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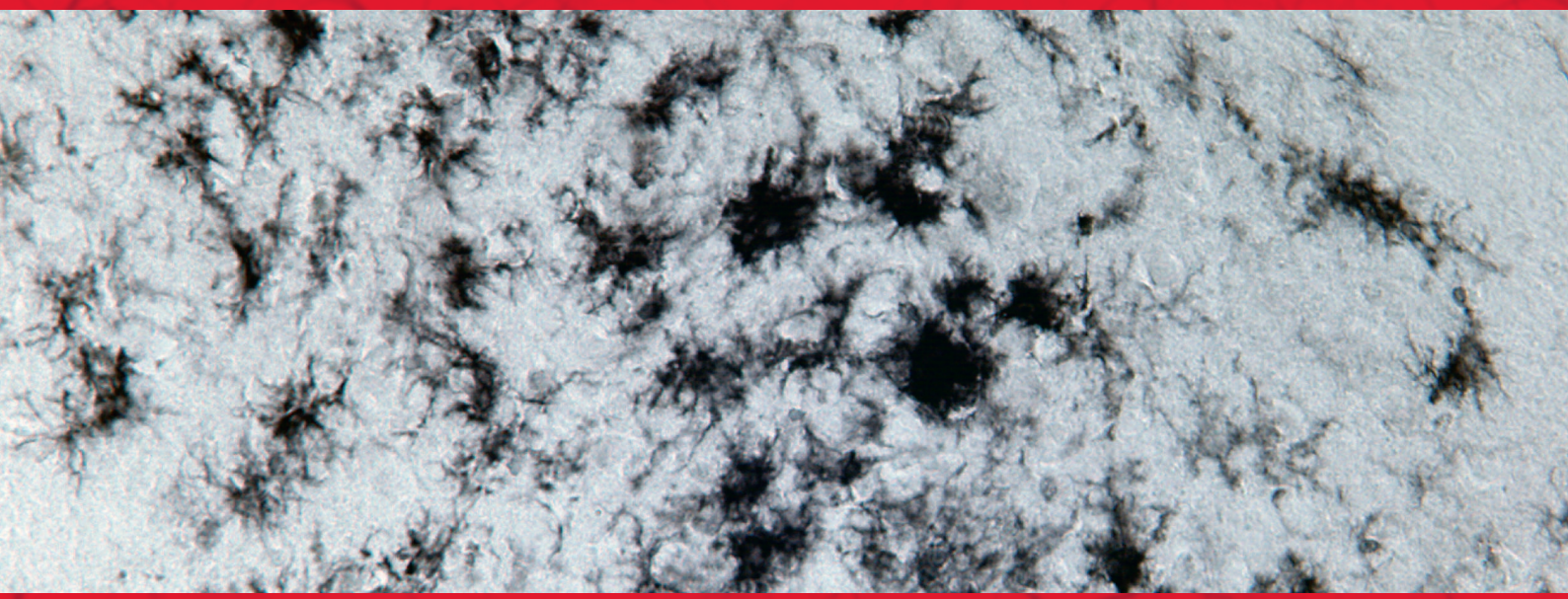

25th Biennial Meeting of the International Society for Neurochemistry jointly with the 13th Meeting of the Asian-Pacific Society for Neurochemistry in conjunction with the 35th Meeting of the Australasian Neuroscience Society

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Front cover: CD11c immunopositive microglia were observed in the subiculum in a brain section from the APPSwDI/mNos2-/- mouse model of Alzheimer's disease. These cells demonstrate genes associated with immunosuppression and express arginase 1. Sections were prepared and immunostained by Angela Everhart and the photograph was taken by Carol Colton using a Nikon Digital Sight DS Qi1mc camera and a Nikon Eclipse TE200 microscope.

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Plenary Lectures

PL01 Plenary Lecture 1 – Professor Yoshinori Ohsumi

PL01

Molecular dissection of autophagy – intracellular recycling system

Y. Ohsumi

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Every cellular process is mediated by balance between synthesis and degradation of proteins. Recently it has become clear that degradation is a highly regulated process, playing critical roles in cell physiology. Proteasome degrades every target protein after strict recognition by ubiquitination reactions, while the lysosome/vacuole system, autophagy, facilitates bulk and non-selective degradation. Under starvation recycling of own proteins becomes crucial for survival. Recent studies indicate that selective elimination of harmful proteins, organelles, and even intracellular bacteria via autophagy plays important roles for cellular homeostasis.

More than 25 years ago I first found autophagy induced by nutrient starvation in the yeast, *S. cerevisiae*, under a light microscope. Taking this advantage of the yeast, we succeeded in isolation of many autophagy-defective mutants. Now it is known that 18 *ATG* genes are essential for starvation-induced autophagy. These Atg proteins function concertedly in the sequestration of cytoplasmic constituents by the formation of a specialized membrane, the autophagosome. The Atg proteins consist of six functional units, including a protein kinase complex, the PI3 kinase complex and two unique ubiquitin-like conjugation systems. Since these core *ATG* genes are well conserved from yeast to mammals, a vast range of studies in autophagy have recently been undertaken in various cell types of many organisms. Now it is getting clear that autophagy is relevant to many diseases such as neurodegeneration, infection and cancer. Autophagy has become popular field, but there are so many questions remaining to be answered.

We are attempting to elucidate the structure and function of these Atg proteins during the unique membrane dynamics during autophagy in yeast. Further comprehensive analyses are required from various points of view. Present our knowledge on the molecular mechanism and physiological roles of autophagy will be presented.

PL02 Plenary Lecture 2 – Professor Franz-Ulrich Hartl

PL02

Molecular chaperones: guardians of the proteome

F. U. Hartl

Max Planck Institute of Biochemistry, Cellular Biochemistry, Martinsried, Germany

The past two decades have witnessed a paradigm shift in our understanding of cellular protein folding. While the three-dimensional structures of functional proteins are determined by their amino acid sequences, it has become clear that within the crowded environment of the cell many proteins depend on molecular chaperones to reach their folded states efficiently and at a biologically relevant time scale. Assistance of protein folding is provided by different types of chaperone which act to prevent misfolding and aggregation, often in an ATP-dependent mechanism. Molecular chaperones also cooperate with the degradation machinery (ubiquitin-proteasome system and autophagy) in the removal of terminally misfolded proteins.

Once folded, many proteins continue to require chaperones to retain their functional state, particularly under conditions of cell stress. Failure of the chaperone network to maintain proteostasis, i.e. the conformational integrity of the cellular proteome, may facilitate the manifestation of diseases in which proteins misfold and are deposited as aggregates, such as Parkinson's and Huntington's disease. A decline in proteostasis capacity occurs during aging, presumably explaining why age is a major risk factor of neurodegenerative pathologies.

I will discuss recent findings from mechanistic and systems-level studies to understand the role of the chaperone network under normal conditions and in models of aggregate deposition disease.

PL03 Plenary Lecture 3 – Professor Ellen Closs

PL03

Why transporters of simple cationic amino acids matter

E. Closs, A. Habermeier, J. Rupp, J.-P. Boissel

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Amino acids are polar compounds that cannot efficiently cross biological membranes by diffusion. Their passage into cells is thus mediated by specialized transport proteins. Cationic amino acids (CAA), such as arginine, lysine, and ornithine, share the same transporters whereas most mammalian cells express two types mediating activities of the so called systems y^+ (specific for CAA) and y^+L (CAA and neutral AA), respectively. Although only distantly related, the two transporter types belong to the same gene family: SLC7. The family members SLC7A1-A3 correspond to the CAT proteins (CAT for CAA transporter) mediating system y^+ activity. They seem to be the major entry path for CAA in most cells. SLC7A4 and A14 have also been attributed to the CAT subfamily. Their function remains however elusive to date. Besides providing cells with CAA for protein synthesis and energy supply, CATs seem to be involved in important signal pathways such as nitric oxide, mTor and neurotransmission. They seem to have specific roles in individual cell types. Our recent data show that induction of CAT-1 is necessary for proper T cell proliferation, whereas CAT-3 missence mutation are associated with autism. SLC7A6 and 7 encode for system y^+L transporter y^+LAT2 and 1, respectively. They catalyze the exchange of CAA against NAA plus Na^+ and thus seem to be rather CAA exporters under physiological conditions. One of their functions in non-epithelial cells seems to be the export of CAA derivatives such as the nitric oxide synthase (NOS) inhibitor asymmetrical dimethyl arginine (ADMA). Reduced activity of y^+LATs leads to ADMA accumulation in endothelial cells and may thus contribute to endothelial dysfunction through NOS inhibition. The role of these exporters in other cell types and for other CAA derivatives still needs to be explored.

PL04 Plenary Lecture 4 – Professor Leslie Vosshall

PL04

Understanding and modulating mosquito attraction to humans

L. Vosshall

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My group is interested in the molecular neurobiology of mosquito host-seeking behavior. Female mosquitoes require a blood meal to complete egg development. In carrying out this innate behavior, mosquitoes spread dangerous infectious diseases such as malaria, dengue fever, and yellow fever. Humans attract mosquitoes via multiple sensory cues including emitted body odor, heat, and carbon dioxide in the breath. The mosquito perceives differences in these cues, both between and within species, to determine which animal or human to target for blood-feeding. We have developed CRISPR/Cas9 genome-editing in the yellow fever and dengue vector mosquito, *Aedes aegypti*, with the goal of understand how sensory cues are integrated by the female mosquito to lead to host-seeking behavior. Some of the questions we are currently addressing are: Why are some people more attractive to mosquitoes than others? How do insect repellents work? How are multiple sensory cues integrated in the mosquito brain to elicit innate behaviors? How do female mosquitoes select a suitable body of water to lay their eggs? Recent advances from my group in analyzing the molecular biology of host-seeking behavior will be discussed.

PL05 Plenary Lecture 5 / Lawrie Austin Lecture – Professor Ashley Bush

PL05

Iron in Alzheimer's disease and Parkinson's disease

A. Bush[1,2]

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[2]*Cooperative Research Center for Mental Health, Carlton South, Australia*

Alzheimer's disease (AD) and Parkinson's disease (PD) are incurable and common neurodegenerative diseases that complicate aging. Therapeutic approaches that focus on the protein aggregates that typify these disorders have been disappointing in clinical trials, suggesting that the neurodegeneration is not merely due to proteinopathy. In both diseases, there is a severe dysregulation of metal homeostasis in affected brain tissue, with iron elevation reported in cortex (AD) and nigra (PD). This likely contributes to severe oxidative damage that characterizes both diseases. Iron also increases in the brain and other tissues with normal aging.

We have determined that the major proteins implicated in AD and PD have important functions in iron transport, and are components of an iron regulatory system that fails in aging. The amyloid protein precursor (APP), like ceruloplasmin (CP), facilitates the export of iron from cells by stabilizing cell surface ferroportin, and prevents dietary iron from accumulating in the brain. Tau impacts on iron export by trafficking APP to the cell surface. Elevated CSF ferritin levels have recently been reported to predict conversion of MCI to AD. Knockout mice for both ceruloplasmin and APP develop iron-mediated PD pathology, remedied by iron chelators. The predisposition of the nigra to PD is explained by the enriched population of neurons that coenrich high concentrations of iron with dopamine. Small molecules that target iron accumulation have been effective in animal models of these diseases, and a recent phase 2 clinical trial of deferiprone in PD lowered nigral iron and improved clinical readouts.

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Young Scientist Lectures
YSL01 Young Scientist Lecture 1 – Michael Fox

YSL01

Extracellular matrix molecules induce inhibitory synapse formation

M. Fox

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Although inhibitory synapses comprise only ~ 20% of the total synapses in the mammalian cerebrum, they play essential roles in controlling neural activity. In fact, perturbing inhibitory synapse assembly or function has been associated with autism, epilepsy and schizophrenia. Although many types of inhibitory synapses exist, these developmental disorders have been strongly linked to defects in inhibitory synapses formed by parvalbumin (PV)-expressing interneurons. Despite their importance we lack a complete understanding of the mechanisms that underlie the formation of these inhibitory synapses. With that in mind our attention has been drawn to collagen XIX, an unconventional, nonfibril-forming collagen expressed by interneurons during synaptogenesis. Here we show that mice lacking collagen XIX exhibit spontaneous seizures, are more susceptible to drug-induced seizures, and exhibit schizophrenia-related behaviors – all phenotypes associated with defects in inhibitory signaling. Moreover, collagen XIX-deficient mice exhibit defects in PV+ synapse formation in subiculum, visual cortex and prefrontal cortex. Like other unconventional collagens, the C-terminal domain of collagen XIX is proteolytically shed and functions as a *matricryptin* (i.e. a fragment of an extracellular matrix molecule that exhibits a unique function from the full length molecule from which it was released from). Since matricryptins from other ECM molecules are synaptogenic, we speculated that collagen XIX-derived matricryptins (termed NC1[XIX]) are also synaptogenic. Indeed, *in vitro* assays show that NC1[XIX] triggers the formation of functionally active inhibitory nerve terminals and is sufficient to rescue synaptic defects in the absence of full-length collagen XIX. The synaptogenic activity of NC1[XIX] can be blocked with RGD-containing peptides, function-blocking integrin antibodies, and transcription inhibiting drugs, revealing that integrins and transcription are necessary for NC1[XIX] function. Taken together, these results reveal a novel set of mechanisms governing inhibitory synapse formation.

YSL02 Young Scientist Lecture 2 – Jess Nithianantharajah

YSL02

Evolution of synaptic genes, cognition and disease susceptibility

J. Nithianantharajah

Florey Institute of Neuroscience and Mental Health, Synapse Biology and Cognition laboratory, Parkville, Australia

The origins and evolution of higher cognitive functions including complex forms of learning, attention and executive functions are unknown. A potential mechanism driving the evolution of vertebrate cognition early in the vertebrate lineage (~ 550 million years ago) was genome duplication and subsequent diversification of postsynaptic genes. The postsynaptic density (PSD) of excitatory synapses contains over 1500 proteins, which have been highly conserved between humans and mice, and mutations in these proteins have been implicated in over 130 brain diseases that involve cognitive dysfunction as a core component. Modelling the complex cognitive functions that are routinely assessed in humans and have been conserved amongst vertebrates has been challenging in animal models. The recently developed touchscreen methodology, however, provides an innovative behavioural tool for dissecting higher cognitive functions in rodents. Using this technology, I will discuss our recent research that provides the first evidence that genomic evolution of a vertebrate synaptic gene family was a driving force in increasing cognitive diversity. Moreover, these data highlight the translational capacity of this technology by being the first to assess mice and humans with the same mutations in analogous cognitive tests using touchscreens, providing evidence for conservation of gene function in both these species. Combining innovative behavioural methodologies with novel disease-relevant mouse models provides a new approach to uncovering fundamental insights into the role of synaptic genes in regulating distinct cognitive processes. Moreover, this approach will pave the way forward for understanding the genetic basis of different aspects of cognition to build links to the genetic basis of cognitive diseases.

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Symposia
S01 History of Neurochemistry

S01-01
Neurochemical activities 20 years before the establishment of ISN
F. Fonnum

University of Oslo, Department of Biochemistry Insitute of Basal Medicine, Oslo, Norway

The paper will discuss the activities and techniques used in the years prior to the first meeting of ISN. The first international gathering of neurochemist took place in Bristol in 1952 and at this meeting they decided to organize the first international symposium of neurochemistry. In all 5 such symposia were organised before ISN was established in 1967. From this period I will show some of the microtechniques developed by OH Lowry, H Hyden and E Giacobini. It involves microdissection, analysis of single cells and their application to memory. Further, this is the first confrontation between groups involved in subcellular fractination. This is also the period when A Carlsson suggested that dopamine was not only a precursor for adrenaline and dopamine, but that it was a transmitter candidate. Soon after several laboratories showed the importance of loss of dopamine in Parkinson's patients and the importance of DOPA for the treatment.

S01-02
Roger Rossiter: much more than the first ISN president
P. Beart

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

Roger James Rossiter (born Glenelg, South Australia, 1913), eldest son of an itinerant Methodist schoolmaster, excelled academically and athletically, graduating with a BSc in chemistry and mathematics at the University of Western Australia (1934). He held a Rhodes Scholarship at the University of Oxford, where he graduated DPhil (1940), BM, BCh (1941) and MA (1942). Rossiter's DPhil thesis was entitled, "The Effect of Hormones and Vitamins on Metabolism, with especial reference to the Thyroid Hormones and Vitamins of the B group". After completing his DPhil he engaged in teaching and research at Oxford. His notable first publications were in Biochemical Journal (1939) with eminent biochemists Rudolph Peters and Severo Ochoa. As Major Rossiter, he served in the British Army during WWII where he was drawn into war-related research on burns, trauma, malaria and subsequently nutrition. Returning to Oxford (1946), he was recruited to the University of Western Ontario (1947) where he served as Professor of Biochemistry, publishing his first neurochemical papers on brain lipids e.g. Nature (1949): "Lipids of peripheral nerve during Wallerian degeneration". Rossiter developed an active neurochemistry group working on lipids, and phosphorus metabolism in neuropathies, establishing an international reputation for his research on the chemistry of myelin and demyelination. His total publications exceeded 200 and he served on the Editorial Board of the Journal of Neurochemistry from 1956 to 69. Professor Rossiter became part of an international group of neurochemists whose discussions from 1962, eventually led to the formation of ISN. He was elected Chairman at the first Council meeting on July 27th 1967 and subsequently served on the ISN Membership and Publication Committees. Professor Rossiter made important contributions to neurochemistry internationally though his service to the World Federation of Neurology's Commission on Neurochemistry (1960), as well as contributing to all levels of medical research in Canada (Fellow of Royal Society of Canada, 1954). Eventually Rossiter became involved in administration and health sciences. He died of heart failure in Helsinki in 1976 whilst gathering data on health care.

S01-03
The first ISN meeting in Australia and the formation of APSN
G. Johnston

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The 13th Biennial ISN meeting in Sydney in July 1991 was an outstanding success scientifically, socially and financially. Sydney was at its sparkling best, the excellent weather enabling neurochemists to fully enjoy the many attractions of the Darling Harbour site. Despite the Gulf war being raged at the time of registration and submission of abstracts, we had 833 registrants. More than 150 Australians attended the meeting. As we had only 26 ISN members in Australia at the time, this indicates that the meeting was very successful in stimulating local interest in neurochemistry.

Neurochemistry interest was further stimulated in the region at the Sydney ISN meeting by the formation of the Asian Pacific Society for Neurochemistry largely at the initiative of the Japanese Society for Neurochemistry and modelled on the American and European regional neurochemistry societies, holding meetings every 2 years in the years when an ISN meeting is not held.

We had made the bid in 1988 for an ISN meeting on behalf of the Australian Neuroscience Society. The bid was accepted in 1989 for the 14th meeting is 1993. However the hosts of the 1991 meeting proposed for Montpellier requested more time to organise their meeting and Sydney ended up hosting the 1991 meeting at rather shorter notice than usual. Our only real hiccup in organising the meeting was when the truck carrying the abstract booklets was highjacked en route from New York to the airport.

The Sydney meeting was cosponsored by the Australian Neuroscience Society who used its share of the profits to further stimulate neurochemistry in Australia, and to encourage continued interaction with the ISN and between neurochemists in the Asian Pacific region. The APSN held its 14th meeting in South Korea in 2014.

It was my privileged to be Chair of the Organising Committee for the Sydney ISN meeting and first President of the APSN.

S01-04

Traditional medicine partnerships for cultural preservation, drug discovery and capability strengthening

J. Jamie, S. Vemulpad

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Approximately 80% of people in developing countries rely on traditional medicine for their primary healthcare. The study of traditional medicinal knowledge is also a successful avenue for drug discovery. For example, approximately 80% of all plant-derived drugs that are in current use globally were discovered as a direct result of the study of plants used by humans as medicines (ethnomedical use) and are used for the same or related ailments as the original medicinal plant.

Australian Aboriginal people have a vast knowledge of their regional flora and have used plants as medicines for thousands of years. Despite the biological uniqueness, cultural significance and medicinal potential of these native flora, there has been limited first-hand (ethnobiological) documentation of Australian Aboriginal traditional medicines and limited bioactivity/chemical investigations to provide evidence for their medicinal uses.

The Indigenous Bioresources Research Group (IBRG) of Macquarie University, Sydney, Australia, work in collaborative partnership with Indigenous people to document and preserve first-hand traditional medicinal knowledge; identify flora of significant medicinal potential and evaluate their effectiveness; identify the major bioactive components responsible for the medicinal properties of the flora; and provide capability strengthening opportunities for the Indigenous community partners. The IBRG have well established partnerships with Indigenous people, including of over a decade with Yaegl Aboriginal Elders of northern New South Wales.

This presentation provides an overview of the importance of traditional medicines and a brief review of New South Wales traditional medicines, including those used for headaches, sores, wounds, skin infections and for general wellbeing. It additionally presents some cultural, scientific and educational goals and achievements through showcasing of its partnership with Yaegl Aboriginal Elders. This case study will particularly highlight the immense reward that truly collaborative partnerships between academics and Indigenous communities can bring, such as the two-way exchange of knowledge and skills and various capability strengthening outcomes, including towards the general health and wellbeing of Indigenous people.

S02 GSK3 Signaling in Alzheimer's Disease Pathogenesis and Drug Development

S02-01

The central role of GSK3β in AMPA receptor endocytosis/ LTD and its implication in ad memory impairments

Y. T. Wang

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Although glycogen synthase kinase 3β (GSK3β) has been implicated in the neuropathogenesis of Alzheimer's Disease (AD), the detailed underlying mechanisms remain largely unknown. Here, we present some evidence that strongly supports a hypothesis that GSK3β may at least in part contribute to AD pathogenesis by increasing endocytosis of AMPA subtype glutamate receptors (AMPARs), and hence disrupting the fine balance between long-term potentiation (LTP) and long-term depression (LTD), the two most well-characterized forms of synaptic plasticity. We found that the GSK3β signaling pathway is required for facilitated AMPA receptor endocytosis and the expression of LTD. Moreover, this GSK3β-mediated AMPAR endocytosis is actively inhibited during LTP induction through activating PI3K/Akt phosphorylation and inhibition of GSK3β. These results demonstrate that GSK3β may play an essential role in mediating AMPAR endocytosis, thereby impairing LTP maintenance and/or promoting LTD production. As increased GSK3β activity has been implicated in AD animal models and patients, we have also investigated if increased AMPAR endocytosis can be at least in part attributed to deficits of synaptic plasticity and memory in an AD model of APP23/PS45 double transgenic mice. We found that there is a severe impairment in the maintenance of hippocampal CA1 LTP and special memory in these mice, and importantly both synaptic impairments and memory deficits can be largely reversed by inhibiting AMPAR endocytosis with $GluA2_{3Y}$ peptide, a well-characterized inhibitor for facilitated AMPAR endocytosis/LTD expression. Our results suggest that GSK3β overactivation may contribute to AD pathogenesis by promoting AMPAR endocytosis and that AMPAR endocytosis provides valuable novel targets for the development of new therapeutics for AD.

S02-02

Regulation of BACE1 expression and amyloidogenesis

R. Vassar

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The β-secretase, β-site amyloid precursor protein (APP) cleaving enzyme 1 (BACE1), is the first of two proteases (the second being γ-secretase) to cut APP to generate the β-amyloid peptide (Aβ) implicated in Alzheimer's disease (AD) pathogenesis. As such, BACE1 is a prime therapeutic target for lowering cerebral Aβ levels as a treatment strategy for AD. The highest levels of BACE1 in the body are found in neurons of the brain, where it is localized within endosomal compartments of cell bodies and presynaptic terminals under non-pathologic conditions. However, in AD brain, BACE1 levels increase several fold, and vesicles containing the enzyme accumulate within swollen, distended axons that pass near amyloid plaques. These peri-plaque dystrophic axons also accumulate APP, suggesting the possibility that increased enzyme and substrate might accelerate Aβ production near amyloid deposits, thus exacerbating plaque growth and AD progression. I will present evidence that BACE1 accumulation in dystrophic axons is associated with increased levels of BACE1-cleaved fragments of APP and Aβ in our 5XFAD mouse model of AD amyloidosis. Possible mechanisms of Aβ-induced BACE1 accumulation will be discussed. We conclude that Aβ causes increased levels of BACE1 that in turn lead to further Aβ production, instigating a vicious cycle of AD pathogenesis.

S02-03

Role of glycogen synthase kinase 3β in alzheimer's neurodegeneration and the potential intervention

W. Jian-Zhi

Key Laboratory of Ministry of Education of China for Neurological Disorders, Tongji Medical College, Huazhong University of Science and Technology, Pathophysiology, Wuhan, China

Glycogen synthase kinase 3b (GSK-3b) is the first identified tau kinase. Our group has demonstrated that can phosphorylate tau at multiple Alzheimer-associated sites. Activation of GSK-3b both pharmacologically and genetically induces spatial memory deficits in mice and in rats accompany with neuronal apoptosis, while the simultaneous tau hyperphosphorylation makes the cells more resistant to GSK-3b-induced cell death. We also found that upregulation of GSK-3b inhibits long-term potentiation with suppression of presynaptic vesicle exocytosis by phosphorylating P/Q-type calcium channel and interrupting SNARE complex formation. Phosphorylated of tau by GSK-3b promotes reciprocally tau SUMOylation, which inhibits tau ubiquitination and prohibits tau degradation. GSK-3b activation aggrevates beta-amyloid-induced neuronal damages, inhibits protein phosphatase-2A, a crucial tau phosphatase that can dephosphorylate Alzheimer-tau at multiple sites, and mediates the olfactory deficit-induced hippocampal impairment. The disease-modified GSK-3b intervention arrests the pathology and memory deficits in an Alzheimer's animal model. Our studies provide new insights for the crucial role of GSK-3b in Alzheimer's neurodegeneration and memory impairment, which also shed light for potential intervention.

