the cell membrane induce hyperpolarization and it would cancel the excitation. ATP-sensitive potassium channel (K_{ATP}) is an inwardly rectifying potassium channel that suppresses depolarization in both cell membrane and mitochondria, and has an important role to make and keep resting membrane potential. Minoxidil opens this channel. It has been reported that opening of K_{ATP} protected the cells from ischemic damage in heart and brain. However, the underlying mechanism is not clear. Here, we have investigated that how minoxidil suppresses ischemic damage and excitotoxicity. Transient ischemia model mice were prepared by 1-h middle cerebral artery occlusion using 6-week old male C57/BL mice. Injection of minoxidil immediately after the operation prevented the damage in a concentration-dependent manner. Thus it was suggested that minoxidil protected the neuronal tissues against excitotoxicity. In the primary cultured neurons, N-methyl-D-aspartic acid (NMDA) induced mitochondrial depolarization and neurodegeneration, following an increase in calcium influx into the cytosol. In contrast, minoxidil suppressed the depolarization and the neurodegeneration. In addition, minoxidil lowered intracellular potassium levels, which was measured using Asante Potassium Green-2 AM, and suppressed an increase in the cleaved caspase level, which was measured by Western blotting. It was suggested that minoxidil decreased mitochondrial depolarization-dependent neuronal apoptotic cell death following decrease in an intracellular potassium levels by opening of K_{ATP} channels.

WTH09-16

A ginseng berry extract improves cognitive function via up-regulation of choline acetyltransferase expression and neuroprotection

S. HyeRim^{1, 2}, J. S. Kil³, H. Kwak¹, D. W. Seo^{1, 2}, E. S. An², E.-K. Choi^{1, 2}, S. S. Joo³, Y.-B. Kim^{1, 2}

¹Chungbuk National University, College of Veterinary Medicine, Cheongju, Korea South

²Designed Cells, nonclinical research team, Cheongju, Korea South

³Gangneung-Wonju National University, Marine Molecular Biotechnology, Gangneung, Korea South

In Alzheimer disease (AD), amyloid-beta (A β) peptides induce the degeneration of presynaptic cholinergic system, in which decreased activity of enzyme choline acetyltransferase (ChAT) responsible for acetylcholine synthesis is observed. Ginseng berry contains diverse ginsenosides including Rb1, higher than ginseng

root, which is a well-known ingredient improving human cognitive function. We investigated the effects of an ethanolic extract of ginseng berries (GBE) on learning and memory function of mice challenged with an A β 1-42 peptide and the underlying mechanisms *in vitro*. GBE significantly inhibited brain acetylcholinesterase activity *in vitro* (1 μ g/mL), and protected against A β 1-42-induced cytotoxicity in F3.ChAT human neural stem cells (≥ 0.1 μ g/mL) and enhanced the ChAT gene expression as well (1 μ g/mL). A β 1-42 injection into the mouse brain led to a loss of learning and memory function in passive avoidance and Morris water-maze performances. Such an impaired cognitive function was recovered by oral administration of GBE (≥ 100 mg/kg), wherein GBE was superior to epigallocatechin gallate (EGCG), a well-known cognition-enhancing ingredient of green tea. In addition, GBE and EGCG restored brain acetylcholine concentration (a neurotransmitter governing memory acquisition) and attenuated astrocyte activation (an inflammatory response) in the A β -challenged mouse brain. The results demonstrate that GBE administration recovered the cognitive function of AD model animals by enhancing acetylcholine level via ChAT gene expression and neuroprotection.

WTH09-17

An apelin receptor agonist protects against retinal neuronal cell death induced by N-methyl-D-aspartic acid in mice

Y. Ishimaru, H. Konishi, T. Kuriyama, F. Shibagaki, A. Yamamuro, Y. Yoshioka, S. Maeda

*Setsunan University, Pharmaceutical sciences/
Pharmacotherapeutics, Hirakata, Japan*

Glutamate excitotoxicity via N-methyl-D-aspartic acid (NMDA) receptors is associated with the loss of the retinal neuronal cells in retinal diseases, such as glaucoma and diabetic retinopathy. The loss of the retinal neuronal cells causes visual impairment, and eventually irreversible blindness. However, there is no medicine to suppress the loss of the retinal neuronal cells in the retinal diseases yet. Apelin is an endogenous peptide ligand for the G protein-coupled receptor APJ. We have previously reported that APJ is expressed in the retinal ganglion cells and the amacrine cells, which are exquisitely sensitive to glutamate excitotoxicity, and that intravitreal injection of apelin protects against the retinal neuronal cell death induced by NMDA in mice. In the present study, we investigated whether systemic administration of a small molecule APJ agonist prevents retinal neuronal cell death induced by NMDA. The effect of the APJ agonist was assessed by immunohistochemistry and electroretinography. Intravitreal injection of NMDA induced a decrease of both the retinal ganglion cells and the amacrine cells stained with anti-Brn-3a and calretinin antibodies. The decrease of the retinal neuronal cells was suppressed by intraperitoneal administration of the APJ agonist at 1 h before intravitreal injection of NMDA. Consistent with the immunohistochemical findings, electroretinography revealed that the APJ agonist prevented a decrease of electroretinogram amplitudes, which reflect the function of the retinal neuronal cells, induced by NMDA. These results show that systemic administration of APJ agonists protects retinal neurons from glutamate excitotoxicity, suggesting that agents targeting APJ may be a new candidate for treating the retinal diseases.

WTH09-18

Extra-mitochondrial role of PINK1 in regulating BDNF signaling

S. K. Soman, D. Tingle, R. K. Dagda

University of Nevada Reno, Pharmacology, Reno, USA

Background: Parkinson's disease (PD) is characterized by the loss of dopaminergic neurons in the midbrain. In addition to classical motor symptoms, PD patients also undergo a cognitive decline. Mutation in the PINK1 gene causes early-onset PD in humans and our studies show decreased levels of BDNF in PINK1^{-/-} mice.

Methods/Results: Western blot analysis shows progressive expression of cytosolic-PINK1 in mouse brain during various stages of postnatal development. Immunohistochemical analysis of PINK1^{-/-} mice shows a significant reduction in the intracellular levels of BDNF and a concomitant reduction in dendrite length of midbrain dopamine neurons. Conversely, PINK1-deficient neurons treated with recombinant human BDNF rescues dendrite length but not in the presence of inhibitors of the BDNF receptor (TrkB), suggesting that the decrease in BDNF contributes to loss of dendrites in PINK1^{-/-} mice. In addition, the immunocytochemical analysis in primary cortical neurons and SH-SY5Y neuroblastoma cells treated with kinetin, a pharmacological activator of PINK1, showed enhanced levels of intracellular BDNF and markers of synaptic plasticity, indicating that PINK1 regulates BDNF production and neuronal development. In PINK1-deficient cortical neurons transfection of wild-type but not PD-associated mutations of PINK1 restored BDNF levels as in wild-type neurons. The regulation of BDNF by PINK1 is mediated by downstream Protein kinase A (PKA) signaling as transfection of SH-SY5Y cells with PKA inhibitor (PKI) blocks the ability of PINK1 to enhance BDNF levels.

Conclusion: Overall, our data suggest a novel role of PINK1 in regulating neuronal plasticity. Secondly, our studies suggest that stimulating PINK1 signaling can restore BDNF levels and neuronal connectivity in the brain in PD patients harboring PINK1 mutations and plausibly suppress cognitive loss in PD patients. Funding: Pennington Foundation (Nevada) and NIH grant R01 NS105783.

WTH09-20

Determination of amyloid- β oligomers effects on the CBLN1-neurexin synaptic organizing complex in Alzheimer's disease

H. Khaled^{1,2}, J. Lacouchie-Payen², A. Lee^{2,3}, S. Bichara-Allard², H. Takahashi^{1,2,3}

¹*University of Montreal, Molecular Biology program, Faculty of Medicine, Montreal, Canada*

²*Institut de recherches cliniques de Montréal, Synapse Development and Plasticity, Montreal, Canada*

³*McGill University, Integrated Program in Neuroscience, Montreal, Canada*

Amyloid- β (A β) is a key molecule involved in the pathogenesis of Alzheimer's disease (AD), which is one of the most common neurodegenerative diseases. Both *in vitro* and *in vivo* studies have demonstrated that synapses are vulnerable to increasing concentrations of soluble A β oligomers. Cerebellin 1 (Cbln1) is an important synaptic organizer that promotes the formation of excitatory synapses by binding to both the presynaptic Neurexins (NRX) with the splicing site 4 (S4) and post-synaptic glutamate receptor delta 2

(GluD2) in the cerebellum. Interestingly, we have previously identified that A β oligomers bind to various NRX isoforms via the S4 site. We thus hypothesize that A β oligomers compete with Cbln1 for the interaction with NRX isoforms with the S4 site (NRXS4 +). Indeed, our preliminary cell surface binding assay using NRX1 α S4 + -transfected COS7 cells treated with A β oligomers in presence of HA-Cbln1 recombinant proteins suggests that A β oligomers and HA-Cbln1 compete with each other for binding with NRX1 α S4 + . This result suggests that A β oligomers may inhibit the synaptogenic ability of Cbln1 to drive excitatory presynaptic assembly through binding with NRXS4 + isoforms. This study may uncover a new A β -induced pathological mechanism in AD and enable the development of new therapeutic targets in the fight against AD.

WTH09-21

Expression of the endoplasmic reticulum chaperone GRP78 in a retinal degeneration model induced by blue led exposure

I.-B. Kim^{1,2,3}, Y. S. Park^{1,2}

¹The Catholic University of Korea, Anatomy, Seoul, Korea South

²The Catholic University of Korea, Catholic Neuroscience Institute, Seoul, Korea South

Glucose-regulated protein (GRP78) is an endoplasmic reticulum (ER) stress marker which belongs to heat shock protein family. This study aims to examine GRP78 expression and its possible function in retinal degeneration (RD) induced by blue LED exposure. To generate RD, mice were exposed to 1800 lx of the blue LED for 2 h. GRP78 was prominently labeled in the rod and cone (R/C) layer, the inner nuclear layer (INL), and the ganglion cell layer (GCL). At 24 h after light exposure, GRP78 expression was decreased in the R/C layer, while its expression in other layers was not apparently changed. After 72 h, GRP78 expression was increased in the inner plexiform layer (IPL) and the outer nuclear layer (ONL). Increased GRP78 in the IPL and the ONL was co-localized with glutamine synthase (GS) in the RD retina at 72 h after light exposure. Double-labeled Müller cells were found in the middle INL (normal position) and the ONL (ectopic position). Also, increased microglial cells labeled with anti-IBA1 in the ONL and subretinal space showed strong GRP78 immunoreactivity. Subcellular localization of GRP78 in ER was confirmed with immunoelectron microscopy. These results demonstrate that GRP78 expression was increased in the retinas of blue LED-induced RD. The cellular identities of GRP78 expression are Müller cells and microglia. At subcellular level, GRP78 was exclusively located on ER of the each retinal cell. These findings suggest that ER stress might be closely related to glial responses in RD and GRP78 play a role in the pathogenesis of RD.

WTH09-22

Possible protective effect of L-theanine on neurons by induction of hyperpolarization

N. Kuramoto¹, R. Nakamura¹, H. Higashi¹, T. Kinjo¹, K. Uno¹, Y. Yoneda²

¹Setsunan University, Faculty of Pharmaceutical Sciences, Molecular pharmacology, Hirakata City, Japan

²Osaka University, Graduate School of Dentistry, Pharmacology, Osaka, Japan

L-theanine is an amino acid ingredient in green tea with a chemical structure close to L-glutamic acid and L-glutamine. It is available on market for sales as a dietary supplement beneficial for inducing good sleep and relaxation. We have shown that L-theanine enhanced the proliferation of neural progenitor cells (NPCs) through a mechanism relevant to the glutamine transporter Slc38a1, which highly expresses in neuronal tissues, after the activation of intracellular mTOR phosphorylation cascades. L-theanine also exhibited a promoting effect on differentiation to neurons in NPCs. Here, we investigated the direct actions of L-theanine on neurons rather than NPCs. Primary neuronal culture was prepared from cerebral cortex of mouse embryo (E14.5). Cytosine arabinoside (AraC) was added at 2 days *in vitro* for one day to suppress growth of proliferative cells. Cells were cultured in Neurobasal medium supplemented with GlutaMAX, NeuroBrew-21 (Miltenyi Biotec) and several antibiotics. L-theanine (Taiyo Kagaku) was cultured with cells at a concentration of 100 or 1000 mM. In the neuron-rich cultures, L-theanine treatment for 9 days did not significantly affect the ability of MTT reduction, nor b-tubulin and cleaved caspase levels. Neurons were highly damaged by N-methyl-D-aspartate at 100 mM, while pre-treatment with L-theanine suppressed the damage by this excitotoxin. L-theanine was effective in increasing the fluorescence intensity of Asante potassium green-2 used for determination of the intracellular potassium level. It was thus suggested that neurons would be hyperpolarized by L-theanine treatment for deteriorating the damage by excitotoxin in neurons in a particular situation.

WTH09-23

Comorbidity of covert stroke in a transgenic animal model: impact on Alzheimer's disease pathogenesis and functional outcomes

M. Liu^{1,2}, T. Beckett³, L. Thomason³, A. Dorr³, B. Stefanovic^{3,4}, J. McLaurin^{1,2}

¹Sunnybrook Research Institute, Biological Sciences, Toronto, Canada

²University of Toronto, Department of Laboratory Medicine and Pathobiology, Toronto, Canada

³Sunnybrook Research Institute, Physical Sciences, Toronto, Canada

⁴University of Toronto, Department of Medical Biophysics, Toronto, Canada

Although Alzheimer's disease is often characterized as a degeneration of the brain while covert stroke is a disease of the vasculature –incidence rates for both greatly increase as a result of aging. AD and covert stroke share several important risk factors, including smoking, diabetes and hypertension. Moreover, stroke survivors are more than twice as likely to develop dementia compared to healthy age-matched individuals. Thus, we decided to

investigate the impact of covert stroke on AD pathogenesis. The endothelin-1 (ET1) model was used to induce the focal subclinical ischemia, while the transgenic APPsi-tTA mouse model of AD was used for temporal control of APP gene expression. T2-structural MRI was conducted 1 week post-surgery, to assess the size and location of the covert stroke. After APP expression, ET1-APPsi-tTA animals showed significant deficits in activities of daily living when compared to tTA control animals, but were similar to sham-APPsi-tTA animals. When compared to tTA controls, ET1-APPsi-tTA animals exhibit a significant increase in locomotion, cognitive and spatial memory impairment post-APP expression. APPsi-tTA animals exhibited an increase in microglial and astrocyte cortical coverage when compared to tTA control animals. Amyloid load for APPsi-tTA animals was examined through 6F3D staining. In a system, in which, the covert stroke and AD occurred sequentially, APP over-expression dominate the cognitive and pathological effects both in the presence and absence of a single covert stroke.

WTH09-24

Understanding the human neuromuscular junction in aging and neurodegenerative disorders with a novel muscle biopsy method (BeeNMJ)

S. Marchand¹, J. Vallée¹, C. Pion², J. Morais³, M. Bélanger², M. Aubertin-Leheudre², R. Robitaille¹

¹Université de Montréal, Neurosciences, Montreal, Canada

²Groupe de recherche en Activités Physiques Adaptées, Faculté des Sciences, Montreal, Canada

³Division of Geriatrics Medicine, McGill University Health Centre, Montreal, Canada

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterised by a rapid and progressive loss of motoneurons from the cerebral cortex, the brainstem and the spinal cord leading to muscle atrophy and weakness followed by death due to respiratory muscle denervation. Many factors contribute to disease progression, one key element being the denervation of the neuromuscular junction (NMJ) at striated muscles. Many studies have focused on that structure in ALS in animal models such as SOD1 mice, but the human NMJ remains largely understudied. We have developed a minimally invasive needle biopsy method guided by surface electrostimulation electrodes called BeeNMJ (Biopsy using Electrostimulation for Enhanced NMJ sampling). Young adult and old healthy males have been recruited for this initial phase of the study. This provides us with NMJ-enriched muscle tissue that enables a thorough morphological characterisation of the human NMJs following immunohistochemistry staining for presynaptic motoneuron terminal (SV2/NF-M), postsynaptic acetylcholine receptors (a-btx), perisynaptic Schwann cells (PSCs, s100b) and muscle fiber type (MHC I). We have observed that the morphology of human NMJs is significantly different than the one of mice, especially regarding postsynaptic apparatus distribution and PSC morphology. This method could be used with ALS patients to help understand the mechanisms underlying disease progression at their NMJs and to extrapolate data from the mice to the humans to be able to refine treatment research to be more adapted to the human NMJ components.

WTH09-25

Degeneration of the nigro-striatal dopaminergic neurons in a rat model of chronic hyperglycemia

M. G. Martinoli¹, J. Renaud¹, J. Beaulieu¹, V. Bassareo², A. Pinna³, N. Simola²

¹Université du Québec TR, Medical Biology, Cellular Neurobiology, Trois-Rivières, Canada

²Università di Cagliari, Department of Biomedical Sciences, Cagliari, Italy

³National Research Council of Italy ^{CNR}, Neuroscience Institute, Cagliari, Italy

An accumulating number of epidemiological studies support a link between diabetes and neurodegenerative disorders. Owing to the preponderant expression of glucose transporter 1 (GLUT1) at the blood-brain barrier and glucose transporter 3 (GLUT3) at the neuron plasma membrane, glucose uptake overwhelmingly occurs in an insulin-independent fashion. Recently, our team established that elevated levels of glucose lead to oxidative stress and apoptosis in dopaminergic neurons and several early studies report dopaminergic alterations in diabetes or acute hyperglycemia. The aim of this study was to characterize the effects of long-term hyperglycemia in dopaminergic pathways. In the nicotinamide-streptozotocin rat model of hyperglycemia, the nigrostriatal motor pathway and the reward-associated mesocorticolimbic pathway, both composed of dopaminergic neurons, were specifically investigated. Neuronal and glial alterations were evaluated 3 and 6 months after hyperglycemia induction. Our results demonstrate preferential degeneration of the nigrostriatal pathway associated with astrogliosis and loss of microglial cells after 6 months. Behavioural alterations were assessed in a series of tasks designed to uncover motor deficits in rodent models of PD. Long-term hyperglycaemic rats manifested signs of bradykinesia and gait disturbances reminiscent of parkinsonian motor impairments. Interestingly, motor deficits and dampened dorsostriatal dopamine release were apparent before neurodegeneration could be discerned, suggesting possible functional impairments of the nigrostriatal pathway before of neuronal death. These results provide refreshing insight on the higher occurrence of Parkinson's disease in diabetic patients increasingly acknowledged by medical authorities.

WTH09-26

Probing amyloid-β protofibrils with a conformation-selective antibody

M. Nichols

University of Missouri-St. Louis, Chemistry & Biochemistry, St. Louis, USA

Antibodies are critical tools throughout every aspect of Alzheimer's disease (AD) including research, diagnostics, and therapy. Additionally, antibodies provide the only clues for the existence of various conformational species in tissue specimens from humans or mouse models. One important target of antibodies in AD is the amyloid-β peptide (Aβ), the primary component of senile plaques in the brain and trigger for AD onset. Aβ, particularly Aβ42, is prone to aggregation forming a variety of soluble and insoluble conformational species during the process. Our studies show that one soluble aggregated species, termed protofibrils, significantly interacts with microglia and is highly proinflammatory. Due to the importance of this Aβ42 species, we developed an

antibody that selectively recognizes protofibrils over other A β forms. Immunization of rabbits with isolated A β 42 protofibrils generated a high-titer anti serum with a high affinity and a strong selectivity for A β 42 protofibrils over A β 42 monomers and fibrils. AbSL did not react with amyloid precursor protein and recognized distinct pathological features in AD transgenic mouse brain slices. The serum was affinity-purified over an A β 42 protofibril column to yield apAbSL. Furthermore, a monoclonal form of AbSL (mAbSL) was developed and both apAbSL and mAbSL antibodies were integrated into numerous ELISA formats that are sensitive and selective for A β 42 protofibrils. Antibody competition studies indicate that the conformational epitope for AbSL resides in the N-terminal region of A β 42 monomers that incorporate into the protofibril structure. By targeting protofibrils, the AbSL antibody may have potential diagnostic and therapeutic uses in AD tissue and patients.

WTH09-27

Obesity-induced cirrhosis results in more complex and poor neurological performance related to hepatic encephalopathy in cirrhosis

R. Ochoa-Sanchez¹, M. Tremblay^{1, 2}, C. F. Rose¹

¹CRCHUM, -, Montreal, Canada

²Université de Montréal, Medicine, Montreal, Canada

Hepatic encephalopathy (HE) is a neuropsychiatric syndrome in chronic liver disease (CLD/cirrhosis). With an increasing prevalence of obesity-induced cirrhosis and evidence linking blood-derived lipids to neurological impairment, we hypothesize that obesity increases the risk, severity and progression of HE.

Aim: Development of an animal model of cirrhosis and obesity to investigate the synergistic effect of obesity and CLD on the development of neurological impairment and HE.

Materials and Methods: Model of CLD and HE: 6-week bile-duct ligation (BDL) rats and Sham-controls were used. Inducing obesity: High-fat diet (HFD) was given for 3 weeks before BDL or Sham surgery. Obese-BDL received HFD for 3 weeks pre-BDL and regular-diet (RD) for 6 weeks post-BDL; Lean-BDL received RD pre-/post-BDL; Lean-Sham received RD pre-/post-Sham. Recognition-memory, motor-coordination, muscular-strength and body-composition were assessed before, 3 and 6 weeks post-surgery. Before surgery, body weight (BW) and fat-mass of rats on HFD (Obese-BDL) were increased vs rats on RD (Lean-BDL/Lean-Sham). 3 weeks post-surgery, BW, fat- and lean-mass were increased in Obese-BDL vs Lean-BDL. Long-term memory was reduced in Obese-BDL, but not in Lean-BDL, vs Lean-Sham. 6 weeks post-surgery, similar to Lean-BDL, Obese-BDL lost BW, fat- and lean-mass against Lean-Sham. Motor-coordination, fore-limb strength and long-term memory were impaired in Obese-BDL vs Lean-BDL or Lean-Sham, whereas hind-limb strength and short-term memory were impaired in Obese- and Lean-BDL.

Conclusion: HFD induces obesity features in healthy non-cirrhotic rats. Such effects are maintained in cirrhotic-BDL rats. Some neurological impairments are detected in Obese-BDL but not in Lean-BDL, while others are exacerbated. A synergistic effect of obesity and CLD accelerates/worsens the disease-associated abnormalities in HE, suggesting more neurological susceptibility in obese-induced cirrhosis.

WTH09-28

A-beta mediated inhibition of choline uptake is independent of cell surface levels of the choline transporter

O. Ojiakor, A. Rai, J. Rylett

Western University, Roberts Research Institute, London, Canada

Alzheimer's disease (AD) is a neurodegenerative disorder defined by cognitive decline. Cognitive processes impaired in AD are mediated by cholinergic neurons related to decreased release of acetylcholine (ACh). The high-affinity choline transporter (CHT) is present at the presynaptic cholinergic nerve terminal and is responsible for reuptake of choline. Disruption of CHT function leads to decreased choline uptake and ACh synthesis, resulting in impaired cholinergic neurotransmission. Our laboratory reported that β -amyloid (A β) alters CHT function in a manner that decreases its cell surface levels and choline uptake activity, and that clathrin-mediated endocytosis of CHT is dependent on a dileucine-like motif (L531-V532). However, the mechanisms underlying A β -induced alteration of CHT function are not well understood. Here we investigated the possibility of a correlation between A β -mediated decrease in choline uptake activity and cell-surface expression of CHT. HEK293 cells stably expressing wild-type human CHT or an L531A mutant were treated with A β -containing conditioned medium for 24 h followed by a hemicholinium-dependent choline uptake assay. We report that an increase in cell surface CHT levels does not significantly prevent A β -induced decrease in choline uptake activity. This suggests that A β -mediated decrease in choline uptake activity is independent of CHT cell surface levels. Based on these results, we hypothesize that interaction with A β triggers a change in the conformational state of CHT required for solute binding, as has been observed for both the glutamate and glycine agonist recognition sites of the NMDA receptor. Understanding the factors that regulate CHT activity and retention on the plasma membrane may be useful for enhancing cholinergic transmission in diseases like AD, when ACh synthesis and release are attenuated but cholinergic neurons remain viable.