S02-04

GSK3 signaling regulates app processing and Aβ production and its pharmaceutical potential for alzheimer's disease

W. Song

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Alzheimer's Disease (AD) is the most common neurodegenerative disorder leading to dementia. Deposition of amyloid β protein

(Aβ) to form neuritic plaques in the brains is the unique pathological feature of AD. Aβ is generated from sequential cleavages of the β-amyloid precursor protein (APP) by the β- and γ-secretases. Beta-site APP cleaving enzyme 1 (BACE1) is the β-secretase essential for Aβ generation. Increased Aβ levels could facilitate AD pathogenesis and inhibition of Aβ generation may have therapeutic implications for AD treatment. Our studies showed that regulation of BACE1 expression plays an important role in AD pathogenesis and could be a valid target for AD drug development. BACE1 tightly controlled APP processing and Aβ production in normal condition, and selection of β-secretase cleavage site by BACE1 had a dramatic effect on Aβ production in the pathological condition. Previous studies have indicated that glycogen synthase kinase 3 (GSK3) may play a role in APP processing by modulating γ-secretase activity, thereby facilitating Aβ production. We found that specific inhibition of GSK3β reduced BACE1-mediated cleavage of APP and Aβ production by decreasing BACE1 gene transcription and expression. The regulation of BACE1 gene expression by GSK3β was dependent on NFκB signaling. Inhibition of GSK3 signaling markedly reduced Aβ deposition and neuritic plaque formation, and rescued memory deficits in the double transgenic AD model mice. Our study suggests that interventions that specifically target the β-isoform of GSK3 may be a safe and effective approach for treating AD.

S03 A Glial Spin on CNS Regeneration: Mechanisms that Regulate Oligodendrocyte Remyelination

S03-01

Multiple roles of fibroblast growth factor receptor signaling during development, myelination and remyelination

R. Bansal

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Myelination is a multistep process where oligodendrocytes progress through a well-coordinated differentiation program regulated by multiple extracellular growth and differentiation signals. By the analysis of a series of mice lacking Fibroblast Growth Factor Receptor-1 and -2 (FGFR1/2) at different stages of myelinogenesis we have shown that FGFR1/2 signaling is first required for embryonic induction of oligodendrocyte progenitors and then later for the growth of the myelin sheath during myelin formation and maintenance, thus revealing a biphasic requirement of these receptors during myelination (Furusho et al., 2011; 2012; Ishii et al., 2013; 2014). Our ongoing studies show that downstream of FGFRs, Erk1/2 play a key role in driving myelin growth. In addition to their multiple roles during developmental myelination and myelin maintenance, FGFRs also play an important role during remyelination. We have established acute and chronic cuprizone-induced mouse models of demyelination in mice lacking both *Fgfr1* and *Fgfr2* in oligodendrocytes and their progenitors. The data suggest that in the acute model, the overall effect of FGFR1/FGFR2 signaling is neither beneficial nor detrimental for oligodendrocyte differentiation. In contrast, in the chronic model, it is beneficial in promoting the endogenous repair process. Thus, FGF-based therapies have potential value in stimulating oligodendrocyte and myelin regeneration in late-stage disease. [Supported by NIH grant NS38878].

S03-02

BDNF exerts distinct influences upon neurons and oligodendrocytes to promote myelination

J. Xiao, A. Wong, R. Wood, T. Kilpatrick, S. Murray

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Brain-derived neurotrophic factor (BDNF) has been implicated in promoting myelination during development and remyelination following injury. However, the precise mechanisms that BDNF utilizes to regulate CNS myelination remain unclear. The analysis of BDNF heterozygous (±) mice suggests that BDNF regulates both the initiation of myelination and the thickness of myelin membrane in the CNS *in vivo*. We have found that oligodendrocyte-expressed TrkB regulates the thickness of the myelin membrane, as ablating TrkB selectively from oligodendrocytes *in vivo* ($TrkB^{fl/fl}$ MBPcre mice) results in significantly thinner layers of myelin, a phenotype also observed in BDNF± mice. Concordant with this, we have further demonstrated that BDNF promotes myelination *in vitro* via activating oligodendroglial Erk1/2, key factors known to control myelin wrapping *in vivo*. Interestingly, oligodendrocytes in the $TrkB^{fl/fl}$ MBPcre mice make normal initial contact with axons and ensheath the same number of axons as the control mice, demonstrating TrkB expression in oligodendrocytes exerts no influence upon the initiation of myelination. Myelin formation is tightly controlled by reciprocal interactions between neurons and oligodendrocytes. Our observations in the $TrkB^{fl/fl}$ MBPCre mice suggest that BDNF must signal through another cell type in the CNS to promote the initiation of myelination. We subsequently generated neuronal-specific TrkB conditional knockout mice ($TrkB^{fl/fl}$ NFL-Cre). The loss of TrkB expression in neurons resulted in significantly impaired initiation of myelination *in vivo* and *in vitro*, a complementary phenotype to the $TrkB^{fl/fl}$ MBPcre mice. Importantly, the impaired initiation of myelination in $TrkB^{fl/fl}$ NFLCre mice is not confounded by either neuronal loss or change in axonal caliber size. Our data therefore demonstrate that neuronal TrkB is required for the initiation of CNS myelination during early postnatal development. Together, these findings indicate that BDNF regulates CNS myelination via distinct cellular mechanisms - regulating the initiation of myelination via neuronal TrkB, while regulating the thickness of myelin membrane via oligodendroglial TrkB.

S03-03

The role of OPC developmental heterogeneity in remyelination

A. Crawford[1], R. Tripathi[2], W. Richardson[2], R. Franklin[1]

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[2]*University College London, Wolfson Institute for Biomedical Research and Research Department of Cell and Developmental Biology, London, United Kingdom*

Within the developing central nervous system oligodendrocyte progenitor cells (OPCs) emerge as discrete populations, ventral and dorsal, raising the question of whether these may represent functionally distinct populations. To determine the relative roles of the dorsal and ventral OPC populations in remyelination a transgenic mouse line has been developed in which the populations can be genetically traced due to their differential expression of fluorescent proteins. Lysolecithin injection into the spinal cord and corpus callosum was performed to create focal white matter demyelinating lesions, and the remyelination process then monitored to 60 days post injection. The dorsal OPC population demonstrated an increased propensity to contribute to remyelination, with a greater proliferative response and a significant increase in cell number within the lesioned tissue. In culture, dorsal OPCs showed enhanced migration and increased efficiency of differentiation into mature oligodendrocytes when compared with ventral OPCs. However, when the toxin model was repeated using aged animals, the dorsal population underwent an age-associated decline in differentiation efficiency. Comparative gene expression analysis

has identified genes differentially expressed between the 2 populations. Determining which of these genes may contribute to the dorsal cells' enhanced migration and differentiation abilities, but importantly also their increased susceptibility to age related differentiation failure, may reveal new targets for the therapeutic enhancement of endogenous remyelination.

S03-04

Adeno-associated gene transfer viral vectors for remyelination therapies in genetic leukodystrophies

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Patients with genetic leukodystrophies such as Krabbe disease, metachromatic leukodystrophy and other undergo degeneration of myelinating oligodendrocytes, impacting on the entire functional architecture of the central nervous system. Krabbe disease is caused by the deficiency of galactosyl-ceramidase (GALC), a lysosomal enzyme that degrades galactosyl-sphingolipids. Krabbe patients accumulate high levels of galactosyl-sphingosine or psychosine. Psychosine associates with lipid rafts where it disrupts membrane architecture and alters crucial cell signaling such as the IGF-1 pathway. The successful treatment of Krabbe disease requires preventing the accumulation of psychosine in oligodendrocytes, and normalization of associated pathogenic signaling. In this study, we have evaluated side-by-side different pseudotyped adeno-associated viral (AAV) vectors to deliver therapeutic expression of GALC to oligodendrocytes and other neural cells *in vitro*. Results showed major benefits of the AAV9-type vectors to deliver expression of corrective enzyme to neurons and other non-myelinating glia. Other vectors like AAV-001 successfully targeted expression cassettes to oligodendrocytes. These vectors appear as potentially powerful tools for global gene therapy in a variety of leukodystrophies. We will present data of their efficacy to treat demyelination in a mouse model of Krabbe disease.

This work was supported by grants from NIH and the Legacy of Angels Foundation to ERB.

S04 Autophagy and Mitophagy in Neurodegeneration: From Basic Mechanisms to Prospects for Therapy

S04-01

Autophagy and neurodegeneration

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Intracellular protein aggregation is a feature of many late-onset neurodegenerative diseases, including Parkinson's disease, tauopathies, and polyglutamine expansion diseases (like Huntington's disease (HD)). Many of these mutant proteins, like that causing HD, cause disease via toxic gain-of-function mechanisms. Therefore, the factors regulating their clearance are crucial for understanding disease pathogenesis and for developing rational therapeutic strategies.

The two major intracellular protein degradation pathways are the ubiquitin-proteasome system and (macro) autophagy. Autophagy is initiated by double-membraned structures, which engulf portions of cytoplasm. The resulting autophagosomes ultimately fuse with lysosomes, where their contents are degraded.

I will briefly describe the basic biology of autophagy before outlining its roles in neurodegeneration. We showed that the autophagy inducer, rapamycin, reduced the levels of mutant huntingtin and attenuated its toxicity in cells, and in *Drosophila* and mouse HD models. We have extended the range of intracellular proteinopathy substrates that are cleared by autophagy to other related neurodegenerative disease targets, like Parkinson's disease. While autophagy induction is protective in models of various neurodegenerative diseases, certain other conditions are associated with compromised autophagy. I will discuss how two genetic variants in Parkinson's disease and Alzheimer's disease impact on autophagosome biogenesis.

S04-02

Mitophagy in parkinson's disease: brakes and accelerators

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The elimination of damaged mitochondria by mitophagy represents a major mechanism ensuring the maintenance of high quality mitochondria in neurons. We found that mitophagy is triggered in multiple models of Parkinson's disease to include complex I inhibitors, oxidative neurotoxins, PINK1 deficiency and mutant LRRK2 expression. However, excessive mitochondrial clearance from dendrites that is not balanced by adequate replacement results in neurodegeneration. PINK1 and protein kinase A play multiple roles in maintaining dendritic mitochondrial homeostasis, in part by regulating mitochondrial calcium, transport and mitophagy.

S04-03

Mitophagy: the roles of PINK1 and PARKIN

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PINK1 and *PARKIN* are the causal genes responsible for hereditary recessive early-onset Parkinsonism. PINK1 is a mitochondrial Ser/Thr kinase whereas Parkin is a ubiquitin-protein ligase (HECT/RING hybrid E3) that catalyzes ubiquitylation of diverse mitochondrial outer membranous proteins (Matsuda et al., JCB 2010). PINK1 and Parkin have been shown to cooperate in the recognition, labeling, and clearance of damaged (i.e., depolarized) mitochondria by selective autophagy (mitophagy). Loss of transmembrane potential ($\Delta\Psi$m) causes the accumulation due to escape from $\Delta\Psi$m-dependent destruction and the activation via self-phosphorylation of PINK1 on depolarized mitochondria (Okatsu et al., Nat Commun 2012, JBC 2013, JCS 2015). Previously we and other groups revealed that PINK1 acts as an upstream factor for Parkin and is essential for both the activation of latent Parkin and recruiting Parkin onto depolarized mitochondria. However, how PINK1 activates latent Parkin and recruits cytoplasmic Parkin to damaged mitochondria remain to be fully elucidated. Recently we found that PINK1 phosphorylates both Parkin and ubiquitin that are sufficient for full activation of Parkin E3 activity, and indeed phosphomimetic ubiquitin bypassed PINK1-dependent activation of a phosphomimetic Parkin mutant in cells. Thus it is clear that the phosphorylated ubiquitin is a Parkin activator (Iguchi et al., JBC 2013, Koyano et al., Nature 2014). Subsequently we revealed that a phosphomimetic ubiquitin chain recruits Parkin to energized mitochondria in the absence of PINK1, whereas a wiled-type ubiquitin chain does not, and that a cellular ubiquitin replacement system confirmed that ubiquitin phosphorylation is indispensable for Parkin translocation. Furthermore, physical interactions between phosphomimetic Parkin and phosphorylated polyubiquitin-chain were detected by immunoprecipitation *in vivo* and *in vitro*. We thus propose that the phosphorylated ubiquitin chain functions as the genuine Parkin receptor for recruitment to depolarized mitochondria (Okatsu et al., JCB 2015).

S04-04

Mechanisms of mitochondrial quality control in autosomal recessive parkinson's disease

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Parkinson's disease is a common, most often sporadic neurodegenerative disorder, caused in nearly 10% of the cases by mutations in single genes with autosomal dominant or recessive inheritance. The genes encoding the E3 ubiquitin protein ligase Parkin (*PARK2*) and the mitochondrial serine/threonine kinase PINK1 (*PARK6*)

account for clinically similar, autosomal recessive early onset forms. Over the past 10 years, a number of studies in different model systems demonstrated that PINK1 and Parkin regulate jointly several processes relevant to maintenance of mitochondrial quality, including mitochondrial trafficking and dynamics, mitophagy and degradation or biogenesis of specific mitochondrial components.

Our team is interested in dissecting the mechanisms by which these proteins maintain mitochondrial quality and evaluate the consequence of their dysfunction in Parkinson's disease. By using a combination of approaches of cell biology, confocal imaging and biochemistry in different cell models, we recently showed that loss of protein import efficiency triggers recruitment of Parkin by PINK1 in proximity of the translocase of outer mitochondrial membrane (TOM). We provided evidence that the degradation of specific TOM subunits plays a key role in initiating the autophagic degradation of damaged mitochondria. We also showed that PINK1 and Parkin interact with the TOM machinery on polarized mitochondria. This interaction may modulate the import of mitochondrial proteins essential to mitochondrial maintenance, such as the multifunctional matrix enzyme 17beta-hydroxysteroid dehydrogenase 10, which is depleted in Parkin-deficient mice and Parkinson's disease patients. Finally, the use of electron and confocal microscopy and calcium imaging techniques to characterize structurally and functionally the endoplasmic reticulum-mitochondria interface, a compartment previously linked to neurodegeneration, revealed enhanced interaction between these organelles in cells from Parkin-deficient mice and patients with *PARK2* mutations. Our current work aims at investigating the relevance of mitochondrial quality control mechanisms regulated by PINK1 and Parkin in different cell types of the central nervous system, to better understand their contribution to the physiopathology of autosomal recessive Parkinson's disease.

S05 Harnessing Human Genetics to Define the Biochemical Pathways involved in Brain Development

S05-01

Studying copy-number variation in human neurological syndromes to identify a novel gene regulatory mechanism for brain development

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Recent improvements in genome sequencing technologies have empowered researchers and clinicians with a means to investigate the genetic basis for neurological disorders that result from copy-number variation (CNV). However, what continues to remain a challenge is to establish the pathogenicity of genomic abnormalities, such as CNVs, and their causative effects on nervous system impairment. Here, we clarify possible genotype-phenotype relationships in human subjects with brain developmental disorder which are associated with microdeletions to 1q43–44. Our investigation has led to the identification of *ZNF238* as a critical gene for brain development. Using a range of molecular and cellular approaches combined with *in utero* electroporation with mice, we demonstrate that loss of *ZNF238* expression leads to impairments in the production of cerebral cortical neurons during fetal development. Our studies further reveal a novel gene regulatory function for ZNF238 in the placement of newborn cerebral cortical neurons as they develop to form functional circuits. Thus, we conclude that loss of *ZNF238* causes impairments in neuronal development and lead to brain developmental disorder in humans. This work leads to an improved understanding of the molecular basis for 1q43–44 CNVs in human health and mental dysfunction.

S05-02

Comprehensive approach to understand pathophysiological role of SIL1, a gene causing intellectual disability

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While many different biological causes have been implicated in the etiologies of neurodevelopmental disorders such as autism-spectrum disorders and intellectual disability (ID), genetic factors are considered to be the most important. Thus, it is essential to clarify the physiological and pathophysiological significance of respective disease-related genes in the brain development and diseases, respectively. To address this issue, we have established an analytical battery of *in utero* electroporation-based *ex vivo* and *in vitro* observations (cortical neuron migration, axon elongation, dendrite development, spine morphogenesis and live-imaging) as well as cell biological and biochemical analyses.

Here we chose Marinesco-Sjogren syndrome (MSS), a rare autosomal recessive disorder with ID, as a disease model. By use of the test battery, we examined the pathophysiological role of the causative gene, SIL1, encoding an endoplasmic reticulum resident cochaperone.

SIL1-silencing caused neuronal migration delay during corticogenesis *ex vivo*. While RNAi-resistant SIL1 rescued the defects, MSS-causing SIL1 mutants did not. When SIL1 was silenced in cortical neurons in one hemisphere, axon growth to the contralateral hemisphere was delayed. MSS-causing mutants had lower affinities to the binding partner chaperone HSPA5 *in vitro*, and SIL1-HSPA5 interaction was crucial for neuronal migration *ex vivo*. Furthermore, time-lapse imaging with a confocal microscope revealed morphological disorganization is associated with abnormal migration of SIL1-deficient neurons. On the other hand, cell cycle of neuronal progenitor cells at ventricular zone was not affected by SIL1-silencing. These results suggest that the MSS-causing mutations prevent SIL1 from interacting with and regulating HSPA5, leading to abnormal neuronal morphology and migration, which may contribute to abnormal brain development and cause ID in MSS.

S05-03

Unexpected activities of the complement pathway in migrating neurons

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During recent years the notion that the immune system participates in regulation of complex behavior and it's malfunctioning may result in autism spectrum disorder has emerged. However, the possibility that immune molecules function in migrating neurons in the developing brain has not been well explored. The innate immune complement pathway is composed of a cascade of proteases, which cleave complement proteins resulting in cell surface bound and receptor activating molecules, leading to cell destruction as a defense response to pathogens. We postulated that this pathway works in a limited fashion to regulate radial neuronal migration. Neuronal migration impairment is observed in complement C3 knockout and knockdown brains. Migration was rescued following addition of C3 mimicry cleavage products. C3 cleavage is downstream to either the classical or the lectin arms of the cascade; whereas interventions in the classical pathway mildly affected neuronal migration, knockout or knockdown of the lectin pathway components MASP1 or MASP2 resulted in severe migration

deficits. Addition of C3 mimicry cleavage products or agonists of the downstream receptors C3aR and C5aR rescued MASP2 knockdown, corroborating that complement pathway activity is required for neuronal migration progression. These findings are important in explaining part of the pathophysiology of autism spectrum disorders and 3MC syndrome.

S05-04

The role of the tubulin gene family in development and disease

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The generation of a human brain requires the birth, migration and differentiation of neurons. Each of these complex cellular events is dependent on the microtubule cytoskeleton. Microtubules are required for interkinetic nuclear migration, the separation of sister chromatids in mitosis, nuclear translocation during migration and the extension of axons. The importance of microtubules in brain development is underpined by the finding that mutations in their structural subunits, the alpha and beta tubulins, cause a spectrum of neurological diseases collectively known as the tubulinopathies. These diseases range from lissencephaly (TUBA1A); to microcephaly (TUBB5); to polymicrogyria (TUBB2B); and to motor neuron disease (TUBA4A). This talk focuses on the role mouse models have played in elucidating the underlying molecular pathology associated with these disease states.

S06 Biological and Therapeutic Roles of Glycine Receptors

S06-01

New biological roles for glycine receptors containing the α2 and α4 subunits

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Glycine receptors (GlyRs) are ligand-gated ion channels that mediate inhibitory synaptic transmission in the spinal cord, brainstem, cerebellum and retina. The major adult GlyR isoform, consisting of α1 and β subunits, has a major role the control of spinal motor reflex circuits. Mutations in the genes encoding this GlyR subtype cause startle disease/hyperekplexia, which affects newborn children and is characterised by noise or touch-induced seizures that result in muscle stiffness and neonatal apnoea episodes. However, it has recently emerged that other GlyR subtypes containing the α2, α3 and α4 subunits may play more diverse biological roles. For example, the GlyR α3 subunit acts as a molecular switch, modulating G-protein-coupled receptor mediated signaling pathways involved in inflammatory pain sensitization and rhythmic breathing. Although GlyR α2 subunit knockout mice were initially reported as lacking a behavioural or morphological phenotype, we recently demonstrated that GlyR α2 is involved in cerebral cortical neurogenesis. Extrasynaptic activation of GlyRs containing the α2 subunit in cortical interneurons activates voltage-gated Ca^{2+} channels that modulate actomyosin contractility to fine-tune neuronal migration in the developing cortical wall. Loss of GlyR α2 subunits leads to a selective depletion of cortical neurons and microcephaly in newborn *Glra2* knockout mice. Lastly, although the GlyR α4 subunit gene is considered to be a pseudogene in humans, using structure-function and mutagenesis methods, we have found that GlyRs containing the α4 subunit are fully functional in other species, including zebrafish, mice and apes. Gene knockdown, dominant-negative mutants and genetraps for *glra4a* in zebrafish suggest that GlyRs containing the α4 subunit underpin startle-evoked escape responses, leading to the intriguing hypothesis that humans respond to startle differently to other species. *Supported by the Medical Research Council, Action Medical Research and UCL Impact studentships.*

S06-02

Plasticity of glycinergic synapses and related behaviors in zebrafish

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Neurotransmitter receptors accumulate at postsynaptic sites and this clustering is necessary for efficient transmission. It has been shown that glycinergic synapses are formed and regulated in an activity-dependent manner in cultured neurons and that this synaptic plasticity is primarily mediated by diffusion of glycine receptors (GlyRs) in synaptic and extrasynaptic sites on the plasma membrane. To assess the formation and plasticity of glycinergic synapses *in vivo*, we studied postsynaptic clustering of GlyRs in zebrafish. We performed chemical screening to search for drugs that affect glycinergic synapse formation in zebrafish larvae and found that L-type calcium channels and CaMKII play important roles in the clustering of GlyRs. Our GAL4/UAS-mediated targeted gene expression revealed that CaMKII regulates the accumulation of GlyRs at synaptic sites. We also developed an acoustic-induced behavioral assay in zebrafish larvae and confirmed a previous finding that application of repetitive sound reduces the probability of acoustic-induced escape response in fish (Oda et al., 1998). Furthermore, we established imaging of GlyRs in live zebrafish and demonstrated that acoustic-induced behavioral plasticity is attributable to CaMKII-mediated synaptic clustering of GlyRs in Mauthner neurons, which trigger the escape response. Taken together, our results reveal the molecular mechanisms underlying glycinergic synapse formation and plasticity.

S06-03

New small molecule analgesics that target the ALPHA3 glycine receptor

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Novel pharmacophores for treating chronic pain are desperately needed. Glycine receptor (GlyR) Cl channels mediate inhibitory neurotransmission in the spinal cord and some brain regions. The vast majority of glycinergic synapses exclusively incorporate α1 GlyR subunits. However, glycinergic synapses on pain sensory neurons in the spinal cord are unique in that they incorporate α3 GlyR subunits. Inflammatory mediators (e.g., prostaglandin E2) induce chronic inflammatory pain by phosphorylating, and inhibiting, α3 GlyRs. This reduces the magnitude of glycinergic inhibitory synaptic currents in spinal pain sensory neurons, thereby 'disinhibiting' these neurons and increasing the rate of transmission of pain impulses to the brain. This mechanism, which provides a paradigm for understanding chronic pain sensitisation, implies that drugs that selectively enhance (i.e., restore) α3 GlyR function should be analgesic. Here I describe the development of a small molecule, picomolar-potent α3-specific enhancer that elicits potent analgesia in animal models of chronic pain.

S06-04

GLYR mouse models and startle disease

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Disturbances in the balance between inhibition and excitation lead to different forms of disease in the central nervous system. Startle disease/Hyperekplexia is a rare human neuromotor disorder due to defects in glycinergic neurotransmission. The major symptoms are exaggerated startle reflexes and loss of postural control. Mutations in genes encoding for the glycine receptors (GlyRs), which are localized in membranes of motoneurons, are the major underlying causes for the observed pathology. Recently, we identified disturbed neuronal ER-Golgi trafficking of mutated glycine receptors as a novel pathway in human hyperekplexia. Mutations in loops B and D/beta1-2 of the extracellular N-terminal domain affect either ligand-binding or the overall biogenesis of the receptor complex. Moreover, mice carrying a mutation in an extracellular loop structure do resemble a hyperekplexia-like phenotype similar to human patients. Loop D/beta1-2 was depicted as an essential component for GlyR biogenesis with only a small fraction of mutated receptors being transported and integrated into the outer cell membrane. GlyR variants of the large intracellular TM3-4 loop and TM4 affect receptor assembly (e.g. pentamerization) or desensitization of the ion channel.

Nonsense mutations accompanied with generation of an early STOP codon are functional NULL mutants. In the mouse model oscillator, we were able to show a functional rescue of a truncated variant when the lacking part was co-expressed in the same cell. Efficacy of rescue in terms of regeneration of glycine-gated chloride currents was determined with 50% compared to wild type. A similar truncation has also been identified from human patients. Here, again a functional restoration of the ion channel complex of both for themselves non-functional domains was demonstrated. These results provide further evidence for the assembly of receptors from independent folding domains.

S07 Microglia-Vasculature Interactions as Determinants of Neurologic Diseases and Stroke

S07-01

Live imaging of innate immune response following ischemic injury: distinct microglia activation profiles in young and aged brains

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Microglial activation is a hallmark of brain inflammatory response to ischemic injury and increasing evidence suggest that any changes in activated microglial phenotype may significantly affect the outcome after stroke. Importantly, microglial activation after stroke is characterized by a robust up-regulation of the Toll like receptor 2. To characterize cellular activation profiles we took advantage of transgenic mouse model, a Toll-like receptor 2 (TLR2) –luc/GFP reporter model developed in our laboratory. In this model, microglial activation and innate immune response can be visualized in real time from the brains of live animals using *in vivo* bioluminescence/biophotonic imaging and high resolution CCD camera. Using this model-system, we investigated how microglial cells change their activation profiles in model of aging in female brains and in response to ischemic injury. The analysis of *in vivo* imaging results in control conditions and after ischemia revealed aberrant activation of TLR2 response together with over-induction of IL-6 JAK/STAT pathway and significant decrease in galectin-3 levels. Intriguingly, *in vivo* imaging analysis of innate immune response in neonatal brains revealed an early decrease in TLR2 signals in response to immune challenge. Taken together, our results strongly suggest development of distinct, age- dependent microglia activation profiles and innate immune responses in the brain response to ischemic injury and immune challenges.

S07-02

Microglial cells as protectants of neurovascular integrity in neonatal stroke

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Microglial cells were considered notoriously toxic after stroke for a long time but there is mounting evidence to also suggest a protective role for microglia. The "diversity" of the microglial phenotypes and their contribution to brain repair after stroke is being increasingly recognized. Emerging evidence indicates that the timing of ischemic injury during brain development has a major impact on the pathophysiology of ischemia. For example, in a comparative study we showed that blood-brain barrier (BBB) integrity is strikingly better preserved in acute neonatal stroke than in adult stroke induced by a transient MCA occlusion (tMCAO). We discovered that microglial cells serve as endogenous neuroprotectants in neonatal arterial stroke, as pharmacological depletion of microglial cells greatly enhances the excitotoxic and inflammatory responses and injury. Furthermore, microglial depletion adversely affects BBB integrity after neonatal stroke and induces hemorrhagic transformation 24–72 h after tMCAO in postnatal day 10 mice. We will discuss the mechanisms by which microglial cells exert neurovascular protection and limit neuroinflammation after neonatal stroke. We will demonstrate that activated microglia can affect BBB integrity in injured immature brain in several ways, including limiting vascular degeneration, affecting astrocyte function and leukocyte trafficking ($CCr2^{RFP/+}$ monocytes and $Ly6G^{+}$ neutrophils). We will then discuss the contribution of TLR2 activation in injury after neonatal stroke using MRI and biophotonic imaging of TLR2 in neonatal *luc/GFP*-TLR2 mice.

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S07-03

Neurovascular interactions: mechanisms, imaging, therapeutics

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Protection of the CNS from leakage of plasma proteins by the blood-brain barrier (BBB) is lifted in a wide range of neuroimmune and neurodegenerative diseases, as well as after traumatic injury. However, whether plasma proteins contribute to neuroinflammation and neuronal damage remains poorly understood. Our laboratory has discovered pleiotropic functions for the plasma protein fibrinogen in the activation of innate immunity in the CNS, neurodegenration, and inhibition of repair. Such diverse functions have mechanistic underpinnings on the unique structure of fibrinogen, which contains multiple binding sites for cellular receptors and proteins expressed in the nervous system. Fibrinogen is a potent pro-inflammatory mediator in the nervous system by activating the CD11b/CD18 integrin receptor (also known as Mac-1 and complement receptor 3) in microglial cells. Using *in vivo* imaging in the mouse spinal cord using two-photon microscopy, we showed that in Experimental Autoimmune Encephalomyelitis (EAE), a model of multiple sclerosis (MS), microglia rapidly perform constant surveillance of blood vessel walls and specifically cluster around blood vessels with fibrin deposition. Pharmacologic or genetic disruption of the fibrinogen/CD11b interaction suppresses microglial cluster formation, neurologic symptoms, inflammation, demyelination, and axonal damage in EAE. These studies identified fibrinogen as a novel molecular link between BBB disruption, activation of CNS innate immunity, and neurodegeneration. Fibrinogen has the potential for selective drug targeting to suppress its damaging functions in the nervous system without affecting its beneficial effects in hemostasis. Strategies for fibrin-selective inhibition of innate immunity and neuroprotection will be discussed.

S07-04

Cellular, subcellular and molecular *in vivo* imaging of the diseased nervous system

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Here, I want to discuss how advances in *in vivo* microscopy and mouse genetics can improve our understanding of the cellular, subcellular and molecular mechanisms that mediate inflammatory tissue damage in the nervous system. To illustrate this approach I will use our recent insights into the *in vivo* pathogenesis of immune-mediated axon damage as an example. Immune-mediated axon damage plays a crucial role in inflammatory diseases of the central nervous system (CNS) like multiple sclerosis (MS), as we know by now that the number of axons damaged by immune cells critically determines the clinical disability of MS patients. However we still understand very little about the process that leads to axon damage. Recently, we have used an *in vivo* imaging approach to investigate the pathogenesis of immune-mediated axon damage in an animal model of multiple sclerosis. By combined time-lapse imaging of fluorescently labeled macrophages/microglia and axons we could follow the slow and spatially restricted degeneration of axons in inflammatory CNS lesions. This "focal axonal degeneration" appears to be a novel type of axonal degeneration that is characterized by intermediated stages that can persist for several days and progress either to the degeneration or full recovery of the affected axons. *In vivo* imaging approaches now allow us to address the following key aspects of the axon degeneration process: First, to identify the molecular mechanisms that drive axonal degeneration, we can now reveal the actions of key damage mediators, in particular the influx of calcium and the release of reactive species, *in vivo*. Second, to better understand the relation between structural and functional axon damage in neuroinflammatory lesions, we can directly measure axonal transport in individual spinal axons. Using these examples, I hope to illustrate how recent advances in light microscopy can help us to reveal and mechanistically dissect the interactions of activated immune cells and CNS target cells as they happen in the living CNS.

S08 Neuroimaging of Dense Core Vesicle Trafficking and Release

S08-01

Quantifying DCV trafficking and release by combining TIRF microscopy and patch-clamp measurements in immune cells

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Killing of virally infected cells or tumor cells by cytotoxic T lymphocytes requires targeting of lytic granules to the junction between the CTL and the target. We used whole-cell patch clamp to measure the cell capacitance at fixed intracellular [Ca^{2+}] to study fusion of lytic granules in human CTLs. Expression of a fluorescently labelled human granzyme B construct allowed identification of lytic granule fusion using total internal reflection fluorescence microscopy. In this way capacitance steps due to lytic granule fusion were identified. Our goal was to determine the size of fusing lytic granules and to describe their behavior at the plasma membrane. On average, 5.02 $\pm$ 3.09 (mean $\pm$ s.d.) lytic granules were released per CTL. The amplitude of lytic granule fusion events was ~ 3.3 fF consistent with a diameter of about 325 nm. Fusion latency was biphasic with time constants of 15.9 and 106 s. The dwell time of fusing lytic granules was exponentially distributed with a mean dwell time of 28.5 s. Fusion ended in spite of the continued presence of granules at the immune synapse. The mobility of fusing granules at the membrane was indistinguishable from that of lytic granules which failed to fuse. While dwelling at the plasma membrane lytic granules exhibit mobility consistent with docking interspersed with short periods of greater mobility. The failure of lytic granules to fuse when docked at the membrane may indicate that the priming reaction is rate limiting.

S08-02

Molecular regulation of the fusion pore in neuroendocrine secretion

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Adrenal neuroendocrine chromaffin cells receive excitatory synaptic input from the splanchnic nerve of the sympathetic nervous system and secrete hormones into the peripheral circulation. Under basal sympathetic firing, modest, yet physiologically relevant amounts of catecholamine are released through the fusion pore formed between the secretory granule and plasma membrane. This short-lived pore is approximately 7–9 nm in diameter and acts as a size-exclusion filter for small freely-soluble catecholamines. Upon elevated splanchnic firing under the sympatho-adrenal stress reflex, increased stimulation drives fusion adrenal chromaffin cell pore expansion, resulting in increased catecholamine secretion and facilitating release of co-packaged peptide hormones. Thus, activity-regulated expansion of the secretory fusion pore is a point of regulation for differential-hormone release of the sympatho-adrenal stress response. We present work demonstrating the central role of dynamin and its phosphorylation status in regulation of fusion pore expansion. Increased sympathetic drive elevates cytosolic Ca^{2+} levels to initiate a calcineurin-dependent dephosphorylation of dynamin 1 at a key residue, serine 774. We show that this dephosphorylation event fundamentally alters fusion behavior and drives an active expansion of the fusion pore. Dynamin 1 initiates pore expansion through an interaction with syndapin 1 and subsequent signaling through syndapin binding partners N-WASP and Arp2/3. Ultimately, Arp2/3 facilitates F-actin assembly near the site of granule fusion and an actin/myosin process drives fusion pore expansion. Thus, activity-dependent dynamin dephosphorylation represents a primary molecular control point for the fight-or-flight acute stress response.

S08-03

Molecular mechanism of DCV maturation and exocytosis

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Peptide hormones and neuropeptides are packaged and stored in specialized intracellular organelles called secretory granules (SGs, also known as dene core vesicles, DCVs). The molecular mechanisms involved in DCV maturation and exocytosis are largely elusive. The nematode C. elegans provides a powerful model system for exploring the molecular basis of synaptogenesis and neurotransmission. Employing C. elegens model system, we have developed genetic screen specific for DCV exocytosis. We have studied a few genes that are involved in several steps of DCV exocytosis from maturation, docking to priming.

S08-04

Trafficking and fusion of neuropeptide-containing dense core vesicles in mammalian CNS neurons

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The regulated secretion of chemical signals in the brain occurs principally from two organelles, synaptic vesicles and dense core vesicles (DCVs). DCVs contain a diverse collection of cargo, including many neuropeptides that trigger a multitude of modulatory effects with quite robust impact, for instance on memory, mood, pain, appetite or social behavior. However, many fundamental questions remain open on DCV trafficking and secretion. My lab has established new photonic approaches to quantitatively characterize DCV-trafficking and fusion of many cargo types in living mammalian CNS neurons with single vesicle resolution. In this lecture I will present our most recent findings using these approaches on DCV trafficking and secretion, the molecular factors involved and the cellular locations where DCV fusion occurs, the SNARE-complex and SM-protein memebers involved in DCV fusion (syntaxin, SNAP-25, synaptobrevin, Munc18), role of priming factors Munc13 and CAPS, and the role modulatory pathways, such as PKC and PKA dependent pathways in DCV trafficking and fusion.

S09 Control of Cognition and Emotion at the Neuron-Matrix Interface

S09-01

Regulation of anxiety by extracellular proteolysis in the amygdala

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It is well-established that stress can trigger maladaptive forms of neuronal plasticity and lead to high anxiety. Anxiety disorders affect about 25% of adults at least once in their lives. Such a high prevalence of anxiety disorders, combined with high co-morbidity with depression, generates an enormous personal, social and economic burden.

Extracellular proteases such as the tissue plasminogen activator, plasmin or neuropsin are uniquely poised to remodel the neuron-extracellular matrix interface and may facilitate fear and anxiety. Two important groups of molecules that are subject to modulation by extracellular proteases are Eph-receptor tyrosine kinases and protease-activated receptors, such as PAR-1. Both groups are enriched in highly plastic areas of the brain, such as the amygdala and the hippocampus, where they promote neuronal plasticity and modulate animal's behavior.

Neuropsin (KLK8) is a kallikrein-like serine protease highly expressed in the amygdala and hippocampus. We found that upon stress neuropsin promotes stress-related anxiety in the amygdala by increasing the dynamics of EphB2/NMDA interaction that drives the expression of an anxiety-related gene, *Fkbp5*. Consistent with this finding; neuropsin-deficient mice do not show stress-related EphB2 cleavage, induction of the *Fkbp5* gene and stress-induced anxiety.

On the other hand we found, that PAR-1 can either promote fear or protect from it depending on the previous "emotional history" of an animal by dynamically switching its coupling to distinct G-protein coupling partners.

Our findings establish novel neuronal mechanisms linking stress-induced proteolysis in the amygdala to anxiety. These novel pathways open new possibilities for treatment of stress-associated disorders, including various forms of anxiety disorders.

S09-02

Matrix metalloproteinase 9: a focal point of synaptic plasticity in neuropsychiatric disorders

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Matrix metalloproteinase 9, MMP-9 is an extracellularly operating enzyme that has been demonstrated as important regulatory molecule in control of synaptic plasticity, learning and memory. Either genetic or pharmacological inhibition of MMP-9 impairs late phase of long-term potentiation at various pathways, as well as appetitive and spatial memory formation, although aversive learning remains apparently intact in MMP-9 KO mice. MMP-9 is locally translated and released from the excitatory synapses in response to neuronal activity. Extrasynaptic MMP-9 is required for growth and maturation of the dendritic spines to accumulate and immobilize AMPA receptors, making the excitatory synapses more efficacious. Animal studies have implicated MMP-9 in such neuropsychiatric conditions, as e.g., epileptogenesis, autism spectrum disorders, development of addiction, and depression. In humans, MMP-9 appears to contribute to epilepsy, alcohol addiction, Fragile X Syndrome, schizophrenia and bipolar disorder. In aggregate, all those conditions may be considered as relying on alterations of dendritic spines/excitatory synapses and thus understanding the role played by MMP-9 in the synaptic plasticity may allow to elucidate the underpinnings of major neuropsychiatric disorders.

S09-03

Mechanisms of cognitive vulnerability to stress:synapses, Spine and a symphony of mediators

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Learning and memory processes carried out within the hippocampus are influenced by stress in a complex manner, and the mechanisms by which stress modulates the physiology of the hippocampus are not fully understood. Here we demonstrate that short (hours-long) modern-life like stress consisting of concurrent psychological and physical stresses affect memory profoundly.Among the several stress-mediators involved, we focus on the release of the neuropeptide corticotropin-releasing hormone (CRH) within the hippocampus during stress and the mechanisms by which it influences synaptic structure and hippocampal function. These involve both actin dynamics and activity driven, calcium-dependent processes. Future challenges are to uncover how the dynamic actions of CRH integrate with the well-established roles of adrenal-derived steroid stress hormones to shape the cognitive functions of the hippocampus in response to stress.

S09-04

A role for integrins in controlling neural circuit activity

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Homeostatic synaptic plasticity adjusts neuronal excitability and network activity when global activity perturbation is imposed. Although homeostatic synaptic plasticity is suggested to function in the developing brain by tuning synaptic strengths in response to changes in sensory activity and also in the mature brain to control network activity under some pathological conditions, the precise physiological role of homeostatic synaptic plasticity remains to be clarified. We have previously demonstrated that postsynaptic β3 integrin, a member of a family of transmembrane receptors for the extracellular matrix proteins, was required for homeostatic synaptic scaling of AMPA receptors in cultured networks (1,2). Constitutive loss of β3 integrin expression in mice resulted in altered anxiety-like

behavior, which, in pilot experiments, was restored to control levels by re-expressing β3 integrin in the ventral hippocampus of adult knock-out mice (3). In contrast, conditioned fear learning and Hebbian forms of synaptic plasticity, such as LTP and LTD, were unchanged upon loss of β3 integrin (3). These findings raise the question of whether β3 integrin may have important, unexplored effects on circuit functions that contribute to the processing of anxiety-related information in the ventral hippocampus. We have therefore addressed if and how the ability of β3 integrin to adjust synaptic AMPA receptors influence ventral hippocampal network activity, and to this end, our current efforts are focused towards delineating the mechanism by which β3 integrin modulates oscillatory network activity.

References:
1. Cingolani et al., Neuron (2008)
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S10 Novel Mechanisms in Synaptic Plasticity

S10-01

Postsynaptic complexes and mental illness

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The postsynaptic proteome of brain synapses is disrupted by mutations causing over 130 brain diseases. Postsynaptic proteins are organized into multiprotein complexes and studies of PSD95 complexes shows polygenic disorders including schizophrenia, autism and intellectual disability converge on these complexes.

Studies of individual mutations in mice shows disruption of postsynaptic proteins cause electrophysiological and behavioural changes. Because these experiments have been reported as single gene studies and not performed in a standardized manner, it is impossible to compare results and determine quantitative differences between mutations.

We have analysed over 50 lines of mice carrying mutations in postsynaptic proteins in a standardized behavioural test battery that assesses the innate and learned behavoural repertoire. In addition, synaptic physiology was examined using standardized procedures. This study represents the largest genetic study of the vertebrate synapses and reveals new principles underlying the organisation of behavior and its relevance to brain disease.

S10-02

Myosin II dependent loss of stable F-actin from dendritic spines by LTP induction

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The neuronal actin-binding protein drebrin A forms a stable structure with F-actin in dendritic spines. NMDA receptor activation causes an exodus of F-actin bound by drebrin A (DA-actin) from dendritic spines, suggesting a pivotal role for DA-actin exodus in synaptic plasticity. In the present study, we first used stochastic optical reconstruction microscopy to reveal localization of drebrin in nanoscale. We prepared primary hippocampal neuronal culture from embryonic day-18 rat brain using Banker's method. Drebrin A is localized at the central region of dendritic spine heads at rest. We then quantitatively assessed DA-actin accumulation in spines, and found that chemical long-term potentiation (LTP) stimulation induces rapid DA-actin exodus and subsequent DA-actin re-entry in dendritic spines and that Ca^{2+} influx through NMDA receptors regulates the exodus and the basal accumulation of DA-actin. DA-actin exodus is blocked by myosin II ATPase inhibitor, but is not blocked by myosin light chain kinase (MLCK) or Rho-associated kinase (ROCK) inhibitors. These results indicate that myosin II mediates the interaction between NMDA receptor activation and DA-actin exodus in LTP induction. Furthermore, myosin II seems to be activated by a rapid actin-linked mechanism rather than slow MLC phosphorylation. Thus the myosin-II mediated DA-actin exodus might be an initial event in LTP induction, triggering actin polymerization and spine enlargement.

S10-03

Epigenetic remodeling drives the switch in synaptic NMDA receptors during brain development

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NMDARs (*N*-methyl-D-aspartate receptors) mediate excitatory transmission and are critical to synaptogenesis, formation of neural circuitry and to higher cognitive functions. A hallmark feature of NMDARs is a developmental switch in receptor phenotype from primarily GluN2B- to GluN2A-containing during postnatal brain development. Although the developmental switch in NMDARs has been an area of intense interest for nearly two decades, mechanisms that regulate the switch are, as yet, unclear. The overall objective of this study was to identify the mechanism underlying the switch in synaptic NMDARs phenotype during postnatal development. Here we show a novel role for the repressor element 1 silencing transcription factor (REST, also known as NRSF) in the developmental switch of NMDARs at hippocampal synapses. REST is activated at a critical window of time and acts via epigenetic remodeling to repress g*rin2b* (the gene encoding GluN2B) expression and alter NMDAR properties at rat hippocampal synapses. Knockdown of REST *in vivo* prevented the decline in GluN2B and developmental switch in NMDARs. Maternal deprivation impaired REST activation and acquisition of the mature NMDAR phenotype. This is significant in that GluN2B expression can restrict synaptic incorporation of AMPA receptors, reduce the threshold for and enhance the magnitude of long-term potentiation and promote hippocampus-dependent learning, plasticity-induced spine growth and dendritic patterning critical to information processing. Moreover, GluN2B-containing NMDARs exhibit slower decay times1, carry more Ca^{2+} current per unit charge and preferentially tether to the plasticity protein CaMKII. These findings highlight the importance of maintaining correct GluN2A and GluN2B amounts in adults and document a previously unappreciated role for REST in experience-dependent fine-tuning of genes involved in synaptic plasticity.

S10-04

Molecular mechanisms of AMPA receptor delivery during LTP

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Despite decades of research, the molecular mechanisms underlying NMDA receptor-dependent LTP remain poorly understood. In this lecture, I will review molecular replacement experiments that address the mechanisms by which AMPA receptors (AMPARs) are delivered to synapses during LTP. Our primary experimental strategy utilizes *in vivo* viral-mediated expression of shRNAs that encode postsynaptic proteins previously not implicated in LTP. Evidence will be presented that a unique assembly of SNARE proteins is required for the exocytosis of AMPARs during LTP. I

will also present evidence that specific families of synaptic cell adhesion proteins, including leucine-rich repeat transmembrane proteins, are likely required for the synaptic stabilization of these recently delivered AMPARs. At the conclusion, I will present a model summarizing current understanding of the molecular mechanisms underlying AMPAR delivery and stabilization during LTP.

S11 The Impact of Nutrition and Gut Microbiota on Multiple Sclerosis and other Neurodegenerative Diseases: A Story yet to be Written

S11-01

Nature plus nurture: intestinal ignition of brain autoimmunity

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We have used a spontaneous variant of relapsing-remitting experimental autoimmune encephalomyelitis (EAE) to study the triggering events of brain autoimmunity. RR mice are single transgenic SJL/J mice expressing a MOG autoreactive T cell receptor in about 70% of their $CD4^+$ T cell repertoire. Within 4–6 months of age, close to 100% develop successive bouts of autoimmune brain attacks, mostly against the cerebellum/brain base or the spinal cord/optic nerve. Disease onset critically depends on the genetic background of the mice, and on an intact intestinal microbiome. Transgenic mice on the MHC compatible B10.S background remain completely protected from EAE, and the same is true for SJL/J bred RR mice maintained in germfree conditions. Disease risk is modulated by modification of the microbiota, such as by antibiotic treatment or by dietary interventions. We currently explore the effect of microbial samples from people with MS on initiation of EAE in the RR-mouse model.

S11-02

Nutrition facts in multiple sclerosis

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Multiple sclerosis (MS) is a chronic and autoimmune disease of the central nervous system, leading to focal breakdown of the myelin sheath and axonal damage. There are two main forms of MS: relapsing-remitting (RRMS) and primary-progressive (PPMS). Both forms are inflammatory in nature, but disease-modifying therapies are currently available only for RRMS, not for PPMS. However, MS is a complex and multifactorial disease, with a possible influence of environmental factors, including dietary habits and lifestyle, and it may be expected now that they may exacerbate or ameliorate MS symptoms by modulating the inflammatory status of the disease, both in RRMS and in PPMS. This can be achieved by controlling the metabolic and inflammatory pathways in the human cell, as well as the composition of commensal gut microbiota and intestinal inflammation. What increases inflammation are energy-dense Western-style diets, characterized by high salt, animal fat, red meat, sugar-sweetened drinks, fried food, low fiber, and lack of physical exercise. The persistence of this type of diet, on one hand up-regulates the metabolism of human cells toward biosynthetic pathways, including the synthesis of pro-inflammatory molecules, on the other hand leads to a dysbiotic gut microbiota, alteration of intestinal immunity, and low-grade systemic inflammation. Conversely, exercise and calorie-restricted diets based on the assumption of vegetables, fruit, legumes, and fish act on nuclear receptors and enzymes that up-regulate oxidative metabolism, while down-regulating the synthesis of pro-inflammatory molecules, and restoring or maintaining a healthy symbiotic gut microbiota. Dietary supplements, such as polyphenols, omega-3 long-chain polyunsaturated fatty acids, alpha-lipoic acid, vitamin D, vitamin A, niacin, vitamin C, prebiotics and probiotics may be added to the diet to achieve a more robust anti-inflammatory nutritional intervention. Taken together, we have now a better knowledge of the possible influence of dietary factors on cell metabolism and gut microbiota, and thus on their possible effects on MS. Nutritional clinical trials are needed, but in the meantime it is possible to provide nutritional guidance and physical activity opportunities to MS patients helping them to stay healthy.

S11-03

The effects of vitamin D on T cells, the microbiota and immune mediated disease

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Evidence from animal models of immune mediated diseases and epidemiological studies in human patients support a role for vitamin D in the pathogenesis of diseases including multiple sclerosis and inflammatory bowel disease (IBD). The active form of vitamin D ($1,25(OH)_2D_3$) inhibits the development of experimental autoimmune encephalomyelitis (EAE) and IBD. $1,25(OH)_2D_3$ directly inhibits IFN-γ and IL-17 production from Th1 and Th17 cells. In addition, vitamin D regulates the development of several regulatory T cell types including FoxP3 + T regulatory cells, TCR$\alpha\beta$/CD8$\alpha\alpha$ intraepithelial lymphocytes and invariant NKT cells. Paradoxically infections that require Th1 and Th17 cells for protection are not affected by $1,25(OH)_2D_3$ treatments *in vivo*. Instead our data suggests that vitamin D is a late regulator of effector T cells and is critical for turning off IFN- γ and IL-17 production. In EAE and IBD, the antigens are persistent and the ability to turn off Th1 and Th17 responses are critical.

Other effects of vitamin D on immune mediated disease include regulation of the microbiota. The effectiveness of vitamin D to regulate IBD was inhibited by disruptions of the microbiota using broad spectrum antibiotics. More specifically the Helicobacteraceae family members within the Proteobacteria pyhlum were higher in vitamin D deficient mice and this was associated with more severe colitis. $1,25(OH)_2D_3$ or antibiotics treatment reduced Helicobacteraceae numbers and was associated with less severe disease. In addition, the ability of the host to produce $1,25(OH)_2D_3$ depends on the microbiota. Colonization of germfree mice resulted in induction of the enzyme that produces active $1,25(OH)_2D_3$ and as a result significantly higher amounts of $1,25(OH)_2D_3$ in the blood. The symbiotic relationship between two environmental factors (vitamin D and the microbiota) that both influence the development of immune mediated diseases has important and unappreciated implications for patients with MS and IBD.

S11-04

The GUT -(CD39)- brain axis: role of the microbiota regulating inflammatory CNS demyelination

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Recent evidence shows that the gut microbiota is essential for the normal development and function of the immune system. Microbiome alterations due to both environmental and genetic factors can be associated with experimental autoimmunity. We reported that the polysaccharide-A (PSA), a symbiont factor produced by the human commensal *B. fragilis*, protects against murine EAE. Oral treatment with PSA induces IL-10-producing CD39 + FoxP3 + Tregs that accumulate in the CNS of EAE mice and functionally suppress the inflammatory response. Others showed that circulating CD39 + regulatory T cells (Tregs) are reduced in both number and capacity to suppress IL-17 by CD4 + T cells in those with relapsing MS. CD39 is an ecto-enzyme that dephosphorylates ATP to adenosine. We now demonstrate that the increase frequency of CD39 + Tregs in circulating blood persists for months after the last treatment with PSA. The enhanced ability of PSA-treated mice to promote a regulatory phenotype was confirmed *in vitro*. CD4 + T cells from cultured PSA-treated peripheral blood mononuclear cells (PBMCs) show a significant increase in the expression of FoxP3 and CD39 when compared to those from PBS-treated mice. When PBS-treated PBMCs were cultured with PSA, FoxP3 and CD39 expression levels matched those observed in PSA-treated cells. Furthermore, *in vitro* human studies show PSA can convert and amplify CD39 + expression by naïve human CD4 + T cells. In addition, we now have preliminary data that demonstrate that some of the approved oral and infused IMD therapies for relapsing MS promote an enhanced frequency of GALT derived CD39 + Tregs. Our data and those of others lead us to hypothesize that GALT derived CD39 + Tregs are a key immunoregulatory factor that IMD therapies promote in the gut, and constitute a common mechanism of action for the protective effects of both oral and infusion based therapies currently approved to treat MS.

S12 Activity-Driven Epigenetic Mechanisms in the Brain

S12-01

Epitranscriptomic mechanisms of memory stability

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RNA modification has emerged as a novel layer of epigenetic control over gene expression that is perfectly suited to serve as a key post-transcriptional regulator in the fine-tuning of gene expression related to adaptation. One of the most prevalent and widely conserved RNA modifications, N6-methyladenosine (m6A), has been shown to be both dynamic and reversible, and is present in neurons. However, whether this epitranscriptomic mechanism is fundamental for the regulation of gene expression underlying learning and memory, has not been explored in any detail. Using m6A capture followed by RNA sequencing, we have discovered an experience-dependent redistribution of m6A in the prefrontal cortex of mice following fear conditioning. This epigenetic mark accumulates in specific regions of the transcriptome and early evidence suggests that it may dictate the fate of messenger RNAs and/or mRNA stability. Importantly, we have also found that the RNA demethylase, FTO, contributes to the formation and maintenance of fear-related memory Thus, our data demonstrate the dynamic nature of the epitranscriptome and suggest that, like DNA methylation, m6A is involved in the regulation of genes directly related to learning and memory.