WTH09-29

P-hydroxyamphetamine causes prepulse inhibition disruptions in mice: contribution of catecholamine and serotonin neurotransmission

H. Onogi¹, O. Nakagawasai², W. Nemoto², S. Mitazaki³, K. Tan-No², T. Tadano⁴

¹Tohoku Fukushi University, Department of Nursing, Faculty of Health Sciences, Sendai, Japan

²Tohoku Medical and Pharmaceutical University, Department of Pharmacology, Faculty of Pharmaceutical Sciences, Sendai, Japan

³Takasaki University of Health and Welfare, Laboratory of Molecular Neuroscience, Faculty of Pharmacy, Takasaki, Japan

⁴Kanazawa University, Complementary and Alternative Medicine Clinical Research and Development, Graduate School of Medicine Sciences, Kanazawa, Japan

p-Hydroxyamphetamine (*p*-OHA), an amphetamine metabolite, has been shown to have a number of pharmacological actions, including causing psychostimulant-induced behaviors such as those induced by drugs of abuse. To reveal the characteristics of *p*-OHA on sensorimotor function in rodents, we tested the effects of *p*-OHA on prepulse inhibition (PPI) in mice. Intracerebroventricular

administration of *p*-OHA dose-dependently induced PPI disruptions for all prepulse intervals tested. *p*-OHA-induced PPI disruptions were attenuated by pretreatment with haloperidol (a typical antipsychotic), clozapine (an atypical antipsychotic), L-741,626 (a selective D₂ receptor antagonist), L-745,870 (a selective D₄ receptor antagonist), 6-hydroxydopamine (a neurotoxin which targets DA-containing neurons), ketanserin (a 5-HT_{2A/2C} receptor antagonist), MDL100,907 (a selective 5-HT_{2A} receptor antagonist), 5,7-dihydroxytryptamine (a neurotoxin which targets serotonin-containing neurons), *p*-chlorophenylalanine (a serotonin synthesis inhibitor), and prazosin (a selective α_1 receptor antagonist). *p*-OHA-treated mice also showed increased homovanillic acid/dopamine (HVA/DA) ratio in the whole brain. These results indicate that *p*-OHA induces PPI disruptions via catecholamine and serotonin neurotransmission.

WTH09-30

Comparative analysis of proteomic and glial cells decoding between resistant and vulnerable neuromuscular junction in ALS

F. Provost, D. Arbour, M.-S. Gauthier, B. Coulombe, R. Robitaille

Université de Montréal, Neuroscience, Montréal, Canada

Amyotrophic lateral sclerosis (ALS) is a neuromuscular disease characterized by the progression loss of motor neurons (MNs) and muscular paralysis. The denervation of the neuromuscular junctions (NMJs) at striated muscles is an early event that appeared before MNs' loss in the spinal cord. Recent data revealed an alteration of synaptic transmission, morphological instability and inappropriate repair in NMJ of SOD1 mice model before the apparition of the motor symptoms. Interestingly, these mechanisms are mainly regulated by the glial cells at the NMJ, Perisynaptic Schwann cells (PSCs) which suggest that the alteration of the PSC functions may contribute to NMJ vulnerability. Numerous studies demonstrated a motor unit type-dependent susceptibility to denervation and surprisingly, the extraocular muscles seem resistant to disease progression. We hypothesized that PSC functions are adapted at the extraocular NMJs, conferring resistance to ALS progression. NMJ morphological analysis, functional properties of PSCs and a comparative proteomic analysis will be performed between resistant and vulnerable muscles in mouse model SOD1G37R. At this point, healthy NMJ's morphology is observed in SOD1 in the extraocular muscle at late symptomatic stage. Typical glial marker of reparation is also found. Further experimentation to understand the functional differences between vulnerable and resistant NMJs is necessary. It will help to provide insights into the denervation mechanisms involved and to identify targets for therapeutic manipulation.

WTH09-31

Effect of lithium on Na⁺/K⁺-ATPase activity in forebrain cortex and hippocampus of sleep-deprived rats

L. Roubalova¹, M. Vosahlikova¹, J. Slaninova¹, M. Alda^{2,3}, P. Svoboda¹

¹*Institute of Physiology of the Czech Academy of Sciences,*

Department of Biomathematics, Prague, Czech Republic

²*Dalhousie University, Department of Psychiatry, Halifax, Canada*

³*National Institute of Mental Health, Klecany, Czech Republic*

Lithium (Li) is one of the few options for successful treatment of bipolar disorder. In this study, we chose a specific model of mania

induced by sleep deprivation and compared the acute, short-term and chronic effects of the therapeutic Li concentration on forebrain cortex and hippocampus of Wistar rats. As a marker of such effects, we determined the activity of Na⁺/K⁺-ATPase - membrane pump, which is responsible for the active transport of sodium and potassium ions and is crucial to the proper maintaining of the membrane electrochemical gradient.

Rats were kept on control or lithium carbonate containing diet for 1, 7 and 28 days. Half of both control and Li treated animals were subsequently subjected to sleep deprivation for 3 days. Plasma membrane and post-nuclear supernatant preparations were isolated from forebrain cortex, hippocampus, and kidney of all experimental groups of animals. The acute, short-term, as well as chronic treatment of rats with Li, resulted in a decrease of Na⁺/K⁺-ATPase activity in forebrain cortex and hippocampus of experimental animals. Sleep deprivation in control rats resulted in an increase in this enzyme activity. This increase in activity after sleep deprivation was not detected in Li-treated animals. Results of these measurements were compared with Na⁺/K⁺-ATPase activity determined in preparations from the kidney of all experimental groups of animals.

WTH09-32

Association between vitamin D status and neurocognitive function in dementia, depression, schizophrenia, and ADHD: review & synthesis

N. Sampat¹, S. Al-Adawi², M. M. Essa¹

¹*Sultan Qaboos University, Food Science and Nutrition, Muscat, Oman*

²*Sultan Qaboos University, Behavioral Medicine, Muscat, Oman*

Vitamin D deficiency has been estimated to affect roughly 30% to 50% of the population and it is pandemic in some parts of the world. In addition to its role in skeletal and calcium homeostasis, Vitamin D has been implicated to have a role in brain functioning in both preclinical and human. Those findings have also expanded to various neurodevelopmental and neuropsychiatric conditions such as dementia, major depressive disorder (MDD), schizophrenia and attention and hyperactive disorder (ADHD). In addition to behavioral, neurological and emotional symptoms, these conditions tend also to display neuropsychological impairment. There is also indication that neuropsychological symptoms tend to be more pervasive and refractory. The role of vitamin D in such neurodevelopmental and neuropsychiatric condition has received scant attention. The aims of the present review is to examine the consequences of Vitamin D deficiency in brain functioning and its impact on various Neurological Disease for low levels of vitamin D could be a cause of the multitude of other health outcomes and diseases. Correcting this deficiency could have an impact on reduction of health cost worldwide.

WTH09-33

Enhanced mGluR5 signaling in excitatory neurons promotes rapid antidepressant effects via ampa receptor activation

T. Serchov¹, A. Holz², F. Mülsch², M. Bartos³, C. Normann², K. Biber², D. van Calker²

¹University Medical Center Freiburg, Stereotactic and Functional Neurosurgery, Freiburg, Germany

²University Medical Center Freiburg, Psychiatry, Freiburg, Germany

³University of Freiburg, Institute of Physiology I, Freiburg, Germany

Conventional antidepressants have limited efficacy and many side effects, highlighting the need of fast-acting and specific medications. Induction of the synaptic protein Homer1a mediates the effects of different antidepressant treatments, including the rapid action of ketamine and sleep deprivation (SD). We show here that mimicking Homer1a up-regulation via intravenous injection of cell-membrane permeable TAT-Homer1a elicits rapid antidepressant effects in various tests. Similarly to ketamine and SD, *in vitro* and *in vivo* application of TAT-Homer1a enhances mGluR5 signaling resulting in increased mTOR pathway phosphorylation and up-regulates synaptic AMPA receptor expression and activity. The antidepressant action of SD and Homer1a induction depends on mGluR5 activation specifically in excitatory CaMK2a neurons and requires enhanced AMPA receptor activity, translation and exocytosis. Moreover, our data demonstrate a pronounced therapeutic potential of different TAT-fused peptides, which directly modulate mGluR5 and AMPA receptor activity and thus might provide a novel strategy for rapid and effective antidepressant treatment.

WTH09-34

Neuroprotective effect of the endogenous peptide apelin on the retinal ganglion cell death in diabetes model mouse F. Shibagaki, Y. Ishimaru, M. Suzuki, A. Yamamuro, Y. Yoshioka, S. Maeda

Setsunan University, Pharmaceutical Science/ Pharmacotherapeutics, Hirakata, Japan

Diabetic retinopathy, a secondary microvascular complication of diabetes, is the leading cause of irreversible blindness. Retinal microvascular dysfunction causes retinal macular edema, pathological angiogenesis and the loss of the retinal ganglion cells (RGCs). It has been shown that intravitreal injection of anti-vascular endothelial growth factor (VEGF) antibody suppresses retinal microvascular impairment in diabetic retinopathy and thereby preventing vision loss. However, VEGF inhibitors cannot prevent the loss of RGCs in diabetic retinopathy. It is necessary to develop a new medicine that suppresses RGC degeneration in diabetic retinopathy. We have previously shown that intravitreal injection of apelin prevents N-methyl-D-aspartate (NMDA)-induced RGCs loss, and that apelin deficiency facilitates RGCs loss induced by NMDA. In the present study, we investigated the effect of endogenous apelin on RGCs loss in the retina of the diabetes model mouse fed a high fat diet (HFD). We used Insulin2 mutant (Ins2 +/−) Akita mouse, which is a mouse model of type 1 diabetes. Akita mice fed the HFD from 5 weeks after birth. The light-evoked responses of the RGCs were measured by electroretinography. Akita mice fed the HFD for 4 weeks exhibited a significant decrease in both the electro-response and the

number of Brn-3a positive RGCs. These reductions were exacerbated by deletion of the endogenous apelin. These results suggest that apelin may play a protective role against RGC death in the diabetic retinopathy.

WTH09-35

NSI-189 enhances neurite outgrowth and mitochondrial function in sensory neurons and reverses peripheral neuropathy in ZDF rats

D. Smith¹, G. Sanchez², M. R. Aghanoori^{1,4}, K. Johe³, N. Calcutt², C. Jolivald², P. Fernyhough^{1, 4}

¹St. Boniface Research Centre, Division of Neurodegenerative Disorders, Winnipeg, Canada

²UCSD, Pathology, La Jolla, USA

³Neuralstem Inc., Germantown, USA

⁴University of Manitoba, Dept of Pharmacology & Therapeutics, Winnipeg, Canada

Benzylpiperazine-aminopyridine NSI-189 stimulates neurogenesis in hippocampus-derived neural stem cells *in vitro* and *in vivo*. We tested the neurotogenic capacity of NSI-189 in cultured adult neurons derived from normal or type 1 (streptozotocin-induced) diabetic rats. We hypothesized that any impact on neurite outgrowth would correspond with augmented mitochondrial function. Dorsal root ganglia (DRG) sensory neurons were cultured under defined conditions. Neuronal bioenergetics was assessed using the Seahorse XF24. NSI-189 (1-3 mM) induced a 2-fold elevation ($p < 0.05$) in neurite outgrowth. In neurons from diabetic rats, NSI-189 (3 mM) raised maximal respiration and spare respiratory capacity by 2-fold ($p < 0.005$). We also characterized effects of NSI-189 on AMP-activated protein kinase (AMPK), a regulator of bioenergetics. In neurons from diabetic rats, NSI-189 (3 mM) elevated AMPK gene expression by 2-fold ($p < 0.01$). We extended our study to *in vivo* efficacy of NSI-189 in the ZDF rat model of type 2 diabetes. NSI-189 (30 mg/kg/day) was given for 16 weeks starting at 24 weeks of age. Peripheral neuropathy was indicated by significant ($p < 0.05$) paw heat hypoalgesia and sensory NCV slowing. Within 4 weeks of treatment, NSI-189 normalized both indices of sensory neuropathy without impacting the diabetic state. DRG from ZDF rats revealed elevated P-AMPK with NS-189 treatment. NSI-189 showed neurotogenic properties in sensory neurons in concert with enhancement of mitochondrial function and reversed indices of sensory neuropathy in diabetic rats. Supported by SBIR award R44NS103703 (KJ and CJ) and Neuralstem Inc.

WTH09-36

SUMOylation affects synaptic function and Alzheimer disease pathology**H. Takamura^{1,2}, S. Matsuzaki^{2,3}, T. Katayama², O. Arancio⁴, P. Fraser^{1,5}**¹University of Toronto, Tanz Centre for Research in Neurodegenerative Diseases, Toronto, Canada²Osaka University, United Graduate School of Child Development, Osaka, Japan³Wakayama Medical University, Department of Pharmacology, Wakayama, Japan⁴Columbia University, Department of Pathology and Cell Biology and Taub Institute for Research on Alzheimer's Disease and the Aging Brain, New York, USA⁵University of Toronto, Department of Medical Biophysics, Toronto, Canada

Small ubiquitin-like modifiers (SUMOs) conjugated to target proteins can affect a number of roles in cellular events. Recent evidence has shown SUMOylation contributions to neuronal function and has been suggested to play important roles in the amyloid plaque and neurofibrillary tangle pathology of Alzheimer disease (AD) and related neurodegenerative diseases. We previously demonstrated in a transgenic mouse model that over-expression of SUMO1 results in an impairment of synaptic development leading to cognitive deficits. In contrast, comparable SUMO2 transgenic animals display normal brain functions. There has been a debate on the effects of SUMO1 and SUMO2 on amyloid pathology and, to resolve this issue, we have recently generated double transgenic mice over-expressing human SUMO1 and a mutant APP as an *in vivo* model. The SUMO1-APP mice displayed normal APP processing but exhibited increased insoluble A β and plaque density accompanied by increased synaptic loss, more pronounced synaptic and cognitive deficits. These findings suggest a potential impairment in A β clearance. In contrast, SUMO2-APP double transgenic mice showed a more beneficial response of SUMO2 to the AD-related stress conditions. Cumulatively, our findings indicate a more detrimental impact of SUMO1 on amyloid pathology and a possible protective effect associated with higher levels of expression for SUMO2.

WTH09-37

Investigating the role of TP53INP1 in neuronal autophagy
E. Tennyson, S. Cregan

Robarts Research Institute, Neuroscience, London, Canada

Autophagy is the process by which cells are able to degrade dysfunctional components, including damaged organelles and misfolded proteins. This is an essential quality control mechanism, and is especially important for the maintenance of neuronal function. Dysregulation of autophagy is thought to play a role in neurodegeneration, present in disorders such as Parkinson's Disease, Alzheimer's Disease, and Huntington's; as well as in cerebral ischemia. Tumour-protein P53-inducible nuclear protein 1 (Tp53INP1) is a stress-induced protein that has been described as a dual regulator of gene transcription and autophagy in non-neuronal cells, though its role in the CNS remains unclear. We have found that Tp53INP1 is up-regulated in response to oxidative stress, in conditions such as hypoxia and oxygen-glucose deprivation in neurons. Therefore we investigated whether Tp53INP1 modulates stress-induced neuronal autophagy and survival, in a cellular model of cerebral ischemia. Primary cortical neurons from INP1

wildtype (INP1^{+/+}) and INP1-null (INP1^{-/-}) embryonic mice were extracted and exposed to stressors including hypoxic or oxygen-glucose deprived conditions, with varying durations of reperfusion. The cultures were subsequently assessed for levels of autophagy using LC3 immunofluorescence, and LC3-II accumulation and p62 degradation through western blot analysis. Together, our results demonstrate an attenuation of hypoxia and OGD-induced neuronal autophagy in the absence of Tp53INP1, implicating this protein as a novel player in the regulation of neuronal survival.

WTH09-38

Ammonia induces Alzheimer's disease pathology in astrocytes**M. Terunuma, A. Komatsu, S. Kishikawa**

Niigata University Graduate School of Medical and Dental Sciences, Oral Biochemistry, Niigata, Japan

Alzheimer's disease (AD) is a neurodegenerative disease and the leading cause of cognitive impairment. Excessive amount of ammonia has been detected in the brain and serum of AD patients, and the toxicity of ammonia has been thought as a factor contributing to the progression of AD. In the central nervous system, astrocytes play a key role in ammonia metabolism by expressing the enzyme called glutamine synthetase, which catalyzes the synthesis of glutamine from glutamate and ammonia, and supply glutamine to neurons for the production of neurotransmitters. Here we report that prolonged NH₄Cl treatment induces the expression of amyloid precursor protein (APP) in astrocytes. APP gene expression was not induced by NH₄Cl treatment however, the elongated half-life of APP was determined. Enhanced APP expression was detected on both the plasma membranes and intracellular compartments. Immunocytochemistry revealed that NH₄Cl induces the accumulation of APP in the endoplasmic reticulum, Golgi apparatus and recycling endosome. To examine if amyloid beta (A β) production is induced by NH₄Cl, we examined the expression of enzymes BACE1 and presenilins (PS1 and PS2) which involve in APP cleavage and the amount of intracellular A β 40 and A β 42. The NH₄Cl treatment did not alter the levels of BACE1 and presenilins (PS1 and PS2) and A β 40, however, the amount of A β 42 was significantly increased. Together, our data suggests that ammonia induces the production and accumulation of APP and A β in astrocytes, which is the underlying causes and pathology of AD.

WTH09-39

Inhibition of BACH1 as a novel therapeutic strategy for Parkinson's disease**B. Thomas¹, M. Ahuja¹, N. A. Kaidery¹, I. Gaisina², K. Igarishi³, O. Attucks⁴**¹Medical University of South Carolina, Darby Research Institute, Charleston, USA²University of Illinois, Medicinal Chemistry, Illinois, USA³Tohoku University, Biochemistry, Sendai, Japan⁴vTv Therapeutics, Preclinical Research, High Point, USA

Parkinson's disease (PD) is a progressive neurodegenerative movement disorder. Except for the palliative treatment, there is no cure available for PD. Based on pathophysiological findings, aberrant oxidative stress and inflammation are extensively targeted for developing PD therapies. The nuclear-factor-E2-related factor 2 (Nrf2)/antioxidant response element (ARE) signaling pathway is a

promising target for neurotherapeutics. However, canonical Nrf2 activators are electrophilic and thus potentially result in oxidative stress on chronic usage. Transcription factor Bach1 [BTB and CNC homology 1] binds to ARE-like sequences, functioning as a transcriptional repressor, thus antagonizing the activation of Nrf2 and potentially targeted to develop ARE activators. We investigated the effects of Bach1 inhibition (both genetic and pharmacological) on Nrf2/ARE signaling *in vitro* and *in vivo* and its ability to block MPTP-induced dopaminergic neurotoxicity, associated oxidative damage, and inflammation in mice. We observed that both genetic (Bach1 null mice) and pharmacological inhibition (Bach1 inhibitor) of Bach1 attenuated MPTP-neurotoxicity. The neuroprotective effects in Bach1 null and Bach1 inhibitor treated wild type mice against MPTP was not due to differences in conversion of MPTP to MPP⁺. Assessment of mRNA and protein levels of Nrf2 pathway target genes in Bach1 null mice and Bach1 inhibitor treated wild type mice exhibited marked induction of both antioxidant and anti-inflammatory genes. Most importantly, we found the pharmacological Bach1 inhibitor to be a non-electrophile. Our results suggest that Bach1 could serve as a novel non-electrophilic target for therapeutic intervention in PD.

WTH09-40

Systemic treatment with a muscarinic antagonist does not improve nmj function and reinnervation in an ALS mouse model

E. Tremblay, D. Arbour, R. Robitaille

Université de Montréal, Neurosciences, Montréal, Canada

Background: Glial cells at the neuromuscular junction (NMJ), perisynaptic Schwann cell (PSC), present an abnormal decoding of synaptic transmission in amyotrophic lateral sclerosis (ALS). Indeed, PSCs show a persistent increase in Ca²⁺ excitability due to over-activation of their muscarinic receptors. Normally, NMJ neurotransmission deficits lead to a down-regulation of muscarinic signaling, which results in PSC sprouting and reparation of the NMJ. Therefore, the persistently elevated PSC muscarinic receptor activation in ALS could alter their ability to repair NMJs during disease progression.

Objectives: We hypothesized that a dampening of muscarinic excitability with a muscarinic antagonist could lead to an improvement of NMJ innervation and neuromuscular function in ALS.

Methods: We administered orally to SOD1^{G37R} and WT mice the selective M3 muscarinic antagonist Darifenacin (10 mg/kg) or a saline (control), 5 days a week, starting at P400 (pre-onset) or P450 (onset), until P520 (endstage). Grip strength, weight, hang time and rotarod tests were performed twice a week. Calcium imaging of PSCs and neuromuscular function were measured on the slow-twitch Soleus (SOL) muscle. Immunohistochemistry was performed on the SOL and EDL muscles.

Results: Darifenacin produced a dose-dependent decrease in PSC Ca²⁺ response evoked by nerve stimulation and bath-applied muscarinic agonist application. Importantly, Ca²⁺ imaging experiments showed that the treatment successfully dampened PSC muscarinic excitability. In addition, we observed signs of NMJ repair in treated SOD1 mice, namely PSC sprouting, but this was insufficient to rescue denervation and neuromuscular function.

Discussion: As a whole, these results reveal that the excessive PSC muscarinic activity in ALS can be successfully dampened *in vivo*. This study will provide a better understanding of the role of muscarinic signaling at the NMJ in ALS.

WTH09-41

Striatal Shati/Nat8 I induce vulnerability to onset of depression in mice

K. Uno^{1, 2}, H. Miyanishi², T. Miyazaki², K. Sodeyama², S.-i. Muramatsu^{3, 4}, T. Kinjo¹, N. Kuramoto¹, A. Nitta²

¹Setsunan University, Laboratory of Molecular Pharmacology, Faculty of Pharmaceutical Sciences, Hirakata, Japan

²The University of Toyama, Department of Pharmaceutical Therapy and Neuropharmacology, Faculty of Pharmaceutical Sciences, Graduate School of Medicine and Pharmaceutical Sciences, Toyama, Japan

³Jichi Medical University, Division of Neurology, Shimoyasu, Japan

⁴The University of Tokyo, Center for Gene & Cell Therapy, the Institute of Medical Science, Tokyo, Japan

The number of patients that have depressive disorder is increasing. However, the mechanism of depression onsets has not been completely revealed. We have previously identified Shati/Nat8 I (Shati), an N-acetyltransferase, in the brain using a mouse model of psychosis. We reported that Shati is related to developmental disorder and methamphetamine dependence. In this study, we revealed the involvement of Shati in the vulnerability of major depression. We generated the Shati-over-expressed mice by injecting an adeno-associated virus into the dorsal striatum, followed by exposed the subthreshold micro social defeat stress (MSDS). Shati-over-expressed mice showed the impairment of social interaction and sucrose preference after the MSDS. These depression-like behaviors were restored by fluvoxamine and LY341495 injection prior these tests. Furthermore, the intracerebral administration of fluvoxamine restored. Taken together, Shati in the striatum has an important role in the vulnerability of depression onsets by regulating serotonergic neuronal system. Our study suggested the new pathways induce depression like-behaviors, and Shati in the striatum might be a new target for medical tools for depression.

WTH09-42

Na⁺/K⁺-ATPase and lipid peroxidation products in the forebrain cortex of sleep-deprived rats treated with therapeutic lithium

M. Vosahlikova¹, L. Roubalova¹, M. Hlouskova¹, K. Cechova¹, S. Musil², I. Miksik³, M. Alda^{4, 5}, P. Svoboda¹

¹Institute of Physiology CAS, Department of Biomathematics, Praha, Czech Republic

²Institute of Analytical Chemistry CAS, Department of Trace Element Analysis, Brno, Czech Republic

³Institute of Physiology CAS, Department of Translation Metabolism, Praha, Czech Republic

⁴Dalhousie University, Department of Psychiatry, Halifax, Canada

⁵National Institute of Mental Health, Applied Neurosciences and Brain Imaging, Klecany, Czech Republic

Lithium (Li) is a typical mood stabilizer representing the first choice for treatment of bipolar disorder. Its mechanisms of action remain debated. We used sleep deprivation (SD) as an animal model of mania and compared the 1, 7 and 28 days effects of the therapeutic Li concentration on Na⁺/K⁺-ATPase properties and lipid peroxidation products (LPP) amount in the forebrain cortex (FBC) of rats. Na⁺/K⁺-ATPase was determined using [³H]ouabain binding,

immunoblot and activity assay. LPP were determined by immunoblot and HPLC. Application of therapeutic Li for 7 and 28 days resulted in a decrease in expression and activity of Na^+/K^+ -ATPase. SD in control Li-untreated rats resulted in Na^+/K^+ -ATPase increase. This increase was attenuated in Li-treated rats. The concentration of LPP was significantly lower in FBC of animals treated with Li for 7 or 28 days and also in animals pre-treated with Li before SD. LPP

concentration in FBC of control rats exposed to SD was higher than in SD-unexposed rats. We can conclude that SD alone up-regulates Na^+/K^+ -ATPase together with increased LPP and that pre-treatment of rats with Li before SD for 7 or 28 days protects the FBC tissue against this type of damage and decreases Na^+/K^+ -ATPase level back to control level.