S12-02

Nucleosome remodeling: a key epigenetic mechanism underlying memory and intellectual disability disorders

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Recent human exome sequencing studies have implicated polymorphic Brg1-Associated Factor (BAF) complexes (mammalian SWI/SNF chromatin remodeling complexes) in several intellectual disabilities and cognitive disorders, including autism. However, it remains unclear how mutations in BAF complexes result in impaired cognitive function. Post-mitotic neurons express a neuron-specific assembly, nBAF, characterized by the neuron-specific subunit BAF53b. Mice harboring mutations of BAF53b show severe defects in long-term memory and long-lasting forms of synaptic plasticity, yet the neuron-specific function of BAF53b is unknown. BAF53b shares 93% sequence similarity with its non-neuronal homologue BAF53a, which is expressed in all non-neuronal cells. The most divergent region between BAF53b and BAF53a is within subdomain 2, suggesting an essential contribution for this domain in neuronal function. To examine the role of subdomain 2 of BAF53b in long-term memory and synaptic plasticity, we generated transgenic animals that over-express a dominant negative BAF53b with a deletion of subdomain 2 (BAF53bΔSB2). BAF53bΔSB2 mice and wildtype littermates were examined using learning and memory tasks, synaptic plasticity protocols, and next generation RNA sequencing. BAF53bΔSB2 animals show impaired long-term memory, maintenance of long-term potentiation and activity-dependent gene expression. BAF53bΔSB2 animals also fail to display learning-induced increases in phosphorylated Cofilin at the post synaptic density indicating a failure in synaptic plasticity. Over-expression of a phospho-mimic of Cofilin in the BAF53bΔSB2 animals rescued long-term memory formation. Together our findings suggest a potential causal link between actin cytoskeletal remodeling at the dendritic spine, BAF53b-mediated gene expression and long-term memory formation. This work is the first to examine how the neuron-specific subdomain of BAF53b contributes to nBAF function in the adult brain and provides insight into why human mutations in nBAF subunits severely impair cognitive function.

S12-03

Targets of histone acetylation important for memory storage

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Transcriptional activation is thought to be a key process in long-lasting forms of memory and synaptic plasticity. This activation is directed by transcription factors and their coactivators, which regulate gene expression via chromatin remodeling, histone modification and interactions with the basal transcription machinery. One type of histone modification associated with transcriptional activation is acetylation, which is regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs) that add or remove acetyl groups from histones, respectively. The transcriptional coactivator CREB-binding protein (CBP), a potent HAT, is involved in specific forms of long-term memory and synaptic plasticity. Mutant mice in which CBP activity in neurons is reduced either by the transgenic expression of an inhibitory form of *cbp* lacking the HAT domain or by knocking in a mutation of the CREB transcription factor-binding KIX domain of *cbp* exhibit deficits in spatial and contextual memory and in long-lasting forms of hippocampal synaptic plasticity. A complementary method to study the role of histone acetylation in synaptic plasticity and memory is to examine the effects of HDAC inhibitors, which increase the level of histone acetylation that correlates with transcriptional activation. We found that increasing histone acetylation using the HDAC inhibitor TSA enhances long-term contextual memory and facilitates synaptic plasticity via the transcription factor CREB. Using genetic approaches, we have found that conditional deletion of the transcriptional corepressor Sin3a results in enhanced contextual memory, consistent with the idea that HDACs are recruited to specific genes by Sin3a-containing complexes. We have identified a family of nuclear receptors that appears to be among the gene targets of HDAC inhibition critical for this cognitive enhancing activity. Histone acetylation may provide an epigenetic mechanism for establishing gene-specific modifications that result in the coordinate

expression of genes required for long-term memory storage and HDAC inhibitors may provide a novel therapeutic approach to treat the cognitive deficits that accompany many psychiatric disorders.

S12-04

Interplay between transcriptional and epigenetic mechanisms in activity-driven gene expression

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The acetylation of lysine residues at the histone tails is an epigenetic modification of the chromatin associated with active transcription. The process is regulated by the opposing activities of lysine acetyltransferase (KAT) and histone deacetylase (HDAC) enzymes and it is thought to play a relevant role in neuronal plasticity, memory and diverse brain pathologies ranging from intellectual disability syndromes to neurodegenerative diseases. Consistent with this view, compounds that inhibit HDAC activity (HDACi) have been show to enhance memory in wild-type animals and to ameliorate cognitive deficits and neurodegeneration in animal models of neurological diseases. I will present a number of genomic analyses aimed to elucidate the interplay between transcription, particularly activity-driven transcription, and lysine acetylation in neurons in the adult brain. Towards this end, we focus on the hippocampus, given the relevance of this brain region for memory processes, and determine the genomic profiles for several acetylation marks both in the basal condition and after manipulating lysine acetylation levels by pharmacological or genetic means. Among other findings, our experiments indicate that understanding the mechanisms of action of HDACi and the role of lysine acetylation in neuronal plasticity, memory and brain pathology will require a clear distinction between epigenetic and transcriptional mechanisms.

S13 New Advances in Tau Regulation and Function

S13-01

New functions for tau

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Tau was initially discovered as a microtubule-associated protein whose ability to stabilize microtubules was decreased when tau was phosphorylated. Following the identification of hyperphosphorylated tau as the primary component of the neurofibrillary tangles of Alzheimer's disease, investigations into new tau functions have significance towards neurodegenerative disease as well as basic mechanisms in neurons. Several years ago, we reported an interaction between tau and non-receptor Src family tyrosine kinases, mediated by a proline rich motif in tau and the SH3 domain of the kinase. We found that tau was capable of potentiating the activation of Src and Fyn activity and that tau was tyrosine phosphorylated by Src and Fyn. In addition, tyrosine phosphorylated tau occurred during embryonic development but became absent 2 weeks after birth. Interestingly, tyrosine phosphorylated tau occurred in Alzheimer's disease. Currently, we are pursuing two avenues of research. First, in examining the regulation of tyrosine phosphorylated tau, we have found that tau interacts with SHP2, a protein tyrosine phosphatase which has been thought to be a proto-oncogene. The significance of the interaction is being investigated. Secondly, we are creating a mouse model that is depleted of both tau and Fyn. Such a model would be useful in examining the importance of the tau-Fyn interaction in normal mice and during neurodegeneration. Properties of the tau-Fyn double knockout are being investigated.

S13-02

Post-synaptic functions of tau in Alzheimer's disease

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Alzheimer's disease is characterized by deposition of the microtubule-associated protein tau in neurons, forming neurofibrillary tangle. We and others have shown that tau plays a role in neuronal dysfunction prior to its deposition. In the present study, we addressed the role of tau in acute and chronic hyperexcitation to understand molecular mechanisms of early brain damage in Alzheimer's disease. Therefore, we used both existing and novel genetic mouse models of Alzheimer's disease as well as tau-deficient strains to investigate the effects of excitotoxicity on neuronal function and survival. We showed that reducing tau levels prevents premature death and memory deficits in mouse models of Alzheimer's disease by interfering with NMDAR-dependent excitotoxic signaling. Biochemical and histological methods were used to determine the role of individual signaling pathways in disease, driven by pathway mapping and next generation mRNA sequencing. Thereby, we identified novel molecular players contributing to tau-dependent excitotoxic brain damage and immediate early gene activation. Taken together, we revealed that tau is critically involved in mediating excitotoxicity in chronic brain damage in Alzheimer's disease, providing possibly new approaches for therapeutic intervention.

S13-03

Re-evaluation of physiological and pathological phosphorylation of tau *in vitro* to human brains

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Tau aggregates called neurofibrillary tangles are one of hallmarks in Alzheimer disease. Tau is a microtubule-associated protein, whose physiological functions such as microtubule-polymerizing or -stabilizing ability is regulated by phosphorylation. Further, tau is hyper-phosphorylated in pathological aggregates, and the hyper-phosphorylation is found in many neurodegenerative diseases, which are collectively called Tauopathy. More than thirty phosphorylation sites are reported and many protein kinases are involved in those phosphorylation. However, it is not known yet how tau is hyperphosphorylated and what is a consequence of hyperphosphorylation. Tau phosphorylation has previously been analyzed using phosphorylation site-specific antibodies, which are available for almost all phosphorylation sites on tau. Those antibodies are so sensitive to detect a small change in phosphorylation. However, it is difficult to estimate the absolute extent of phosphorylation and to analyze combinatory phosphorylation in tau. To reevaluate the phosphorylation of tau more quantitatively, we employed Phos-tag SDS-PAGE, a new method that separates proteins in SDS-PAGE depending on their phosphorylation states, followed by immunoblotting with a phosphorylation-independent antibody. Tau shifted upward depending on the number and sites of phosphorylation in Phos-tag SDS-PAGE more than Laemmli's SDS-PAGE. We have analyzed tau phosphorylation *in vitro*, in cultured cells, mouse brains and human brains. There were many tau species with different phosphorylation states in brains. While tau in fetal mouse brains is highly phosphorylated as was previously reported, unexpectedly there were substantial amount of unphosphorylated tau in adult mouse and human brains. We would like to report several new interesting insights on tau phosphorylation obtained by the method.

S13-04

New insights into the regulation of tau by protein phosphatase 2A

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Protein Phosphatase 2A (PP2A) is a major brain Ser/Thr phosphatase that binds to and dephosphorylates tau. Notably, we have shown that methylation of PP2A catalytic subunit at Leu-309, which modulates PP2A substrate specificity, is down-regulated in Alzheimer's disease (AD). We have reported that dietary, drug or

genetic alterations in folate and homocysteine metabolism in several mouse models can lead to brain region specific impairment of neuronal PP2A methylation, and concomitant accumulation of AD-like phosphorylated tau. PP2A methylation also critically modulates tau distribution and protein-protein interactions. Here, we provide evidence for novel functional interrelationships between specific AD-related protein kinases and PP2A, which in turn affect tau phosphorylation. Our findings unveil a complex interplay between neuronal signal transduction cascades and metabolic pathways that converge on the regulation of PP2A and tau.

S14 Emerging Principles of Astroglia-Neuron Networking

S14-01

Induction of epileptiform activity rapidly alters astrocyte morphology *in vitro*

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Astrocytes actively contribute to the functioning of neuronal networks. Their close contact to thousands of neurons enables them to modulate and maintain neuronal function effectively by, for example, buffering potassium or supplying energy. A disruption of this spatial relationship could be of pathophysiological significance. Indeed, astrocyte dysfunction and long-term morphology changes have been implicated in numerous diseases including epilepsy. How rapidly astrocyte morphology responds to epileptiform activity and to what degree that contributes to aberrant network activity is largely unknown. Using established protocols of *in vitro* hippocampal epileptogenesis, electrophysiology and two-photon excitation fluorescence imaging, we detected morphology changes in the astrocyte periphery within minutes after epileptiform discharges appeared in CA1. These morphology changes outlasted the induction of epileptiform activity and where sensitive to inhibition of Rho-associated protein kinase (ROCK, Y-27632). Interestingly, ROCK inhibition also reduced epileptiform activity. Similar astrocyte morphology changes could be induced by pharmacological activation of the adenylyl cyclase (NKH477), which also resulted in spontaneous epileptiform activity. These observations indicate that development of epileptiform activity and astrocyte morphology changes are tightly linked and occur with little delay. In parallel to morphology changes, gap junction-dependent dye coupling between neighboring astrocytes decreased. Astrocyte gap junction coupling is important for potassium buffering, is reduced in epilepsy and its absence leads to spontaneous epileptiform activity *in vitro*. We therefore speculate that the observed rapid astrocyte morphology changes may impair diffusion within the coupled astrocyte network and thereby contribute to epileptiform activity.

S14-02

The astrocytic control of D-serine and adenosine during sleep/wake cycles

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We have previously demonstrated the important role of astrocytes in the control of sleep homeostasis. In this presentation I will discuss how wakefulness regulates the extracellular levels of adenosine and D-serine. Using a combination of synaptic physiology and biosensors to monitor adenosine and D-serine our studies show that both astrocyte-derived adenosine and D-serine are subjected to wakefulness dependent regulation in the extracellular space. When brain slices are isolated during the light phase (subjective nighttime) both adenosine and D-serine are low. In contrast in brain slices isolated during the dark phase (subjective daytime) their levels are elevated. Changes in the levels of D-serine, an endogenous co-agonist of the NMDA receptor, lead to alterations in synaptic NMDA receptor activity. To begin to identify the pathways that mediate these changes in D-serine activity we are using pharmacological approaches to probe the role of different transmitter systems that are known to have elevated activity during wakefulness.We have previously demonstrated the important role of astrocytes in the control of sleep homeostasis. In this presentation I will discuss how wakefulness regulates the extracellular levels of adenosine and D-serine. Using a combination of synaptic physiology and biosensors to monitor adenosine and D-serine our studies show that both astrocyte-derived adenosine and D-serine are subjected to wakefulness dependent regulation in the extracellular space. When brain slices are isolated during the light phase (subjective nighttime) both adenosine and D-serine are low. In contrast in brain slices isolated during the dark phase (subjective daytime) their levels are elevated. Changes in the levels of D-serine, an endogenous co-agonist of the NMDA receptor, lead to alterations in synaptic NMDA receptor activity. To begin to identify the pathways that mediate these changes in D-serine activity we are using pharmacological approaches to probe the role of different transmitter systems that are known to have elevated activity during wakefulness.

S14-03

Surface trafficking of astroglial GLT-1 glutamate transporter

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Control of the glutamate timecourse in the synapse is crucial for excitatory transmission. This process is mainly ensured by astrocytic transporters, of which high expression is essential to compensate for their slow transport cycle. Although molecular mechanisms regulating transporter intracellular trafficking have been identified, the relationship between surface transporter dynamics and synaptic function remains unexplored. We have investigated this issue and demonstrated that GLT-1 transporters were highly mobile on astrocytes. Surface diffusion of GLT-1 is sensitive to neuronal and glial cell activities, and is strongly reduced in the vicinity of glutamatergic synapses, favoring transporter retention. Remarkably, glutamate uncaging at synaptic sites increases GLT-1 diffusion, displacing transporters away from this compartment. Functionally, impairing GLT-1 membrane diffusion through cross-linking *in vitro* and *in vivo* slows the kinetics of excitatory postsynaptic currents indicative of prolonged timecourse of synaptic glutamate. These data provide the first evidence for a physiological role of GLT-1 surface diffusion in shaping synaptic transmission.

S14-04

Homeostatic plasticity in astrocyte-synapse relationships

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Memory formation in the brain is thought to rely on the remodelling of synaptic connections which eventually results in neural network rewiring. This remodelling is likely to involve astroglial protrusions which often occur in the vicinity of excitatory synapses. Indeed, rapidly emerging experimental evidence has associated astroglial Ca^{2+} activity with diverse molecular signals influencing synaptic connectivity. The phenomenology, cellular mechanisms and causal relationships of use-dependent astroglial restructuring remain however poorly understood. Here, to monitor rapid nanoscopic rearrangement of astroglia upon induction of long-term potentiation (LTP), we combined electrophysiology with two-photon excitation microscopy and uncaging. We document NMDA receptor dependent-withdrawal of astroglial processes from the vicinity of synapses following LTP induction. This reduction in synaptic astroglial coverage facilitates escape of synaptic glutamate thus boosting NMDA receptor-mediated cross-talk among synapses. The molecular mechanisms behind astroglial restructuring require local Ca^{2+} elevations but do not appear to involve mGluRs or IP_3-receptor signalling. In the quest to unravel the Ca^{2+} machinery involved, we have developed a dye concentration-independent fluorescence lifetime imaging method sensitive to nanomolar Ca^{2+}. High-resolution mapping of resting Ca^{2+} inside astrocytes in acute slices has revealed heterogeneous Ca^{2+} landscapes, their developmental and use-dependent plasticity, and distinct groups of astroglia with respect to their resting Ca^{2+}. Experiments are underway to build a conceptual understanding of how induction of synaptic plasticity engages astroglia to remodel local microenvironment.

S15 Neurochemistry of Decision Making & Reward-Seeking

S15-01

The effect of reward-related cues on reward seeking

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Evidence suggests that two forms of incentive process modulate reward seeking: (i) the experienced incentive value derived from consummatory experience, and (ii) the predicted incentive value derived from cues that predict reward. With regard to the later we have found that the influence of predictive learning on choice depends critically on basolateral amygdala afferents on the nucleus accumbens shell. Like other parts of the striatum, the shell is composed primarily of two populations of spiny projection neurons (SPNs) that differentially express dopamine D1 and D2 receptors. In addition, these D1 and D2 SPNs release distinct opioid ligands, dynorphin and enkephalin respectively. In recent studies we have found that the influence of predictive learning on choice depends on the interaction of D1 MSNs with a delta-opioid receptor process in the shell. Subsequent studies have established that this interaction is controlled by cholinergic interneurons; we found that delta-receptors translocate to the membrane of cholinergic interneurons in response to predictive learning – perhaps the first evidence of learning related translocation of a GPCR – where they modulate the release of acetycholine and, hence, the activity of D1-repector expressing SPNs to influence the effect of predictive learning on choice. Interestingly, the translocation event appears to be influenced by BLA afferents on the shell and serves to modulate both excitatory and inhibitory predictions on choice.

S15-02

Transient synaptic plasticity induced by drug cues is reversed by drug relapse

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Cues reminding an addict of drug use are powerful motivators to seek and continue drug use. In the rodent, this is modeled by using cocaine-conditioned cues to reinstate lever pressing after withdrawal from self-administration. We recently found that this cue-induced reinstatement of cocaine seeking produces a rapid induction of transient synaptic plasticity (tSP) within 15 min quantified by increases in dendritic spine diameter and AMPA glutamate currents in nucleus accumbens core (NAcc). This synaptic potentiation correlates with initial intensity of cocaine seeking (lever presses) and disappears in parallel with lever pressing over the first 45 min after initiating cued reinstatement. The present study investigated the effects of cocaine re-exposure on this tSP. We describe a new animal model to evaluate the role of tSP in regulating the motivation to seek cocaine that incorporates three anthropomorphic characteristics of cocaine seeking: (i) cue-induced lever pressing, (ii) delayed access to self-administered cocaine, and (iii) loss of access to cocaine after 45 min of use. Animals were sacrificed at discrete time points along this reinstatement time course to examine changes in tSP measured by quantification of the diameter and density of diolistically labeled dendritic spines or AMPA/NMDA ratio in NAcc. Ten minutes of access to cocaine after initiation of 10 min of cue-induced reinstatement (t = 20 min) reduced spine head diameter and AMPA/NMDA ratio compared to a control saline access group. In the controls, the potentiated spine head diameter at t = 20 min positively correlated with initial cue-induced lever pressing. Conversely, no such relationship was found with the cocaine access group. Dendritic spine diameter was suppressed as long as cocaine was available, but removal of cocaine access after 45 min of use rapidly repotentiated spine head diameter within 10 min commensurate with an increase in lever pressing. These results suggest that the increase in spine head diameter tracks the level of motivation the animal manifested to seek cocaine. Moreover this paradigm provides a more biologically relevant approach to study drug relapse in the rodent.

S15-03

Corticostriatal control of rewarded learning

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Abnormalities in various forms of cognition, including rewarded learning, are found in neuropsychiatric conditions including drug addictions. Prior studies have identified subregions of prefrontal cortex and dorsal striatum in specific cognitive processes that subserve rewarded learning, but the precise neuroanatomical circuits and neurochemical mechanisms involved remain unclear. This presentation will show novel data obtained from techniques including *in vivo* electrophysiology and optogenetics demonstrating roles of the ventromedial prefrontal cortex and dorsolateral striatum in a mouse rewarded discrimination and reversal paradigm. In addition, findings will be shown from *in vivo* fast-scan voltammetric measurements of dopamine release in the dorsolateral and ventral striatum during learning. These findings will be considered together in the context of current models of the role of corticostriatal circuits in guiding rewarded learning, with implications for understanding how these circuits are functionally compromised in addiction. Research supported by the National Institute on Alcohol Abuse and Alcoholism Intramural Research Program.

S15-04

Targeting glucocorticoid receptors that promote resilience in the treatment of addiction

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There is strong evidence to suggest that the combination of alcohol and chronic repetitive stress leads to long-lasting effects on brain function, specifically areas associated with stress, motivation

and decision-making such as the amygdala, nucleus accumbens and prefrontal cortex. Alcohol and stress together facilitate the imprinting of long-lasting memories. The molecular mechanisms and circuits involved are being studied but are not fully understood. Current evidence suggests that corticosterone (animals) or cortisol (humans), in addition to direct transcriptional effects on the genome, can directly regulate pre- and postsynaptic synaptic transmission through membrane bound glucocorticoid receptors (GR). Indeed, corticosterone-sensitive synaptic receptors may be critical sites for stress regulation of synaptic responses. Direct modulation of synaptic transmission by corticosterone may contribute to the regulation of synaptic plasticity and memory during stress (Johnson *et al.*, 2005; Prager *et al.*, 2010). Specifically, previous data has shown that long term alcohol (1) increases the expression of NR2B-containing NMDA receptors at glutamate synapses, (2) changes receptor density, and (3) changes morphology of dendritic spines (Prendergast and Mulholland; 2012). During alcohol withdrawal these changes are associated with increased glucocorticoid signalling and increased neuronal excitability. It has therefore been proposed that these synapse changes lead to the anxiety and alcohol craving associated with withdrawal (Prendergast and Mulholland; 2012). My lab is targeting this receptor system and the amygdala in order to understand the effect of combining alcohol and stress on these pathways. Lastly, we are testing GR specific compounds as potential new medications to promote the development of resilience to developing addiction.

S16 Synaptic Defects in Intellectual Disability Syndromes and Autism

S16-01

The impact of pathogenic SYNGAP1 mutations on brain development

G. Rumbaugh

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Tremendous progress has been made into the etiology of neurodevelopmental disorders. While this research has lead to translatable therapeutic targets, it remains unclear how genetic mutations known to cause childhood developmental disorders disrupt brain development. Therfore, we will discuss recent progress on the patho-neurobiology of *Syngap1* haploinsufficiency, which causes one of the most common forms of sporadic intellectual disability. *Syngap1* has also been identified as a high-risk locus for ASD.

In the first set of studies, we found that re-expressing *wt* levels of SynGAP protein globally in the CNS immediately after birth protected mutant animals from developing cognitive and behavioral abnormalities when tested in adulthood. Interestingly, genetic reversal studies performed at PND21 were less successful than neonatal reversal. These animals demonstrated improvements in some behaviors but no improvement in others. When reversal was carried out in adulthood, we could not detect improvement in any of the behaviors used to assess brain function in *Syngap1* mutants. These studies indicate that *Syngap1* mutations disrupt key aspects of brain development, though distinct behavioral abnormalities emerge at different periods of periods of postnatal development. In contrast to behavioral studies, adult genetic reversal completely reversed severe hippocampal LTP deficits, suggesting the intriguing possibility that adult-initiated repair of pathogenic *Syngap1* mutations may improve aspects of cognition that have yet to be tested in mice.

In the second set of studies, we found that pathogenic *Syngap1* mutations had a particularly damaging impact on developing pyramidal neurons. *Syngap1* haploinsufficiency restricted to forebrain glutamatergic neurons was sufficient to disrupt cognition and excitability, while removing mutations from this population prevented the emergence of cognitive abnormalities in mice born with *Syngap1* haploinsufficiency. In contrast, manipulating *Syngap1* function in GABAergic neurons had no effect on cognition, excitability, or neurotransmission, highlighting the specificity of *Syngap1* mutations within forebrain excitatory neurons. Interestingly, cognitive abnormalities were reliably predicted by L2 pyramidal cell synaptic excitability, indicating that the gradual dysfunction of L2 pyramidal cells over development is a biomarker of cognitive deficits in *Syngap1* mutants.

S16-02

Planar cell polarity proteins and their role in neurodevelopmental disorders

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In recent years, a growing amount of data have accumulated implicating Planar Cell Polarity (PCP) signaling in neuronal development, including mechanisms controlling asymmetric division, neuronal migration, neuronal polarity, axonal guidance, dendrite morphogenesis, and synaptogenesis, to cite a few. Given their major role in development, it is not surprising to find them associated with neurodevelopmental disorders. In this presentation, I will focus on mutations of PCP genes and their correlation with neurodevelopmental diseases, including Autism spectrum disorder (ASD) or intellectual disabilities (ID) disorders. Although most of these inherited or de novo human mutations associated with ASD or ID give rise to multisystemic phenotypes, some mutations or deletion are involved in monogenic disorders. With the recent development of single nucleotide polymorphism (SNP) association, global rare copy number variation, rare de novo variants or de novo mutations, PCP genes appear as good candidate as susceptibility genes in ASD and ID syndromes. I will present new evidences showing that PCP signaling is implicated in the regulation of synaptic plasticity, and that it impacts on learning and memory but also ASD-like behavior. Together these data reveal the central role of PCP signaling in brain development and associated disorders.

S16-03

Genes and synapses in autism

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The diagnosis of autism spectrum disorders (ASD) is based on impairments in reciprocal social communication, and repetitive behaviors, but beyond this unifying definition, there is an extreme degree of clinical and genetic heterogeneity. The genetic architecture of ASD is therefore complex made of a combination of common and rare variants. Our previous studies pointed at one biological pathway associated with ASD related to the synapse. Among the causative genes, synaptic cell adhesion molecules (neuroligins and neurexins) and scaffolding proteins (SHANK) are crucial for synapse formation/maintenance as well as correct balance between inhibitory and excitatory synaptic currents. In this presentation, I will discuss our recent results coming from human studies in large populations and genetic isolates as well as mouse studies that shed new light on the inheritance of ASD. I will first detail our recent studies on the contribution of SHANK genes in ASD based on results obtained in humans and mouse models. I will then briefly introduce our results on the link between common variants and the diversity of the human brain neuroanatomy. Finally, I will present a

genetic and functional study on a new susceptibility gene for ASD that shed new light on the sensory-motor abnormalities in ASD.

S16-04

Neuronal dysfunctions underlying phelan–mcdermid syndrome and their pharmacological rescue in mouse and IPS cells

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Haploinsufficiency of the SHANK3/PROSAP2 gene is very likely to be an essential cause of the major neurological features associated with Phelan-McDermid Syndrome (PMS) including severe expressive language and speech delay, hypotonia, global developmental delay, and autistic behaviour. SHANK3 is a synaptic scaffolding protein enriched in the postsynaptic density of excitatory synapses, and plays important roles in the formation, maturation, and maintenance of synapses. We demonstrated that in rat neuronal cell cultures, mGlu5 receptor expression is strongly affected when Shank3 expression is down-regulated by specific shRNA. This knock down led to impairment of mGlu5 signaling at synapse that is rescued using the positive allosteric modulator of mGlu5, CDPPB.

Considering that the Shank3-Homer complex is essential to link the mGlu5 to its down stream signal pathways we measured calcium signaling after DHPG stimulation in neurons KO for Shank3 and we found a reduction of intracellular calcium release. Morphologically this defect is associated with alteration of Homer 1b/c cluster formation

To test our hypothesis *in vivo* we measured mGlu5 and Homer protein expression in different brain areas of Shank3 KO mice and we found a reduction in the expression of both mGlu5 and of Homer1/b protein expression specifically in the striatum.

To be closer to the human pathology we differentiated iPSCs from two PMS patients into neurons and we found a reduction in mGlu5 and Homer protein expression and clustering compared with neurons derived from iPSCs of healthy donors.

Our data strongly suggest that alterations in mGlu5 signaling pathways, due to disassembly of the mGlu5-Homer-Shank complex are involved in the pathogenesis of PMS and that positive modulators of mGlu5 could represent a new strategy for pharmacological treatment of patients with Shank3 mutations.

S17 CNS Myelination: an Update on Novel Regulatory Mechanisms

S17-01

White matter plasticity in response to functional activity

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Structural changes in myelin are traditionally regarded in the context of pathology, but increasingly a wide range of studies from a number of laboratories suggests that white matter can change in response to functional activity, environmental experience, and learning. The possibility that activity-dependent effects on myelin could contribute to information processing by optimizing the timing of spike time arrival at relay points in neural networks will be discussed. The molecular mechanisms that have been identified for activity-dependent effects on myelin will be reviewed.

S17-02

MYRF promotes myelination through both positive regulation of myelin genes and micro-RNA mediated repression of OPC genes

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The differentiation of oligodendrocyte progenitor cells (OPCs) into myelinating oligodendrocytes is tightly coordinated by the opposing expression of positive regulators of differentiation (such as Sox10 and Myrf) as well as negative regulators (such as Hes5, Sox5 and Sox6). In addition, oligodendrocyte development is mediated by the activity of several microRNAs that are induced during differentiation and serve to repress these negative regulators. We have previously demonstrated that Myrf acts to directly promote the expression of many myelin genes during oligodendrocyte differentiation. Here, we find that in addition to its direct positive regulation of myelin genes Myrf is also required for the induction of miR-219 and miR-338 in differentiating oligodendrocytes. This induction is direct, with Myrf binding enhancer regions proximal to the two miRNAs during oligodendrocyte differentiation and promoting transcription from these elements. In the absence of Myrf, miR-219 and 338 fail to be expressed in the differentiating oligodendrocytes, leading to aberrant maintained expression of the inhibitors of differentiation Hes5, Sox5 and Sox6. These results identify Myrf-mediated induction of microRNAs as a mechanism to couple the induction of myelin genes with the repression of negative regulators of differentiation, ensuring a well-coordinated differentiation process.

S17-03

New mechanisms regulating myelination in the central nervous system: the role of GPR17 and of a novel micro-RNA

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In the CNS, generation of myelin is orchestrated by membrane receptors responding to extracellular signals, their intracellular pathways and key transcription factors mediating their effects. How these extrinsic and intrinsic factors are intimately intertwined with each other in the regulatory network for myelination is still largely unclear. We have previously shown that the membrane GPR17 receptor is crucially needed to start Oligodendrocyte Precursors (OPCs) differentiation; however, in immature oligodendrocytes (OLs), GPR17 has to be downregulated to allow cells' terminal maturation and myelination (Daniele S *et al.*, Cell Signal. 26:1310–25, 2014). We have shown this to occur via the phosphorylation/desensitization of GPR17 by G-protein Receptor Kinases followed by GPR17 internalization (ibidem; Fratangeli *et al.*, J Biol Chem. 288:5241–56, 2013). However, how additional intrinsic mechanisms contribute to GPR17 desensitization and OPC progression to mature myelinating stages is still matter of speculation. In this respect, we have recently identified a new microRNA (miR-X) critically involved in OPC differentiation. Both the forced expression and the silencing of miR-X strongly altered OPC maturation in culture in parallel to marked changes of several key OL genes, including GPR17, MAP5 and MBP. Levels of miR-X were altered in both spinal cord tissues from experimental autoimmune encephalomyelitis (EAE) mice (a rodent model of human multiple sclerosis, MS) and in the cerebrospinal fluid of MS patients, suggesting that it could be a hallmark of the disease. These data suggest that miR-X participates to OPC maturation by regulating key OL genes and that its dysregulation may contribute to demyelination/defective remyelination. Understanding the molecular links between miR-X and myelin genes will provide novel therapeutic means to enhance endogenous CNS reparative capabilities in both MS and other demyelinating neurodegenerative diseases.

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S17-04

Glutamate as a regulator of oligodendrocyte morphogenesis

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Oligodendrocytes (OLGs), the myelinating cells of the central nervous system (CNS), undergo extensive changes in morphology when they mature first from bipolar OLG progenitors into premyelinating OLGs extending a complex and expanded process network and then into mature OLGs generating a fully functional myelin sheath. Such OLG morphogenesis is to a large extent driven by changes in the actin cytoskeleton, which occur during development spatially as well as temporally well-coordinated. Currently, however, little is known about the extracellular factors and downstream signaling pathways that are involved in orchestrating these morphological aspects of CNS myelination.

We introduce here a novel signaling cascade that involves the activation of sodium-dependent glutamate transporters and regulation of the actin-binding/bundling domain of calcium/calmodulin-dependent protein kinase IIβ (CaMKIIβ) as a potential modulator of OLG morphogenesis and CNS myelination. More specifically, our findings so far demonstrate that glutamate transporter-mediated promotion of OLG morphogenesis is associated with a transient increase in intracellular calcium levels and a transient phosphorylation event within CaMKIIβ's actin-binding/bundling domain. Importantly such changes in CaMKIIβ phosphorylation were found to correlate with changes in CaMKIIβ-actin-binding. These findings are consistent with the idea that transient and cyclic changes in actin cytoskeletal organization allow morphological remodeling without causing actin cytoskeletal collapse. In the context of our results, it is of further note that cells of the OLG lineage have been shown by us and others to express sodium-dependent glutamate transporters, and that there is increasing evidence for a role of sodium-dependent glutamate transporters beyond their well-described functions in regulating extracellular glutamate concentrations. Thus, our studies point toward the existence of a cyclic acting sodium-dependent glutamate transporter-CaMKIIβ-actin cytoskeleton axis within differentiating OLGs that plays a critical modulatory role in the regulation of CNS myelination.

S18 Extracellular Vesicles: Their Role in Neuronal Signalling in Health and Disease

S18-01

Exosomes as a novel way for interneuronal communications

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Exosomes are small extracellular vesicles, which stem from endosomes fusing with the plasma membrane, and can be recaptured by receiving cells. They contain lipids, proteins and RNAs able to modify the physiology of receiving cells. Functioning of the brain relies on intercellular communication between neural cells. These communications can modulate the strength of responses at sparse groups of specific synapses, to modulate circuits underlying associations and memory. Expression of new genes must then follow to stabilize the long term modifications of the synaptic response. Local changes of the physiology of synapses from one neuron driven by another, have so far been explained by classical signal transduction to modulate transcription, translation and posttranslational modifications. *In vitro* evidence now demonstrates that exosomes are released by neurons in a way depending on synaptic activity. (Lachenal *et al.* 2011) These exosomes which contain specific sets of miRNAs can be retaken by other neurons (Chivet *et al.* 2014) suggesting a novel way for interneuronal communication. Exosomes could thus represent an ideal mechanism for inter-neuronal transfer of information allowing anterograde and retrograde signaling across synapses necessary for plasticity. They might also allow spreading across the nervous system of pathological proteins like PrPsc, APP fragments, phosphorylated Tau or Alpha-synuclein.

S18-02

Pathogenic role of microglia-derived microvesicles in neuroinflammation and neurodegeneration

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Cells communicate not only via chemical signals, but also via extracellular membrane microvesicles (EVs) which are released into the intercellular space and which can carry signals, either on their membrane or in their lumen. Beside representing important mediators of intercellular communication, EVs are emerging as new biomarkers of tissue damage.

Our previous results indicated that reactive microglia shed EVs, both *in vitro* and *in vivo*. EVs store inflammatory signals, such as IL1-beta protein and mRNA, propagate an inflammatory response among glial cells and acutely modulate neurotransmission by causing excitatory-inhibitory unbalance (Bianco *et al.*, 2009; Verderio *et al.*, 2012; Antonucci *et al.*, 2012; Gabrielli *et al.*, 2015). A recently proposed function of EVs produced by brain cells is the transfer and processing of pathogenic proteins associated to Alzheimer's disease (AD). Given microgliosis is a central event in AD and production of EVs increases upon microglial activation, we evaluated whether production of EVs increases in AD patients and explored the possibility that EVs represent a mechanism by which reactive microglia may contribute to AD neuronal damage.

Methods and Results: By using flow cytometry to quantify myeloid EVs in the cerebrospinal fluid (CSF) collected from human subjects we found strikingly high levels of microglial EVs in patients with AD as compared to healthy controls. Furthermore, we observed that EVs isolated from AD patients display strong neurotoxic activity *in vitro*, when exposed to hippocampal neurons maintained in primary culture. The molecular mechanisms by which microglia-derived EVs interact with neurons and may propagate neuronal damage will be the focus of the presentation.

S18-03

How stem cells speak with immune cells

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Advances in stem cell biology have raised great expectations that diseases of the central nervous system may be ameliorated by the development of non-haematopoietic stem cell medicines. Yet, the application of stem cells as therapeutics is challenging and the interpretation of some of the outcomes ambiguous. The initial idea that stem cell transplants work only via structural cell replacement has been challenged by the observation of consistent intercellular information exchange between the graft and the host. Sustained stem cell graft-to-host exchange of signals has led to remarkable trophic effects on endogenous brain cells and beneficial modulatory actions on innate and adaptive immune responses that ultimately promote the healing of the injured CNS. Among a number of promising candidate stem cell sources, mesenchymal/stromal stem cells (MSCs) and neural stem/precursor cells (NPCs) are being extensively investigated for their capacities to *signal* to the immune system upon transplantation in experimental CNS diseases.

Here, we focused on defining whether the form of cellular signalling mediated by extracellular membrane vesicles (EVs) exists for neural stem/precursor cells (NPCs), and on its molecular signature and functional relevance on target cells. We also investigated whether the EV cargo molecules are modulated by extracellular pro- or anti-inflammatory cytokines, determined the key elements responsible for this novel mechanism of EV-mediated intercellular communication, and finally reflected on the forthcoming challenges related to the translation of these exciting experimental proofs into ready-to-use clinical medicines for inflammatory CNS diseases.

S18-04

Signal-mediated transfer of exosomes from glia to neurons: "care packages" for neuronal support?

E.-M. Krämer-Albers, C. Frühbeis, D. Fröhlich, W. P. Kuo

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Continuous communication between neurons and oligodendrocytes is essential for myelination and the maintenance of axonal integrity. Exosomes are universally secreted vesicles that mediate cell communication in multiple tissues, including the CNS. Exosomes deliver a specific set of biomolecules including proteins, lipids and small non-coding RNAs modulating the phenotypic behavior of recipient cells. Our recent work revealed that exosomes are involved in bidirectional neuron-glia interaction. Exosomes are released by oligodendrocytes in response to neurotransmitter signalling by activation of glial ionotropic glutamate receptors. These exosomes are internalized by neurons via endocytosis and the exosome cargo is functionally recovered by the recipient neurons. Neurons appear to benefit from exosome internalization by increased resistance to stress conditions such as oxidative stress, starvation or ischemia. Oligodendroglial exosomes have the ability to modulate a broad spectrum of neuronal functions. Treatment of cultured neurons with isolated glial exosomes affects action potential firing and axonal transport, activates signal transduction pathways, and regulates neuronal gene expression. To determine the specific role of exosomes in glial support, we studied PLP- and CNP-deficient mice characterized by secondary axonal degeneration. Intriguingly, exosomes released from PLP- and CNP-deficient oligodendrocytes exhibit quantitative, qualitative, and functional abnormalities. In summary, we propose that oligodendroglial exosomes function as vehicles for the transfer of biomolecules from oligodendrocytes to neurons and are implicated in neuroprotection and glial maintenance of axonal functions. Funded by DFG.

Relevant Publications:

Fröhlich D, Kuo WP, Frühbeis C, *et al.* (2014) Multifaceted effects of oligodendroglial exosomes on neurons: impact on neuronal firing rate, signal transduction, and gene regulation. *Philos Trans R Soc Lond B Biol Sci.*, Sep 26;369

Frühbeis C. Fröhlich D, Kuo WP, *et al.* (2013) Neurotransmitter-triggered transfer of exosomes mediates oligodendrocyte-neuron communication, *PLOS Biol* 11(7):e1001604.

Frühbeis C, Fröhlich D, Kuo WP, and Krämer-Albers EM (2013) Extracellular vesicles as mediators of neuron-glia communication. *Front Cell Neurosci.* 7:182.

S19 Molecular Determinants of Homeostatic Plasticity in the Brain

S19-01

An unbiased screen identifies the adaptor protein complex AP-3A as a mediator of synaptic scaling up

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Synaptic scaling is a form of homeostatic synaptic plasticity that stabilizes neuronal firing through the regulation of synaptic AMPAR abundance. This process is transcription-dependent but the pathways that translate a change in firing into transcription-dependent changes in AMPAR trafficking are unknown. Here we devised a cell-type specific screen that identified 33 transcripts with altered expression after scaling; two of these coded for μ subunits (μ3A and μ4) of the adaptor protein complexes AP-3A and AP-4, both known to associate with AMPAR. Further characterization of μ3A revealed that μ3A expression was upregulated *in vitro* by activity blockade; this upregulation enhanced the sorting of AMPAR to recycling endosomes, and was essential for the regulated delivery of AMPAR to the surface during synaptic scaling up. Together these data provide the first insight into the transcriptional regulation of synaptic scaling, and identify μ3A as an essential transcription-dependent switch point that can direct AMPAR to RE to enable homeostatic plasticity.

S19-02

Messages from the extracellular space: ECM during homeostatic plasticity

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During late neuronal development a condensed, brain-specific extracellular matrix (ECM) peculiarity rich in chondroitin sulfate proteoglycans such as brevican is formed. This specialized structure that assembles around hyaluronic acid in the extracellular space affects long- as well as short-term plasticity and has been found to function in synapse and neuronal network stabilization. Neuronal networks are balanced by mechanisms of homeostatic plasticity, which adjusts synaptic strength via molecular and morphological changes in the pre- and post-synapse. In my presentation I will discuss the involvement of the hyaluronic acid-based extracellular matrix (ECM) of the brain in mechanisms of homeostatic plasticity. We hypothesize that the ECM may be be remodeled to allow for structural and molecular changes during conditions of homeostatic plasticity. Indeed, we found that the ECM component brevican is proteolycally cleaved after inducing homeostatic plasticity in neuronal cell cultures by prolonged network inactivation. Specific protease inhibitors could block this effect and influence regulation of synaptic protein levels. Thus, the ECM degradation may liberate synapses to allow for a higher degree of structural and functional plasticity.

S19-03

Control of synaptic connectivity and homeostasis by astrocytes

C. Eroglu

Duke University Medical Center, Cell Biology and Neurobiology, Durham, USA

How are synaptic networks formed during development, maintained throughout life and remodeled during learning and disease? This is the main question that drives the research in our laboratory. We investigate the roles of *glial cells, particularly astrocytes,* in the development, remodeling and function of synaptic circuits. In this talk I will summarize our recent findings that revealed that astrocytes through secreted synaptogenic protein hevin controls the formation and maintenance of thalamocortical synapses.

S19-04

Synaptic tenacity – beyond one molecule or another

N. Ziv

Technion, Faculty of Medicine, Haifa, Israel

Activity-dependent modifications to synaptic connections – synaptic plasticity – is widely believed to represent a fundamental mechanism for altering network function, giving rise to emergent phenomena commonly referred to as learning and memory. This belief also implies, however, that synapses, when *not* driven to change their properties by physiologically relevant stimuli, should retain these properties over time. Otherwise, physiologically relevant modifications would be gradually lost amidst spurious changes and spontaneous drift. We refer to the expected tendency of synapses to hold onto their properties as "synaptic tenacity".

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

S20 Microglia Plasticity – Molecular Characteristics of Different Phenotypes

S20-01

Microglia plasticity – molecular characteristics of different phenotypes

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Microglia are CNS resident myeloid cells which respond to signals originating in the injured, infected or dysfunctional brain. Under some pathological conditions blood-derived macrophages contribute to the heterogeneity of myeloid population. Also astrocytic brain tumors (gliomas) attract microglia and infiltrating macrophages, which instead of initiating anti-tumor and inflammatory responses, are re-programmed into cells promoting tumor progression. The molecular signature of glioma-associated myeloid cells, and individual roles of microglia and peripheral macrophages are still unclear. Lack of robust markers, which can differentiate microglia from infiltrating macrophages, and overlapping profiles, makes it less feasible. Global gene expression profiling, functional assays and a comprehensive analysis of biochemical pathways in primary microglia cultures stimulated with lipopolysaccharide or glioma-secreted factors (GCM) revealed activation of non-overlapping transcriptional, signaling and metabolic pathways. We found that glioma secreted factors enhance cytoprotective and "healing" properties of microglia. Assessment of transcriptional regulation in various myeloid populations demonstrated that microglia are unique and distinct from other macrophage cell types. However, our studies of the "putative" microglial and macrophage marker expression in microglia exposed to glioma or LPS demonstrated rather a phenotype switching and polarization than a fixed profile. GCM increased the microglia-specific genes, while LPS down-regulated these markers compared to controls. Conversely, LPS increased the "macrophage specific" markers in microglia. Those results indicate the functional plasticity of microglia. Gene expression studies in sorted microglia and infiltrating macrophages from murine experimental gliomas indicate that blood-derived macrophages first acquire the M1 inflammatory phenotype and become re-programmed into pro-tumorigenic M2-like cells in glioma microenvironment. The similar phenomenon was shown in rat gliomas, however, microglia were found to be the predominant glioma-associated myeloid population. A recently developed protocol to isolate human microglia from resected gliomas allowed us to characterize glioma-associated myeloid cells and define different phenotypes in the context of tumor malignancy. Our data demonstrate distinct functional properties and transcriptional networks of microglia activation *in vivo*, that suggests its distinct functions in cell damage, cytoprotection and repair processes.

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S20-02

Human microglial biology

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Microglia are critical cells in brain physiology and are also involved in many brain disorders, both psychiatric and neurological. Understanding the biology of these cells is critical to developing microglial-focused medications to treat brain disorders. We have been studying the biology of microglia derived from adult human brain with the aim of shedding light on their biology. Our studies have shown that adult human brain microglia show very low basal proliferation and that M-CSF is able to increase human microglial proliferation and phagocytosis. We have also demonstrated that the transcription factor PU.1, which we first showed expressed in rodent microglia is also strongly expressed by human microglia under basal conditions. M-CSF enhances human microglial PU.1 expression and phagocytosis, and also induces CEBPβ. PU.1 knock-down with siRNA inhibits human microglial phagocytosis and survival, and has major effects on human microglial gene expression. PU.1/CEBPβ dimers may be critical for proliferation/survival and phagocytic activity of adult human microglia. Species similarities (eg: PU.1 expression) and differences exist in microglial biology, and some of these will be discussed in this presentation. For example, valproic acid, commonly used in bipolar disorder and epilepsy, is known to be a strong activator of rodent microglial caspase 3-mediated apoptosis and also phagocytosis. In contrast, in human microglial cultures it does not cause caspase 3-mediated apoptosis and inhibits, rather than stimulates, phagocytosis. The reasons for these different species-specific actions are currently not clear but suggest that more studies of human microglial biology are needed to fully understand the roles of these critical cells in brain physiology and pathology.

S20-03

Microglia in neuroprotection and CNS immunosenescence

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Microglial cells activated in response to CNS injury are essential for providing post-injury neuronal protection, restoring homeostasis and promoting healing of injured tissues. They do so via increased production of growth factors and pro-regenerative cytokines, as well as via phagocytosis of dead cells and debris. Although activated microglia have long been implicated as cytotoxic immune effector cells promoting neuronal degeneration in Alzheimer's disease (AD), we have not been able to detect activated microglia in association with neurofibrillary degeneration (NFD) in human AD brain, but instead have found a conspicuous co-localization of dystrophic microglia and degenerating neurons. In contrast to activated microglia which are readily identified due to their cytoplasmic

hypertrophy, dystrophic cells show structural deformities, such as gnarling, twisting, beading and fragmentation of their cytoplasmic processes, changes that are increasingly prevalent with aging and therefore representative of senescent microglial degeneration. We have also found that presence of dystrophic microglia precedes the occurrence of NFD and we therefore hypothesize that NFD occurs as a consequence of CNS immune senescence. Additional support for this hypothesis comes from studies showing that when indeed microglial activation occurs as a result of infection or trauma the onset or extent of NFD is unaffected.

S20-04

Microglial cells promote glioma growth

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We have studied the interactions between microglial cells and glioma cells. Based on our first finding that microglia promotes glioma growth (Markovic *et al.*, 2005, J Neuropathol Exp Neurol. 64:754–762), we have identified mechanisms of interaction between these cells. We found that glioma cells induce microglia to express a metalloprotease, MT1-MMP which is necessary for the activation of MMP-2. Glioma cells release MMP-2 in an inactive form which is then activated by the microglial MT1-MMP (Markovic *et al*., 2009, Proc Natl Acad Sci USA. 106:12530–12535). The metalloproteases degrade extracellular matrix and thereby promote glioma invasion and expansion. In a collaboration with Seija Lehnhardt, we found that this signalling between microglia and glioma cells is mediated by Toll -like receptor 2 (Vinnakota *et al*., 2013, Neuro Oncology 15:1457–1468). As ligand for Toll -like receptor 2 we recently identified versican which is released from mouse and human glioma cells (Hu *et al*., 2015, Neuro Oncol. 17:200–210). Microglial cells also upregulate and release MMP-9 triggered by the same pathway (Hu *et al*., 2014, Int J Cancer. 135:2569–2578). Microglial cells accumulate around and within glioma. As chemoattractant for microglia we identified GDNF which is released from the glioma cells and attracts microglia (Ku *et al*., 2013, Acta Neuropathol 125:609–620). With a microarray screen, we also found that glioma cells induce a specific phenotype in microglia which does neither fit into the M1 nor in the M2 classification (Szulzewsky *et al*. 2015, PLOSone, in press).

S21 What do HCN Channels Teach us about Health and Disease?

S21-01

Epigenetic regulation of HCN channels: a window into neuroplasticity?

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The mechanisms generating epileptic neuronal networks following insults such as severe seizures are unknown. We have previously shown a down-regulation of hyperpolarizing cAMP-dependent cation-nonselective (HCN) channel type 1 (HCN1) during the process of seizure-induced epilepsy (Brewster *et al*., 2002), a finding confirmed almost universally by numerous groups around the world. We therefore sought the mechanisms regulating HCN1 repression as a potential unifying or common principle in the neuroplasticity that converts a normal neuron into an epileptic one. HCN1 repression required the actionf of a key neuronal transcription facotr, NRSF/REST. Further, interfering with the function of the neuron-restrictive silencer factor (NRSF/REST), an important transcription factor that influences neuronal phenotype, attenuated development of epilepsy after a provoking insult (McClelland *et al*., 2011). Epilepsy-provoking seizures increase the low NRSF levels in mature hippocampus several fold yet surprisingly, provoked repression of only a subset (~10%) of potential NRSF target genes. Accordingly, the repressed gene-set was rescued when NRSF binding to chromatin was blocked. Unexpectedly, genes selectively repressed by NRSF had midrange binding frequencies to the repressor, a property that rendered them sensitive to moderate fluctuations of NRSF levels. Genes selectively regulated by NRSF during epileptogenesis coded for ion channels, receptors and other crucial contributors to neuronal function (McClelland *et al*., eLife 2014). Thus, dynamic, selective regulation of NRSF target genes may play a role in influencing neuronal properties in pathological and physiological contexts, a lesson learned from the regulation of HCN channels (Noam *et al*., 2011)

S21-02

The identification and characterization of novel HCN2 mutation found in febrile seizure patients

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Hyperpolarization-activated cyclic nucleotide-gated (HCN) currents (so called I_h) play an important role in the stabilization of resting membrane potential in neuron. In the human brain, HCN1 and HCN2 proteins make heterotetramer which produces I_h. Recent studies report that HCN channels are implicated in febrile seizures. We found the HCN2 amino acid substitution (S126L) from two febrile seizure patients and examined the characterization of the HCN2 mutant protein. An *in-vitro* experiment exhibited that little difference in the activation curve between wildtype and mutant at room temperature (25°C). Next we examined the effect of temperature raises in the physiological range (35–40°C). During hyperthermic condition (mimicking a febrile situation), the half maximal activation voltage ($V_{1/2}$) shifted to depolarized side, making it larger in the mutant than in the wildtype. The current density of mutant channels at -140 mV was significant larger than that of wildtype. There was no notable difference between the wildtype and the mutant in the application of cyclicAMP. These results indicate that novel HCN2 mutation increased I_h in hyperthermic situation and the temperature sensitivity. This mutation might contribute to neuronal hyperexcitability, thus leading to febrile seizure in patients.

S21-03

pre-synaptic HCN channel plasticity in epilepsy

M. Shah

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The entorhinal cortex (EC) is situated adjacent to the hippocampus. Like the cortex, it is made up of 6 layers. The axons of the neurons within the superficial EC layers I-III project to the hippocampus. In contrast, neurons within the deeper layers (layer V) receive projections from the hippocampus and their axons project to other cortical regions. Thus, alterations in excitability within the EC are likely to have profound effects on the activity of the hippocampus as well as other cortical areas.