WTH10 Intracellular trafficking & proteostasis (Session B)

WTH10-01

Protein components of NMDA receptor channel subunits in mouse brain

K. Sakimura¹, Y. Suzuki^{1, 3}, C. Nakamoto^{1, 2}, T. Sasaoka³, M. Abe^{1, 2}

¹Brain Res. Inst., Niigata Univ., Cell. Neurobiol., Niigata, Japan

²Brain Res Inst., Niigata Univ., Animal Model Development, Niigata, Japan

³Brain Res Inst., Niigata Univ., Comp. Exp. Med., Niigata, Japan

The main NMDARs are composed of GluN1 and GluN2 subunits. The GluN1 subunit is encoded by a single gene, but exists as several splice variants. Focusing on these C-terminal side patterns, two types of GluN1, C2 and C2', have been identified. On the other hand, four GluN2A-2D subunits are encoded by separate genes. Although it is very important to know the quantity of each subunit in various brain regions for the understanding of NMDAR function, no quantitative analysis has been made. We newly developed a quantitative western blot method to determine the amount of two main variants GluN1-C2 and GluN1-2C' and four GluN2 subunits in the cortex, hippocampus and cerebellum. In the crude fractions, there were abundant GluN1-C2 and GluN1-2C' subunits in the cortex and hippocampus (1-C2 : 1-2C' : 2A : 2B : 2C : 2D = 1.0 : 2.4 : 0.77 : 1.37 : 0 : 0.05 in the cortex, 1.0 : 3.8 : 1.1 : 1.6 : 0 : 0.09 in the hippocampus, respectively), whereas GluN1-2C' subunits were abundant in the cerebellum (1-C2 : 1-2C' : 2A : 2B : 2C : 2D = 1.0 : 10.3 : 1.4 : 0 : 3.0 : 0.63). On the other hand, in the synaptosomal fractions, the ratio of GluN1 to GluN2 subunits was almost the same in all the regions, showing that relative amounts of GluN1 subunits decreased during trafficking to synaptic membranes and resulted in the formation of functional GluN1/N2 (1:1).

WTH10-02

A critical role for glutamate transporters in the locomotor responses to amphetamines

S. Underhill, S. Millan, T. Wigstrom, S. Amara

NIMH, LMCN, Bethesda, USA

AMPH increases extracellular dopamine concentrations through its actions on the dopamine transporter (DAT), however it has also been established to regulate glutamatergic neurotransmission by increasing endocytosis of the neuronal glutamate transporter, EAAT3. Using various model systems, we characterized the mechanism by which AMPH activates EAAT3 internalization to potentiate glutamatergic tone. Confocal and TIRF microscopy of cells transiently co-transfected with DAT and eGFP-tagged EAAT3, demonstrate that AMPH increases internalization of EAAT3. In primary midbrain cultures, AMPH treatment results in a decrease in EAAT3-mediated transport. Moreover, acute application of AMPH to midbrain slices decreases the amount of surface EAAT3 by 30-50% when assessed by cell-surface biotinylation assays, but the surface expression of several other membrane markers was unaltered. Through mutational analyses we identified a unique five residue sequence on the C-terminus of EAAT3 that dictates its sensitivity to AMPH. When these residues are replaced with alanines, EAAT3 remains at the cell surface after AMPH treatment. EAAT3 trafficking was blocked by introducing a cell-permeable peptide that effectively outcompetes the endogenous C-terminus of EAAT3 and decreases internalization. Using a rat line expressing Cre through a DAT promoter, we stereotactically injected a virus that expresses this short interfering domain in VTA dopamine neurons. Baseline behavior of these animals remains unchanged, however after IP injection of AMPH, animals which express the peptide in dopamine neurons show a decreased response to AMPH. Expression of the peptide prevented endocytosis of EAAT3 in dopamine neurons and led to a profound decrease in AMPH-mediated locomotor behaviors, revealing a critical role for glutamate transporter regulation in the actions of AMPH. These data suggest new opportunities for the development of drugs that modulate attention-deficit hyperactivity disorders.

WTH11 Glial cells (Session B)

WTH11-01

Characterization of novel kainic acid analogs as inhibitors of select microglial functions

M. Alford¹, Z. Tian², F. Menard², A. Klegeris¹

¹University of British Columbia, Biology, Kelowna, Canada

²University of British Columbia, Chemistry, Kelowna, Canada

Alzheimer's disease (AD) is characterized by abnormal accumulation of extracellular amyloid beta protein (A β) plaques and intracellular neurofibrillary tangles, as well as by a state of chronic inflammation in the central nervous system (CNS). Adverse activation of microglia, the brain immune cells, is believed to contribute to AD pathology including excessive neuronal death. Thus, normalizing immune functions of microglia could slow neurodegeneration, and identification of novel compounds capable of modifying microglial functions is an important goal. Since kainic acid (KA) has been shown to modulate microglial morphology and immune functions, we synthesized six new KA analogs (KAAs) and tested their effects on select microglial functions by using three different cell types as microglia models. Four of the KAAs at low micromolar concentrations inhibited secretion of cytotoxins, monocyte chemoattractant protein (MCP)–1, reactive oxygen species (ROS) and nitric oxide (NO) by immune-stimulated microglia-like cells. We hypothesize that the effects of the novel KAAs on microglia-like cells are not mediated by KA receptors since their biological activity was distinct from that of KA in all assays performed. A structural similarity search identified aldose reductase (AR) as a potential target for the novel KAAs. This hypothesis was supported by use of AR inhibitor zopolrestat, which abolished the inhibitory effects of two KAAs on microglial secretion of NO. Since the newly developed KAAs inhibited pro-inflammatory and cytotoxic functions of microglia, they should be further investigated for their potential beneficial effect on neuroinflammation and neurodegeneration in AD animal models.

WTH11-02

HIF1A drives hypoxia-mediated suppression of oligodendrocyte formation through induction of bHLH transcription factor ASCL2

K. Allan, L. Hu, S. Hung, B. Clayton, M. Scavuzzo, C. Bartels, P. Tesar

Case Western Reserve University, Genetics and Genome Sciences, Cleveland, USA

One out of every ten infants is born prematurely in the United States. Ten percent of these children harbor damage to white matter often resulting in sustained neurodevelopmental deficits. Diffuse white matter injury (DWMI) is the most common form of white matter injury in premature children and results from an incurable complication of hypoxic injury to the developing central nervous system (CNS). This hypoxic injury is mediated by Hypoxia Inducible Factors (HIFs), transcription factors that are master regulators of the response to hypoxia and impair oligodendrocyte progenitor cell (OPC) maturation to myelinating oligodendrocytes. The mechanism by which HIFs inhibit the generation of oligodendrocytes from OPCs remains elusive and could offer novel targets

for therapeutic intervention. Here we demonstrate that HIF1a directs a global rewiring of the chromatin state in OPCs, thereby maintaining them in a stem cell state and preventing their differentiation into oligodendrocytes. ChIP-seq of HIF1a combined with chromatin and transcript profiling identified Ascl2, a bHLH transcription factor not normally expressed in the developing or adult CNS, as a direct HIF1a target up-regulated in hypoxic OPCs. CRISPR-mediated activation of Ascl2 demonstrated that Ascl2 alone was sufficient to block the generation of oligodendrocytes from OPCs even in normoxic conditions. Rearing mouse pups in mild chronic hypoxia demonstrated that Ascl2 is induced *in vivo* in the neonatal developing brain in response to hypoxic injury. Collectively this work suggests a novel function for Ascl2 in the CNS in response to hypoxia and offers a node for future therapeutic development for DWMI.

WTH11-03

Astrocytic TFEB as a regulator of brain homeostasis

H. Antikainen

Rutgers University, Biology, Newark, USA

Transcription factor EB (TFEB) is a central regulator of lysosomal biogenesis and autophagy that responds to nutrient signaling and cellular stress. We have previously described an Alzheimer's disease-like phenotype with neuronal death and aggregating toxic proteins such as beta-amyloid and phospho-tau in TFEB fl/fl-nestin cre mice, which lack TFEB in neuronal progenitor cells. In this mouse line, three different mature cell lineages are affected by the genetic manipulation: neurons, oligodendrocytes and astrocytes. In the current study, we are examining the effects of TFEB loss in each of these cell types and their contributions to the disease-like phenotype. Oligodendrocytes seem to retain the ability to myelinate despite the loss of TFEB in TFEB fl/fl-nestin cre mice as seen by myelin basic protein stains. Astrocytes are more reactive in TFEB fl/fl-nestin cre in the corpus callosum, striatum and cortical layers. In these regions, also microglia exhibit increased reactivity, despite not being directly affected by the genetic manipulation. The microglia could be triggered by stimuli in the environment caused by the loss of TFEB, or by altered astroglia-to-microglia signaling. We will use astrocyte-specific TFEB knockout mice and knockdown primary cell culture to address whether the loss of TFEB increases astrocyte reactivity and pro-inflammatory signaling directly, and to assess what the contribution of TFEB loss in astrocytes is to the disease-like phenotype in the brain.

WTH11-04

Early morpho-functional changes in reactive astrocytes after juvenile mild traumatic brain injury

J. Badaut¹, A. Ichkova¹, T. Clement¹, A. Delahaye², J. Aussudre¹, M.-L. Fournier¹, M. Boccazzi², J. Van-Steel-Winckel¹, P. Gressens², U.-V. Nagerl³

¹CNRS-University of Bordeaux, CNRS UMR 5287, Bordeaux, France

²INSERM, INSERM U1141, Paris, France

³CNRS-University of Bordeaux, CNRS 5297, Bordeaux, France

Mild-Traumatic brain injury (mTBI) is the first cause for emergency department visits in the kids. Even for mTBI, pediatric patients suffer long-term cognitive impairments but the underlying cellular and molecular mechanisms are unknown. Astrocytes are responding to an injury by becoming “reactive”. We hypothesized that early astrocyte-dysfunction is contributing to the poor outcome in the long-term. We analyzed the morphology, gene expression and calcium signals of astrocytes after juvenile mild-TBI (jmTBI). We modeled jmTBI in postnatal-day-17 CD1-mice. The injury was induced over the left parietal cortex using an electromagnetic-impactor with no skull-fracture. Morphological changes in astrocytes were assessed with GFAP-immunofluorescence, and calcium-signals were recorded with 2-photon-microscopy 1 and 3 days after injury. Gene expression changes after jmTBI were evaluated with RNA-Seq on isolated astrocytes obtained using MACS with GLAST. Increase in GFAP-expression and in the number and the length of astrocytic processes was observed in astrocytes after jmTBI. Genes of pan-reactive astrocytes were up-regulated together with other genes from the astrocyte A1 and A2 sub-category after jmTBI. An analysis of the up-regulated-genes revealed an overrepresentation of genes associated with energy metabolism and extracellular-matrix in the jmTBI. These changes went hand in hand with transient alterations in astrocytic-calcium signals in response to ATP, which were characterized by a delayed and longer-lasting time course in jmTBI compared to sham control at day-1. jmTBI induces phenotypic changes in astrocytes that share characteristics of reactive astrocytes, suggesting that could be a target for drug development.

WTH11-05

Fyn tyrosine kinase interactions that regulate oligodendroglia migration and differentiation

B. Badillo, D. Osterhout

SUNY Upstate Medical University, Cell and Developmental Biology, Syracuse, USA

Oligodendroglial progenitor cells (OPCs) are produced in the ventral neuroepithelium at later stages in the ontogenesis of the cortex. They migrate into the brain parenchyma, where they will contact axons and differentiate. As they undergo terminal differentiation, OPCs undergo dramatic morphological changes, transforming from a simple bipolar cell to a cell with multiple complex processes extending from the cell body. Once in contact with an axon, the oligodendrocyte process expands and begins to form the myelin membrane, which will wrap and ensheath the axon. Fyn tyrosine kinase is an important signaling pathway that can regulate both migration and differentiation of oligodendroglial progenitor cells. PDGF will stimulate migration of OPCs through the activation of Fyn. Fyn also regulates the morphological differentiation of these

cells, initiating process outgrowth and myelin sheet formation *in vitro*. Fyn activity is important early in differentiation and up-regulated before any changes in cellular morphology are observed. In Fyn deficient mice, myelin formation is markedly reduced, demonstrating the importance of this kinase in myelination. Fyn can interact with many downstream effectors, including molecular signaling pathways that interact with the cytoskeleton that regulate cell morphology and movement. One important interaction involves the adaptor protein Dab1. We have demonstrated that Fyn-Dab1 interactions are important for OPC migration, as animals deficient in either Fyn or Dab1 show reduced OPC migration from the subventricular zone *in vivo*. Further *in vitro* studies reveal more components of this pathway. Inhibition of Fyn-Dab1 interactions will reduce OPC migration and process outgrowth. Downstream targets of Fyn and Dab1 include Cdk 5, which may be important for migration, but not process outgrowth. Fyn interactions with additional cytoskeleton elements influence the dynamic morphological changes during OPC differentiation.

WTH11-06

Deletion of voltage-gated Ca⁺⁺ channels in reactive astrocytes reduces brain inflammation and promotes remyelination in mice

V. Cheli, D. S. González, N. Zamora, R. Wang, P. Paez

SUNY University at Buffalo, Hunter James Kelly Research Institute, Department of Pharmacology and Toxicology, Buffalo, USA

We have previously reported that voltage-gated Ca⁺⁺ channels containing the Cav1.2 alpha subunit are centrally involved in triggering astrocyte reactivity *in vitro*. To determine whether Cav1.2 Ca⁺⁺ channels contributes to astrocyte activation *in vivo*, we generated an inducible conditional knockout mouse in which the Cav1.2 alpha subunit was deleted in GFAP positive astrocytes. This astrocytic Cav1.2 knockout mouse was tested in the cuprizone model of myelin injury and repair which causes astrocyte and microglia activation in the absence of a lymphocytic response. Deletion of Cav1.2 channels in GFAP positive astrocytes during cuprizone-induced demyelination lead to a significant reduction in the degree of astrocyte and microglia activation and proliferation. Concomitantly, the production of pro-inflammatory factors such as TNF α , IL1 β and TGF β 1 was significantly decreased in the corpus callosum and cortex of Cav1.2 knockout mice through demyelination. Furthermore, this mild inflammatory environment promoted oligodendrocyte progenitor cells maturation and myelin regeneration across the remyelination phase of the cuprizone model. Similar results were found in mice treated with nimodipine, a Cav1.2 Ca⁺⁺ channel inhibitor with high affinity to the CNS. Animals injected with nimodipine during the demyelination stage of the CPZ treatment displayed a reduce number of astrocyte and microglial cells activation and proliferation as well as a faster and more efficient brain remyelination. Together, these results indicate that Cav1.2 Ca⁺⁺ channels play a crucial role in the induction and proliferation of reactive astrocytes during demyelination; and attenuation of astrocytic voltage-gated Ca⁺⁺ influx may be an effective therapy to reduce brain inflammation and promote myelin recovery in demyelinating diseases.

WTH11-07

Differentiation of oligodendrocyte progenitors is not the dominant mechanism of small molecule enhanced oligodendrocyte formation**B. Clayton, D. Allimuthu, P. Tesar, D. Adams***Case Western Reserve University, Genetics and Genome Sciences, Cleveland, USA*

Diseases of myelin, both genetic and acquired, are a significant cause of morbidity and mortality. Establishing safe and effective therapies to promote myelination is a major clinical goal for multiple sclerosis and other neurological disorders. Multiple high throughput screening studies have identified and validated small molecules capable of enhancing the generation of rodent myelinating oligodendrocytes by what was thought to be stimulation of oligodendrocyte progenitor cell (OPC) differentiation. Nevertheless, we show that nearly all of these promyelinating compounds do not actually promote OPC differentiation. By integrating quantitative immunohistochemistry and single cell RNAseq analysis throughout the cellular stages of oligodendrocyte generation, we reveal that nearly all validated promyelinating compounds do not stimulate or accelerate the formation of new oligodendrocytes from OPCs but rather function specifically to enhance a later transition to mature myelin protein-expressing oligodendrocytes. To understand if other molecules might stimulate OPC differentiation, we performed a modified high throughput 3,000 compound screen specifically focused on the early OPC to new oligodendrocyte transition. Surprisingly, validated hits from this screen only consisted of molecules annotated to modulate thyroid hormone receptor. In addition, oligodendrocyte enhancing effects of thyroid hormone combined with promyelinating drugs is additive showing unique mechanism and stage of action for these compounds. Collectively, these results highlight the existence of at least two cellular stages that can be targeted through distinct mechanisms to effectively enhance the generation of oligodendrocytes and will inform discovery and implementation of clinical remyelination strategies for the diversity of myelin disorders.

WTH11-08

The functional role of transferrin receptor-1 in peripheral nerve myelination**J. DeGeer, D. Gerber, C. Sparano, U. Suter***ETH Zurich, Institute of Molecular Health Sciences, Department of Biology, Zurich, Switzerland*

Iron is an essential element, upon which all biological life depends. While perinatal iron deficiency results in impaired CNS myelination, mechanisms of iron uptake by myelinating glia remain to be deciphered *in vivo*. Schwann cells of peripheral nerves synthesize enormous amounts of plasma membrane to ensheath axons during myelination. Since a multitude of enzymes involved in protein synthesis and lipid biogenesis require iron as a cofactor, we hypothesize that Schwann cells require a steady supply of iron during myelination. Iron bound to the serum glycoprotein Transferrin (Tf) represents a major non-Heme cellular iron source. Tf-bound iron gains entry into the cell in association with Tf receptor 1 (TfR1), and in Schwann cells we have shown that this process is Dynamin 2-dependent. To address the importance of Tf-bound iron during PNS myelination, we generated a mouse line deficient of TfR1 expression in Schwann cells using Mpz (P0)-driven Cre

expression. TfR1 mutant sciatic nerves initially myelinated normally, while adult nerves were hypomyelinated at 2 months of age. Global gene expression analysis of early postnatal mutant Schwann cells revealed enhanced glycolytic and pro-angiogenic gene signatures—a finding supported by increased lactate concentrations and vasculogenesis in age-matched nerves. Ongoing efforts are being made to characterize the metabolic changes in promyelinating Schwann cells *ex vivo* in relation to iron availability and redox status both developmentally and into adulthood. Together our data indicate that TfR1 expression regulates Schwann cell metabolism and myelin growth, revealing a developmental axis of communication between Schwann cells and the endothelium.

WTH11-09

Role of astrocyte-derived GDNF in neuronal protection and brain recovery after ischemic stroke**S. Ding***University of Missouri, Dalton Cardiovascular Research Center, Columbia, USA*

Astrocytes play a non-cell autonomous role in neuroprotection after ischemic stroke. Glial cell-derived neurotrophic factor (GDNF) was originally isolated from a rat glioma cell-line supernatant and is a potent survival neurotrophic factor. Reactive gliosis and glial scar formation is a hallmark of focal ischemic stroke (FIS). Here, we investigated the effect of reactive astrocytes-derived GDNF on neuronal death and brain damage after FIS using inducible and astrocyte-specific *Gdnf* conditional knockout (cKO), i.e., *Glast-Gdnf*^{-/-} cKO mice. Under non-ischemic conditions, we found that *Glast-Gdnf*^{-/-} cKO mice exhibited significant lower number of Brdu+ and Ki67 cells as well as DCX+ cells in the dentate gyrus (DG) in hippocampus in adult mice, indicating astrocytic GDNF can promote adult neurogenesis. Under ischemic conditions, *Glast-Gdnf*^{-/-} cKO mice had a significant reduction in infarct volume after 2, 4 and 14 days after PT as compared with WT litter mates after PT. *Glast-Gdnf*^{-/-} cKO mice also exhibited a significant reduction in FJB+ degenerating neurons in PIR. Moreover, *Glast-Gdnf*^{-/-} cKO mice had lower densities of Brdu+ and Ki67 + cell in the PIR than WT mice, indicating that astrocytic GDNF can promote cell proliferation in the PIR. Furthermore, behavioral tests showed that deletion of GDNF caused more motor deficits after stroke and low recovery. In summary, our study suggests that reactive astrocytes-derived GDNF plays important roles in reducing neuronal death and brain damage through neural regeneration in PIR after PT, and promoting endogenous neurotrophic factor release from reactive astrocytes might be a potential therapeutic approach in stroke therapy.

WTH11-10

Extracellular matrix-associated molecules gene expression in astrocytes during early postnatal development**I. Dominova, L. Klimaviciusa, N. Filiakova, E. Sotnikov***Immanuel Kant Baltic Federal University, Laboratory of Neurobiology, Kaliningrad, Russia*

It is well-known astrocytes are active participants in the tripartite synapse. However, there is an opinion that synapse represents the

tetrapartite structure, which includes pre- and postsynaptic neurons, astrocytes and the extracellular matrix (ECM), which is formed by glycoproteins and proteoglycans, released by neurons and astrocytes. Thus, ECM affects the synapse stability and function and the development of the astrocytes. Our aim was to study age-dependent diversity in the expression values (EVs) of ECM-associated molecules (ECM-AMs) in astrocytes. Analysis of transcriptomic data of astrocytes from Ctx, Bst and Hip of P3 and P11/12 rats demonstrated the EVs decreasing of ECM-AMs genes during development, but at the same time in Ctx EVs of some genes increased to P11/12. Further studies of the gene families of ECM-receptor interaction pathway by comparison P3 and P11/12 rats showed, that in P3 rat EVs of TnC was up-regulated in Bst and Ctx and TnR was down-regulated in Bst and Hip. For Lam family EVs in P3 rats in Bst were up-regulated, while in Ctx they were down-regulated, but in Hip, genes were up- and down-regulated simultaneously. Analysis of Sdc EVs in P3 rats demonstrated decreasing EVs in Bst and Hip, but in Ctx Sdc1 and Sdc4 were up- and down-regulated, respectively. Furthermore, EVs of Acan, Bcan, Ncan were increased, but expressions of Agrn, Reln, Vcan were down-regulated in P11/12 rats. Altogether obtained data indicate on the significant contribution of astrocytes in releasing of ECM-AMs and formation ECM in the brain, as well as on an ongoing postnatal maturation of astrocytes. This study was financially supported by the Grant of the Russian Foundation for Basic Research 18-34-00152.

WTH11-11

Erasure of polycomb repression in Schwann cell after injury

P. Duong^{1,2,3}, K. Ma^{1,2,3}, J. Moran¹, S. Song¹, J. Svaren^{1,3}

¹University of Wisconsin-Madison, Waisman Center, Madison, USA

²University of Wisconsin-Madison, Cellular and Molecular Pathology Graduate Program, Madison, USA

³University of Wisconsin-Madison, Dept. Of Comparative Biosciences, Madison, USA

Schwann cells are able to reprogram themselves after peripheral nerve injury through an epithelial mesenchymal transition-like program and enter into a proliferative and regenerative state. This remarkable switch from terminally differentiated Schwann cells to a pro-regenerative state is poorly understood, however. Our preliminary data indicate that Schwann cell's reprogramming to the repair mode may require manipulation of epigenomic pathways. We identified that many nerve repair genes are regulated by the repressive histone H3K27 methylation (H3K27me3) which is deposited by Polycomb repression complex 2 (PRC2). Interestingly, ChIP analysis showed that H3K27me3 and PRC1-induced histone modifications are removed after nerve injury from nerve repair genes, such as sonic hedgehog and glial-derived neurotrophic factor (Shh and Gdnf), which are required for the pro-regenerative activities of Schwann cells. Additionally, our study shows that inactivation of PRC2 via Schwann-cell specific Eed knockout resulted in delayed proliferation and regeneration partly through the dysfunctional regulation of CDKN2A. Therefore, we have developed the Schwann cell specific double knockouts of H3K27 demethylases JMJD3/KDM6B and UTX/KDM6a to test if they are required to promote reversal of histone methylation on H3K27 and trigger activation of injury genes. Analysis of Schwann cell-specific knockouts of these demethylases showed that they are not required for the development of Schwann cells nor for activation of

most genes post injury. Therefore, additional mechanisms to bypass polycomb repression after nerve injury may be involved.