The EC is one of the key brain regions involved in seizure initiation and maintenance during epilepsy. Hence, it is critical to understand how excitability within the EC alters during epilepsy. We have shown that the EC is hyperexcitable *in vivo* following seizures in an animal model of epilepsy (Shah *et al*., Neuron, 2004; Huang *et al*., Journal of Neuroscience, 2009). *In vitro* electrophysiological experiments also revealed that EC layer III pyramidal neurons have enhanced excitabiility following seizure induction (Shah *et al*., Neuron, 2004). This hyperactivity is at least partially due to a decrease in expression of the hyperpolarization-activated cyclic nucleotide gated (HCN) channels (Shah *et al*., Neuron, 2004; Huang *et al*., Journal of Neuroscience, 2009). HCN channels are concentrated in EC pyramidal dendrites and indeed, EC layer III pyramidal cell dendritic HCN channel function is reduced following seizure activity in an animal model of epilepsy.

Our recent work, though, shows that HCN channels are also localized to a subset of pre-synaptic glutamatergic terminals which exclusively synapse onto EC layer III pyramids (Huang *et al*., Nature Neuroscience, 2011). Here, they inhibit synaptic transmission by reducing pre-synaptic T-type Ca^{2+} channel function. Interestingly, we found that seizure activity also reduces pre-synaptic HCN channel function. In this talk, I will discuss the implications for this for EC neuronal circuit activity during epilepsy.

S21-04

Generalised seizure–-mediated changes in HCN channels associates with learning deficits

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The $GABA_A\gamma2$(R43Q) mouse is an established model of absence epilepsy displaying spontaneous spike-and-wave discharges (SWD) and associated behavioral arrest. Absence epilepsy typically results from cortico-thalamic networks. Nevertheless, there is increasing evidence for changes in hippocampal metabolism and electrical behaviour, consistent with a link between absence seizures and hippocampus-related co-morbidities. Hyperpolarization-activated -Cyclic -Nucleotide –gated (HCN) channels are known to be transcriptionally regulated in a number of seizure models. Here we investigate the expression and function of these channels in the hippocampus of the genetic epilepsy model. A reduction in HCN1, but not HCN2 transcript, was observed in $GABA_A\gamma2$(R43Q) mice relative to their littermate controls. In contrast, no change in HCN1 transcript was noted at an age prior to seizure expression or in a SWD-free model in which the R43Q mutation has been crossed into a seizure-resistant genetic background. Whole-cell recordings from CA1 pyramidal neurons confirm a reduction in I_h in the $GABA_A\gamma2$ (R43Q) mouse. Further, a left-shift in half-activation of the I_h conductance-voltage relationship is consistent with a reduction in HCN1 with no change in HCN2 channel expression. Behavioral analysis using the Morris water maze indicates that $GABA_A\gamma2$ (R43Q) mice are unable to learn as effectively as their wildtype littermates suggesting a deficit in hippocampal-based learning. SWD-free mice harboring the R43Q mutation had no learning deficit. We conclude that SWDs reduce hippocampal HCN1 expression and function, and that the reduction associates with a spatial learning deficit.

S22 Synaptic Cell Adhesion in Development and Plasticity

S22-01

Neuroplastins, IG-like cams with differential functions in inhibitory and excitatory synapses

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Neuroplastins are highly glycosylated cell adhesion molecules of the immunoglobulin superfamily [1]. They come in different isoforms bearing two (np55) or three (np65) Ig domains. While np55 is widely distributed in many tissues, np65 is primarily expressed in the nervous system. Np65 is a regulator of synaptic plasticity and, as reported recently by Desrivieres and colleagues, polymorphisms in the human NPTN are associated with cortical thickness and intellectual abilities in adolescents [2]. Studies on primary neuronal cultures derived from neuroplastin-deficient mice revealed differential effects on the formation of glutamatergic and GABAergic synapses [3]. While excitatory synapses display delayed synaptogenesis, inhibitory synapses contain altered subunit composition of $GABA_A$ receptors as compared to wild types. The latter phenotype may be explained by direct interaction between neuroplastins and certain subtypes of $GABA_A$ receptors. Np65s can homophilically interact in trans via their most N-terminal Ig domains. Disturbance of this interaction via function-blocking antibodies or competition with peptides or recombinant fusion constructs causes phenotypes similar to knock outs. Homophilic interaction induces intracellular down-stream signaling. One candidate pathway includes interaction with the adaptor protein Traf6. The role of this interaction and the thereby induced signaling pathway is currently under investigation.

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S22-02

Neuroligins at inhibitory synapses – mechanisms of synaptogenesis and dysfunction in autism

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Neuroligin-2 and Neuroligin-4 are synaptic cell adhesion proteins of the Neuroligin family and are preferentially localised to inhibitory synapses. Loss of function mutations in Neuroligin-4 cause monogenic heritable forms of autism in humans and autism-like behavioral defects in mice.

I will present biochemical, structural biology, cell biological, and electrophysiological data demonstrating that Neuroligin-2 and Neuroligin-4 regulate the recruitment of $GABA_A$-receptors to nascent inhibitory synapses by activating the signaling/scaffold protein Collybistin. Functional defects resulting from Neuroligin-4 loss in mice include reductions in $GABA_A$-receptor signaling in several brain regions, such as the CA3 region of the hippocampus, and may be the cause for the autism-like behavioral defects seen upon Neuroligin-4 mutation.

S22-03

Role of NGL-3 in the regulation of synapse formation and synaptic plasticity

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Synaptic adhesion molecules play important roles in the regulation of synapse development, including initial axo-dendritic contacts, early synapse formation, maturation and plasticity of these synapses. The netrin-G ligand (NGL; also known as LRRC4) family of synaptic adhesion molecules contains three known members (NGL-1/LRRC4C, NGL-2/LRRC4, and NGL-3/LRRC4B). They share a similar domain structure, including the leucine-rich repeats and an Ig-like domain in the extracellular region, a transmembrane domain, and a C-terminal PDZ-binding motif, which directly interacts with the PDZ domains of the excitatory postsynaptic scaffolding protein PSD-95. Functionally, however, these adhesion molecules have distinct properties, contributing to different aspects of synapse development through unique mechanisms. Postsynaptic NGL-1 and NGL-2 bind to presynaptic netrin-G1 and netrin-G2, respectively, whereas postsynaptic NGL-3 binds to presynaptic LAR, a family of receptor tyrosine phosphatases with three known members (LAR, PTPσ, and PTPδ). In addition, NGL-3 induces presynaptic differentiation that is much stronger than that observed in NGL-1 or NGL-2. In contrast, NGL-1 and NGL-2 seem to promote lamina-specific subdendritic segmentation instructed by presynaptic axons containing netrin-G1 and netrin-G2, respectively. While the *in vitro* and *in vivo* roles of NGL-1 and NGL-2 have recently been reported, NGL-3 has been explored to a lesser extent. In this presentation, I will discuss *in vitro* and *in vivo* functions of NGL-3, including its potential role in the regulation of excitatory synaptic transmission and plasticity.

S22-04

New perspectives on NMDA receptor function

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The NMDA subtype of ionotropic glutamate receptors (NMDA receptors) are formed by tetrameric complexes of GluN1 and GluN2 subunits and play central roles in synapse maturation and synaptic plasticity. Their functions in excitatory synaptic transmission, postsynaptic signaling, long term potentiation and long term depression have been extensively studied for many years. We have searched for novel functions of NMDA receptors by comprehensively analyzing gene expression changes when basal activity of NMDA receptors are blocked by NMDA receptor antagonists (APV or GluN2A- or GluN2B-preferring inhibitors). These studies have led to the discovery of unexpected roles for NMDA receptors in the regulation of neuronal morphology, neuronal survival and even non-cell autonomous effects on glial cells.

S23 Lipids in Normal and Pathological Neuronal Function

S23-01

Cholesterol in the nervous system: different roles of an essential lipid

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In the brain, several functions have been attributed to cholesterol, such as neuronal differentiation, neurite outgrowth, and synaptogenesis. However, the bulk amount of cholesterol in the brain resides in myelin. About 25% of the free cholesterol of an entire adult mouse is incorporated in myelin membranes. In the brain, myelin even accounts for about 80% of the brain cholesterol content. Given that sterol synthesis is a complex and energy consuming process, the animal devotes substantial effort to establish and maintain this large and quite homogenous pool of cholesterol. This suggests that cholesterol in myelin serves essential brain functions. Cholesterol influences myelination at many steps, from the differentiation of myelinating glial cells, over the process of myelin membrane biogenesis, to the functionality of mature myelin. Cholesterol emerged as the only integral myelin component that is essential and rate-limiting for the development of myelin in the central and peripheral nervous system. Moreover, disorders that interfere with sterol synthesis or intracellular trafficking of cholesterol and other lipids cause hypomyelination and neurodegeneration.

S23-02

PLD1 generated phosphatidic acid and neuronal function in normal and pathological condition

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Exocytosis of neurotransmitters and hormones occurs through the fusion of secretory vesicles with the plasma membrane. This highly regulated process involves key proteins such as SNAREs but also specific lipids at sites of membrane fusion. Phospholipases D (PLD1/2) have recently emerged as promoters of membrane fusion in various exocytotic events potentially by providing fusogenic cone-shaped phosphatidic acid (PA). Overexpression and silencing approaches have suggested that PLD1 plays a positive role in secretory granule exocytosis. Using novel pharmacological and genetic approaches we now strengthen this model. Amperometric recordings from mice PLD1−/− chromaffin cells in culture suggest that PA favors a late step in membrane fusion. Using a molecular probe for PA, I will also show that the fusogenic lipid accumulates at the plasma membrane facing chromaffin granules that appeared morphologically docked at the electronic microscopy level, a process that is regulated by the ribosomal S6 kinase 2 (RSK2). I will also show that neurons cultured from mice deficient for *Rsk2*, a model for the Coffin-Lowry syndrome (CLS), a syndromic form of mental retardation with growth defect, exhibit a significant delay in development in a pattern very similar to that shown by neurons cultured from *Pld1* knockout mice. We found that gene silencing of *Pld1* or *Rsk2,* as well as acute pharmacological inhibition of PLD1 or RSK2 strongly impaired neuronal growth factor (NGF)-induced neurite outgrowth from PC12 cells. NGF triggered RSK2-dependent phosphorylation of PLD1 leading to the synthesis of phosphatidic acid at the site of neurite growth. NGF-induced neurite outgrowth was severely inhibited in PC12 cells silenced for RSK2 expression, but expression of a phosphomimetic PLD1 mutant was able to rescue the growth defect, revealing that PLD1 is the major target of RSK2 in neurite formation. Finally PLD1 and RSK2 inhibitors altered NGF-induced VAMP-7 vesicle exocytosis. These findings suggest that the loss of function mutations in *RSK2* that lead to CLS and neuronal deficits are related to defects in hormonal release and neuronal growth due to impaired RSK2-dependent PLD1 activity.

S23-03

Role of sphingomyelin in neuronal physiology and pathology

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Sphingomyelin is the most abundant sphingolipid especially enriched in neurons. By compartmentalizing the membranes, sphingomyelin contributes to cellular signalling together with cholesterol. However, its specific roles are poorly understood. To address this issue we have used mice that lack the acid sphingomyelinase (ASMko) and present high sphingomyelin levels at the lysosomal, plasma and synaptic membranes of neurons. These mice mimic Niemann Pick disease type A, a rare disease caused by mutations in the ASM gene leading to severe neurological alterations and early death. Our studies show a relevant influence of sphingomyelin in processes such as autophagy, calcium regulation and synaptic plasticity. We have characterized the molecular mechanisms underlying these influences, which involve lysosomal membrane permeabilization, the plasma membrane calcium ATPase or the dendritic spine actin cytoskeleton. This knowledge has allowed us to test strategies to revert the aberrant neuronal phenotypes in the ASMko mice. The results not only offer insight on sphingomyelin function but also open therapeutical perspectives for a currently untreatable disease like Niemann Pick type A.

S23-04

Resident caps (CADPS) on dense-core vesicles primes vesicles for exocytosis

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CAPS (aka CADPS) plays a central role in preparing the membrane fusion machinery for calcium-triggered dense-core vesicle exocytosis. Prior *in vitro* studies found that CAPS binds $PI(4,5)P_2$ and syntaxin-1/SNAP-25 t-SNARE complexes, key

plasma membrane constituents for exocytosis, via its PH and MHD1 domain, respectively. Cell studies confirmed the importance of CAPS PH and MHD1 domains for evoked vesicle exocytosis. However, direct studies of CAPS at sites of vesicle exocytosis in live cells have not been reported. We expressed a fluorescent CAPS-mKate2 protein in PC12 cells to determine its location before, during and following exocytosis. Expressed BDNF-eGFP in vesicles was used to detect exocytosis by TIRF microscopy. CAPS clusters composed of ~9 molecules were found to align with BDNF-eGFP-containing vesicles in resting cells. Importantly, CAPS clusters moved precisely with any mobile vesicles in the TIRF field, and newly-recruited vesicles delivered CAPS to membrane-proximal sites during vesicle docking. Confocal z sectioning of digitonin-permeabilized, fixed PC12 cells incubated with CAPS antibody revealed that most dense-core vesicles carried CAPS as a resident. In live cell studies, CAPS-mKate2 clusters were present at virtually all vesicle exocytic events involving either resident or recruited vesicles. At moderate calcium elevations (400 μM) that are optimal for evoking vesicle exocytosis, CAPS-mKate2 clusters persisted during and following exocytosis. However, at greater calcium elevations (800 μM), CAPS-mKate2 dissociated from vesicles that underwent exocytosis. To determine if CAPS functioned as a vesicle resident protein, we utilized a CAPS(ΔC135) protein that fails to localize to dense-core vesicles. In PC12 cells depleted for CAPS by shRNA knockdown, there are few evoked dense-core vesicle exocytic events. These were restored by expression of a wild-type CAPS but not by expression of CAPS(ΔC135). We conclude that CAPS acts to enable exocytosis while bound to dense-core vesicles. Studies to identify proteins that mediate CAPS binding to dense-core vesicles are in progress.

S24 Neuroglia in Ageing and Neurodegeneration

S24-01

Morphological and metabolical changes in neuroglia during the progression of Alzheimer's disease and ageing

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Neuroglial cells are fundamental for brain homeostasis and therefore represent the intrinsic brain defence system. Thus, ageing and all forms of neuropathological processes inevitably involve glial cells. Neurodegenerative diseases, including Alzheimer's disease (AD) disrupt brain connectivity and feunction, therefore, affecting neuronal-neuronal, neuronal-glial and glial-glial interaction. Furthermore, neurodegenerative processes trigger universal and conserved glial reactions classically, but not exclusive, represented by astrogliosis and microglial activation. The recently acquired knowledge allows us to regard ageing and neurodegenerative diseases as primarily gliodegenerative processes, in which glial cells differentially determine the progression and outcome of neuropathological processes such as ageing and AD among others. We have recently probed this active pathological role, by showing: (i) an astroglial generalised atrophy with a concomitant astrogliosis just restricted to Ab plaques presence in the case of AD, whilst global hypertrophic behaviour in ageing processes, except in the entorhinal cortex, ii) alterations in glutamate glial metabolism and changes in S-100b trophic factor in both ageing and AD and finally (iii) an early resting microglial recruitment in AD affected areas, even before the presence of activated/macrophagic microglial cells. These glial alterations, which are complex and region dependent are fundamental for the disruption of neural networks connectivity as well as with the neurotransmitters imbalance that underlie the mnesic deficits associated with AD and ageing, even if they show a differential cellular pattern regional distribution and behaviour in both processes. All this, could trigger new insights into the search for a potential therapeutic treatments for pathological ageing and AD.

S24-02

Changes in microglial response to glutamate and thyroid hormone in neurodegenration

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Microglia express various neurotransmitter receptors and hormone receptors. We have investigated the function of AMPA-type of glutamate (Glu) receptors (AMPAR) and thyroid hormone (TH) receptors both in cultured microglia and *in vivo*. Majority of microglial AMPAR are Ca^{2+} impermeable due to the expression of GluA2. Low expression of GluA2 was reported in some neurodegenerative diseases. On the other hand, impairment of THs, such as hypothyroidism and hyperthyroidism, in adult brain can cause neurological dysfunctions or psychosis. Therefore, $GluA2^{-/-}$ mice and TH receptor α (THRα−/−) mice or TH-injected mice were used to show changes of microglial response to Glu/kainate (KA) or TH.

Glu-induced currents in the presence of an inhibitor of AMPAR desensitization, showed time-dependent decrease after activation of microglia with lipopolysaccharide (LPS) in $GluA2^{+/+}$ microglia, but not in $GluA2^{-/-}$ microglia. Upon activation of microglia, expression level of GluA2 subunits significantly increased, while expression of GluA1, A3 and A4 subunits on membrane surface significantly decreased, showing little Glu-induced currents. However, $GluA2^{-/-}$ microglia showed higher Ca^{2+}-permeability, consequently inducing significant increase in the release of proinflammatory cytokine, such as TNF-α. These results suggest that dysfunction or decreased expression of GluA2 may accelerate Glu neurotoxicity via excess release of proinflammatory cytokines from microglia.

As for the TH function in microglia, exposure to TH increased migration, membrane ruffling and phagocytosis of primary cultured mouse microglia. TH-induced activation of glial cells in cortex and hippocampus were dependent on sex and age of the mice.

These results may help to understand how dysfunction of AMPAR or TH in microglia contributes to neurodegenerative diseases or age-related neuronal dysfunction.

S24-03

Early signs of neuroinflammation and vascular dysfunction in experimental Alzheimer's disease. is the hilus mainly susceptible?

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder without effective therapy. Brain amyloid deposits are classical histopathological hallmarks that generate an inflammatory reaction affecting neuronal and glial function. We studied early alterations of hippocampal glia and microvasculature besides their progression during the neuropathology in PDAPP-J20 transgenic mice, recognized AD model. At 3 m, before deposits formation but assessable levels of soluble Aβ1–40 and 1–42, microglial Iba1+ cells from transgenic mice already exhibited signs of activation and larger soma size in the hilus of the dentate gyrus, alterations appearing later on stratum radiatum. Iba1 immunohistochemistry revealed increased cell density and immunoreactive area in PDAPP mice from 9 m onwards selectively in the hilus, in coincidence with prominent amyloid Congo red + deposition. The microvasculature is also affected in AD showing morphological aberrations in the hippocampus. At pre-plaque stages we detected a decreased presence of the tight junction protein occludin in the hilus, suggestive of premature brain blood barrier disruption. Our work emphasizes the role of brain vessels and glia- as key constituents of the neurovascular unit- from the onset of the disease in an AD transgenic mice and focuses on a hippocampal subfield that revealed a special vulnerability as well as seems to be early implicated.

S24-04

Dual role of reactive astrogliosis in CNS diseases - from animal models to molecular targets

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Reactive gliosis or reactive astrogliosis is a term used to describe the morphological and functional changes of astroglial cells responding to CNS injury or other neurological diseases. Reactive astrocytes show altered expression of many genes, and the upregulation of GFAP, the main constituent of astrocyte intermediate filaments (nanofilaments), is commonly used as a hallmark of reactive astrocytes. Cytokines, e.g. TGF-α, CNTF, IL-6, LIF, are known to trigger astrocyte activation, either directly via STAT3 signaling in astrocytes or, indirectly, via other cell types e.g. microglia, neurons or endothelial cells.We will demonstrate that this defensive reaction of astrocytes helps to handle the acute stress, limit tissue damage and restore homeostasis, at least in some situations, but it may also inhibit adaptive neural plasticity mechanisms underlying recovery of function.Understanding the multifaceted roles of astrocytes in the healthy and diseased CNS should contribute to the development of treatment strategies that might - in a context-dependent manner and at suitable time points - modulate reactive astrogliosis in order to promote brain repair and reduce the neurological impairment.

S25 Cellular and Molecular Approaches to Study Pathomechanisms of Familial PD

S25-01

The role of LRRK2 in cell biological function

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Parkinson's disease (PD) is a common currently incurable disorder causing primarily motor dysfunction as well as psychiatric symptoms and cognitive impairment. Mutations in *LRRK2* are a common cause of familial and idiopathic PD. LRRK2 has been implicated to function in several cell biological processes and signalling pathways including cytoskeletal function, membrane trafficking, autophagy, innate immune responses as well as Wnt and Ca^{2+} signalling. Determining the specific cell biological and signalling functions of LRRK2 will support the search for therapeutic targets for a disease modifying PD treatment.

We showed that changes in LRRK2 activity cause alterations in Wnt signalling. The two main outputs of Wnt signalling are transcriptional regulation and effects on the cytoskeleton. Deregulated Wnt signalling has been increasingly linked to a number of late-onset neurodegenerative diseases. Interestingly, several cell biological functions disrupted in PD for example cytoskeletal changes are partially controlled by Wnt signalling cascades. Our observations indicate that LRRK2 functions as a scaffold protein in membrane and cytoplasmic Wnt signalling complexes. *LRRK2* mutations reduce the interaction with the membrane LRP6 Wnt signalling co-receptor and reduce LRRK2 mediated Wnt signal activation. Thus our data implicate LRRK2 as a central scaffold protein in canonical Wnt signalling, and suggest that *LRRK2* mutations contribute to the pathogenesis of PD by altering this crucial signal transduction pathway. We also demonstrated an interaction between LRRK2 and β-tubulin relevant for microtubule dynamics. The interaction site is in close proximity to the acetylation site in α-tubulin potentially affecting the dynamic instability of microtubules by changes in tubulin acetylation. Therefore, our data suggests that alterations in microtubule stability caused by LRRK2 mutations also contribute to PD pathogenesis.

Both Wnt signalling components and microtubule stability can be targeted therapeutically for example with Wnt activators such as DKK1 inhibitors or microtubule stabilising agents to modify PD progression.

S25-02

The central role of alpha-synuclein in Parkinson's disease

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The first causal mutation for Parkinson's disease (PD) was found in the alpha-synuclein gene and this was quickly followed by data showing that the pathonomic round, fibrillar Lewy body inclusions that are found in the majority of PD cases contained the alpha-synuclein protein as the main constituent of their core fibrils. These findings alone put alpha-synuclein processes as central to the main pathology of PD. Alpha-synuclein is usually a soluble protein that concentrates in the synpase and coordinates membrane events and events between the nucleus and synapse. Lewy bodies form from a build up of punctate membrane aggregates of phosphorylated alpha-synuclein that coalesce into loosely packed filaments that undergo ubiquitination but not degradation, rather "maturing" by truncation and compaction. These alpha-synuclein inclusion bodies occur to a small degree in the elderly (now considered preclinical) and in patients with diverse neurological and psychiatric disorders, but the largest numbers of patients with Lewy bodies are those with PD. There is considerable evidence that these abnormal aggregates of alpha-synuclein spread in a characteristic pattern through the brain suggesting that the pathology is passed from one cell to another. Patients with PD consitently have Lewy pathologies in the brainstem with infiltration into limbic and then neocortical brain regions occurring over time. Assessment of the timing of spread of pathology reveals a slow pace of relatively restricted regional Lewy body involvement in PD. A number of new animal models that transmit pathological forms of alpha-synuclein between brain regions confirm the central role this protein and are revealing the mechanisms by which such pathology may spread in PD.

S25-03

Parkinson-associated VPS35 mutations alter retromer cellular functions

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Endosomal sorting is a highly orchestrated cellular process. The retromer is a highly conserved heterotrimeric complex which associates with endosomal membrane and facilitates the retrograde sorting of multiple receptors, including the cation-independent mannose-6-phosphate receptor. The cycling of retromer on and off the endosomal membrane is regulated by a well-established network of retromer-interacting proteins. Here, we find that Parkinson's disease-associated Vps35 variant, R524W, and not P316S, is a loss-of-function mutation as marked by a reduced association with this regulatory network, dysregulation of endosomal receptor sorting and the accumulation of intracellular α-synuclein positive aggregates called Lewy Bodies, a hallmark of PD.

S25-04

Kinase signaling between MARK2 and PINK1 – a link between the pathophysiologies of alzheimer and parkinson diseases

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PTEN-induced kinase1 (PINK1) acts at multiple levels to promote mitochondrial health, including regulatory influence on

ATP-synthesis, protein quality control, apoptosis, mitochondrial transport, and destiny. PINK1 mutations are linked to Parkinson disease (PD) and mostly result in loss of kinase activity. But the molecular events responsible for neuronal death as well as the physiological targets and regulators of PINK1 are still a matter of debate. We will discuss recent progress revealing the cellular functions of the cytosolic pool of PINK1 in mitochondrial trafficking and neuronal differentiation. Regulation of PINK1 signaling occurs by mitochondrial processing to truncated forms of PINK1, differentially targeted to several subcellular compartments. The first identified activating kinase of PINK1 is MAP/microtubule affinity regulating kinase2 (MARK2), which phosphorylates T313, a frequent mutation site linked to PD. Kinases of the MARK2 family perform diverse functions in neuronal polarity, transport, migration, and neurodegeneration such as Alzheimer disease (AD); in particular they regulate the interactions between microtubules and microtubule-associated proteins such as tau. This MARK-PINK kinase signaling axis might provide a link between neurodegenerative processes in AD and PD and opens novel possibilities of targeting pathological signaling processes. – Supported by MPG, DZNE, KNDD (BMBF).

S26 What is Glycogen doing in the Brain? – Biochemistry, Physiology, Pathology

S26-01

Two isoforms of glycogen phosphorylase activated by the same signaling trains – how and why?

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While the brain as an organ relies on glucose from the blood, astrocytes contain a small, albeit metabolically active amount of glycogen. Intriguingly, glycogen is not a static molecule but highly dynamic being degraded and rebuild continuously – a process that seem important for a number of processes including neurotransmitter glutamate uptake and memory formation. Interestingly, it seems to be perturbed in some pathologies including diabetes. In astrocytes, two isozymes of glycogen phosphorylase, GPMM and GPBB, degrade glycogen. The distinct roles played by these two isozymes are still somewhat enigmatic as is the non receptor-mediated regulation of glycogen dynamics; so the question is then, what are these two isozymes doing in the brain? To address this, we have employed cultured mouse astrocytes in which we distinctly knocked down the two isozymes employing a siRNA approach. The data suggest that GPBB is acutely activated by an increase in the cytosolic AMP level, whereas GPMM is fully activated by reversible phosphorylation. In addition, it seems that the pyruvate pools derived from breakdown of glycogen mediated by the two isozymes, respectively, are not equivalent in terms of fueling metabolic processes; i.e. faulty signaling-metabolism coupling distinctly affecting the two isozymes may to some extent underlie the metabolic dysfunction observed in some diseases such as diabetes. In a parallel study, we have found that store-operated Ca^{2+} entry, a process involved in re-filling the endoplasmic reticulum (ER) Ca^{2+} pool following signaling events, induces glycogen breakdown in a cAMP-dependent manner. This suggests the involvement of an adenylate cyclase isoform activated by Ca^{2+}. Which of the two isozymes of GP is responsible for this observation remains to be determined. Finally, pharmacological inhibition of glycogen breakdown depleted the ER Ca^{2+} pool, suggesting that ATP derived from breakdown of glycogen supports accumulation of Ca^{2+} into the ER. These findings are interesting from both a neurochemical viewpoint as well as from a disease perspective, and future studies will investigate if faulty signaling-metabolism coupling plays a role in neurological diseases.

S26-02

Role of brain glycogen in long-term potentiation, associative learning, hypoxia, and hippocampal seizures in alert behaving mice

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During the past 25 years, the Seville's group has developed electrophysiological techniques for *in vivo* recording of field synaptic potentials and local field potentials during performance of spontaneous behaviors and/or during the acquisition of associative and non-associative learning tasks. Indeed, glycogen is the only carbohydrate reserve of the brain, but its overall contribution to brain functions remains unclear. Although it has traditionally been considered as an emergency energetic reservoir, increasing evidence points to a role of glycogen in the normal activity of the brain. To address this long-standing question, the Barcelona's group generated a brain-specific Glycogen Synthase knockout ($GYS1^{Nestin\text{-}KO}$) mouse and studied the functional consequences of the lack of glycogen in the brain under alert behaving conditions. These animals showed a significant deficiency in the acquisition of an associative learning task and in the concomitant activity-dependent changes in hippocampal synaptic strength. Long-term potentiation (LTP) evoked in the hippocampal CA3-CA1 synapse was also decreased in behaving $GYS1^{Nestin\text{-}KO}$ mice. In addition, $GYS1^{Nestin\text{-}KO}$ mice presented significantly smaller power spectra of hippocampal local field potentials than controls in both normal and hypoxic conditions, and a larger susceptibility to generate hippocampal seizures and myoclonus following the administration of kainate and/or a brief train stimulation of Schaffer collaterals. These results unequivocally show a key role of brain glycogen in the proper acquisition of new motor and cognitive abilities and in the underlying changes in synaptic strength. Brain glycogen could also play a protective role both in hypoxic situations and in the prevention of brain seizures.

S26-03

Role of glycogen-derived lactate in synaptic plasticity and memory

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Until recently, lactate has been viewed as a by-product of glycolysis with no relevant role in brain functions. Our laboratory has provided evidence that lactate plays a significant role in neuroenergetics. In particular, we have proposed the existence of the so-called "astrocyte-neuron lactate shuttle" according to which, during neuronal activation lactate is released by astrocytes following the glycolytic processing of glucose and is transported to neurons to meet their energy needs.

More recently, we have provided evidence for a role played by glycogen-derived lactate release by astrocytes for the establishment of long-term memory (LTM) in an inhibitory avoidance paradigm (Suzuki *et al.*, 2011). This key role of lactate in neuronal plasticity mechanisms was demonstrated in experiments in which specific pharmacological and gene expression down-regulation interventions were implemented to prevent the production of lactate from glycogen – which is exclusively localized in astrocytes – and its release from these cells in the hippocampus during behavioral training. Such interventions completely prevented the establishment of LTM and their effect was fully reversed by the intrahippocampal administration of lactate during the training session. Glucose at equicaloric concentrations only marginally mimicked the rescuing effect of lactate suggesting that the action of lactate was independent of its ability to act as an energy substrate.

We then went on to elucidate the molecular mechanism of the effects of lactate on plasticity and could demonstrate that lactate promotes the expression of the synaptic plasticity-related genes Arc, c-Fos, and Zif268 in neurons (Yang *et al.*, 2014). This effect was mediated through a mechanism involving NMDA receptor activity and its downstream signaling cascade Erk1/2. In parallel to this, lactate increased intracellular levels of NADH, thereby modulating the redox state of neurons. These results provide insights for the understanding of the molecular mechanisms underlying the critical role of astrocyte-derived lactate in LTM formation. This set of data reveals a previously unidentified action of lactate as a signaling molecule for neuronal plasticity.

S26-04

Glycogen content and metabolism studied by *in vivo* 13C magnetic resonance spectroscopy in healthy and diabetic human brain

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Animal and culture studies implicated cerebral glycogen in supporting the function and survival of neurons during glucose deprivation, as well as during normal brain activity. *In vivo* ^{13}C magnetic resonance spectroscopy (MRS) methodology was developed in late 1990s to assess cerebral glycogen metabolism noninvasively in the rodent brain and was later translated to humans. To date, ^{13}C MRS in conjunction with intravenous administration of [1-^{13}C]glucose to isotopically label glycogen remains the only method to detect glycogen and estimate its content and turnover rate in the living human brain. Using this method, we have shown that the human brain stores several fold higher glucose levels (3–4 μmol/g) in the form of glycogen relative to free glucose (~1 μmol/g) and that it mobilizes this store during hypoglycemia. We have further observed increased glycogen synthesis following a single episode of hypoglycemia versus euglycemia, supporting the hypothesis that glycogen may supercompensate after hypoglycemia and thereby contribute to the pathogenesis of hypoglycemia unawareness (HU) syndrome, a condition where patients with type 1 diabetes (T1D) who experience recurrent hypoglycemia develop defective glucose counter-regulation and become unable to sense hypoglycemia. However, ^{13}C MRS has also shown that patients with T1D and HU do not have higher levels of glycogen than healthy controls. More recent experiments during which ^{13}C-labeled glycogen was monitored for 3 + days in healthy humans following pre-conditioning with recurrent eu- or hypoglycemia revealed even higher glycogen content (~6–7 μmol/g) in the human brain, further underlining its functional importance for cerebral metabolism, however questioned a major role for cerebral glycogen in the development of HU.

S27 Temporal Evolution of Microglial Function and Phenotype in Ischemic Injury

S27-01

Effects of ischemic preconditioning on microglial phenotype and transcriptome

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Background: Ischemic preconditioning (IPC) is a robust neuroprotective phenomenon in which a brief period of cerebral ischemia confers transient tolerance to subsequent ischemic challenge. Microglia are critical in stroke pathophysiology however their role in IPC is unknown.

Methods: We performed middle cerebral artery occlusion (MCAO) or sham surgery on 12–14 week old *wild-type* male mice following established paradigms for IPC ± stroke. We assessed infarct volume with 2,3,5-triphenyltetrazolium staining and used *ex vivo* flow cytometry (*ex*FC) to quantify immune cell populations in cortex following IPC. We performed immunofluorescent microscopy (IFM) with stereology (optical dissector), Cavalieri point counting and Ki67 staining to characterize ionized calcium-binding adapter 1 molecule (IBA1)-positive immune cell number, morphology and proliferation, respectively. Finally, we performed mouse ST gene arrays (Affymetrix) on RNA isolated from sorted cortical microglia and carried out bioinformatic (Ingenuity Systems) and promoter analyses to characterize the effects of IPC on the microglial transcriptome.

Results: IPC reduced infarct volume from 53 ± 6 (mean ± SEM) to 26 ± 8 mm^3 ($p < 0.01$, $n = \geq 5$ mice per group) and induced robust increases in the number of ipsilateral cortical microglia and macrophages relative to both sham and contralateral controls ($n = \geq 13$ and ≥ 4 mice per group for *ex*FC and IFM, respectively). IPC also induced marked increases in $IBA1^+$ cell volume (processes and somata). Microarray analysis demonstrated marked changes in microglial transcriptome [2,230 gene probesets (6.5% of total) were significantly regulated]. The top three IPC-induced canonical gene expression pathways were: (i) DNA replication, recombination and repair, (ii) Cellular assembly and (iii) Cell cycle. We also found marked increases in interferon stimulated gene expression.

Conclusions: Our novel *in vivo* microglia-specific, IPC-targeted, genomic dataset indicates profound activation of cell proliferation-related gene expression. This finding correlates well with the IPC-induced increases we found in microglia/macrophage cell number. These unexpected findings indicate that cell cycle regulation of CNS immune cells may be important in IPC-mediated neuroprotection.

S27-02

Chronic stress exacerbates neuronal loss associated with secondary neurodegeneration and suppresses microglial-like cells following focal motor cortex ischemia in the mouse

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Post-stroke patients describe suffering from persistent and unremitting levels of distress. Using an experimental model of focal cortical ischemia in adult male C57BL/6 mice, we examined whether exposure to chronic stress could modify the development of secondary thalamic neurodegeneration (STND), which is commonly reported to be associated with impaired functional recovery. We were particularly focused on the modulatory role of microglia-like cells, as several clinical studies have linked microglial activation to the development of STND. One month following the induction of cortical ischemia we identified that numbers of microglial-like cells, as well as putative markers of microglial structural reorganization (Iba-1), complement processing (CD11b), phagocytosis (CD68), and antigen presentation (MHC-II) were all significantly elevated in response to occlusion. We further identified that these changes co-occurred with a decrease in the numbers of mature neurons within the thalamus. Occluded animals that were also exposed to chronic stress exhibited significantly lower levels of Iba-1 positive cells and a reduced expression of Iba-1 and CD11b compared to the 'occlusion-alone' group. Interestingly, the dampened expression of microglial/monocyte markers observed in stressed animals was associated with significant additional loss of neurons. These findings indicate that the process of STND can be negatively modified, potentially in a microglial dependent manner, by exposure to chronic stress.

S27-03

Microglia are activated to a neurotoxic phenotype and exacerbate delayed neuronal death after cardiac arrest

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The role of activated microglia for neuronal death and resulting functional deficit after cardiac arrest and resuscitation remains controversial, partly because it is difficult to selectively manipulate microglia. Attempts at modulating microglial activation with agents such as minocycline have produced conflicting results, inconsistently affecting neuronal death. Microglia are phenotypically activated early after reperfusion in our mouse model of cardiac arrest. Microglial activation precedes neuronal death, which is delayed by several days after cardiac arrest, providing opportunity for microglia to contribute to the delayed death of injured neurons. Activated microglia rapidly up-regulate expression of pro-inflam-

matory cytokines after cardiac arrest. This pro-inflammatory phenotype is associated with an increasing ability of microglia isolated after cardiac arrest to exacerbate cell death in cultured neurons, which suggests that microglia are activated to a neurotoxic phenotype. Using a novel genetically engineered mouse, we were able to selectively ablate microglia in adult mice. We found that neuronal survival after cardiac arrest is drastically improved when microglia are absent, supporting that microglial activation is indeed detrimental *in vivo*. The neurotoxic microglial transformation is induced in response to danger signals that are released by injured neurons after cardiac arrest. We identified a novel danger molecule that is released into the cerebrospinal fluid after cardiac arrest and induces pro-inflammatory and neurotoxic microglial transformation. This transformation depends in part on Toll-like receptor mediated signaling. Targeting the danger molecule-induced neurotoxic microglial transformation after cardiac arrest provides a new therapeutic opportunity to improve neuronal survival and reduce functional deficit in survivors.

S27-04

Microglia phagocytose stressed neurons resulting in delayed neuronal death by phagoptosis after brain ischaemia or inflammation

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Microglia are resident brain macrophages that become highly phagocytic when activated, and are known to phagocytose dead and dying neurons. However, we find that activated microglia can also phagocytose stressed or damaged neurons and thereby kill them, a form of cell death called "phagoptosis". Stressed-but-viable neurons exposed the "eat-me" signal phosphatidylserine, which induced phagocytosis via the opsonin MFG-E8 and phagocytic receptors VNR and MerTK on microglia. Sub-toxic levels of glutamate or oxidants induced reversible phosphatidylserine exposure on viable neurons, but in the presence of activated microglia this phosphatidylserine exposure induced phagocytosis of the neurons. Inflammatory activation of neuronal-glial co-cultures, by nanomolar b-amyloid, LPS, TNFa or rotenone, resulted in progressive loss of neurons (without any apoptosis or necrosis), accompanied by microglial phagocytosis of neurons, and prevented by blocking phagocytosis. LPS-induced neuronal loss *in vivo* was reduced by co-injection of phagocytosis inhibitors or in MFG-E8 knockout mice.

Transient brain ischaemia is known to induce glutamate release and reversible phosphatidylserine exposure *in vivo*, as well as microglial activation and delayed neuronal loss. We induced mild, transient brain ischaemia in rats and mice by injection of endothelin-1, and 3–7 days later found microglial activation, induction of MFG-E8 and MerTK, and large numbers of neuronal nuclei inside microglia. MFG-E8 knockout mice and MerTK mutant rats had: reduced microglial phagocytosis of neurons at 3 days and reduced brain atrophy and motor deficits at 28 days. Overall, blocking phagocytosis strongly protected against brain damage induced by transient ischaemia or inflammation, and therefore phagocytic receptors are potential targets for therapy.

S28 Neuroinformatics Tools and Neurochemical Atlases for Global Neuroscience Collaboration

S28-01

Brain atlasing and the role of the international neuroinformatics coordinating facility (INCF) in global neuroscience research

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With a new generation of three-dimensional digital brain atlases, new solutions for analyzing and integrating brain data are being developed. Digital brain atlases will serve as frameworks for services similar to current online geographical atlases, such as Google Maps and Google Earth, which provide interactive access to huge amounts of high resolution image data, together with additional information and detailed visualizations. The key aim of the INCF program on digital atlasing is to coordinate and improve the impact of atlasing projects, with a focus on the rodent brain. Several of the INCF national nodes contribute to development of infrastructures for brain atlasing in a combined effort with the European Union ICT Future Emerging Technologies Flagship project, the Human Brain Project.

The templates used for digital brain atlases can be high resolution 3-D histology data or other volumetric data sets acquired with, e.g., magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI). The templates can re-sliced and viewed in arbitrary angles without loss of image quality. Additional data modalities required to delineate brain regions can be added by registering high resolution microscopic images from slices through the brain to the templates. With these new atlas frameworks, and a set of tools to interact with the frameworks, research groups can connect their data to atlas space, share the data through online data systems, and search and find other relevant data through the same systems. The main role of the INCF in these efforts is to continue to support the establishment of standards for nomenclatures and spatial coordinate systems (Waxholm Space), and coordinate the development of data systems delivering services to the community. Examples of efforts at the national level include the Scalable Brain Atlas (Dutch node of the INCF) and the Rodent Brain WorkBench (Norwegian node of the INCF). New INCF special interest groups are being established, aiming at reaching out to the broader science community.

S28-02

Second generation brain atlases – the impact of histochemistry, developmental gene expression, and MRI

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The period 1980–2010 saw the emergence of a set of stereotaxic atlases for virtually all rodents and primates that are used for neuroscience studies. These atlases are almost all based on the analysis of Nissl and acetlycholinesterase sections. In the past 15 years new technologies have emerged that have further enhanced the accuracy of brain mapping. These approaches include supplementing standard stains with the use of a panel of immunohistochemical markers, incorporating information from developmental gene expression, and magnetic resonance imaging. These new technologies have increased the quality of the standard atlases, providing more precise nuclear boundaries and, through the use of MRI, an opportunity to map the brain of a single animal over time. Atlases using a panel of markers are now available for the rat, mouse, marmoset, and chick brains. These second generation atlases have taken advantage of information from developmental gene expression (particularly data from site-specific recombinases) to make the presentation of adult brain anatomy consistent with the new evidence of segmentation and migration during embryonic development. We are currently producing an MRI rat brain atlas that makes use of technologies generating an isotropic resolution of around 25 μm. The use of a range of MRI contrasts provides a series of images equivalent to those obtained by different histological stains, and the averaging of a number of brains greatly reduces noise artefact. The image planes in this forthcoming rat brain MRI atlas have been aligned to match those in the most widely used histological atlas of the rat brain.

S28-03

Mapping neural connections in primates – an expanding role for cortical connectivity atlases

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Building a comprehensive map of connectivity in a mammalian brain requires the registration of multiple specimens to a common atlas. The goals of this study are to establish an automated workflow for mapping marmoset monkey neural connectivity data (from retrograde tracer injections) into a reference template space (stereotaxic atlas), and to assess a reliability of these procedures.

To map the data obtained from a single specimen into the atlas space, the locations of labelled cells marked on fluorescence sections are initially transferred to neighbouring Nissl sections. Afterwards, the Nissl-stained sections are stacked and reconstructed into volumetric form and registered to the stereotactic atlas. The reconstruction is performed with affine transformations followed by deformable warping. The latter step reduces section specific distortions and allows for more reliable subsequent deformable mapping into the atlas space. This process yields a set of transformations which are then applied to the actual cell locations. In the final step, the individual cells are assigned to a particular brain structure based on the atlas parcellation. The workflow was applied to data obtained from nine test cases and resulted in a database of the cells' coordinates in the atlas space.

The cell mapping accuracy was assessed by comparing the number of the cells in each cortical area indicated by the automated approach with the count determined manually by an anatomist. Additionally, the discrepancy between locations of the injection sites used in the experiment and recovered from mapping was used as another benchmark.

The established workflow allows for streamlined processing of the cases to produce a spatially defined connectivity map of the marmoset cortex, independent of anatomical parcellation scheme unlike the traditional method relying on prior assignment of data to discrete anatomical structures. Furthermore, this map can be used as a gold standard in DTI validation studies.

S28-04

Towards a multimodal human brain atlas – cytoarchitecture, neurotransmitters and fiber tracts

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The human brain has a multimodal organization, and includes different spatial and temporal scales. New mapping strategies such as 3D Polarized Light Imaging provide new insights into the fiber architecture, and maps of the distribution of neurotransmitter receptors reveal the molecular organization of the brain. To combine the different levels of brain organization into an atlas requires modern ICT and high performance computing, and opens new perspectives to develop brain models at cellular resolution.

S29 Optogenetics and Optopharmacology to Illuminate the Brain

S29-01

Channelrhodopsin *et al.*: natural and engineered photoreceptors for optogenetic applications

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We could show that some microbial photoreceptors are ideal tools to manipulate animal cells by illumination. We discovered the Channelrhodopsins from the unicellular green alga *C. reinhardtii* which are Light-gated cation channels, allowing fast light-induced depolarization of the plasma membrane (1,2). Mutations led to a slower photocycle and therefore to Channelrhodopsins with higher light sensitivity. Neuronal expression of Channelrhodopsin-2 (ChR2) yields Light-induced action potentials and Light-manipulated behaviour in *C. elegans* (3). The Light-activated chloride pump halorhodopsin (HR) from the archaeum *Natronomonas pharaonis* hyperpolarizes the plasma membrane and therefore allows Light-induced silencing of neurons (4). These two antagonistic rhodopsins may even be expressed in the same cell and still specifically be light-activated with 460 nm for ChR2 and 580 nm for HR. Recently we found a ChR2 mutant with increased expression and high light sensitivity (ChR2-XXL) which allows light modulation of deep brain neurons in adult *Drosphila* flies, even without feeding the chromophor all-trans retinal (5).

We heterologously expressed Photoactivated Adenylyl Cyclases (PAC) from *Euglena gracilis* (6,7) or bacteria (8,9), flavoproteins which quickly elevate cytoplasmic cyclic AMP by illumination with blue light in cultured cells and in living animals or plants. Recently new genes for opsins with an attached guanylyl cyclase domain were found in fungi by others (10). We characterized these Cyclase Opsins (Cyclops) in-vivo and *in-vitro* and determined tight light-regulation of cyclase activity. Expression of Cyclop in *C. elegans* yields fast light-activated cGMP production and allows reversible, non-invasive light-manipulation of behaviour.

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S29-02

Optovins convert endogenous TRPA1 channels into photoreceptors

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Optogenetic technologies enable high-resolution optical control of neuronal activity, but most techniques require transgenic expression of opsins or other exogenous photoreceptors. To circumvent this requirement, we used high-throughput behavioral screening in zebrafish to identify optovin, a small molecule that enables optogenetic-like activation of neurons without introduction of exogenous photoreceptors. Optovin functions by converting endogenous TRPA1 cation channels into photoreceptors and allows repeated photoactivation of motor behaviors in wild-type zebrafish and mice. TRPA1 is both necessary and sufficient for the optovin response. Optovin activates human TRPA1 via structure-dependent photochemical reactions with redox-sensitive cysteine residues. In animals with severed spinal cords, optovin treatment enables control of motor activity in the paralyzed extremities by localized illumination. These studies identify a light-based strategy for controlling endogenous TRPA1 receptors *in vivo*, with potential clinical and research applications in nontransgenic animals, including humans.

S29-03

An allosteric modulator to control endogenous g protein-coupled receptors with light

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Controlling drug activity with light offers the possibility of enhancing pharmacological selectivity with spatial and temporal regulation, thus enabling highly localized.

Therapeutic effects and precise dosing patterns. We have developed and characterized the first photoswitchable allosteric modulator of a G protein–coupled receptor. Alloswitch-1 is selective for the metabotropic glutamate receptor mGlu5 and enables the optical control of endogenous mGlu receptors.

S29-04

Multicoloured optogenetic manipulation of synaptic function

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The development of optogenetic techniques has changed the way scientists can manipulate neurocircuitry to study functional effects. We have previously developed channelrhodopsin variants that permit the direct excitation of neurons with different wavelengths of light and novel optogenetic approach to inhibit protein functions

through chromophore assisted light inactivation (CALI). In this talk, we will discuss how these technologies have been implemented to manipulate synaptic release both *in vivo* and *in vitro*. With the blue light activated channelrhodopsin ChIEF, we have been able to induce long-term depression (LTD) and long-term potentiation (LTP) in specific synapses. The red-light activatable channelrhodopsin variant ReaChR can be used with ChR2 to independently control synaptic release from two sets of presynaptic terminals in the same region. CALI of synaptic function can be achieved by tethering a weakly fluorescent protein to proteins essential for synaptic release. We will also discuss potential approaches to expand the wavelengths of light can be used to conduct CALI experiments with newly developed fluorescent protein or the use of synthetic chemicals.

S30 Nucleotide Repeat Sequences in Neurodegeneration

S30-01

Repeat expansions: mutagenesis, pathogenesis, and therapeutics

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Since 1991 more than 40 neurological, neurodegenerative, and neuromuscular diseases have been found to be caused by the genetic expansion of any one of a series of gene-specific repeat sequences. Diseases include myotonic dystrophy, Huntington's disease, fragile X mental retardation, and most recently, amyotrophic lateral sclerosis and frontotemporal dementia. Many paths exist by which repeat expansions can cause disease (transcription loss, toxic-RNAs, toxic proteins with repeating amino acids, or toxic-RAN translated peptides). Many of these pathways are the focus of current therapeutic approaches. However, several diseases suffer multiple pathogenic paths. The relative disease contribution of any one path is unclear. Common to all diseases are repeat expansions – the root of disease. Disease age-of-onset and disease severity correlate with the inherited size of the repeat expansion, transmitted by their parents. Ongoing repeat expansions occurring in affected tissues correlate with disease age-of onset, severity, and progression. Dramatic repeat length variations exist between tissues of the same individual, with differences > 5000 repeats, with the largest expansions in heart, cerebral cortex and striatum. The considerably larger expansions in the clinically affected tissues of individuals suggest that ongoing somatic expansions contribute to disease onset, severity and progression. In both mutagenic and pathogenenic processes, unusual nucleic acid structures and various pathways (DNA repair, splicing, translation etc.) have been identified as critical. Understanding the paths of repeat mutations, and the processes of pathogenesis are revealing new therapeutic avenues.

S30-02

Understanding how mouse and human neurons cope with mutant huntingtin

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People with Huntington's disease carry a mutation in a gene that encodes the protein huntingtin (Htt) and makes it prone to aggregate. Aggregated deposits of Htt accumulate in the brain, suggesting a mismatch in Htt production and clearance. The mutation is present since birth, but patients often don't manifest symptoms until midlife, suggesting that neurons have coping responses to mitigate the accumulation and deleterious effects of mutant Htt. We developed robotic microscopes that can do high-throughput automated longitudinal single-cell analysis and specialized optical tools to observe protein homeostasis in live murine or human neurons made from stem cells. These studies suggest that mutant Htt adopts structures that can promote neurodegeneration but that the capacity of the cell to cope with mutant Htt is a major determinant of its susceptibility. In this talk, we will describe some of these coping mechanisms and their roles in neurodegeneration induced by mutant Htt. We will also discuss important differences in protein homeostasis pathways in neurons and other cell types that affect their susceptibility to protein misfolding and neurodegenerative disease.

S30-03

Molecular mediators, environmental modulators and gene-environment interactions in huntington's disease

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Huntington's disease (HD) is a tandem repeat (CAG) expansion disorder involving a triad of psychiatric, cognitive and motor symptoms. In a transgenic mouse model of HD (R6/1 line) we have shown that expansion of the 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 (which enhances sensory stimulation, cognitive activity and physical exercise) can delay onset of the affective, cognitive and motor endophenotypes. Environmental enrichment and physical exercise induce changes in gene expression, which exhibit temporal specificity and regional selectivity. Our results suggest that the timing and duration of these environmental manipulations are critical in terms of their ability to modify gene expression.

We have investigated these mice as a model of both depression and dementia in HD, and have discovered various molecular abnormalities, including specific deficits in neurotrophin, glutamatergic, serotonergic and dopaminergic signalling pathways. A selective subset of these molecular changes have been found to be sexually dimorphic and, along with a possible role of sex hormones, may help explain the depression-like behaviours in these female HD mice.

These findings have been extended to additional environmental factors (including the negative effects of stress) and neuroendocrine modulators. We have recently provided the first evidence that chronic stress can accelerate onset of HD, identifying specific symptoms including cognitive deficits which are most vulnerable to this environmental modifier.

Together with epidemiological studies and clinical trials, this research is informing the design of ongoing intervention studies for HD. These approaches may also facilitate the development of "enviromimetics" for a variety of brain disorders known to be modulated by enhanced cognitive activity and physical exercise.