WTH11-12

Modulation of microglia by IGF1 and motor improvement in aged rats

E. Falomir-Lockhart¹, F. J. C. Dolcetti¹, M. L. Herrera², L. M. G. Segura³, C. B. Hereñu², M. J. Bellini¹

¹INIBIOLP, Universidad Nacional de La Plata, Facultad de Ciencias Médicas, La Plata, Argentina

²Universidad Nacional de Córdoba, Facultad de Ciencias Químicas, IFEC-CONICET, Córdoba, Argentina

³Instituto Cajal, Csic, Neuroactive Steroids, Madrid, Spain

In the last decades, the average lifespan has increased due to the improvement on medical care and social and cultural conditions. This fact comes together with the consequent motor and cognitive impairment as well as an increment in the incidence of age-related pathologies, such as Parkinson's disease. Aging presents a loss of brain homeostasis and a chronic neuroinflammation, caused by senescent microglia, which are polarized towards a pro-inflammatory phenotype, generating thus an exacerbated immune response. In fact, the progression of many neurodegenerative diseases is dependent on microglia activation. Therefore, it is of great interest to design strategies that allow modulating these glial cells phenotype. One possibility to modulate microglia activation in the aged brain is the use of neurotrophic factors. Many neurotrophic factors produced by glial cells, such as IGF1, are able to polarize them into a more neurotrophic/neuroprotective phenotype, promoting neuronal survival. Therefore, we implemented IGF1 gene therapy in aged rats and studied who the motor improvement (previously observed in our laboratory) and microglia activation could be related. We demonstrated that the therapy modulated microglia number and activation in an area-dependent manner. Moreover, IGF1-therapy efficiently polarized microglia in the Striatum into an anti-inflammatory phenotype. Finally, IGF1 increased microglial phagocytic activity in the Striatum, promoting the elimination of neurons expression Homer1. These results suggest that IGF1 gene therapy could be an effective treatment to modulate microglia activation and promote motor improvement.

WTH11-13

Astrocytes as a target for nogo-a and implications for synapse formation in-vitro and in a model of acute demyelination

F. Gomes¹, S. Espírito-Santo^{2,1}, V. Coutinho¹, R. Dezone¹, J. Stipursky¹, A. Rodrigues⁴, C. Batista¹, R. Paes-de-Carvalho⁴, B. Fuss³

¹Federal University of Rio de Janeiro, Institute of Biomedical Sciences, Rio de Janeiro, Brazil

²Universidade do Estado de Minas Gerais, Ciências Biológicas, Ubu, Brazil

³Virginia Commonwealth University School of Medicine, Department of Anatomy and Neurobiology, Richmond, USA

⁴Universidade Federal Fluminense, Ciências Biomédicas, Niteroi, Brazil

Astrocytes comprise one outstanding regulator of synapse development and plasticity, through the release of pro- and anti-

synaptogenic factors. Myelin and its associated proteins, such as Nogo-A, directly affect synapses in an inhibitory fashion and, thereby, contribute to neural circuitry stabilization. However, potential roles of Nogo-A-astrocyte interactions and their implications on synapse development have not been characterized. We aimed to investigate whether Nogo-A can affect the capacity of astrocytes to induce synaptogenesis. Our *in-vitro* data show that cortical astrocytes respond to Nogo-A by RhoA pathway activation, actin stress fibers formation and morphological change. This phenotype was associated with reduced levels of GLAST protein and aspartate uptake, decreased mRNA levels of the synaptogenesis-associated genes, Hevin, glypican-4, TGF- β 1 and BDNF, as well as decreased and increased protein levels for Hevin and SPARC, respectively. Conditioned medium of Nogo-A treated astrocytes suppressed the formation of synapses in cortical neuronal cultures. During cuprizone-induced acute demyelination, reduced immunostaining for Nogo-A in the visual cortex was accompanied by higher levels of Hevin expression in astrocytes and an increase in excitatory synapse density. Hence, we suggest that interactions between Nogo-A and astrocytes might represent an important pathway of plasticity regulation and could be a target for therapeutic intervention in demyelinating diseases.

WTH11-14

Translating thyroid hormone action into therapy for CNS myelin repair

M. Hartley¹, T. Banerji¹, I. Tagge², P. Chaudhary³, E. Calkins³, M. DeBell¹, M. Shokat¹, B. Emery^{3, 4}, D. Bourdette³, T. Scanlan¹

¹Oregon Health & Science University, Department of Physiology and Pharmacology and Program in Chemical Biology, Portland, OR, USA

²Oregon Health & Science University, Advanced Imaging Research Center, Portland, OR, USA

³Oregon Health & Science University, Department of Neurology, Portland, OR, USA

⁴Oregon Health & Science University, Jungers Center for Neuroscience Research, Portland, OR, USA

Current clinical therapies for multiple sclerosis target the inflammatory component of the disease, but no approved therapies address demyelination by directly promoting myelin repair. Thyroid hormone induces oligodendrocyte differentiation and myelination during neurodevelopment and our strategy utilizes this endogenous pathway to promote repair in adult disease. Thyromimetics are synthetic thyroid hormone agonists that avoid the adverse effects associated with hyperthyroidism through isoform specificity and selective tissue distribution. Recently, a new class of prodrug thyromimetics has been developed that improve delivery of a parent thyromimetic to the CNS. To test thyromimetics as agents of myelin repair, we have characterized the remyelination phase of a mouse model of demyelination based on genetic ablation of Myrf, a transcription factor critical for oligodendrocyte health and maintenance. The iCKO-Myrf model has advantages over standard demyelination models including a clinical behavioral phenotype and clear phases of demyelination and remyelination. Treatment with thyromimetics improved clinical motor deficits and increased myelin as measured by both histology and small animal MRI. Further studies compared the efficacy of parent thyromimetics with CNS-penetrating prodrug derivatives. These studies validate the use of a thyromimetic strategy for promoting myelin repair and form the scientific basis for translation to the clinic. C.

WTH11-15

Chondroitin sulfate protects oligodendrocytes from oxidative stress and regulates oligodendrocyte differentiation

H. Hashimoto¹, D. Verden¹, P. Herson², W. Macklin¹

¹University of Colorado Denver School of Medicine, Department of Cell and Developmental Biology, Aurora, USA

²University of Colorado Denver, Department of Anesthesiology, Aurora, USA

Oligodendrocytes, the myelinating glial cells of the central nervous system, are crucial for normal nervous system function. In the juvenile mouse brain, peak myelination occurs post-weaning, yet our earlier studies establish that myelinating oligodendrocytes at this age are resistant to ischemic stroke. The current studies investigate a possible mechanism of myelinating oligodendrocyte protection following ischemic stroke. We focus on molecules of the extracellular matrix, such as chondroitin sulfate (CS), which is expressed following ischemic damage. Following 45 min middle cerebral artery occlusion in P25 mice, CS was up-regulated at 1-3 days recovery, and decreased at 7 days. CS was expressed by NG2-positive cells and Iba1 positive microglia. Treatment of oligodendrocyte progenitor cells (OPCs) *in vitro* with exogenous CS promoted OPC proliferation and differentiation, and it impacted oligodendrocyte survival and differentiation following oxidative stress. Oligodendrocytes pre-incubated with CS were more resistant to H₂O₂-induced oxidative stress, and this pre-treatment increased Akt phosphorylation and HO-1 expression. This suggests that CS protects oligodendrocytes from oxidative stress, possibly by Akt phosphorylation and increased HO-1 expression. Since CS is expressed by oligodendrocytes themselves, we studied whether its down-regulation impacted oligodendrocytes. In fact, down-regulating CS synthesis inhibited OPC differentiation and induced cell death following H₂O₂ treatment. Taken together, these studies suggest that CS production protects oligodendrocytes from oxidative stress, possible via its barrier function or by altering oligodendrocyte signaling to enhance protection. The support for this project is American Heart Association/Bugher Center Grant.

WTH11-16

Profiling astrocyte-specific transcriptional changes after ischemic stroke

V. Hernandez, K. J. Lechtenberg, M. S. Buckwalter

Stanford School of Medicine, Neurology and Neurological Sciences, Stanford, USA

Ischemic stroke is a leading cause of death and long term disability. Neuroinflammation after stroke can significantly affect stroke outcomes, as it can induce tissue repair, but can also exacerbate cell death. After stroke, astrocytes enter a reactive phase of astrogliosis, which is characterized by astrocytic proliferation, formation of an astrocytic scar, and release of pro-inflammatory cytokines. However, the exact astrocytic signaling pathways regulating neuroinflammation after stroke are unresolved. A barrier to understanding this has been the challenge of parsing the astrocytic response from that of infiltrating inflammatory cell types in the brain after stroke. To address this, we used the RiboTag technique to obtain astrocyte-derived transcripts after stroke. By crossing the RiboTag and Aldh1 l1-CreER mouse models, we expressed a hemagglutinin tag on ribosomes only in astrocytes, enabling

immunoprecipitation of astrocytic ribosomes and isolation of actively translating astrocytic mRNA. Targeted gene expression analysis showed that astrocyte transcripts obtained from stroked mice (N = 6) using this technique were highly significantly enriched in astrocyte-specific genes such as GFAP (19.66 fold higher) and de-enriched in characteristic genes from other brain cell types, such as Cx3cr1 (15.79 fold lower) and Tubb3 (24.86 fold lower) compared to whole cortex transcripts. We verified by immunohistochemistry that this tag is expressed by 99% of astrocytes following recombination, and 99% of tag-expressing cells also express GFAP. Our data indicates that the RiboTag technique can effectively profile the astrocyte translome after stroke. Using this technique, we will comprehensively define the astrocyte-specific translome response at specific time points after stroke.

WTH11-17

Glutamate and glucose uptake in Bergmann glia cells are modified by bisphenol-a and 17 beta-estradiol L. Hernandez-Kelly¹, L. Cid², A. Ortega², F. Castelán³

¹Universidad Autonoma de Tlaxcala, Centro traxcala de Biología de la Conducta, Tlaxcala, Mexico

²Cinvestav-IPN, Departamento de Toxicología, Mexico, Mexico

³Universidad Nacional Autonoma de Mexico, Instituto de Investigaciones Biomédicas, Mexico, Mexico

Glutamate (Glu) is the major excitatory transmitter in the vertebrate brain. Glu removal from the synaptic cleft is a crucial factor of Glu recycling and an essential mechanism to prevent Glu receptors over-stimulation that results in neuronal death. Glu uptake is achieved mainly by glia Glu transporters, known as excitatory amino acids transporters (EAATs). EAAT1, also named glutamate/aspartate transporter (GLAST) is highly expressed in cerebellar Bergmann glia cells. Glu entrance activates glutamine synthetase, an energy demanding event, increasing glia cells glucose uptake. Estrogens have been described as neuroprotective through the activation different subtypes of receptors: nuclear and plasma membrane receptors. Bisphenol A (BPA) was developed as a synthetic estrogen and today is commonly used in many products such as reusable plastic bottles, internal coating of foods cans and thermal paper. In this work we analyzed the effect of the exposure of both 17b-estradiol and the estrogen-like pollutant BPA in [³H]D-aspartate uptake activity and in [³H]2-DeoxyD-glucose (DOG) uptake activity in cultured chick cerebellar Bergmann glia cells. Upon treatment of Bergmann glia cells with 17b-estradiol a time and dose-dependent increase in [³H]D-aspartate uptake activity was found. This augmentation is related to an increase of GLAST transporters in the plasma membrane. Likewise, glucose uptake into Bergmann glia cells is also increase upon estradiol stimulation. These results suggest that the well-established neuroprotective effect of estradiol might be related to its effect in Glu turnover in which glia cells are critically involved.

WTH11-18

A unifying mechanism for many small molecule enhancers of oligodendrocyte formation

Z. Hubler¹, D. Allimuthu¹, I. Bederman¹, Matthew. S. Elitt¹, M. Madhavan¹, K. C. Allan¹, H. E. Shick¹, E. Garrison², M. Karl², D. C. Factor¹, Z. S. Nevin¹, M. Giera³, F. Bracher⁴, R. H. Miller², P. J. Tesar¹, D. J. Adams¹

¹Case Western Reserve University, Genetics and Genome Sciences, Cleveland, USA

²George Washington University School of Medicine and Health Sciences, Department of Anatomy and Regenerative Biology, Washington DC, USA

³Leiden University Medical Center, Leiden, The Netherlands

⁴Ludwig-Maximilians University of Munich, Department of Pharmacy - Center for Drug Research, Munich, Germany

Loss of myelin-producing oligodendrocytes in the central nervous system underlies a number of neurological diseases, including multiple sclerosis. In adults, the primary source of oligodendrocytes is oligodendrocyte progenitor cells (OPCs). To discover novel therapies for demyelinating disorders, we and others have performed *in vitro* drug screens for small molecules that enhance oligodendrocyte formation from OPCs. Our high-throughput screening hits were mechanistically diverse and their canonical targets could not be ascribed to any known oligodendrocyte biology. Surprisingly, we found that as opposed to functioning via their canonical targets, our screening hits enhance oligodendrocyte formation through a unifying off-target effect of inhibiting three enzymes in the cholesterol biosynthesis pathway, CYP51, EBP, and TM7SF2. Selective small molecule inhibitors of these enzymes enhance oligodendrocytes formation *in vitro*. Accumulation of the 8,9-unsaturated sterols, the substrates of these enzymes, is a key mechanistic node for enhanced oligodendrocyte formation. Inhibitors of CYP51, EBP, and TM7SF2 enhance remyelination *in vivo* and lead to an accumulation of 8,9 unsaturated sterols in the brains of mice. Our work describes a unifying hypothesis for dozens of small molecules which enhance oligodendrocyte formation and illuminates a novel pathway for therapeutic targeting.

WTH11-19

The role of mtor in oligodendrocyte susceptibility to demyelination and efficiency of remyelination

M. Jeffries¹, J. Bourne², W. Macklin², T. Wood¹

¹Rutgers New Jersey Medical School, Pharmacology, Physiology, & Neuroscience, Newark, USA

²University of Colorado School of Medicine, Cell & Developmental Biology, Aurora, USA

In demyelinating diseases such as multiple sclerosis, myelin loss and subsequent neuronal degeneration decrease patient functionality, making it critical to determine mechanisms to limit demyelination and promote remyelination. We previously showed that mTOR deletion in the oligodendrocyte (OL) lineage in the brain during development results in no developmental phenotype but delayed myelin loss with age. We therefore tested the hypothesis that mTOR signaling regulates susceptibility to demyelination and efficiency of remyelination in the adult central nervous system. To assess myelin integrity over time, we examined *Cnp-Cre;mTOR^{fl/fl}* mouse brains at 12 weeks of age, one month after normal myelination was previously observed by electron microscopy.

Surprisingly, these mice displayed significantly reduced mature OL numbers and myelination in the callosum, suggesting an important role for mTOR in preventing endogenous demyelination. In order to examine remyelination, we used a *Ng2-Cre^{ERT};mTOR^{fl/fl}* mouse line to conditionally delete *mTOR* from adult oligodendrocyte precursor cells (OPCs) that give rise to new OLs responsible for remyelination. Cuprizone was administered via dietary intake over 6 weeks to induce demyelination, followed by remyelination upon cuprizone removal. During initial remyelination, we observed thinner myelin in mice lacking mTOR in adult OPCs, suggesting a deficiency in early remyelination, although proliferation and differentiation was unaffected. Surprisingly, myelin thickness recovered by late-stage remyelination 4 weeks after removal of cuprizone treatment, suggesting a transient role in initial myelin wrapping and/or compensation by other signaling pathways. Taken together, our results suggest that mTOR signaling promotes normal myelin maintenance and efficient remyelination in the adult brain.

WTH11-20

Immunohistochemical and transcriptional characterization of spleen glia

T. Lucas¹, L. Zhu¹, M. Rao^{2,3}, M. Buckwalter¹

¹Stanford University, Neurology and Neurological Sciences, Stanford, CA, USA

²Boston Children's Hospital, Pediatrics, Boston, MA, USA

³Harvard Medical School, Pediatrics, Boston, MA, USA

With the exception of enteric glia, visceral non-myelinating Schwann cells are not well studied. Moreover, the organs they reside in are innervated by autonomic nerves. Sympathetic nerves provide the sole innervation to lymph nodes and the spleen. Stress, which activates sympathetic nerves, has well-described effects on inflammation and autoimmune diseases. Given the role of glia in neuro-immune communication throughout the nervous system we hypothesize that glia in immune organs play an important role in sympathetically-driven immune changes, and decided to characterize their anatomy and gene transcription. We found that tyrosine hydroxylase positive nerves are intertwined with GFAP, S100b, and PLP1-expressing non-myelinating glia throughout the spleen. Using mice in which GFAP drives cre or PLP1 drives the expression of GFP in reporter animals, we validated that GFP expression is limited to spleen glia with > 99% concordance. We observe that nerves course the outside of arterioles that penetrate T and B cell areas, with spleen glia uniformly associated, making glia optimally located to receive neurotransmitters and signal to immune cells. To determine the molecular composition of spleen glia and compare their similarity to other glial cell types, we dissociated the spleens of mice into single cell suspensions and extracted RNA from size-purified glia. We observe by qPCR that spleen glia express peripheral glial markers; GFAP, S100b, and Sox10. Additionally, they express ATP1B2, which is also in astrocytes and oligodendrocyte precursor cells, and KCNA1 (enteric glia and oligodendrocytes). Ongoing RNA-seq analysis will shed light on whether there are distinct markers of spleen glia and how they communicate with neurons and immune cells.

WTH11-21

Microglial clusters after facial nerve axotomy injury are related to proliferation

G. Manich, A. Kakoulidou, N. Villacampa, B. Almolda, B. González, B. Castellano

Autonomous University of Barcelona, Cell Biology, Physiology and Immunology, Bellaterra, Spain

After facial nerve axotomy (FNA), microglial activation and facial motor neuron (FMN) degeneration are triggered. In this process microglia proliferate, wrap FMN and form clusters. Also, microglia are involved in phagocytosis and interaction with infiltrated T-lymphocytes. Previous studies in our group revealed that transgenic mice (Tg) overproducing interleukin-6 under the GFAP promoter (GFAP-IL6Tg) showed less microglial clusters and higher FMN death compared to axotomized wild-type (WT) mice. Contrarily, Tg overproducing interleukin-10 (GFAP-IL10Tg) showed more clusters and enhanced FMN survival. In here, we aimed to investigate: 1) the role of both IL-6 or IL-10 in microglia proliferation after FNA; 2) the involvement of proliferation in cluster formation. Therefore, GFAP-IL6Tg, GFAP-IL10Tg and WT mice were axotomized and samples from 3 to 28 days post-injury (dpi) were stained by immunohistochemistry against Pu.1 and phosphohistone-H3 (PHH3). Additionally, another group of mice were daily administered with BrdU for 14 days after FNA and sacrificed immediately. Results showed that, in all groups, microglial density increased until 14dpi and subsequently decreased until 28dpi. In GFAP-IL6Tg, higher microglia density was found in basal conditions respect to WT. GFAP-IL-10Tg only showed higher microglial density than WT at 14dpi. Regarding proliferation, a peak in PHH3 + cells was found at 3dpi, and PHH3 + cells decreased until 14dpi and disappeared later in all genotypes. Noticeably, at 3dpi, PHH3 + cell number was higher in both Tg than WT. BrdU administration revealed that some, but not all, clusters contained BrdU+ microglia and, interestingly, microglia wrapping FMN were strongly BrdU +. Overall, proliferation is involved in cluster formation, and IL-6 or IL-10 overproduction doesn't importantly modify microglia proliferation after FNA.

WTH11-22

Exosomes as nanobiological carriers of apotransferrin for therapeutic use in demyelinating diseases

V. Mattera^{1,2,3}

¹Biological and Physical Chemistry, Biological Chemistry, Buenos Aires, Argentina

²Biomedical Research in Retroviruses and AIDS, Microbiology, Buenos Aires, Argentina

³Nanobiotechnology, Pharmaceutical Technology, Buenos Aires, Argentina

⁴Fleni, Neuroimmunology, Buenos Aires, Argentina

Exosomes are extracellular vesicles with a diameter ranging between 20 and 100 nm and their capacity for biologic information transfer makes them an attractive tool as therapeutic agents for nanodelivery to specific target cells. In addition, their structure is biocompatible, protecting the cargo from degradation. Also, this vesicles are able to cross the blood brain barrier and increase cargo molecule stability, solubility and bioavailability. Previous studies in our laboratory demonstrated the pro-myelinating and differentiating effect of apoTransferrin (aTf) in the CNS. The present work focuses

on the development of an aTf nanoencapsulation system to be targeted to oligodendroglial cells by intranasal administration in cuprizone- demyelinated mice. Exosomes isolated from different sources such as plasma, glial and neuronal cell lines and primary cultures were characterized by Western blot, scanning electron microscopy and dynamic light scattering to verify their purity, structural integrity and average size. Given that some exosomes contain the Tf receptor 1 (TfR1), our interest is to find an easy and quick pathway to load them with aTf and use them as a delivery particle of this growth factor to the target cells. Western blot analyses of experiments conducted using exosomes isolated from cell line OLN93 demonstrate that exosomes are able to be charged with aTf. Flow cytometry experiments showed that exosomes isolated from human plasma are able to bind exogenous aTf to TfR1. Altogether, our studies succeeded in thoroughly characterizing the isolated nanoparticles are exosomes and show that, due to the presence of TfR1, these growth factor are able to bind aTf and function as nanocarriers of this protein in an animal demyelination model.

WTH11-23

Quantification of D-amino acid release from primary rat astrocytes using enzymatic electrochemical biosensors S. Moussa¹, T. Kennedy², L. Pollegioni³, J. Mauzeroll¹

¹McGill University, Chemistry, Montreal, Canada

²McGill University, Montreal Neurological Institute, Montreal, Canada

³University of Insubria, Biotechnology and Life Sciences, Varese, Italy

D-serine level fluctuations have been implicated in various neurodegenerative disorders such as Alzheimer's and Amyotrophic Lateral Sclerosis. This amino acid is now recognized for its role in various brain functions particularly in the hippocampus, a region of the brain involved in cognitive function and memory formation, but the mechanism of D-serine release and the stimuli governing this release remain a mystery. The aim of this work is to demonstrate the successful development of miniaturized and optimized permselective poly(*meta*)-phenylenediamine (PPD) based enzymatic D-amino acid detecting biosensors and their application in studying D-serine release *in vitro*. The sensors, based on a PPD surface-modified 10 µm platinum microelectrode, are crosslinked with an *Rhodotorula gracilis* D-amino acid oxidase (RgDAAO) film. We have confirmed the reproducibility and lifetime of these sensors, with optimized storage conditions. These biosensors were then been employed for electrochemical detection of D-serine release from primary rat astrocytes. Thus far, we observed that D-serine is released from these astrocytes and that L-serine and D-serine loading into these cells result in an imbalance in the availability of D-serine in these cultures. Future work includes performing scanning electrochemical microscopy imaging on live cells for the on-line detection of D-serine release.

WTH11-24

Increased ratio of large myelin protein zero (L-MPZ) in myelin leads to Charcot-Marie-Tooth disease-like neuropathy

Y. Otani^{1, 2}, N. Ohno^{3, 4}, Y. Yamaguchi², J.-J. Cui², H. Baba²

¹Shimane University, Faculty of medicine, Department of Anatomy and Neuroscience, Izumo, Japan

²Tokyo university of Pharmacy and Lifesciences, Department of Molecular Neurobiology, Tokyo, Japan

³Jichi Medical University, Department of Anatomy, Division of Histology and Cell Biology, Shimotsuke, Japan

⁴National Institute for Physiological Sciences, 4. Division of Neurobiology and Bioinformatics, Okazaki, Japan

Translational readthrough is known as a key mechanism that expands the coding potential of the genomes from viruses to drosophila. Recently, in mammals, Translational readthrough proteins including large myelin protein zero (L-MPZ) (Yamaguchi et al., 2012), vascular endothelial growth factor extra form (VEGF-Ax) (Eswarappa et al., 2014), and aquaporin 4 extended isoform (AQP4ex) (De Bellis et al., 2017) have been identified in mammals. This indicates the importance in higher animals as well. L-MPZ is an isoform of myelin protein zero (P0), containing additional 63 amino acids at C-terminus by translational readthrough mechanism in various species including human. While L-MPZ is localized in the PNS myelin like P0, a role of this protein is still uncertain. The aim of this study was to clarify L-MPZ function in the PNS. We generated a mouse line (L-MPZ mice) that synthesizes only L-MPZ by CRSPR-Cas9 system. L-MPZ mice caused neuropathy like Charcot-Marie-Tooth disease (CMT). L-MPZ was not compensated function of P0 protein. This indicated that the function of L-MPZ was different from the function of P0. Heterozygote mice those had increased L-MPZ and decreased P0 levels demonstrated various conditions from normal to neuropathy phenotypes. Thus, increased ratio of L-MPZ/P0 may cause CMT phenotype. Our study indicates a new possibility of CMT pathogenesis caused by alteration of the L-MPZ/P0 ratio.

WTH11-25

Iron metabolism in the brain: the role of ferritin and transferrin receptor in oligodendrocyte maturation and myelination

P. Paez, R. Wang, S. Roseblum, D. S. González, V. Cheli

School of Medicine and Biomedical Sciences, SUNY at Buffalo, Department of Pharmacology and Toxicology, Buffalo, USA

There is a critical relationship between oligodendrocyte progenitor cells (OPCs) development, myelin production, and iron bioavailability. Iron deficiency leads to hypomyelination both in humans and animal models, and the neurological sequelae of hypomyelination are significant. However, iron absorption and management in OPCs is poorly understood. To gain a mechanistic understanding on iron homeostasis in OPCs and on how iron participates in the myelination of the postnatal brain, we have created two inducible conditional knock-out mice in which two essential proteins implicated in iron uptake and storage: the transferrin receptor 1 (TfR1) and the ferritin heavy chain (Fth) were postnatally deleted specifically in OPCs. Blocking Fth and TfR1 production reduce oligodendrocyte iron content and significantly delay OPC development in primary cultures. When these proteins were knock-out *in vivo* during the

first postnatal week, the corpus callosum as well as the cortex of both conditional knock-out animals displayed a significant reduction in the synthesis of myelin proteins and in the percentage of myelinated axons. This reduced postnatal myelination was accompanied by a significant decrease in the number of myelinating oligodendrocytes and with a rise in proliferating OPCs. Importantly, Fth as well as Tfr1 knock-out mice showed a substantial reduction in oligodendrocyte iron levels. In contrast, deleting these proteins in mature myelinating oligodendrocytes result in milder phenotypes characterized by small reductions in the percentage of myelinated axons and minor changes in the g-ratio of myelinated axons. These results indicate that Fth and Tfr1 are critical proteins for early postnatal iron uptake and storage in OPCs and as a consequence for the normal myelination of the central nervous system.

WTH11-26

Developmental loss of oligodendrocytes hinders adult CNS remyelination and increases astroglial and microglial activation

A. Pajohesh-Ganji

The George Washington University, Anatomy and Cell Biology, Washington, USA

Multiple sclerosis (MS) is a neurodegenerative autoimmune disease characterized by loss of oligodendrocytes (OLs) and myelin. Although gender, genetics, environment, and race are risk factors for MS, what makes an individual more susceptible to MS remains unknown. Epidemiological data suggest that susceptibility is established early in life and frequently long before diagnosis of the disease. To model this, apoptosis in a subset of Dsred mature OLs in a transgenic mouse model (MBP-iCP9) was achieved by utilizing a chemical inducer of dimerization (CID), during early development. We tested the hypothesis that early ablation of OLs leads to more severe disease when animals are subjected to a "second disease challenge". Data indicate that apoptosis of OLs by CID postnatal injection results in myelin perturbation, leading to transient functional deficits that recovers 10 days after the last CID injection. A second focal insult, using lysolecithin (LPC) in the spinal cord, results in significant increase in lesion volume and a decrease in remyelination in early OL ablated mice. Utilizing Experimental Autoimmune Encephalomyelitis (EAE) model, as the second challenge, results in more severe tissue damage in early OL ablated mice. The increased lack of tissue integrity in early OL ablated mice is not reflected in functional tests. However, the increased tissue damage is accompanied by a significant increase in markers of astroglial and microglial activation. We postulate that ablation of OLs during development alters the micro-environment affecting astroglial and microglial properties resulting in a more severe response after the second insult. Further studies will focus on gene expression profiles of astrocytes and microglia from these tissues, which may provide valuable insight into the question of why some individuals are more susceptible to MS.

WTH11-27

Selective mitochondrial autophagy as key pathway for carbon monoxide cytoprotection

C. Pereira¹, H. Vieira^{1, 2}

¹*CEDOC, Nova medical school/UNL, Cell death and disease, Lisboa, Portugal*

²*Instituto de Biologia Experimental e Tecnológica, IBET, Oeiras, Portugal*

Mitochondria dysfunction with consequent oxidative stress accumulation is implicated in most neurodegenerative diseases. Therefore, the efficient removal of these mitochondria is essential to prevent cellular dysfunction, and occurs through autophagy. Autophagy turnover of mitochondria, named mitophagy, is a key mitochondrial quality-control (MQC) mechanism to avoid cell death in mammals. Low doses of carbon monoxide (CO) is known for exerting anti-apoptotic, anti-inflammatory and anti-proliferative functions, involving ROS signalling. Here, we combined experimental approaches to investigate whether the CO cytoprotective effect against oxidative stress in astrocytes is dependent on MQC process. In primary culture of astrocytes, CO improved cell survival being directly dependent on autophagy, since whenever autophagic process was inhibited CO's cytoprotective effect was reverted. Likewise, CO quickly induced mitophagy (within 1 h) and this was mediated by PINK1/PARKIN and BNIP3/NIX proteins, which may work in a compensation manner. At short time-window (1 h) CO also activates the machinery for the generation of mitochondria. Furthermore, CO also promotes mitochondrial biogenesis (within 24 h), by increasing gene expression of peroxisome proliferator-activated receptor gamma coactivator-1 (PGC-1 α), specific mitochondrial transcription factor, at short periods of treatment. In summary, CO appears as a novel agent modulating mitochondria, promoting clearance of harmful cell components and cytoprotection.

WTH11-28

A novel leukoencephalopathy targets myelination defects due to loss of vacuolar protein sorting (VPS11) function

R. R. Skoff, R. Thummel, D. Bessert, S. Banerjee, X. Luo

Wayne State University, Department of Anatomy and Cell Biology, Detroit, USA

Genetic leukoencephalopathies (gLE) are genetic disorders affecting the white matter of the central nervous system. Our collaborators recently identified a mutation in *VPS11* as a causative allele in the gLE phenotypes observed in individuals from Ashkenazi Jewish families. VPS11 functions in a complex of four C-VPS proteins, which are conserved from yeast to humans, and control critical cellular processes in the endolysosomal and autophagy pathways. Here, we characterize for the first time in mammals the cell type and distribution of Vps11. Vps11 is highly enriched in oligodendrocytes and is closely associated with myelin. At low magnification, Vps11 appears localized to the same compartment as myelin but at higher magnifications, Vps11 and MBP do not co-localize. Rather, Vps11 forms numerous bead-like structures throughout the myelin sheath, suggestive of its localization to Schmidt-Lanterman clefts. Vps11 and Mag are clearly in the inner tongue of myelin, generally separate but with moderate co-localization. Vps11 is not in the axon; however, in longitudinal and cross sections, NF proteins (low and high) co-localize with Vps11. Vps11 is tightly regulated by proteolipid protein as it is significantly

increased in *Plp1* null mutants and significantly decreased in *Plp1* mutants with duplications. Our preliminary observations suggest Vps11 transports axonal and myelin proteins for degradation to oligodendrocyte endosomal and lysosomal structures. Vps11 thus appears to be the first identified protein to shuttle cargo from axonal-myelin compartments back to the cell body.

WTH11-29

Signaling cascades associated to the exposure to silica nanoparticles in retina glial cells

F. Sanchez, A. Rodriguez, A. Ortega

Center for research and advanced studies of the National Polytechnic Institute, Toxicology, Ciudad de México, Mexico

The use of silica nanoparticles is increasing due to their application in various areas of human health. Nevertheless, neurotoxicity studies of these particles have been based mainly on epidemiological studies in which exposure to NPs is associated with neurodegenerative diseases such as Parkinson's disease and some others. Evidence has accumulated in the last decade supporting an active role of glia cells in the regulation of neuronal communication. In this context, in this contribution, we evaluated the effect of NPs-SiO₂ exposure in the function and signaling of glutamate plasma membrane transporters. Monolayers of the cultured cells were exposed to different NPs-SiO₂ concentrations for different time periods. Cell viability was determined by the MTT method and no significant decrease in the integrity of the cultures was found. Subsequently, the cells were exposed to different concentrations of silica NPs (0.4, 4.8, 8, 15 and 20 µg/ml) for different time periods (15, 30, 60 and 90 min). The phosphorylation index of the extracellular-regulated kinase (ERK) was evaluated via Western blot and compared it to the glutamate effect. ERK1/2 were Tyr-phosphorylated after a 30 min treatment with SiNPs- at low concentrations (0.4 and 4.8 µg/ml). Our results suggest that exposure to nanomaterials affect the transcriptional and **translational** control of gene expression in glial cells and by these means disrupt the exquisite glia neuronal coupling that is critically involved in synaptic transmission.

WTH11-30

Iron metabolism in the peripheral nervous system: the role of DMT1, ferritin and transferrin receptor 1 in Schwann cell maturation

D. S. Gonzalez, V. Cheli, R. Wan, P. Paez

University at Buffalo, Pharmacology, Buffalo, USA

Iron is the most abundant transition metal in biology and is an essential cofactor for many cellular enzymes. Iron homeostasis impairment is also a central component of peripheral neuropathies. However, nothing is known about the metabolism of iron in the peripheral nervous system and particularly its role in Schwann cell maturation. To determine whether iron is necessary for Schwann cell maturation and myelination, we created three inducible conditional knockout mice in which three essential proteins for iron incorporation and storage: the divalent metal transporter 1 (DMT1), ferritin and the transferrin receptor 1, were postnatally deleted specifically in Schwann cells. Blocking DMT1, ferritin or transferrin receptor 1 *in vitro* significantly reduce Schwann cell proliferation, maturation and the myelination of dorsal root ganglia axons. This was

accompanied by an important reduction in iron incorporation and storage. *In vivo*, the sciatic nerve of all conditional knockout animals displayed a significant reduction in the synthesis of myelin proteins and in the percentage of myelinated axons at several postnatal time points. Knocking out ferritin produce the most severe phenotype, followed by DMT1 and lastly transferrin receptor 1. Importantly, DMT1 as well as ferritin knockout mice showed substantial motor coordination deficits. These results indicate that DMT1, ferritin and transferrin receptor are critical proteins for postnatal iron uptake and storage in Schwann cells and as a consequence for the normal myelination of the peripheral nervous system.

WTH11-31

Influence of astrocyte heterogeneity to Aβ oligomers toxicity

R. Santos¹, A. Bérnago¹, S. Ferreira², F. Gomes¹

¹*Federal University of Rio de Janeiro, Institute of Biomedical Sciences, RJ, Brazil*

²*Federal University of Rio de Janeiro, Institute of Biophysics, RJ, Brazil*

Introduction: Alzheimer's disease (AD) is the main cause of dementia, and Aβ oligomers (AβOs) are considered its major toxin. AD differentially affects brain regions: while the hippocampus is damaged earlier, the cerebellum is in later stages, suggesting different susceptibility to AβOs'. Astrocytes are essential for brain function. Recently, it has been shown that astrocytes present heterogeneous responses to insults, thus predicting brain vulnerability to neurodegenerative diseases. We have shown that AβOs induce hippocampal astrocytes' deficits, leading to neuronal dysfunction; however, AβOs influence on cerebellar astrocytes is unknown. The goal of this work was to investigate the effect of AβOs in cerebellar astrocytes.

Methods: primary astrocyte cultures were exposed to AβOs (500 nM for 24 h) and processed for immunocytochemistry, PCR, MTT and nitrite measurements.

Results: MTT reduction showed that viability of astrocytes from both regions was not impaired by AβOs. While AβOs increased nitrite levels in hippocampal astrocytes, no effect was observed in cerebellar astrocytes. Further, AβOs did not enhance the levels of lipocalin II, a marker for astrocyte activation, in cerebellar astrocytes. We investigated the effect of AβOs on astrocytic production of synaptogenic molecules. AβOs decreased expression of TGF-β and increased expression of hevin by cerebellar astrocytes, expression of SPARC and glypican-4 was not affected. Meanwhile, hippocampal astrocytes did not show alterations in those genes.

Conclusion: Our results show that cerebellar and hippocampal astrocytes are differently affected by AβOs. Due to the roles of astrocytes in neuronal survival and homeostasis, these may data contribute to understanding the higher resistance of the cerebellum to AβO insults.

WTH11-32

3D ultrastructural morphology of mouse astrocytes in Alzheimer's disease**A. Schober, J. Benjamin Kacerovsky, C. Salmon, N. Alivodej, A. Zhou, B. Phillips, T. Tibuleac, K. Murai***Research Institute of McGill University Health Center, Centre for Research in Neuroscience, Montreal, Canada*

Astrocytes have long been simplistically characterized as a uniform cell population that take on the morphology of either protoplasmic or fibrous. However, emerging evidence indicates that astrocytes comprise a highly complex cell population with diverse morphology and function in both the healthy and diseased brain. Astrocytes have recently been implicated to play a major role in neurodegenerative diseases, including Alzheimer's disease (AD), however the highly specialized anatomical properties that are changed in these cells remains poorly understood. To better understand the morphology of astrocytes in normal and AD mice, we used a multi-level imaging approach with structured illumination microscopy (SIM) and focused ion beam high-resolution scanning electron microscopy (FIB-SEM). Both imaging techniques allow us to visualize astrocyte morphology at the subcellular resolution. Using SIM, we are able to not only visualize the complex branching pattern of whole astrocytes, but also to map the distribution of mitochondria throughout the whole cell. In order to further investigate astrocyte morphology in finer detail, we utilized FIB-SEM to three-dimensionally reconstruct astrocyte processes as well as their organelles throughout sections of brain tissue. Using both SIM and FIB-SEM allowed us to quantitatively compare the surface structure and organelle ultrastructure of astrocyte sub-compartments as well as to assess changes in healthy and diseased tissue. The data provided by these imaging techniques provide an important structural framework for understanding how the brain is organized in not only normal tissue, but also during Alzheimer's Disease and how this organization within astrocytes may relate to brain function.

WTH11-33

Modafinil regulates glutamine synthetase via PI3K-AKT signalling pathway in cerebellum**J. Silva, L. Méndez, E. Bejarano-Pérez, L. C. H. Hernández-Kelly, A. Ortega***Centro de Investigación y de Estudios Avanzados del IPN, Department of Toxicology, Mexico city, Mexico*

Glutamate is the major excitatory transmitter in the Central Nervous System (CNS) of vertebrates, exerts its actions through the activation of specific membrane receptors expressed in neurons and glial cells. Over-stimulation of glutamatergic receptors results in neuronal death, phenomena known as excitotoxicity. Extracellular glutamate levels are tightly regulated by a family high-affinity uptake systems expressed in neurons and glial cells. Most of the uptake process occurs in the glial compartment and is part of a biochemical glia/neuronal coupling known as the glutamate/glutamine shuttle by which this amino acid is recycled. Once internalized into glial cells, glutamate is metabolized to glutamine via Glutamine synthetase (GS) to be released to the vicinity of the presynaptic terminal, which takes it up and converts it back to glutamate, completing the cycle. Inhibition of GS blocks glutamatergic transmission. Among the great variety of CNS stimulants, modafinil is widely used as a wakefulness agent recommended for

the treatment of excessive daytime sleepiness, fatigue, and impaired cognition. Despite the fact that the mechanism of action of this stimulant is still unclear, it increases glutamate extracellular levels. To establish a plausible involvement of glutamine synthetase in the effects of modafinil, we used the model of chick cerebellar Bergmann glia primary cultures. Acute treatment with modafinil results in an increase in GS activity as determined by glutamyl hydroxamate production. Moreover, using a PI3K block, we can determinate the participation of that signaling pathway in effect of modafinil. These results strengthen the notion of an important role of glial cells in glutamate-dependent neurotransmission through the regulation of its compulsory glia-mediated turnover.

WTH11-35

Exposure to manganese induces PI3K/AKT signaling in bergmann glial cells**J. S. Verdugo, M. Castillo-Montesinos, L. M. Sánchez-Palestino, J. G. Tovar-Ramírez, L. C. R. Hernández-Kelly, A. Ortega***Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Toxicología, Mexico City, Mexico*

Manganese (Mn) is an essential trace element that in high doses triggers serious oxidative and neurotoxic effects. An established consequence of Mn neurotoxicity is the disruption of the glutamate/glutamine (Glu/Gln) cycle, leading to an excitotoxic insult. Glu exerts its actions through the activation of specific plasma membrane receptors and transporters present in neurons and glial cells. Their over-activation is the biochemical hallmark of neuronal and oligodendrocyte cell death. The molecular mechanisms mediating Mn-induced neurotoxicity, particularly in the context of the Glu/Gln cycle, have yet to be completely understood. Hence, we decided to investigate the effect of Mn short-term exposure in signaling pathways, such as the phosphatidylinositol 3 kinase (PI3K)/protein kinase B (Akt) cascade that regulate the Glu/Gln shuttle in Bergmann glial cells (BGC) primary cultures, a well-established model of glial/neuronal interactions. Confluent BGC monolayers were exposed to subtoxic concentrations of Mn (MnCl₂: 50-500 μ M) different time periods. Akt, as well as the eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) phosphorylation patterns, were evaluated. Mn exposure increased Akt phosphorylation as early as 5 min exposure and lasted for 30 min in a concentration-dependent manner. Pharmacological PI3K inhibition blocked these effects. Also, the inhibition of the sodium-calcium exchanger diminished Akt phosphorylation induced by Mn. Finally, Mn treatment (200 μ M) augmented 4E-BP1 phosphorylation after 10 to 30 min of exposure. Overall, these findings suggest that an altered intracellular PI3K/Akt signaling may represent an early event in Mn toxic mechanisms and suggest that protein synthesis could be severely affected by Mn. These results strengthen the idea of the critical role that glial cells have in neurotoxicity development.

WTH11-36

Astrocytic responses VIA IL-1 regulate recovery from west NILE virus encephalitis**A. Soung¹, R. Klein^{1,2,3}**¹*Washington University School of Medicine, Internal Medicine, St. Louis, USA*²*Washington University School of Medicine, Pathology and Immunology, St. Louis, USA*³*Washington University School of Medicine, Neuroscience, St. Louis, USA*

West Nile virus (WNV) is an encephalitic arbovirus capable of infecting neurons throughout the central nervous system (CNS), including those within the hippocampus, a structure essential for spatial learning and memory. Although most patients infected with WNV are asymptomatic, many may develop symptoms ranging from a flu-like illness to a more severe neuroinvasive infection (WNND). Approximately half of patients that recover from WNND exhibit long-term cognitive sequelae, including deficits in motor skills, learning, and memory that persist beyond infection clearance. Unfortunately, no treatment is available for these patients, and little is known about the molecular mechanisms that underlie persistent neurological deficits. Astrocytes perform a variety of neuroprotective functions including regulation of synaptic function and neuronal repair and produce neurotrophic factors and anti-inflammatory cytokines. Following viral infections, astrocytes express cytokines that have been shown to regulate neural correlates of memory, including adult neurogenesis and synaptic plasticity. However, few studies have examined how astrocyte activation in response to viral infections impacts these correlates. Our lab has demonstrated that interleukin-1 receptor 1 (IL-1R1) signaling leads to the generation of proinflammatory, reactive astrocytes at the expense of adult neurogenesis within the hippocampus. These newly generated astrocytes become a source of anti-neurogenic cytokines that continuously inhibit neurogenesis repair. Using inducible Cre-lines, we have determined that IL-1R1 signaling in neural stem cells critically regulates this process. This study provides an important understanding into how astrocytic cytokine responses regulate adult neurogenesis during recovery of viral infections and how this may limit repair and promote spatial learning deficits.

WTH11-37

Two-photon *in vivo* Ca²⁺ imaging in oligodendrocytes and oligodendrocyte precursor cells**S. Sugio, R. Ono, H. Wake***Kobe University Graduate School of Medicine, System Neuroscience, Kobe, Japan*

Neurons were sending axons and connecting various regions to carry the sensory/motor information. A part of axons was wrapped by processes of oligodendrocytes (OCs) and formed myelinated axons. Once axons were myelinated, its conduction velocities were dramatically increased, approximately 50-fold higher than those of un-myelinated axons. Since, myelin tightly holds the axons by their complex lamina structure under the electron microscopy, it had been believed that myelin does rarely turn over under healthy condition. However, recent studies have been unveiled that the oligodendrocytes could be a plastic cells that are dynamically adapted their cell fate, morphology and myelination to the changes in neuronal activities, which could plastically modulate neuronal conduction

velocities to alter the activity pattern of neuronal populations. Although, the physiological significances of activity-dependent myelination have been known in recent years, while the bidirectional signaling between neurons and OCs (including oligodendrocyte precursors: OPCs) that regulates activity-dependent myelination (for instance, Ca²⁺ transients via neurotransmitter or gliotransmitter) has not yet studied. To examine the spatiotemporal patterns of Ca²⁺ transients that regulate activity-dependent myelination, here, we performed two photon *in vivo* Ca²⁺ imaging using a transgenic mouse that expressed a fluorescent Ca²⁺ indicator (GCaMP6f) in OC and OPCs, and then compared the changes of Ca²⁺ frequency and amplitude at three different situations; awake, anesthesia and after a motor learning acquisition. We found that the Ca²⁺ responses in processes of OCs/OPCs were varied; approximately 50% of processes in an OCs/OPCs were positively correlated with the changes in neuronal activities, the others were not. Our finding suggested that the Ca²⁺ transients regulating activity-dependent myelination should be lie in the various responses.

WTH11-38

Glutamate inhibits neural oxide nitric synthase degradation in bergmann glial cells**R. Tiburcio¹, A. Ortega¹, J. Luna², A. Hernandez²**¹*Center for Research and Advanced Studies of the National Polytechnic Institute, Genetics and Molecular Biology, Mexico City, Mexico*²*Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Departamento de Immunología, Mexico City, Mexico*

Glutamate is the major excitatory neurotransmitter of the vertebrate brain. It exerts its actions through the activation of specific plasma membrane receptors expressed both in neurons and in glial cells. In addition, glial glutamate transporters are responsible of the majority of the brain glutamate uptake activity. A tight regulation of glutamate extracellular levels prevents neuronal over stimulation and cell death, and it is critically involved in glutamate turnover. Recent evidence has shown that glutamate uptake systems, particularly those enriched in glia cells, trigger biochemical cascades in a similar fashion as receptors. Since a glutamate-dependent increase in cGMP levels has been described in glia cells and that the nitric oxide-cGMP signaling cascade increases their glutamate uptake activity, we characterized herein the effect of the exposure to this excitatory amino acid in the expression levels and activity of the neuronal isoform of nitric oxide synthase in cerebellar Bergmann Glial Cells. A glutamate dependent increase in neuronal nitric oxide synthase protein levels and activity was found upon glutamate exposure. This effect is mediated by glutamate transporters and is related to an increase in the stability of the enzyme. These results strengthen the notion of a complex regulation of glial glutamate uptake that supports neuronal glutamate signaling.

WTH11-39

Oligodendrocytes in the hypermyelinating AKT-dd mouse are resistant to ischemic injury**D. Verden, C. Schroeder, B. Wassermann, P. Herson, W. Macklin***University of Colorado - CU Anschutz, Cell & Developmental Biology, Aurora, USA*

White matter injury is a major component of disability following ischemic stroke. Oligodendrocytes, the myelinating cells of the central nervous system, are acutely vulnerable to ischemic injury in adults. Loss of oligodendrocytes disrupts communication between brain regions along myelinated axons and creates large amounts of myelin debris, hampering repair processes and exacerbating pathology. Previous work from our lab has found oligodendrocytes in the juvenile brain to be uniquely resistant to ischemia. Interestingly, this is a period of peak myelination in brain development. We asked if juvenile oligodendrocyte injury resistance could be due to protective mechanisms taking place during active myelination. The current study utilizes a hypermyelinating Akt-DD mouse model to demonstrate that actively myelinating oligodendrocytes in the adult brain are resistant to ischemia-reperfusion injury. While both WT and Akt-DD mice suffered severe neuronal loss following ischemia, Akt-DD oligodendrocytes survived to a much greater degree than WT counterparts. In addition to oligodendrocyte survival, half of Akt-DD mice exhibited altered inflammatory responses, with reduced astrogliosis and differential microglial/NG2 cell activation. Together, these data suggest that actively myelinating oligodendrocytes are protected from oxidative injuries, and that protecting oligodendrocytes may extend therapeutic windows for managing inflammatory elements of the post-stroke recovery.

WTH11-40

Abnormalities of peripheral myelin development in Charcot-Marie-Tooth (CMT) disease model, I-MPZ mouse
Y. Yamaguchi, Y. Otani, M. Takehara, T. Nakajima, T. Narazaki, J. Cui, H. Baba*Tokyo University of Pharmacy and Life Sciences, Dept Molecular Neurobiology, Hachioji, Japan*

Large myelin protein zero (L-MPZ) is a translational readthrough isoform of myelin protein zero (P0, MPZ). Recently, translational stop codon readthrough has become a key mechanism to modulate canonical gene function in mammals. Previously, we reported that abnormal increase of L-MPZ caused Charcot-Marie-Tooth (CMT) disease-like neuropathy including demyelination and axonal damage, suggesting that imbalance of translational readthrough may cause disease. However, the onset and progression of this neuropathy are still unclear. In order to clarify these disease processes, immunohistological analysis of L-MPZ in normal development and morphological analysis of demyelination models were performed. In sciatic nerves prepared from postnatal day (P) 0-21 and adult ICR mice, L-MPZ-positive signals were colocalized with myelin basic protein (MBP) in compact myelin at any age. These signals were dramatically increased during P0-10 and after that became constant but decreased in lyssolecithin-injected demyelinated fibers. Additionally, L-MPZ signals were enriched in Schmidt-Lanterman incisures (SLI) and paranodal regions in the adult myelinated fibers, while P0 was mainly in compact myelin. These results suggest that L-MPZ is involved in the formation and maintenance of the PNS myelin. Morphological analyses of P7-28 of a mouse line (L-MPZ mouse) synthesized only L-MPZ demonstrated that thinner myelinated fibers and immature Schwann cells were started to increase from P14. Thus, excessive increase of L-MPZ prevents maturation of myelinating Schwann cells in developmental process.

WTH12 Neuron-glia interactions (Session B)

WTH12-01

Axon-myelin pathology following experimental traumatic brain injury: serial 3-dimensional ultrastructural analysis **R. Armstrong¹, K. Radomski¹, X. Zi¹, G. Kidd²**

¹Uniformed Services University, APG, Bethesda, MD, USA

²Renovo Neural Inc, 3D-EM, Cleveland, OH, USA

Myelinated axons crossing through the corpus callosum (CC) are particularly vulnerable to damage from traumatic brain injury (TBI), which results in diffuse axonal injury. Electron microscopy (EM) is the gold standard method for analyzing ultrastructure of axons and myelin sheaths. However, analysis of single plane images limits interpretation of the proximal and distal pathology along individual axons. We address this gap using serial block-face scanning EM to collect sequential high resolution images and generate three-dimensional EM reconstructions (3DEM). Myelinated CC axons were reconstructed at 3 days and 6 weeks following a mild TBI. Adult C57BL/6J male mice received a single impact injury onto the closed skull at bregma. Similar to diffuse axonal injury in human TBI, this TBI model produces damaged axons interspersed among intact axons. Our 3DEM confirmed expected pathology and revealed novel features along individual myelinated axons. Axons with advanced degeneration were fragmented and terminated at enlarged end bulbs. Damaged axons also exhibited swellings and cytoskeletal breakdown, which were surprisingly interspersed among lengths of axon with apparently intact ultrastructure. Importantly, 3DEM demonstrated three forms of myelin pathology after TBI: a) partial myelin sheath loss at paranodal regions, b) demyelination of longer internodal segments, and c) outfoldings of compact myelin extending into the adjacent tissue. Finally, myelin debris within microglia was observed in CC regions with axon damage after TBI, implicating myelin pathology in activation of phagocytosis. This 3DEM analysis illustrates novel features of axon and myelin white matter pathology after TBI. These results are important for interpretation of axon cytoskeletal plasticity, and white matter electrophysiological deficits and neuroimaging findings after TBI.

WTH12-02

Inducing neuroprotection by altering purkinje cell mitochondria dynamics during inflammatory demyelination

K. Atkinson¹, M. Sekyi¹, A. Alluisi¹, S. Noori¹, C. Diaz¹, S. H. Kim², W. Rincon¹, T. Benbarka¹, J. Katzenellenbogen², S. Tiwari-Woodruff¹

¹University of California, Riverside, Division of Biomedical Sciences, Riverside, USA

²University of Illinois at Urbana-Champaign, Department of Chemistry, Champaign, USA

Cerebellar symptoms consisting of tremors, impaired motor control and coordination occur in approximately 80% of multiple sclerosis (MS) patients. There is a crucial need to elucidate the pathophysiology and mechanism of these symptoms and find therapeutics. Alteration of Purkinje cells (PCs) in the cerebellum have been linked to cerebellar dysfunction in MS patients. Loss of metabolic support of demyelinating PC axons contributes to axonal

mitochondrial dysfunction and subsequent neurodegeneration. We hypothesize that PC axonal mitochondrial dysfunction is a result of inflammatory demyelination leading to degenerating PCs. To test this hypothesis, cerebellar pathology was investigated in experimental autoimmune encephalomyelitis (EAE) groups of mice and compared to normal mice and therapeutically (with a known remyelinating estrogen receptor- β ligand, IndCI-*o*-Me and mitochondrial biogenesis inducer, Resveratrol)-treated mice. Behavioral gaitwalk and immunohistochemistry analyses (inflammation, myelination, and mitochondria bioenergetics) was performed. EAE induced altered gait dynamics, along with extensive cerebellar inflammation and demyelination. EAE PCs showed decreased dendritic arborization and increased mitochondria, indicative of mitochondria stress. Cerebellar inflammation and demyelination were decreased with IndCI-*o*-Me but not resveratrol treatment. A decrease in dendritic mitochondria was observed with both IndCI-*o*-Me and resveratrol treated EAE mice, even though only IndCI-*o*-Me PCs demonstrated recovery in arborization and increased myelination. We conclude that remyelination along with alleviation of PC mitochondria stress is required for recovery of PC dysfunction. Future experiments will investigate in detail the timing, structural changes, and function of PC mitochondria in the presence of these drugs.

WTH12-03

Pre-existing mature oligodendrocytes contribute to remyelination

C. Bacmeister, H. Barr, C. McClain, E. Hughes

University of Colorado School of Medicine, Cell and Developmental Biology, Aurora, USA

The contribution of pre-existing mature oligodendrocytes to remyelination remains controversial. Traditionally, rodent models of demyelination indicate no participation of mature oligodendrocytes in remyelination (1–3), but recent evidence in large animal models and MS patients suggests otherwise (4–6). Using longitudinal *in vivo* two-photon imaging of oligodendrocytes expressing EGFP in a cuprizone model of demyelination, we show that surviving oligodendrocytes can contribute to remyelination in the adult mouse motor cortex through the rare addition of new myelin sheaths. Oligodendrocytes that survive a demyelinating insult lose a quarter of their sheaths and rarely add new sheaths. However, after mice are trained in a skilled forelimb reach task, ~90% of surviving cells add new sheaths at a rate that compensates for sheath loss. Two weeks after training, the number of sheaths on a given cell is restored to the starting number of sheaths and is maintained for at least two additional weeks. Furthermore, half of the new sheaths added after training are placed in previously unmyelinated locations, suggesting that learning generates a novel pattern of myelination. In addition, training also promotes retraction of pre-existing sheaths. Therapies designed to engage mature oligodendrocytes in remyelination may therefore be beneficial in demyelinating disorders and could promote adult neuroplasticity.

WTH12-04

Timing of behavioral intervention modulates oligodendrogenesis following demyelinating injury
H. Barr, C. Bacmeister, C. McClain, M. Thornton, E. Hughes

University of Colorado School of Medicine, Cell & Developmental Biology, Aurora, USA

Loss of oligodendrocytes, the myelin-producing cells of the central nervous system (CNS), critically affects axonal health and causes neurological disability, as in the demyelinating disease Multiple Sclerosis (MS). Multiple immunotherapies target MS-attack prevention, yet mechanisms to stimulate the generation of new oligodendrocytes remain limited. Although robust oligodendrogenesis and white matter changes occur in response to motor learning, rehabilitation models have had variable and limited success in MS patients, perhaps due to the critical importance of timing for the efficacy of behavioral interventions following neurological injury. Here, we longitudinally *in vivo* two-photon imaged myelinating oligodendrocytes expressing EGFP in the motor cortex of transgenic mice (*MOBP-EGFP*) throughout learning and rehearsal of a forelimb reach task. We report that learning initially suppresses, then subsequently increases the rate of oligodendrogenesis. We then demyelinated mice using cuprizone, resulting in significant cortical oligodendrocyte loss and motor impairments characteristic of MS. To examine the effects of learning-induced oligodendrogenesis on post-injury remyelination, we trained demyelinated mice in the forelimb reach task. While rehearsal of the task had no effects on remyelination, learning strongly shaped oligodendrogenesis in a timing-dependent manner. Learning occurring immediately after injury impeded generation of new oligodendrocytes, resulting in delayed and decreased repair. However, learning occurring after the onset of remyelination increased oligodendrocyte gain by over 10% relative to control recovery. Given that a single oligodendrocyte can produce over fifty sheaths, increasing the number of newly generated cells during remyelination can have a substantial effect on axonal health and survival. These results demonstrate the critical importance of appropriate timing of endogenous signals to improve recovery outcomes in CNS injury.

WTH12-05

Identification of a novel gene regulating axon caliber growth and myelination

J. Bin, D. Suminaite, M. Madden, S. Benito, M. Livesey, D. Lyons

University of Edinburgh, Centre for Discovery Brain Sciences, Edinburgh, United Kingdom

No single essential signal has been identified that is required for myelination in the central nervous system (CNS). However, the decision as to which axons get myelinated and how much myelin they get is influenced by axon caliber. While axon caliber is a fundamental property of neurons that modulates both myelination and conduction speed, surprisingly very little is known about what regulates axon caliber in the CNS. To identify novel genes important for myelinated axon caliber growth, we performed a forward genetic screen in zebrafish. From this screen, a mutant was discovered that exhibited smaller diameter axons, hypomyelination, and myelinated cell bodies. The causative gene was identified to be a nuclear transport receptor whose cargo and function in the CNS has not previously been explored. To further dissect the cell-autonomous

roles of this receptor in neurons and oligodendrocytes, we generated cell-type specific knockouts using *crispr/cas9* and performed super-resolution live-imaging and followed the dynamics of axon growth and myelination over time. In addition, we have performed electrophysiology recordings from individual neurons with myelinated axons in the spinal cord of mutants and wild type siblings to analyze changes to the shape, fidelity and propagation speed of action potentials, as well as the ability to sustain high frequency firing, when caliber growth and/or myelination are impaired. Together our findings highlight the importance of nuclear transport in regulation of axon caliber growth, myelination, and nervous system function.

WTH12-06

Synthesis, *in vitro* and *in vivo* analysis of analogues of lanthionine ketimine: potential drugs for neurological disorders

T. Denton¹, D. Shen¹

¹*Washington State University, Pharmaceutical Sciences, Spokane, USA*

²*Washington State University, Pharmaceutical Sciences, Spokane, USA*

Lanthionine ketimine (LK) is a natural amino acid metabolite found in mammalian brain tissue. LK, and its synthetic ethyl ester derivative, LKE, have potent neuroprotective, neurotrophic and anti-neuroinflammatory properties and have been shown to increase autophagy in neurons and glia. LKE shows benefits in diverse preclinical models of neurodegenerative diseases. Using LK and LKE as lead compounds, we have recently synthesized a series of 2-substituted-3-phosphono-1-thia-4-aza-2-cyclohexene-5-carboxylates and their corresponding carboxy ethyl esters (LK(E)-P(E)s). The synthesis incorporates multiple reactions in “one-pot.” The structures were confirmed by ¹H, ¹³C and ³¹P NMR and HRMS. The new analogues were assayed for cytotoxicity and autophagy stimulation effects in HeLa and RG2 glioma cell lines. The lead compound 2-n-hexyl-LKE-P was evaluated in a *Drosophila melanogaster* model of Parkinson’s disease (Pink1B9) via oral administration. Behaviors including climbing ability and longevity were recorded and analyzed. Results show that 2-n-hexyl-LKE-P is stable, non-toxic, stimulates autophagy and improves the behavioral deficits the Parkinson’s disease model flies.

WTH12-07

Astrocyte-targeted production of IL-10 does not modify “do-not-eat-me” signalling after facial nerve axotomy in mice

A. R. Gómez-López, G. Manich, B. González, B. Castellano

Universitat Autònoma de Barcelona, Department of Cell Biology, Physiology and Immunology, Barcelona, Spain

Interleukin-10 (IL-10) is an anti-inflammatory cytokine, that is up-regulated under neuroinflammatory conditions, mainly associated with the recovery phase. Research developed in our group showed that, after facial nerve axotomy (FNA), mice over-expressing IL-10 (GFAP-IL10Tg) had an increase in motor neuron survival respect to wild-type (WT) animals, which was related to changes in microglial activation pattern. One of the mechanisms that

regulates microglial activation is the “Do-not-eat-me” signalling, which consists in the interaction of microglial inhibitory receptors (like CD200R, CX3CR1 and CD45) with their corresponding neuronal ligands (CD200, CX3CL1 and CD22, respectively). In this study, we aimed to analyse the regulation of “Do-not-eat-me” signalling after FNA and to explore if IL-10 overproduction modifies it. To accomplish that, adult GFAP-IL10Tg and WT mice were lesioned and sacrificed at 3, 7, 14, 21 and 28 days post-injury. Single and double immunohistochemistry techniques were used to detect and quantify microglial receptors and neuronal ligands. Our results showed that after FNA in WT mice, neuronal CD200, CX3CL1 and CD22 were up-regulated at early stages, followed by a down-regulation at later time-points. Likewise, in WT mice, microglial CD200R and CD45 increased at early time-points and decreased at later stages. After FNA, IL-10 overproduction did not exert any effect on neuronal CD200 signalling, whereas up-regulations in CX3CL1 and CD22 were observed at later time-points. Additionally, no differences were detected in CD45 and CD200R signalling between GFAP-IL10Tg and WT mice. These results suggest that, after FNA, “Do-not-eat-me” signalling intends to regulate microglial activation at early time-points, and that IL-10 does not modify it in FNA.

WTH12-08

Enolase inhibition alters metabolic hormones and inflammatory factors to promote neuroprotection in spinal cord injury

A. Haque, R. Polcyn, M. Capone, D. Matzelle, A. Hossain, R. Chandran, N. Banik

Medical University of South Carolina, Microbiology and Immunology, Charleston, USA

Enolase inhibition is a potential therapeutic strategy currently being investigated for treatment of spinal cord injury (SCI) as it reduces pro-inflammatory cytokines and chemokines, alters metabolic factors, and reduces gliosis in an acute model of SCI. Herein, the role of enolase in sub-acute SCI has been examined to better understand the effects of this enzyme on inflammation, metabolic hormones, glial cell activation, and neuroprotection under these shorter post-injury conditions. Immunohistochemical analyses of inflammatory markers vimentin, Cox-2, and Caspase-1 indicated that enolase inhibition attenuated the elevated levels of inflammation seen following SCI. Regulation of enolase also reduced microglia/astrocyte activation with marked inhibition of Iba1 and GFAP proteins, and protection of neurofilament protein (NFP) in spinal cord tissues from damage due to subacute SCI. An analysis of metabolic hormones revealed that ENOblock treatment significantly up-regulated plasma concentrations of peptide YY, glucagon-like peptide 1, glucose-dependent insulintropic peptide, glucagon, and insulin hormones as compared to vehicle-treated controls. ENOblock did not have a significant effect on plasma concentrations of pancreatic polypeptide. Interestingly, ENOblock treatment inhibited chondroitin sulfate proteoglycan (CSPG), which is produced by activated glia and serves to block regrowth of axons across the lesion site following injury. An increased level of NeuN and MBP was detected in SCI tissues where active Caspase-1 was reduced after ENOblock treatment, suggesting neuroprotection and preservation of myelin respectively. These data indicate a role for enolase in modulating inflammation, metabolism, and gliosis in sub-acute SCI in rats with important implications for clinical

consideration. Supported by the SCIRF and the Veterans Administration.

WTH12-09

Choline transporter-like 1 (CTL1) regulates lipid homeostasis and peripheral nerve myelination *in vivo* C. Heffernan¹, D. Palmar¹, E. Bonder¹, J. Golowasch², D. Ory³, X. Jiang³, H. Kim¹, P. Maurel¹

¹Rutgers, The State University of New Jersey, Biological Sciences, Newark, USA

²New Jersey Institute of Technology, Biological Sciences, Newark, USA

³Washington University in St. Louis, Metabolomics Facility, St. Louis, USA

Choline Transporter-Like 1 (CTL1) is a transmembrane protein that imports extracellular choline to the cytosol for choline-dependent lipid biosynthesis. We have previously described a protein complex in Schwann cells comprising CTL1 that regulates choline import, intracellular choline levels and lipid homeostasis *in vitro*. Furthermore, CTL1-deficient Schwann cells (by shRNA knockdown) were impaired in their ability to myelinate neurites *in vitro*. To investigate whether CTL1 regulates peripheral nerve myelination *in vivo*, we generated a novel floxed CTL1 (CTL1^{flox/flox}) mouse and crossed it with the established desert hedgehog-Cre (dhh^{Cre}) to ablate CTL1 expression in the Schwann cell lineage. Electron microscopy analysis of P30 dhh^{Cre};CTL1^{flox/flox} sciatic nerve highlighted significantly malformed myelin, namely excessive myelin production, myelin infolding, outfolding or delamination, abnormal Schmidt-Lanterman incisures and also degeneration of underlying axons. Since CTL1 regulates choline-dependent lipid homeostasis *in vitro*, we fractionated myelin from the sciatic nerves of dhh^{Cre};CTL1^{flox/flox} mice, performed lipidomic LC-MS/MS mass spectrometry and identified fluctuations in specific lipid subspecies of phosphatidylcholine, phosphatidylinositol, diacylglycerol, sphingomyelin, phosphatidic acid and ceramide. Interestingly, *ex vivo* electrophysiological studies on dissected dhh^{Cre};CTL1^{flox/flox} mice sciatic nerve suggested that nerve conduction thresholds were significantly reduced in dhh^{Cre};CTL1^{flox/flox} mice compared to controls, however nerve conduction velocity was unaffected. Finally, preliminary studies suggest that the mobility of dhh^{Cre};CTL1^{flox/flox} mice (rotarod test) is significantly impaired compared to controls. We conclude that CTL1 function includes the regulation of lipid homeostasis and peripheral nerve myelination *in vivo*.

WTH12-10

Microglia manipulate synapses by surveying and touching them in both homeostatic condition and learning A. Ikegami¹, K. Haruwaka^{1, 2}, H. Wake¹

¹Kobe university, System Neuroscience, Kobe, Japan

²The Graduate University for Advanced Studies, Department of Physiological Sciences, Hayama, Japan

Microglia are the sole immune cells in central nervous system (CNS). They possess characteristic morphology and kinetics; long and ramified processes, and are continuously surveying local environment with these processes. During the surveillance, microglia contact with synapses. Many researches have revealed that

microglia are essential for synaptic modulation both in the developmental and mature periods. Disrupting the immune pathway between microglia and synapses cause the failure of normal neural circuits formation, and microglial BDNF is proved to be necessary for synapse formation that occurs with motor learning. However, the direct evidence of the correlation between microglial surveillance/contact and synaptic turnover and their motility is not given. In this study, we conducted two photon imaging of the same microglia and spines in somatosensory cortex of adult mice for two weeks, and revealed that there were higher rates of synapse formation or elimination where microglia contact more intensely. Further we investigated how microglial process movement to contact synapses in primary motor cortex change over the motor learning. In the later phase of lever-pulling training, microglial process movement gradually converged, indicating they became to contact with more specific places (synapses). Our results showed that microglia were controlling synaptic numbers by contact with synapses *in vivo*, and their motility was changed with the learning processes. This could further explain why altered microglia phenotype cause several neurological/psychiatric conditions with synapse abnormalities.

WTH12-11

Investigating the role of glial TGF β in axon maintenance A. Lassetter, A. E. Sheehan, R. Barria, A. Nicole Fox, M. R. Freeman

Oregon Health & Science University, Vollum Institute, Portland, USA

Axons are metabolically demanding structures responsible for transmitting information throughout the nervous system. In humans, axons in the peripheral nervous system can extend up to a meter, posing a significant challenge for distant cell bodies to support their axons. Most axons throughout the nervous system are ensheathed along their length by glial cells, which have been hypothesized to offer an additional source of support to meet the homeostatic demands of the axons they ensheath. Observations in human demyelinating diseases find that glial cell loss is followed by axon degeneration, further supporting this notion that loss of glial support leads to failure in axon maintenance. The extent and nature of the proposed glial support remains poorly defined and further molecular-genetic analysis is required to characterize the precise roles glia play in long-term axon maintenance. To this end, we conducted a genetic screen to identify glial genes that are required for long-term axon maintenance. We utilized the genetically-tractable *Drosophila* model organism to screen a panel of over 2,000 genes comprised of nearly all transmembrane, secreted, and signaling molecules encoded in the *Drosophila* genome using *in vivo* RNA interference. We systematically knocked down a single gene, exclusively in glia, and evaluated the effect this had on long-term survival of axons *in vivo* where the glia-axon organization remains intact. We identified numerous candidate genes that, when knocked down in glia, cause defects in axon integrity, including several members of the highly conserved transforming growth factor beta (TGF β) superfamily. The TGF β family has established roles during development, including neuron-glia interactions, but its role in adult axon maintenance has not been investigated. Our findings provide evidence for a potentially novel role for glial TGF β signaling as a regulator of axon maintenance.

WTH12-12

Myelin loss disrupts motor cortex circuit function L. Nettles^{1, 2, 4}, H. J. Barr³, E. G. Hughes^{3, 4}, C. G. Welle^{1, 2, 4}

¹University of Colorado Anschutz Medical Campus, Neurosurgery, Aurora, USA

²University of Colorado Anschutz Medical Campus, Physiology and Biophysics, Aurora, USA

³University of Colorado Anschutz Medical Campus, Cell and Developmental Biology, Aurora, USA

⁴University of Colorado Anschutz Medical Campus, Neuroscience Graduate Program, Aurora, USA

Multiple sclerosis (MS) is an autoimmune disorder that destroys oligodendrocytes, which myelinate the central nervous system, resulting in impaired motor behavior. The cellular consequences of demyelination include reduced action potential conduction velocity and shortened axon initial segments, causing hyperexcitability. How myelin loss affects neural circuit function in the intact brain is a remarkably underexplored area. Moreover, motor rehabilitation in MS patients has had limited success in mediating recovery and the mechanisms underlying these variable effects are unknown. This work aims to understand the effects of demyelination on a skilled reach behavior and the corresponding neural activity in primary motor cortex (M1). Using chronically implanted microelectrode arrays, we recorded in M1 of mice during a skilled forelimb reach task. Neural activity was recorded before, during, and after demyelination, using dietary administration of a demyelinating toxin, cuprizone. Demyelination impaired performance of the reach task and produced motor cortex hyperexcitability. We found that while both excitatory and inhibitory neurons had increased firing rates following demyelination, hyperexcitability was primarily driven by movement-responsive neurons located in deeper layers of cortex. Demyelination also decreased modulation of reach-induced firing rate. Rehearsal of a pre-learned task increased behavioral success and normalized firing activity after demyelination, but did not improve deficits in modulation. Overall, our data suggests that myelin loss results in behavioral deficits that correlate with disruption of motor cortex circuit function, and that rehabilitation training may ameliorate some demyelination-induced deficits in neuronal activity.

WTH12-13

Involvement of astrocytic ephrin-B1 in hippocampal excitatory and inhibitory synapse development and maintenance

A. Nguyen¹, J. Koeppen¹, S. Hanna¹, A. Vengala¹, K. Mina¹, A. Obenaus², I. Ethell¹

¹University of California, Riverside, Biomedical Sciences Division, Riverside, USA

²University of California, Irvine, Department of Pediatrics, Irvine, USA

Astrocytes regulate synapse development and maintenance and their dysfunctions are linked to neurodevelopmental disorders such as autism and intellectual disabilities. Our studies suggest a new role for astrocytic ephrin-B1 in regulating hippocampal circuits during synapse growth in early postnatal development and synaptic plasticity in the adult brain. Our previous study suggest that astrocytic ephrin-B1 is involved in pruning of existing synapses or suppressing new synapse formation through interactions with

neuronal EphB receptors and deletion of ephrin-B1 from adult astrocytes elicited synapse formation. In this study, we examined whether deletion of astrocytic ephrin-B1 in *ephrin-B1^{fllox/y}* (WT) and ERT2-Cre^{GFAP} *ephrin-B1^{fllox/y}* (KO) mice during early postnatal development (P14→P28) would also lead to excessive formation of synapses in the developing hippocampus. Adult deletion resulted in significant increase in dendritic spine density and pre-synaptic excitatory vGlut1 puncta with no changes in inhibitory GAD65 puncta in CA1 hippocampus. Interestingly, we observed increased number of immature dendritic spines that coincided with two-fold decrease in synaptic AMPAR levels, decreased AMPAR-mediated current amplitude, decreased AMPA/NMDA EPSC ratio and overall decrease in postsynaptic firing of CA1 hippocampal neurons following stimulation of Schaffer Collaterals in adult KO mice, indicating functionally immature synapses. In contrast, early postnatal deletion resulted in significantly increased postsynaptic fEPSPs and postsynaptic firing of CA1 hippocampal neurons with increased pre-synaptic vGlut1 and GAD65 puncta. We are currently investigating whether the opposing functional effects of astrocytic ephrin-B1 deletion in developing and adult hippocampus can be explained by the changes in AMPA/NMDAR composition or excitatory-inhibitory balance. *This work was supported by the grant MH067121 from NIMH.*

WTH12-14

Gene edited mouse lines with multiple levels of stable hypomyelination

A. Peterson¹, H. Bagheri¹, M. Bechler², M. Swire², A. Jarjour², H. Friedman¹, C. French-Constant²

¹McGill University, Human Genetics, Oncology, Neurology & Neurosurgery, Montreal, Canada

²University of Edinburgh, MRC Centre for Regenerative Medicine, Edinburgh, UK

The longitudinal and radial dimensions of myelin sheaths correlate positively with increasing axon caliber. Here we describe the architecture of CNS myelin sheaths in mice mutated to accumulate low levels of Myelin Basic Protein (MBP). Using CRISPR, transcriptional enhancers of the *Mbp* gene were deleted individually or in combination. Homozygous KO lines accumulating between 62% and 28% of the WT *Mbp* mRNA level demonstrate unremarkable within cage behavior, reproduce normally and are long lived. CNS fibers demonstrate significant reductions in myelin thickness relative to axon calibers consistent with earlier models with limited MBP accumulation (1). Hypomyelination in the mice derived here is maintained through 9 months, the oldest examined, and its extent correlates with *Mbp* mRNA levels. Spinal cord fibres also demonstrate significantly reduced sheath length and anomalies at nodes of Ranvier. Similarly, cortical oligodendrocytes elaborate foreshortened sheaths. Thus, when MBP abundance is suboptimal, myelin sheaths are both thinner and shorter. As these fully viable and seemingly stable mouse models demonstrate different degrees of hypomyelination we expect they will be useful in numerous investigations designed to probe axon-myelin relationships during development and in the mature nervous system. (1) Popko B. et al. Cell. 1987.

WTH12-15

Glutamatergic regulation of remyelination after spinal cord injury

N. Pukos¹, D. McTigue²

¹The Ohio State University, Neuroscience Graduate Program, Columbus, USA

²The Ohio State University, Neuroscience, Columbus, USA

Chronic demyelination, a hallmark of spinal cord injury (SCI), results in slowed conduction velocity and impaired recovery after injury. Spontaneous remyelination of these axons occurs after SCI and is attributed to the robust proliferation of oligodendrocyte progenitor cells (OPCs) that differentiate into mature OLs. Since OPCs are the primary source of new myelin, it is critical to understand OPC behaviors at various times pre and post-injury. Evidence from chemical demyelination studies show glutamate is packaged in vesicular glutamate transporter (Vglut)-positive vesicles and stored along demyelinated axon shafts. Activity-dependent release of glutamate from these axons stimulates OPC migration, differentiation, and ultimately remyelination. Since spinal tracts are mostly glutamatergic, we expect a similar phenomenon to occur after SCI. Here, we tested the hypothesis that Vglut2 accumulates in axons after SCI and predicts OPC-axon contact points. For this, we collected spinal cords from naïve and SCI mice from 7 days to 6 months post-injury (mpi) to examine Vglut2 distribution in axons. This revealed the number of Vglut2 + puncta in spared white matter increased continuously after SCI to a peak 9-fold greater than naïve at 6mpi. The number of Vglut2 + puncta within axons contacted by OPC processes was also quantified. In naïve tissue, ~10% of Vglut2 + puncta were contacted by an OPC process. OPC/Vglut2 contacts increased significantly by 28dpi and rose to a peak at 6mpi when 40% of Vglut2 puncta had an OPC contact. Overall this work shows for the first time that OPCs contact Vglut2-enriched regions of axons after CNS trauma, and suggests that loss of such contacts may contribute to chronic post-SCI demyelination.

WTH12-16

The gap junction nexus: supramolecular structural impacts on intercellular communication

R. Stout¹, D. Tanis¹, S. McCutcheon², V. Modi¹, D. Spray²

¹New York Institute of Technology, Biomedical Sciences, Old Westbury, USA

²Albert Einstein College of Medicine, Dominick P. Purpura Department of Neuroscience, Bronx, USA

We recently found that the mobility of gap junction channels within the supramolecular gap junction nexus organelle is controlled by cysteine residues within the carboxyl-terminus of the connexin proteins that form gap junction (GJ) intercellular channels. We also found that channel mobility is acutely modifiable. We show GJs modify location and mobility of other proteins in diverse manners through multiple underlying mechanisms. In parallel with our demonstration of such a dynamic GJ nexus supramolecular structure, a separate concept has emerged from the work of groups using phospho-specific connexin antibodies and studies indicating anchored kinase regulation of GJ channel activity. This concept leads to a widely held hypothesis in the field of GJ research which can be summarized as: The small percentage (< 10%) of active GJ channels within the larger GJ are spatially ordered via step-wise and

spatially-regulated phosphorylation of the connexin carboxyl-termini. However, such restriction of active and inactive GJ channels to sub-regions of the GJ plaque structure is impossible in an unstably arranged GJ nexus. Therefore, we hypothesize that intercellular communication established by connexins that form stably arranged GJs can be controlled by a form of metaregulation via the stability of the supramolecular structure itself. We used ensemble techniques such as FRAP to study mobility of GJ channels and a suite of interacting proteins. We will present results of spatially realistic cell modelling simulations and describe how we integrate experimental results from our microscopy experiments with published biophysical studies to generate model parameters for the mesoscale computational model of the tripartite synapse.

WTH12-17

Role of ectosomes in establishing the modular organization of the myelinated axon: structural findings **S. Szuchet, S. O'Sullivan, M. Domowicz**

University of Chicago, Department of Neurology MC2030, Chicago, USA

For vertebrates to exist and evolve, an energy efficient mechanism of fast nerve conduction had to be developed. The structure that fulfils this need, and hence sustains the very existence of the vertebrate kingdom, is the myelinated axon, whose modular architecture underlies saltatory conduction. Aided by molecular biology and immuno-histochemical technologies, scientists have been able to show specific protein complexes populating the different axonal domains whose formation and positioning are critical for proper nervous system function. Scientists have also been able to specify neurons and oligodendrocytes (OLGs) as the primary architects of this structure. However, how all of this is coordinated and executed is still unknown. We present structural evidence that: i) upon reaching an axon, the OLG process splits into two branches that embrace the axon, thereby positioning its plasma membrane directly around the axolemma; ii) within a circumscribed area, OLG and neuronal plasma membranes emit ectosomes – known to be carriers of signalling molecules between cells in both prokaryotes and higher eukaryotes. We posit that OLGs and neurons use ectosomes to communicate with one another, to plan, coordinate, and implement the modular organization of the myelinated axon. The concept of ectosomes as potential custodians of active signalling molecules, that guide OLG-neuron interaction, marks a critical turning point in understanding the singularity of their interaction and cooperation. For now, the signalling molecules can

be identified by establishing first the timing of ectosome release during development followed by their purification and content characterization. Deciphering the code of OLG-neuron cross-talk will advance our knowledge of their intercellular communication under physiological conditions and pave the way for unravelling disease-specific biomarkers.

WTH12-18

Investigating the role of fractalkine signalling in postnatal neural and oligodendrocyte precursor cells **A. Watson, B. Wang, T. Footz, A. Voronova**

University of Alberta, Medical Genetics, Edmonton, Canada

Background: In the adult brain, oligodendrocytes are generated by neural precursor cells (NPCs) and oligodendrocyte precursor cells (OPCs) from the subventricular zone (SVZ). We have previously discovered that interneurons secrete cytokine fractalkine (FKN), which directly regulates oligodendrocyte formation from embryonic cortical NPCs during brain development (Voronova et al. 2017 Neuron). Here, we test the hypothesis that FKN has a direct role regulating postnatal NPCs and OPCs.

Methods: We utilized single cell RNA fluorescent *in situ* hybridization (RNA scope) to detect FKN receptor CX3CR1 expression in postnatal brain. We also infused FKN directly conjugated to fluorophore Alexa-647 (FKN-647) to identify which cell types bind FKN *in vivo*. To test the effect of FKN on NPCs and OPCs in culture, we expanded NPCs from murine postnatal SVZ as neurospheres, where only NPC cells propagate and other cells such as microglia, neurons and astrocytes do not survive. Neurosphere cells were then cultured as monolayers to allow glial differentiation, or as secondary neurospheres to measure NPC proliferation.

Results: We show that *Cx3cr1* mRNA is expressed in postnatal and adult SVZ NPCs and parenchymal OPCs, in addition to microglia. Moreover, FKN-647 injected into lateral ventricle of adult murine brain diffuses into the tissue and binds NPCs and OPCs. Our microglia-free NPC cultures demonstrate increased OPC and oligodendrocyte differentiation, but not proliferation, in the presence of FKN. Our initial data suggest FKN may reduce OPC apoptotic cell death.

Conclusions: FKN promotes oligodendroglial cell genesis from postnatal NPCs and may have a protective effect on OPC survival. We are currently investigating whether FKN infusion regulates oligodendrogenesis from adult SVZ NPCs and parenchymal OPCs in normal and demyelinated brain, respectively.

WTH13 Lipids (Session B)

WTH13-01

Ceramide regulates interaction of HSD17B4 with PEX5 and function of peroxisomes in astrocytes

E. Bieberich¹, Z. Zhu¹, J. Chen², P. Tripathi¹, G. Wang¹, H. Qin¹, A. Elsherbini¹, L. Zhong¹, X. Jiang¹, W. Zhi³, S. Spassieva¹, A. Morris⁴

¹University of Kentucky, Physiology, Lexington, USA

²University of Kentucky, Internal Medicine, Lexington, USA

³Augusta University, Biotechnology and Genomic Medicine, Augusta, USA

⁴VA Medical Center Lexington, Veteran Affairs, Lexington, USA

The sphingolipid ceramide regulates beta oxidation of medium and long chain fatty acids in mitochondria. It is not known whether it also regulates oxidation of very long chain fatty acids (VLCFAs) in peroxisomes. Using affinity chromatography, co-immunoprecipitation experiments, and proximity ligation assays we discovered that ceramide interacts with Hsd17b4, an enzyme critical for peroxisomal VLCFA oxidation and docosahexaenoic acid (DHA) generation. Immunocytochemistry with HEK293 cells, N2a cells, and primary cultured astrocytes showed that Hsd17b4 is distributed to ceramide-enriched mitochondrial-associated membranes (CEMAMs). Molecular docking and *in vitro* mutagenesis experiments showed that ceramide binds to the sterol carrier protein 2-like domain in Hsd17b4 adjacent to PTS1, the C-terminal signal for interaction with Pex5, a peroxin mediating transport of Hsd17b4 into peroxisomes. Inhibition of ceramide biosynthesis induced translocation of Hsd17b4 from CEMAMs to peroxisomes, interaction of Hsd17b4 with Pex5, and up-regulation of DHA. This data indicates a novel role of ceramide as molecular switch regulating interaction of Hsd17b4 with Pex5 and peroxisomal function. This work was funded by R01AG034389, R01NS095215, and NSF 1615874 to EB and a VA Merit Award I01CX001550 and P30 GM127211 to AJM.

WTH13-02

DHA esterified to phosphatidylserine is more efficient at targeting the brain than DHA esterified to triacylglycerol

R. Chouinard-Watkins¹, R. J. Scott Lacombe¹, A. H. Metherell¹, M. Masoodi^{1,2}, Richard. P. Bazinet¹

¹University of Toronto, Nutritional Sciences, Toronto, Canada

²Nestlé Research, Lipid Biology, Lausanne, Switzerland

Docosahexaenoic acid (DHA, 22:6n-3) is crucial for optimal neuronal development and function. However, the brain has a poor capacity to synthesise this fatty acid and therefore it is widely believed that humans need to consume DHA in the diet to maintain adequate tissue levels. When consumed acutely esterified to phosphatidylcholine, DHA is more efficient at targeting the brain than when consumed esterified to triacylglycerol. However, the brain DHA bioavailability of other forms of DHA-containing phospholipids, after oral ingestion, is unknown. The objective of this study was to compare brain uptake of DHA after acute gavage with different DHA carriers. Ten-week-old rats were gavaged with ³H-labeled DHA esterified to phosphatidylcholine (DHA-PtdCho), phosphatidylethanolamine (DHA-PtdEtn), phosphatidylserine (DHA-PtdSer) or triacylglycerol (DHA-TG) (n = 7 per group).

Six hours post-gavage, the animals were euthanized and cortex, hippocampus, liver and serum were sampled. Lipids were extracted, separated by thin layer chromatography and radioactivity was quantified by liquid scintillation counting. Radioactivity recovered in cortex total phospholipids was similar between the DHA-PtdCho and DHA-PtdSer groups, and were 5.8 and 6.7 times higher than in the DHA-TG group, respectively. Similar results were obtained for the hippocampus. Liver and serum total lipid radioactivity were higher in the DHA-PtdSer group than in the DHA-PtdCho or DHA-PtdEtn group, but not compared to the DHA-TG group. These results suggest that both DHA-PtdCho and DHA-PtdSer are more efficient at targeting the brain than DHA-TG after acute gavage, but that their peripheral metabolism is different and this will require further investigation. (Funded by Nestec and supported by FRQS).

WTH13-03

Association of A β with astrocyte-derived and ceramide-enriched exosomes mediates A β mitotoxicity in neurons

A. Elsherbini¹, E. Bieberich¹, G. Wang¹, H. Qin¹, s. karki¹, s. Crivelli¹, X. Xiang¹, L. Zhong¹, Z. Zhu¹

¹University of Kentucky, Physiology, Lexington, USA

²Augusta university, Department of neuroscience and regenerative medicine, Augusta, USA

The cause for neurotoxicity of A β in Alzheimer's disease (AD) is still not clear. Here, we show that serum from 5xFAD mice and AD patients, but not serum from wild type mice or healthy human controls contains a proportion of ceramide-enriched and astrocyte-derived (GFAP positive) exosomes termed astrosomes that are associated with A β . Anti ceramide antibody prevented A β association with astrosomes suggesting that ceramide mediate binding of A β to astrosomes. In contrast to A β alone, A β -associated astrosomes increased neurite fragmentation and neuronal cell death by 3-fold, suggesting that association with astrosomes enhanced A β neurotoxicity. A β -associated astrosomes from familial AD (5xFAD) mice and AD patient serum were taken up by mouse Neuro 2a cells and human iPS cell-derived neurons. They were transported to mitochondria and increased the concentration of A β and ceramide, induced mitochondrial clustering, and up-regulated the fission protein Drp-1. We tested if A β -associated astrosomes mediated binding of A β to VDAC1, an ADP/ATP transporter known to form pro-apoptotic pores in mitochondria when bound to A β . A β -associated astrosomes induced complex formation between A β and VDAC1 concurrent with caspase activation. Complex formation and caspase activation were not observed with exosomes from wild type and human control serum or A β alone. Our data suggest that the association of A β with ceramide in astrosomes enhances A β interaction with VDAC1 and mediates A β neurotoxicity in AD. This work is supported by R01AG034389.

WTH13-04

Human remyelination promoting antibody induces astrocytes proliferation modulating the sphingolipid rheostat in primary rat mixed

A. Prinetti¹, S. Grassi¹, S. Prioni¹, D. Button², J. Cao², I. Hakimi², P. Sarmiere², M. Srinivas², L. Cabitta¹, S. Sonnino¹, P. Giussani¹

¹University of Milano, Dept of Medical Biotechnology and Translational Medicine, Milano, Italy

²Acorda Therapeutics, Inc., none, Ardsley, NY, USA

Remyelination promoting human IgMs increase the number of myelinated axons in animal models of multiple sclerosis stimulating myelin production by oligodendrocytes (OLs); however, their exact mechanism of action and, whether they are directly targeting OLs, or other cell types remains to be elucidated. We assessed the effect of remyelination promoting antibody rHlgM22 on the proliferative response and on the ceramide (Cer)/S1P rheostat in mixed glial cell cultures (MGCs). rHlgM22 treatment caused a time-dependent increase in PDGF α R protein in MGCs, and induced a dose-dependent proliferative response in MGCs being the most significant proliferative response associated with astrocytes. In many cell types, the balance between Cer and S1P, is critical in determining the cell fate. rHlgM22 had no effects on Cer levels but increased production and release of S1P in the extracellular milieu of MGC. Release of S1P was strongly reduced by a selective inhibitor of PDGF α R. Increased S1P production was not mediated by regulation of sphingosine kinase 1 and 2, instead, we observed a significant reduction in the levels of sphingosine 1-phosphate lyase 1. Remarkably, rHlgM22 treatment did not induce changes in the production and/or release of S1P in pure astrocyte cultures. Taken together, these data suggest that rHlgM22 indirectly influences the proliferation of astrocytes in MGCs, by affecting the Cer/S1P balance. The specific cell population directly targeted by rHlgM22 remains to be identified, however our study unveils another aspect of the complexity of rHlgM22-induced remyelinating effect.

WTH13-05

Docetaxel increases the levels of neurotoxic deoxysphingolipids in mice DRG

S. Spassieva¹, K. A. Becker², A.-K. Uerschels³, L. G. Goins¹, S. Doolen⁵, J. Bielawski⁴, E. Bieberich¹, B. Taylor⁵, E. Gulbins²

¹University of Kentucky, Physiology, Lexington, USA

²University of Duisburg-Essen, Molecular Biology, Essen, Germany

³University of Duisburg-Essen, Neurosurgery, Essen, Germany

⁴Medical University of South Carolina, Biochemistry and Molecular Biology, Charleston, USA

⁵University of Pittsburgh, Anesthesiology and Preoperative Medicine, Pittsburgh, USA

A major dose-limiting side effect of docetaxel chemotherapy is peripheral neuropathy. Its molecular mechanism is currently not understood, and there are no treatments available. Previously, we have shown an association between neuropathy symptoms of patients treated with paclitaxel and the levels of deoxysphingolipids in their plasma (Kramer *et al.*, FASEB J, 2015). Deoxysphingolipids are produced when the first enzyme of the sphingolipid biosynthetic pathway, serine palmitoyltransferase, uses L-alanine as a substrate instead of its canonical amino acid substrate, L-serine. In the current investigation, we used a mouse model to test whether deoxysphingolipids accumulate in the dorsal root ganglia (DRG) due to docetaxel treatment. In addition, we used live imaging of the actin cytoskeleton to study deoxysphingolipids' toxicity in primary DRG neurons.

We observed significant elevation of deoxysphingolipid levels in the DRG isolated from mice treated with docetaxel. In addition, immunohistochemistry analyses revealed that serine palmitoyltransferase was up-regulated in the DRG of the docetaxel-treated mice. Our results demonstrated that systemic docetaxel treatment affects the sphingolipid metabolic pathway in the periphery. Our experiments with primary DRG neurons showed that deoxysphingolipid toxicity was manifested by actin cytoskeletal changes and neurite swellings. Moreover, those changes and the neurite swellings were attenuated by the addition of the bioactive sphingolipid, sphingosine-1-phosphate, suggesting that, in the neurites, deoxysphingolipids and sphingosine-1-phosphate signaling have opposing effects on the actin cytoskeleton.

WTH14 Other topics (Session B)

WTH14-01

Near-infrared optogenetics by photon upconversion hydrogels

I. Ajioka^{1, 2}, Y. Sasaki⁴, M. Oshikawa^{1,3}, N. Yanai^{2,4}

¹Tokyo Medical and Dental University, Center for Brain Integration Research, Tokyo, Japan

²JST, PRESTO Molecular Technology, Saitama, Japan

³Kanagawa Institute of Industrial Science and Technology, KISTEC, Kanagawa, Japan

⁴Kyushu University, Department of Chemistry and Biochemistry, Fukuoka, Japan

Optogenetics is a technology allowing light-responsive protein to regulate cellular events. Since visible light does not penetrate effectively through biological tissues, near-infrared (NIR) light has more advantages than visible light as a light source for optogenetics in deep tissues. However, the photo-activation of proteins requires much higher energy photons than NIR photons. To overcome this limitation, in this study, we developed NIR light-triggered optogenetics by triplet-triplet annihilation (TTA)- photon upconversion (UC) hydrogels. It has been reported that the triplet lifetime of sensitizers can be elongated by excited state thermal equilibrium with covalently-attached aromatic moieties having long triplet lifetime. We employed this strategy to overcome the above-mentioned issue of limited molecular diffusion in viscous oxygen-blocking matrices. Combined with photoactivatable Cre recombinase (PA-Cre) technology, NIR light stimulation successfully performs genome engineering such as hippocampal dendritic spine formation.

WTH14-02

Targeted over-expression of COX-2 in the mouse hippocampus

A. Andonova, J. Hewett

Syracuse University, Biology, Syracuse, USA

Cyclooxygenase-2 (COX-2) is a key source of prostaglandin production in the brain. These lipid mediators contribute to brain functions, such as learning and memory. Mounting evidence suggests that COX-2 also contributes to the maintenance of the seizure threshold, an innate property of the brain that depends on the balance between excitatory and inhibitory neurotransmission. For example, inhibition of COX-2 shifts the balance toward excitation and increases risk for seizures. COX-2 is expressed constitutively in cell bodies and dendrites of certain populations of glutamatergic neurons, including pyramidal cells in the CA3 region of the hippocampus. It is reasonable to propose that COX-2 expression in the CA3 contributes to maintenance of the seizure threshold. This is being tested by targeted COX-2 over-expression in CA3 pyramidal cells using two single transgenic mouse lines and a Cre/loxP conditional gene-targeting approach. The first transgenic line, termed COE, harbors a COX-2 cDNA transgene construct that can be transcriptionally activated by Cre recombinase. The second transgenic line, termed Grik4, expresses Cre recombinase within the pyramidal neurons of the CA3. The expression is targeted to the CA3 by the endogenous promoter/enhancer elements of the

ionotropic glutamate receptor Grik4 (kainate 4). Initial results show that this approach generates offspring of the four expected genotypes: non-transgenic, both single transgenic and double transgenic mice. Seizure behavior in the resulting progeny is being evaluated in an acute seizure paradigm using pentylenetetrazole (PTZ), a GABA receptor antagonist, that induces a single transient episode of seizure activity. Seizure severity, incidence and latency are being quantified using a behavioral scoring system. Results from this research could contribute to a better understanding of the molecular mechanisms that control seizure threshold and therefore seizure susceptibility.

WTH14-04

Screening of substances for biocompatibility based on the proliferation of C6 glioma cells

C. Arend¹, M. Brandmann¹, T. Harder^{2, 3}, R. Dringen¹

¹University of Bremen, Centre for Biomolecular Interactions Bremen, Faculty 2 Biology/Chemistry, Bremen, Germany

²University of Bremen, Faculty 2 Biology/Chemistry, Bremen, Germany

³Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Bremerhaven, Germany

For numerous purposes it is necessary to screen high numbers of biological samples for toxicity or biocompatibility. For such applications, we developed a low-cost and robust cell culture test system utilizing the C6 glioma cell line for semi-high-throughput screening of the antiproliferative and/or biocompatible potential of test samples which is based on the determination of cellular and extracellular activities of the cytosolic enzyme lactate dehydrogenase (LDH). The toxins formaldehyde, arsenite, copper chloride, rotenone and staurosporine were used to validate the screening protocol. All applied toxins affected proliferation with half-maximal effects observed for concentrations of 60 μ M formaldehyde, 20 μ M arsenite, 600 μ M copper chloride, 10 μ M rotenone and 400 nM staurosporine. In addition, formaldehyde, arsenite and copper chloride, but not the respiratory chain inhibitor rotenone or the apoptosis inducer staurosporine, demonstrated cytotoxic potential. The established screening procedure was applied to screen a large number of algal extracts to test for potential toxicity of compounds in the extracts but also for protection against the detrimental effects induced by the toxins used. None of the applied algal extracts prevented the antiproliferative or toxic effects of the toxins applied, while the cytotoxicity induced by formaldehyde, arsenite and copper chloride was prevented by application of semicarbazide, dimercaptosuccinic acid and bathocuproinedisulfonic acid, respectively. These results show that the established large-scale screening approach is useful to screen a large number of different compounds for their biocompatibility and for potential protection against various types of toxins.

WTH14-05

Evaluation of spinal cord injury in rat model using diffusion tensor imaging**V. Cubínková¹, A.-N. Murgoci¹, T. Smolek¹, L. Baciak³, D. Cizkova^{1, 2}**¹*Institute of Neuroimmunology, Slovak Academy of Science, Center of Regenerative Medicine, Bratislava, Slovakia*²*University of Veterinary Medicine and Pharmacy, Kosice, Slovakia, Department of Anatomy, Histology and Physiology, Kosice, Slovakia*³*SNM University of Technology in Bratislava, Department of NMR Spectroscopy and Mass Spectroscopy, Bratislava, Slovakia*

Spinal cord injury (SCI) is neurological disorder that causes temporary or permanent disability. The consequences of this disorder are devastating, most patients' loss the capacity of movement and sensation below the site of damage. It is important to understand the pathological processes that occur in the lesion site and to find efficient ways to diagnose the severity of injured spinal cord tracts in situ from beginning up to a certain level of recovery following therapeutic interventions. The goal of our study was to set-up the criteria for diffusion tensor imaging (DTI) in order to capture changes of nerve fibre tracts in rat model. For that purpose, DTI parameters including fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) were calculated, and diffusion tensor tractography in the injury site was reconstructed in balloon compression SCI rat model. We compared DTI data with locomotor outcomes obtained *in vivo* with histological results. We tested their consistency and capability of reflecting the lesion development in time. Here we detected the decreasing value of FA for injury site and at the same time higher values of the MD. Both axial and radial diffusivity were increased in comparison to control subjects. Our data confirm that DTI is useful *in vivo* imaging tool capable to determine damaged white matter tracts after mild SCI.

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WTH14-06

Assessing changes in exosomal miRNA expression in degenerating iPSC derived neurons**S. Drake, A. Fournier***McGill University, Montreal Neurological Institute, Montreal, Canada*

Neurodegeneration is the best-known correlate for clinical disease in MS, however for progressive MS which is typified by steadily increasing disease burden, there are neither neuroprotective therapies nor prognostic tests informing long-term disease outcomes. MicroRNAs (miRNA) are small non-coding RNAs that mediate RNA interference gene silencing of messenger RNAs. Interestingly, they are stable outside of cells and are frequently packaged into exosomes for transport outside of cells. These can be found in abundance in patient blood samples. Many studies have identified changes in the miRNA signature of multiple sclerosis patients however it is difficult to relate these changes to the disease pathology. In multiple sclerosis, multiple mechanisms have been linked to alterations in neuronal health including glutamate excitotoxicity, iron dys-homeostasis, and oxidative stress. These can result

in swelling and fragmentation of neurites and changes in gene expression within the neuron. Therefore, we are using *in vitro* neurodegeneration assays in human iPSC-derived cortical neurons to identify changes in the exosomal miRNA signature in neurons experiencing environmental stressors. We are assessing cell viability and neurite degeneration in nitric oxide, glutamate, rotenone, and iron treated neurons to establish consistent neurodegeneration assays. We then will isolate exosomes from treated neurons, extract the miRNAs and send them for sequencing to identify dysregulated miRNAs. We intend to correlate these to what is already known in the literature about miRNA dysregulation in MS to identify key miRNA species that may implicate neurodegenerative processes in the disease progression. Ultimately we aim to identify miRNAs dysregulated by neurotoxic agents relevant to the CNS environment in MS that can be used to help assess and predict a patient's prognostic disease course, and potentially inform neuroprotective therapies for progressive MS.

WTH14-07

Structural description of monoaminergic transporters in D. melanogaster and T. ni and their interaction with substrates**A. Fierro¹, S. Arancibia-Opazo¹, M. Marambio¹, J. Rojas¹, J. Campusano²**¹*Pontificia Universidad Catolica de Chile, Organic Chemistry Department, Santiago, Chile*²*Pontificia Universidad Catolica de Chile, Cell and Molecular Biology Department, Santiago, Chile*

Octopamine, is a major neurotransmitter linked to important biological processes in insects including learning and memory and feeding behavior. As the *monoaminergic system (MS)* in mammals, it is mainly constituted by the neurotransmitter, their receptors, their associated metabolic enzymes and transporters that reuptake the neurotransmitters. Interestingly, one of the molecular entities responsible for octopamine availability, the octopamine transporter (OAT), has not been identified in certain insect species. For instance, no OAT has been reported in the fly *Drosophila melanogaster* (Dm) but it has been described for a common agricultural pest insect, *Trichoplusia ni* (Tni). Here, by using molecular simulation methodologies, we report for the first time a model for an OAT protein and provide new insights into the macromolecules responsible for the reuptake of dopamine, serotonin and octopamine in *T. ni*. Once models for OA, serotonin (5-HT) and dopamine (DA) transporters were obtained, docking studies and molecular dynamic simulations for each substrate into the binding cavity were carried out. We also evaluated structural and electrostatic differences of these proteins as compared to Dm transporters. We further assessed the idea that DmDAT and DmSERT are able to bind octopamine.

These studies provide new information that contributes to our understanding of amine availability in insects and could encourage the development of future generations of molecules for selective control of pests.

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WTH14-08

Electropolymerized poly(3,4-ethylenedioxythiophene) coatings for implantable stimulating microelectrodes *in vivo*

J. Hagler

Polytechnique Montreal, Chemical Engineering, Montreal, Canada

Implantable neural electrodes are important tools for recording and manipulating functions of the nervous system. Neural network interfaces, such as electrodes, have been used to better understand neural network plasticity through recording brain signals, and stimulating neural electrodes have been used clinically for therapeutic and assistive purposes in people with disease and injury. The challenges facing neural interface engineering is to develop materials that can seamlessly interface with the biological environment of the brain over long time periods, consistently provide the desired therapeutic results, and mitigate health risks associated with chronic implantation. Coating electrodes with conductive polymers such as poly (3,4-ethylenedioxythiophene)(PEDOT) has shown to enhance the performance of metal electrodes by decreasing the impedance and increasing the charge storage capacitance. PEDOT is an excellent candidate for interfacing with the brain because of its mixed ionic-electronic conductivity, and electrochemical stability. Here, stimulating platinum-iridium (PtIr) neural microelectrodes were coated with PEDOT:tetrafluoroborate through electrodeposition in the solvent propylene carbonate. Coated and uncoated stimulating electrodes, along with tungsten recording electrodes, were implanted in the hippocampus of mice. The coated/uncoated PtIr electrodes were stimulated daily, the recording electrodes measured the local field potentials generated by the stimulation, and the impedance before and after stimulation was measured at each electrode. The coated electrodes were able to effectively stimulate the brain, record the generated local field potentials, thus demonstrating that PEDOT-coated electrodes are a viable alternative to bare PtIr recording electrodes.

WTH14-09

Effects of acidified drinking water on motor behavior, neuropathology, and gut microbiota in a mouse model of batten disease

A. Kovacs^{1,3}, T. B. Johnson¹, L. M. Langin¹, J. Zhao², J. M. Weimer^{1,3}, D. A. Pearce^{1,3}

¹*Sanford Research, Pediatrics and Rare Diseases Group, Sioux Falls, USA*

²*Sanford Research, Population Health Group, Sioux Falls, USA*

³*Sanford School of Medicine, University of South Dakota, Department of Pediatrics, Sioux Falls, USA*

CLN3 mutations cause the fatal neurodegenerative disorder, CLN3 Batten disease. The *Cln3*-knockout (*Cln3*^{-/-}) mouse model displays pathological and neurological features of the human disease including motor deficits. When mice received acidified drinking water (pH 2.5-2.9) as opposed to normal tap water (pH 8.4) for several generations, the motor skills of *Cln3*^{-/-} mice normalized to control levels, indicating a disease-modifying effect of acidified water. Here we investigated if acidified water administered from postnatal day 21 has therapeutic benefits in *Cln3*^{-/-} mice. Indeed, acidified water temporarily attenuated the motor deficits, had beneficial effects on behavioral parameters and prevented glial activation in the thalamus of *Cln3*^{-/-} mice. Interestingly, in control

mice, acidified drinking water also caused significant changes in motor performance. Since the gut microbiota can influence neurological functions, we examined the gut flora in our disease model and found that the gut microbiota of *Cln3*^{-/-} mice was markedly different from control mice, and acidified water differentially changed the gut microbiota composition of these mice. These results indicate that acidified water may provide therapeutic benefit to CLN3 Batten disease patients, and that the pH of drinking water is a major environmental factor that strongly influences the results of murine behavioral and pathological studies.

WTH14-10

Membrane clustering of syntaxin 3 in hippocampal neurons

J. Lochner, S. Lowenstein, H. Thomson

Lewis & Clark College, Biochemistry & Molecular Biology, Portland, USA

Resolving the precise spatial organization of synapse-associated proteins is providing unprecedented insight into synaptic structure and function. applied super-resolution microscopy to characterize the plasma membrane distribution and patterning of syntaxin 3 (Stx3), a SNARE (soluble N-ethylmaleimide-sensitive factor activating protein receptor) protein, in cultured hippocampal neurons. Our photoactivated localization microscopy (PALM) analysis of Stx3-Dendra2 revealed that Stx3 localized to submicron-sized clusters on the membrane of hippocampal processes. The Stx3 nanoclusters co-existed with randomly distributed non-clustered Stx3 molecules in the soma and in the processes. Stx 3 clusters were particularly prominent in the tips of dendritic filopodia and on the heads of dendritic spines. Accumulating experimental findings indicate that membrane propagated forces initially drive formation of loose clusters of many membrane proteins. For example, electrostatic interactions between the strongly anionic lipid PIP₂ and a highly conserved stretch of basic amino acids adjacent to the transmembrane domain of Stx1 are sufficient to promote Stx1A clustering. When basic residues in the Stx3 polybasic juxtamembrane region were mutated to neutral or acidic residues, Stx3 clustering persisted demonstrating that electrostatic interactions are not the dominant membrane force driving Stx3 clustering. Relying on distinct membrane forces to promote loose clustering and lateral organization of different membrane Stxs may facilitate the spatial separation of fusion machinery needed for driving the distinct fusion processes these proteins facilitate. When the Stx1 polybasic linker was introduced through mutagenesis within the juxtamembrane region of Stx3, dual-color structured illumination microscopy (SIM) analysis revealed that the Stx1 polybasic linker drove Stx3 to colocalize with Stx1.

WTH14-11

Factors influencing d3 vs d2 dopamine receptor subtype selectivity**R. Luedtke¹, H. Hayatshahi², S. Griffin¹, M. Taylor¹, K. Xu², J. Liu², R. Mach³**¹University North Texas HSC, Pharmacology and Neuroscience, Fort Worth, USA²University of North Texas College of Pharmacy, Pharmaceutical Sciences, Fort Worth, USA³University of Pennsylvania Perelman School of Medicine, Radiology, Philadelphia, USA

We have reported on the ability of arylamide phenylpiperazines to bind selectively to the D3 versus the D2 dopamine receptor subtype. Our goal was to investigate how the composition and size of the phenylpiperazine might influence binding affinity at the human D2 and D3 dopamine receptors. Two factors were identified as being important for determining the binding affinity of bitopic arylamide phenylpiperazines at the dopamine D3 receptor subtype. One factor was the strength of the salt bridge between the highly conserved residue Asp^{3.32} in the third extracellular loop (ELIII) with the protonated nitrogen of the nonaromatic ring at the piperazine position. The second factor was the configuration of the unbound ligand in an aqueous solution. These two factors could be useful when designing high affinity subtype selective bitopic ligands. While this model is based upon the interaction of arylamide phenylpiperazines with the D2 and D3 dopamine receptor subtypes, it provides insights into the complexity of the factors that define a bitopic mode of the binding at GPCRs.

WTH14-12

Combining benzoyl chloride derivatization with qualitative and quantitative analysis to identify biomarkers in *slc6a3* zebrafish**K. Malesky, G. Wang, C. Springer, S. Li, S. Thibodeaux**

Novartis Institutes of BioMedical Research, Neuroscience, Cambridge, USA

The dopamine transporter (DAT) is responsible for reuptake of dopamine into presynaptic terminals. Schizophrenia symptoms have been attributed in part to disturbances in dopamine signal transduction with polymorphisms in the DAT gene (*slc6a3*) being associated with treatment-resistant schizophrenia. *slc6a3*^{-/-} zebrafish manifest a consistent repetitive behavior as adults, with a distinctive posture and repetitive digging-like activity. The quantification of neurotransmitter levels is critical to relate behavioral observations to biochemical modulations. Initial analysis of dopamine and dopamine pathway neurotransmitters was applied to zebrafish brain samples following published protocols. Our work has sought to expand these efforts into an exploratory biomarker workflow. The development of a single analytical method to measure a broad range of neurotransmitters can further expand biomarker identification. By applying derivatization strategies, the neurotransmitters' diverse physicochemical properties are homogenized, making them amenable to reverse phase chromatography while also increasing their stability and sensitivity in the mass spectrometer. We employ a quadrupole mass spectrometer utilizing parent ion scanning and MRM to analyze samples and allow us to preliminarily identify masses of interest from *slc6a3*^{-/-} zebrafish brain tissue samples. To aid compound identification, benzoyl chloride was reacted *in silico*

with small molecules from the HMDB, KEGG and Lipidmaps databases. The resulting list of 15K compounds was compared to masses of interest in the samples and used to identify neurotransmitters, both known and unknown, contained in *slc6a3*^{-/-} zebrafish brain samples. When possible, further compound identification was accomplished by comparison to authentic standards. The application of these mass spectrometry methods validated the expected dopamine pathway alterations, identified other potential biomarkers, and revealed gender specific differences in neurotransmitter concentrations.

WTH14-13

Müller glia reprogramming by targeted expression of KLF4**T. Marinho, P. França, V. Valença, M. Martins, M. Silveira**

Federal University of Rio de Janeiro, Carlos Chagas Filho Institute of Biophysics, Rio de Janeiro, Brazil

Müller glia (MG) in the mammalian retina respond poorly to damage and present limited proliferative and differentiation potential, whereas in zebrafish it is the main source of regeneration and restore all neuronal types. For this reason, it represents a promising endogenous regenerative source if properly stimulated in mammals. Previously, we showed that over-expression of Klf4, a pluripotency factor, changes the potency of late progenitors as they reacquire the competence to generate retinal ganglion cells, an early-born cell type. As the transcription profile of MG shares high similarity with late progenitors, we hypothesized that Klf4 could induce in mammalian MG the competence to generate ganglion cells. We used plasmids based on the Cre-lox system to over-express Klf4 conditionally in MG. Eyes of P0 rats were electroporated *in vivo*, and after 10 days the retinas explants were transferred to *in vitro* culture and stimulated with 4OHT and EGF. Upon Klf4 over-expression, we detected a 2.4-fold increase in the proportion of TUBB3 (betaIII-tubulin) positive cells, and a 10-fold increase in NEUN positive cells among the cells that underwent recombination. Thus, we propose that Klf4 favors neurogenesis from MG in this system, as there was an increase in the number of cells which express markers common to retinal ganglion cells and amacrine cells. Moreover, our data suggest that these neurons are likely generated mainly from direct conversion of MG. These results indicate that Klf4 must be studied further *in vivo* as a candidate to new strategies to reprogram MG for regenerative therapies directed to degenerative diseases, such as glaucoma.

WTH14-14

Nanodendrimer-N-acetylcysteine enhances survival and in vivo migration of transplanted allogeneic glial restricted precursor cells**C. Nemeth¹, S. Tomlinson¹, R. Sharma², A. Sharma², M. Rosen¹, M. Johnston^{1, 2}, R. Kannan^{2, 1}, S. Kannan^{2, 1}, A. Fatemi^{1, 2}**¹Kennedy Krieger Institute, Moser Center for Leukodystrophies, Baltimore, USA²Johns Hopkins University School of Medicine, Baltimore, USA

Oligodendrocyte replacement is a promising avenue for the use of glial restricted precursor cells (GRPs); however, limited cell survival reduces integration and functional recovery.

Nanotherapeutic approaches can facilitate stem cell delivery while concurrently delivering factors aimed at enhancing and nourishing stem cells en route to, and at, the target site. Here, survival and migration of GRPs was assessed in a mouse model of neonatal white matter injury with different methods of G4-PAMAM dendrimer nanoparticle support. GRPs isolated from embryonic day 13.5 mice expressing eGFP were purified by A2B5 selection. Three groups underwent injury followed by: 1) vehicle injection at P10 and intracallosal (IC) injection of 100,000 GFP-expressing GRPs at P22; 2) single dose of dendrimer conjugated to the antioxidant/anti-inflammatory N-acetylcysteine (D-NAC; 10 mg/kg, intraperitoneal) at P10, followed by IC GFP-GRP at P22; or 3) P10 vehicle injection and IC GFP-GRPs pretreated for 12 h with D-NAC at P22. At 8w post-transplant, GRPs were detected in 82%, 94%, and 100% of mice receiving GRPs alone, D-NAC at P10 and GRPs, and D-NAC pretreated GRPs, respectively. Migration improved as GRPs from P10 D-NAC mice traveled 25% farther than untreated mice while D-NAC pretreated GRPs traveled a 75% greater distance. At one-week post-transplant, intraperitoneal dendrimer colocalized at injury sites, while dendrimer was detected outside pre-treated GRPs suggesting combination therapies enhance the injury environment independent of administration route. We demonstrate that D-NAC nanoparticles enhance transplanted progenitor survival and migration, suggesting combinatorial therapies may allow engraftment without overt immunosuppression.

WTH14-15

Glutamine synthetase in the blood-brain barrier: role in hindering hyperammonemia-induced neurotoxicity?

M. Oliveira, M. Trembley, C. Rose

CRCHUM - Université de Montreal, Medicine, Montreal, Canada

Introduction: Hepatic encephalopathy (HE) is a neuropsychiatric disorder, a major complication of chronic liver disease (CLD). Hyperammonemia is central in the pathogenesis of HE as ammonia crosses the blood-brain barrier (BBB) causing toxicity. Glutamine synthetase (GS), an enzyme which removes ammonia during the amidation of glutamate to glutamine, is expressed in liver, muscle and brain. Since liver is responsible for regulating blood ammonia levels, extra-hepatic GS plays an important compensatory role during CLD. However, GS expression in endothelial cells (EC) of the BBB has never been explored.

Methods: GS protein and activity was assessed in 1) rat brain microvascular EC (+/- ammonia exposure and plasma from rats with CLD) and 2) isolated cerebral microvessels (CMV) from naïve rats.

Results: GS was co-localized with EC in brain of naïve rats. GS protein and activity was detected in CMV and *in vitro*, but with lower levels compared to brain ($p < 0.05$). EC exposed to ammonia resulted in increased GS activity ($p < 0.05$). However, ECs exposed to plasma from CLD rats resulted in lower GS activity and protein expression compared to controls ($p < 0.05$).

Conclusion: We demonstrate for the first time the presence of GS in EC in both *in vitro* and *in vivo*. Stimulated by ammonia, GS is however reduced following exposure to plasma from hyperammonemic CLD rats. This suggests other systemic factors such as oxidative stress (present in CLD; Bosoi et al., Free Radic Biol Med, 2012) could hinder GS activity. We speculate a down-regulation of GS in the BBB during CLD leads to rapid entry of ammonia into the brain and the development of HE. Hence, up-regulating GS in the BBB could become a new therapeutic target for HE.

WTH14-16

Pomegranate juice protects rat brain from exhaustive exercise induced-oxidative stress

K. Ruamthum

Suranaree University of Technology, Preclinic, Nakhon Ratchasima, Thailand

Exhaustive exercise causes the increase in reactive oxygen species leading to oxidative stress in the brain. Till now, there is no study conducted to find out the effect of high antioxidant Thai pomegranate juice (TPJ) consumption on oxidative status in brain after exhaustive exercise. Therefore, the effects of TPJ on antioxidant enzyme catalase (CAT) activity and malondialdehyde (MDA) level in the rat cerebral cortex after exhaustive exercise were investigated. Rats were orally administered with water, 1% Tween 80, vitamin E (250 mg/kg), and TPJ (36.98, 56.85, and 86.57 mg GAE/kg as low, middle and high doses) for 30 days ($n = 8$ per group). In the last day, rats were forced to swim until exhaustion and the cerebral cortex was harvested for the assessment of CAT activity and MDA level. Another set of rats served as non-exercise control (sedentary without drug, $n = 6$). Exhaustive exercise (exercise + water, exercise + 1% Tween 80) significantly lower CAT activity and significantly increased MDA level, compared with non-exercise control ($p < 0.05$). Vitamin E and TPJ (middle and high doses, but not low dose) significantly elevated the reduced CAT activities induced by exhaustive exercise ($p < 0.05$). Low dose TPJ and high dose TPJ, but not middle dose TPJ and vitamin E, significantly reduced the increased MDA level induced by exhaustive exercise ($p < 0.05$). TPJ could increase antioxidant capacity in brain and could be beneficial for the protection of exhaustive exercise-induced oxidative stress on brain. The antioxidants in TPJ may contribute to the reduction of oxidative stress in brain. Thus, high antioxidant TPJ consumption may be useful for protecting oxidative stress in brain occurring from many causes.

WTH14-17

Lumican is required for axial neural stem cells adhesion gradient and proper spinal cord elongation

M. Shaker

University of Queensland, StemCARE, Brisbane, Australia

During the development, mammalian embryos exhibit a transition from head morphogenesis to trunk elongation to meet the demand of axial elongation. Caudal neural tube (NT) is formed with neural stem cells (NSCs) derived from neuromesodermal progenitors (NMPs) localized at the tail tip. However, molecular and cellular basis of elongating NT morphogenesis is poorly understood. Here, we provide evidence that caudal NSCs exhibit strong adhesion affinity which is gradually decreased along the anteroposterior (AP) axis in mouse embryonic spinal cord. This axial gradient of cell adhesion property is under the control of caudalizing signal Wnt. RNA-seq analysis revealed that the extracellular matrix (ECM) genes, including Lumican (Lum), expressions in caudal NSCs were enriched. Knockdown of Lum perturbed cell adhesion, and collective migration of NSCs, resulting in the failure of NT elongation in the chick. Together, these results suggest that progressive reduction of NSCs' adhesion affinity along the AP axis is under the control of Wnt-Lum molecular networks which is essential for a proper elongation of the spinal cord.

WTH14-18

Comparison of early stage Parkinson's disease with pantothenate kinase associated neurodegeneration
**F. Middleton¹, D. LaRocca¹, S. Baser², C. Muniz⁴, A. Espinoza¹,
 A. Rajan⁶, R. Ericson⁶, K. Wagner⁶, C. Bass⁶, A. Brindle⁶,
 P. Stoeter⁵, D. Mihaila², P. Roa⁴**

¹*SUNY Upstate Medical University, Neuroscience & Physiology, Syracuse, USA*

²*SUNY Upstate Medical University, Neurology, Syracuse, USA*

³*Allegheny General Hospital, Neurology, Pittsburgh, USA*

⁴*CEDIMAT, Neurology, Santo Domingo, Dominican Republic*

⁵*CEDIMAT, Radiology, Santo Domingo, Dominican Republic*

⁶*Quadrant Biosciences, Research, Syracuse, USA*

⁷*MIND R and T, Research, Murrysville, USA*

Parkinson's Disease (PD) affects 2% of the population over age 65 and is mostly idiopathic. Pantothenate Kinase Associated Neurodegeneration (PKAN) is a rare autosomal recessive neurodegenerative disorder affecting 1-2 subjects per million with onset in late childhood. Both diseases are associated with brain iron

accumulation, pathophysiological changes in the basal ganglia, and exhibit partial symptomatic overlap, including bradykinesia, rigidity, postural instability, and facial masking. We compared the symptomatology and molecular signatures in PD and PKAN, using parallel analyses of similarly-sized (n=70) cohorts of PD subjects recruited from the US and PKAN subjects recruited from the southwest Dominican Republic, where PKAN affects 1-2 subjects per thousand. We screened for DNA sequence changes, as well as changes in salivary microRNA. Extensive phenotyping was performed using standardized assessments of motor function as well as computerized assessments. Genetically, almost all PKAN subjects were homozygous for a c.680A>G missense mutation reported previously in this population. PKAN subjects showed significantly higher UPDRS scores than PD subjects while both groups showed significant impairment in simple and procedural reaction time tasks, balance scores, and Go/No-Go task performance. Both PD subjects and PKAN subjects also showed robust correlations between distinct miRNAs and specific demographic, clinical, and behavioral phenotypes.

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