S30-04

Isogenic stem cell-based modelling of huntington disease: towards validation and discovery of therapeutic targets in human neurons

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Efforts to understand how cellular processes are altered in neurodegenerative diseases have long been hampered by the inaccessibility of the chief tissue of interest, namely the brain. However, a number of recent technological advances are helping overcome this hurdle. One is the ability to direct the differentiation of patient-specific human pluripotent stem cells into neuronal and glial types of interest. Another is the possibility to edit specific loci in the genome using programmable sequence-specific nucleases, such as TALENs and CRISPR-Cas9, allowing the precise removal or addition of mutations of interest. In this talk, I will discuss the progress made in developing isogenic stem cell-based models of Huntington disease (HD), a progressive, monogenic and highly dominant form of dementia, and the application of these models to the study of HD-related cellular processes in a physiologically relevant human context.

S31 Non-coding RNAs: Important Regulators in the Nervous System

S31-01

Non-coding RNA deregulation in pain associated with neuropathy and cancer

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The recent years have witnessed a steady increase in our understanding of the importance of noncoding RNAs (ncRNAs) in gene regulation and disease pathogenesis, including pathological pain. Although the initial focus was on analyzing expression and dysregulation of candidate miRNAs, our recent efforts have been focused on elucidation of miRNA-mediated functional mechanisms in pain modulation as well as the discovery of other ncRNA species in nociceptive pathways. Using a comprehensive approach combining genome-wide miRNA screening, molecular and in silico analyses with behavioural approaches and mouse models, we have indentified miRNAs that act at the interface between tumor cells and sensory nerves in the pathogenesis of cancer-associated pain and particularly observed a key role for regulation of a chloride channel in peripheral sensory neurons. Moreover, using genetically segregated in-bred strains of rats with differing levels of neuropathic pain, we have identified miRNAs relevant to nerve lesion-induced pain. Our on-going efforts are dedicated to elucidating the role of ncRNAs in mediating diverse cell-cell interactions in sensory neurons in the pathogenesis of chronic pain.

S31-02

MIR-134-dependent plasticity of nociceptive spinal circuits

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Spinal cord lesions may induce severe neuropathic pain. While more than 8% of the world's population suffers from neuropathic pain, the mechanisms underlying this pain remain unclear. The neuronal actin cytoskeleton is critically involved in morphological plasticity and synaptic reorganization acting as a key player in neuropathic pain mechanisms. LIM Kinase1 (LIMK1) is a protein kinase responsible for actin polymerization by inhibiting Cofilin/ADF (Actin Depolymerisation Factor) activity. LIMK1 expression is controlled by the microRNA, miR-134 that represses LIMK1-mRNA translation. MiR-134 is considered as a negative regulator of dendritic spine volume and LIMK1 has been reported to promote actin polymerization in dendrites. Moreover, LIMK1/cofilin regulates the insertion and trafficking of the AMPA excitatory glutamate receptors (AMPAR) at the synapse. Therefore, it is likely that miR-134/LIMK1 modulates the transmission of nociceptive information in the spinal dorsal horn. Here, we investigate miR-134 distribution in the spinal dorsal horn of both sham and neuropathic animals. We show that miR-134 is preferably localized in the postsynaptic compartments. qRT-PCR analysis also show a decrease of miR-134 expression in neuropathic animals when compared to shams, that is concomitant with an increase of LIMK1. We find that endogenous miR-134 down-regulation in excitatory post-synaptic compartment limits pain sensitization in neuropathic rats. Further experimental decrease of miR-134 even partially normalizes pain threshold. Knocking down miR-134 by intrathecal injection significantly increases pain withdrawal threshold (less pain), observed when tested for evoked (Von Frey test) or spontaneous (dynamic weight bearing test) pain behavior. Regarding the mechanism involved, we show the effect of miR-134 knocking down and LIMK1 overexpression as well on AMPAR insertion to the plasma membrane. Taken together, our results suggest that the antinociceptive effect of miR-134 Knockdown may be due to a decrease in AMPAR insertion at the plasma membrane of excitatory post-synaptic density.

S31-03

Molecular determinants of schizophrenia-associated alteration of miRNA biogenesis

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In the last decade, small non-coding miRNA have emerged as significant players in the regulation of brain development and neural function. These molecules guide ribonucleoprotein complexes controlling the intracellular fate of mRNA by acting as nucleic acid adapter sequences that facilitate homology based target interactions. These molecules and their effector complexes form a multiplicity of regulatory modules with genes and constitute influential nodes in the network architecture supporting complex neural structure and function. The pattern of miRNA expression throughout the brain and within individual neurons is also highly regulated to enable temporospatial segmentation of their regulatory influence. Post-mortem investigations of cortical miRNA expression suggest these molecules are dysregulated in schizophrenia as a consequence of both genetic and epigenetic factors. Understanding these factors in the context of schizophrenia will be important for developing interventions that can modify miRNA and the pathways they regulate.

To this end we dissected cortical grey matter from the dorsolateral prefrontal cortex (DLPFC) and analysed schizophrenia-associated gene and miRNA expression using microarray and qPCR. The results suggest that miRNA regulated pathways are critical in the pathophysiology of the disorder and implicate the miRNA biogenesis pathway including the processing machinery encoded by Dicer and DGCR8. Overexpression of DGCR8 in differentiated human SH-SY5Y neuroblast cells induced a change in miRNA expression consistent with the observation in postmortem schizophrenia, and also reduced the expression of a co-transfected Reelin-UTR reporter construct.

The influence of genetic variation in *MIR137* on its mature miRNA expression was also explored after allelic stratification using TaqMan genotyping assay. This supported recent studies suggesting the risk genotype is associated with reduced cortical expression of the mature miRNA. To investigate the consequences of this change we modelled miR-137 suppression in differentiated human SH-SY5Y cells using a synthetic antagonist and identified widespread involvement of target genes in neural and schizophrenia-associated pathways. This study suggests that dysregulation of cortical miRNA expression is associated with changes in gene expression in schizophrenia, and that genetic and epigenetic factors affecting miRNA genes and the molecules regulating their biogenesis could play a significant role in the neuropathology of the disorder.

S31-04

Non-coding RNAs modulating sensory neuron function and regeneration

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Peripheral nerve injury initiates regenerative processes of neuronal axons but frequently is complicated by a pathological neuroimmune response leading to persisting neuropathic pain and dysfunction of primary nociceptive afferent neurons. The communication pathways linking between signals regulating inflammation, regeneration and pain are still incompletely understood. Here, we report that the interleukin-6 signal transducer gp130 involved in inflammation and neuron regeneration may convey bidirectional body-brain pain messages through microRNA (miRNA) regulators of neuroinflammation and neuroregeneration. Mice with a conditional gp130 null mutation in sensory neurons (SNS-gp130$^{-/-}$) show a delay in peripheral nerve regeneration and a protection from persisting inflammatory, cancer and neuropathic pain. Non-biased sequencing of mouse RNA highlighted tissue-specific miRNA changes induced by spared nerve injury (sni) compared to sham operation, with largest differences in the prefrontal cortex of injured over control mice (295 PFC miRNAs 30% over- or under-regulated compared to 124 hippocampal miRNAs). SNS-gp130$^{-/-}$ mice showed generally limited differences of sni-induced miRNAs in the pain pathway, smaller than the effect of sham operation. This suggests a causal involvement of miRNA changes not only in neuroregeneration but also in neuropathic pain reactions involving pathological neuroimmune interaction. Specifically, we found the nerve growth factor targeted miR-21 significantly up-regulated after sni. miR-21 localized in neuronal cell bodies in DRG cryosections with *in situ* hybridization probes. Reintroduction of gp130 with viral vectors into peripheral neurons in culture recovered expression of nociceptor specific transducer ion channel TRPA1 and the deficit in neurite outgrowth but not the low miR-21 levels associated with gp130 depletion. Our findings demonstrate that specific miRNAs participate in communicating body-brain messages associated with nerve injury and call for testing the potential of micro-RNAs as therapeutic targets for treating nerve injury and chronic pain.

S32 Central Role of Glial Cells in the Pathogenesis of Schizophrenia

S32-01

Astrocytic dopamine modulates neuronal network and cognitive functions in the prefrontal cortex

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Background level of dopamine receptor stimulation in the prefrontal cortex (PFC), sets the responsivity of the network to dopamine and is essential to normal alert conditions enabling optimization of frontal cognitive functions in rodents and humans. In the PFC, extracellular levels of dopamine allowing for optimal stimulation of dopaminergic receptors are maintained within a narrow range of concentrations by a series of cellular mechanisms whose nature remains currently unclear. Here, we report the identification of a subset of astroglial cells in rat, mice and Human that are competent for gliosecretion of dopamine in the PFC. At ultrastructural level the astrocytic processes containing dopamine are positioned in a strategic position between neuronal dopaminergic varicosities and asymmetric synapses, which would allow them to fulfill the crucial role of bridging the gap between sparse neuronal dopamine release and tonic regulation of extracellular dopamine levels in the neuropil. Consistent with these anatomical observations, a conditional and selective deletion of VMAT2 in astrocytes results in a significant reduction of extracellular levels of dopamine and a generalized loss of the dopaminergic tone necessary to regulate basal excitatory neurotransmission in layer 5 pyramidal neurons. Consequently, synaptic plasticity and frontal cognitive performance are significantly impaired in the VMAT2-deficient mice. Furthermore, restoration of VMAT2 using lentivirus encoding VMAT2 in the recombined astrocytes or treatment of VMAT2-deficient mice with L-DOPA exerts a positive effect on synaptic transmisison defects and on frontal cognitive impairments, increasing levels of the extracellular dopamine and restoring normal glioscretion in the PFC. Our results provide a new cellular framework that includes astrocytes in the mechanisms underlying dopaminergic modulation of executive PFC functions. Moreover, they provide new potential targets for therapeutic intervention in many neuropsychiatric disorders characterized by dysregulation of PFC dopaminergic state, such as schizophrenia and autism spectrum disorder.

S32-02

The astrocyte-derived tryptophan metabolite kynurenic acid: neuromodulation and links to schizophrenia

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Several lines of evidence link the endogenous neuromodulator kynurenic acid (KYNA), a major metabolite of the essential amino acid tryptophan and antagonist of both α7 nicotinic and N-methyl-D-aspartate (NMDA) receptors, to deficits seen in individuals with schizophrenia (SZ): 1) brain and cerebrospinal fluid KYNA levels are increased in SZ; 2) α7 nicotinic and NMDA receptors play critical roles in both neurodevelopment and cognition; 3) in adult rats, experimental KYNA elevations cause cognitive dysfunctions reminiscent of SZ; 4) perinatal increases in brain KYNA result in an array of SZ-like abnormalities and vulnerabilities in adult rodents; 5) brain KYNA metabolism is stimulated by stress and immune stimulation during early development; and 6) first results indicate that inhibitors of KYNA biosynthesis (specifically kynurenine aminotransferase II inhibitors) show efficacy in animal preparations that are believed to be informative for SZ pathophysiology. The possible role of endogenous KYNA in SZ has stimulated the study of the metabolite's neurochemical and functional characteristics in the mammalian brain. Thus, using mainly rodents as experimental animals, we and others have identified a number of unexpected neurobiological properties of KYNA. This included the discovery of new receptor targets, such as the G protein-coupled receptor GPR35 and the aryl hydrocarbon receptor, and of KYNA's ability to bi-directionally modulate the extracellular concentration of dopamine, glutamate and GABA in the brain *in vivo*. Notably, cerebral KYNA synthesis is controlled by cellular energy metabolism and is rapidly stimulated in response to seizure activity and early neurodegenerative events. Selective genetic and pharmacological tools now allow investigators to gain more detailed insight into the mechanisms that determine the disposition and function of KYNA in the brain under physiological conditions, and to manipulate brain KYNA to treat SZ and, possibly, other major brain diseases.

S32-03

Activation of microglia and astrocytes in schizophrenia is linked to increases in brain cytokines

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Objective: While schizophrenia may have a progressive component, the evidence for neurodegenerative processes as indicated by reactive microglia and astrocytes is inconclusive. We identified a subgroup of individuals with schizophrenia with increased expression of inflammatory markers in prefrontal cortex. This subgroup encompassed ~40% of people with schizophrenia.

Method: We measured molecular markers of microglia and astrocytes in adult chronic patients with schizophrenia (n = 37) compared to controls (n = 37). We used glial fibrillary acidic protein (GFAP) and Human Leukocyte Antigen (HLA) mRNA and protein measurements from grey matter homogenates and immunohistochemistry with cell density measurements to determine if there were glial changes according to diagnosis or according to cytokine subgroup. We used RNA-Seq data on a subset of patients and

controls (n = 20/group) to ascertain whether other mRNA transcripts associated with astrogliosis and/or blood brain barrier breakdown could be identified in the individuals with active neuroinflammation.

Results: We found evidence of increased microglia in both grey matter and white matter in schizophrenia compared to controls. However, GFAP (mRNA and protein) and astrocyte morphology were not significantly different between people with schizophrenia and controls overall, confirming earlier observations. In contrast, individuals with schizophrenia with neuroinflammation had elevated expression of GFAP mRNA and hypertrophic astrocyte morphology compared to those with schizophrenia who did not show evidence of elevated cytokines. Further, the elevated cytokine group showed significantly elevated expression of three mRNA transcripts previously associated with astrogliosis and mRNA changes indicative of blood brain barrier leakage.

Discussion: We found evidence of changes consistent with neuroinflammation in people with schizophrenia, including mircogliosis, astrogliosis and blood brain barrier disruption in a subset of people with schizophrenia. The observations would be consistent with at least some people with schizophrenia having changes associated with brain tissue damage.

S32-04

Development of "psychosis" and IST prevention following prenatal exposure to maternal inflammation

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The findings that changes in brain structure and function precede full-fledged schizophrenia (SCZ) have raised the possibility that SCZ can be prevented. Unfortunately, given the diagnostic, methodological, and ethical challenges of pharmacological and imaging studies in high-risk (HR) individuals, evaluation of this possibility remains a major challenge, and results remain controversial. Animal models of SCZ are invaluable for investigating such questions. Increasing evidence points out to the involvement of inflammatory processes in schizophrenia, including findings of microglial activation and increased pro-inflammatory cytokines levels in patients, anti-anti-inflammatory action od antipsychotic drugs (APSs), and beneficial actions of antinflammatory drugs like the tetracycline antibiotic minocycline (MINO). We used the prenatal immune stimulation model that is based on the association of prenatal infection and increased risk for SCZ, to test whether inhibition of microglial activation might play a role in the prevention of SCZ. Pregnant rats were injected on gestational day 15 with the viral mimic polyriboinosinic-polyribocytidylic acid (poly-I:C) or saline. Their adolescent offspring (PND34-47) received the atypical APD risperidone (RIS), the tetracycline antibiotic minocycline (MINO), or the anti-oxidant N-acetylcysteine (NAC), and underwent behavioral testing and imaging at adulthood. Brain microglial activation was measured on PNDs 48 and PND 90. All three drugs were effective in preventing both structural and behavioral pathology but with different degrees of efficacy and different effects on controls. Microglial activation was present in the adult brains of poly-I:C offspring but was absent after early intervention. The results suggest that treatment with a range of pharmacological agents with anti-inflammatory, antioxidant and neuroprotective properties can prevent both brain and behavioral pathology and that these actions may be related to their capacity to prevent brain inflammatory processes.

S33 The Endocannabinoid System and Brain Function: Problems and Possibilities

S33-01

Impact of endocannabinoid signaling during adolescence on drug abuse and reward processing

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Puberty is a highly susceptible developmental period during which the organisation and the neuronal maturation of the brain, which began during perinatal development, are completed. During puberty an individual changes from a biologically non-reproductive, infertile juvenile into an adult who can reproduce. This stage is one of the major changes in biology and these maturational events and processes of reorganisation are needed for the occurrence of adult behavioral performance but also render the organism vulnerable to all sorts of disturbances. It is exactly during this developmental period that many neuropsychiatric disorders such as schizophrenia, mood and eating disorders, as well as drug abuse have their onset. Teenagers typically tend to seek out new stimuli during puberty, engange in risky behavior and show an increase in consummatory behavior for appetitive rewards and drugs of abuse. Therefore, puberty has been shown to represent a susceptible period for experimental drug use and it is known that the initation of substance abuse (e.g. alcohol abuse) during this developmental period is strongly associated with a higher risk for the development of addictive behavior in later life. Along with the dopaminergic and the endogenous opioid system, the endocannabinoid system has emerged recently as a key neurochemical mediator of reward processes. It is well known that cannabinoids can induce euphoric and rewarding effects in humans and animals and growing evidence indicates that the endocannabinoid system modulates various aspects of drug and non-drug reward. Results from our studies in rats indicate an important role for the developing endocannabinoid system in the modulation of reward sensitivity and the vulnerability towards alcohol use/abuse in adolescent animals.

S33-02

The effect of cannabinoids on human brain structure, function and neurochemistry

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Chronic cannabis use in humans is associated with altered brain structure and function, with evidence for greater adverse effects of exposure during critical neurodevelopmental periods (e.g. adolescence). We have demonstrated dose-dependent reduction of hippocampal and amygdala volumes in long term heavy cannabis users, alongside elevated psychotic-like symptoms and poorer cognitive function, particularly memory. Cannabis plant matter is comprised of multiple cannabinoid compounds that interact with the endogenous cannabinoid system. Among these, Δ^9-tetrahydrocannabinol (THC) is associated with worse outcomes, while cannabidiol (CBD) may ameliorate the adverse effects of THC and has intriguing therapeutic properties (e.g. anxiolytic, antipsychotic). We have evidence that prolonged exposure to CBD may protect against hippocampal volume loss in chronic users. The mechanisms of neuroprotection by CBD are currently not known. In an ongoing randomized controlled trial of acute administration of THC and CBD to human volunteers (frequent and infrequent cannabis users) we have acquired magnetic resonance spectroscopic data to probe interactions with the brain's predominant excitatory and inhibitory neurotransmitters. Preliminary data indicate that each compound differentially modulates GABA and glutamate levels in the hippocampus. THC decreased while CBD increased hippocampal glutamate levels relative to placebo. Both compounds increased GABA levels. Differential patterns were observed in the posterior cingulate cortex, and between frequent and infrequent users, likely due to an aberrant endocannabinoid system in frequent users. GABA-ergic and glutamatergic response to cannabinoid administration correlated with the brain electrophysiological mismatch negativity component response to cannabinoid administration; this component is impaired in schizophrenia. The implications of these findings overall, and specifically for CBD treatment for schizophrenia and/or cannabis dependence, will be discussed in light of the shifting policies toward legalization of cannabis for medicinal and recreational use.

S33-03

Targeting endocannabinoid regulating enzymes to treat drug addiction in preclinical models of dependence

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The endogenous cannabinoid system, which consists of two predominant ligands, *N*-arachidonoylethanolamine (anandamide; AEA) and 2-arachidonylglycerol (2-AG) that bind cannabinoid CB_1 and CB_2 receptors, plays an integral role in many physiological functions. These receptors as well as enzymes responsible for the regulation of endocannabinoids represent potential targets to treat a variety of disorders, including pain and inflammation, neurodegenerative diseases, anxiety-related disorders, and drug dependence and drug addiction. A growing body of data indicates that inhibitors of fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), the respective primary hydrolytic enzymes for AEA and 2-AG, represent potential strategies to treat drug dependence and other conditions. Inhibitors of these enzymes elevate AEA and 2-AG in brain and show efficacy in laboratory animal models of drug dependence, pain and inflammation, and anxiety and stress, but produce minimal psychomimetic effects associated with Δ^9-tetrahydrocannabinol (THC), the primary psychoactive constituent present in cannabis, and other cannabinoid receptor agonists. This presentation will review research testing FAAH and MAGL inhibitors in rodent models of THC, morphine, and nicotine. Overall, inhibitors of these endocannabinoid hydrolytic enzymes show promise as potential treatments for cannabis, opioid, and nicotine dependence through the activation of CB_1 receptors.

S33-04

Endogenous cannabinoids and emotional resiliency: translational implications for therapeutics development

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Endogenous cannabinoids (eCBs) are lipid signaling molecules that exert biological actions via CB1 and CB1 type cannabinoid receptors. eCBs are highly responsive to changes in affective state, and we have hypothesized that this signaling system represents a resiliency mechanism buffering against the deleterious effects of environmental stress. For example, genetic or pharmacological impairment in eCB signaling increases anxiety and depressive behaviors and neuroendocrine stress responses, and predisposes to the development of stress-related structural and functional changes in the brain. Importantly, pharmacological or genetic augmentation of eCB signaling has protective effects against the deleterious effects of stress. Specifically, we have recently reported a novel eCB augmenting strategy involving substrate-selective inhibition of COX-2, which can reverse stress-induced behavioral dysregulation. Here we will present genetic and pharmacological data supporting the role for eCB signaling as a resiliency factor buffering against the deleterious consequences of environmental stress, and describe translational studies aimed at developing novel therapeutics based on diverse eCB augmentation strategies.

S34 From Mechanisms to Novel Therapeutic Targets in Combatting Epileptogenesis and Epilepsy

S34-01

Emerging role of glial cells in epileptogenesis and as therapeutic targets

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Epilepsy is characterized by the periodic and unpredicted occurrence of seizures. Currently available antiepileptic drugs and therapies are insufficient to control seizure activity in one third of the patients. Thus, there is an urgent need for the development of more efficient anti-epileptogenic therapies. Recent data suggest that dysfunctional astrocytes might be associated with epilepsy. It remains however unclear whether the observed glial changes are causative of the disorder or merely represent a compensatory effect. We asked whether impaired astrocyte gap junction coupling might be involved in the etiology of mesial temporal lobe epilepsy (MTLE).

We have characterized functional properties of astrocytes in hippocampal specimens from MTLE patients without and with hippocampal sclerosis (MTLE-HS) and in a mouse model, unilateral intracortical kainate injection, that reproduces key features of human MTLE-HS. We combined patch-clamp recording, rapid glutamate application, extracellular K^+ concentration analysis, EEG- and video-monitoring, and fate mapping analysis.

We found that the hippocampus of MTLE-HS patients is completely devoid of *bona fide* astrocytes and gap junction coupling, while coupled astrocytes were abundantly present in non-HS specimens. In the MTLE-HS model, uncoupling impairs K^+ buffering and temporally precedes neuronal death and the generation of spontaneous seizures, indicating that uncoupling is a crucial factor in epileptogenesis. Uncoupling was induced through i.p. injection of lipopolysaccharide (LPS), prevented in Toll-like receptor 4 knockout mice and reproduced *in situ* through acute cytokine or LPS incubation. Fate mapping studies confirmed that in the course of MTLE-HS, astrocytes acquire an atypical functional phenotype and lose coupling.

These data challenge the commonly accepted neurocentric view of epileptogenesis and indicate that astrocytes may be a prime cause of MTLE-HS. Our study identifies novel targets for anti-epileptogenic therapeutic intervention.

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S34-02

Targeting tau-based mechanisms as anti-epiletogenic therapy: from animals to humans

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Epilepsy is a common brain disease that affects an estimated 50 million people worldwide. Current anti-epileptic drugs (AEDs) merely suppress seizures, but have not been found to stop or interfere with the process which converts a healthy brain into an epileptic brain following a brain insult¾ a process known as epileptogenesis. Down-regulation of protein phosphatase 2A (PP2A), which causes the hyperphosphorylation of tau, is implicated in neurodegenerative diseases commonly associated with acquired epilepsy, such as Alzheimer's Disease and traumatic brain injury. Here we used the PP2A activator sodium selenate to investigate the role of PP2A in three different rat models of epileptogenesis: amygdala kindling, post-kainic acid status epilepticus (post-SE), and post-traumatic epilepsy (PTE). PP2A activity was significantly decreased, and tau phosphorylation was increased, in epileptogenic brain regions in all three models. Sodium selenate treatment mitigated epileptogenesis and the biochemical abnormalities, and this effect was maintained after drug washout in the post-SE and PTE models. Our study indicates that epileptogenesis is associated with down-regulation of PP2A and the hyperphosphorylation of tau, and that sodium selenate, targeting this mechanism, is a potential anti-epileptogenic therapy.

S34-03

Neuropeptides in epilepsy: molecular basis to treatment options

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Neuropeptides, acting on G-protein coupled receptors, are known as modulators of neuronal functions. One of these roles is the control of neuronal excitability. In line with this, numerous neuropeptides have being suggested to play direct or indirect roles in epilepsy.

Anticonvulsant / antiepileptic effects were suggested for galanin, dynorphins, neuropeptide Y and somatostatin. In contrast tachykinins and enkephalins are considered mostly pro-convulsant. Effects depend on the position and type of receptor stimulated. Noteworthy, neuropeptidergic systems undergo marked changes during epileptogenesis and in epilepsy, which influence their functional role and their suitability as drug targets. Some, like neuropeptide Y and the Y2-receptor, are considered endogenous anticonvulsant mechanisms with increased expression during epilepsy. In contrast, dynorphins are down-regulated inter-ictally, thereby contributing to reduced seizure thresholds, but leaving unoccupied kappa opioid receptors as potential drug targets. Still, the complexity of peptidergic systems

and partially multiple signaling pathways activated through the G-protein coupled receptors make them a difficult terrain.

In recent years pre-clinical experiments of gene-therapy applications refreshed the scene. First line experiments with vectors expressing NPY alone or together with the Y2 receptor are promising and other candidates like galanin are following. Neuropeptides, stored in large dense core vesicles and released "on demand" – i.e. at burst stimulation – in restricted areas are a tempting scenario.

This talk will review the proposed roles of neuropeptide systems in animal models of epilepsy and human patients as well as treatment options.

S34-04

From mechanisms to biomarker identification for epileptogenesis

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A biomarker is "a measure of a disease process", meaning that it can be objectively measured and evaluated as an indicator of pathogenic process(es) related to epileptogenesis. The pathogenic epileptogenic process may include a myriad of network alterations in the brain, including neurodegeneration, neurogenesis, gliosis, axonal damage or sprouting, dendritic plasticity, blood-brain barrier damage, recruitment of inflammatory cells into brain tissue, reorganization of the extracellular matrix, and reorganization of the molecular architecture of individual neuronal cells. The epileptogenic pathology depends on the epileptogenic etiology and may vary even between the patients with the same etiology, which increases the pathological complexity. Moreover, there might be a need to adjust the biomarker search according to the stage of epileptogenesis, which an individual is experiencing during tissue sampling or analysis. Further, each one of the components of circuitry reorganization is regulated by many molecular pathways, which creates both an opportunity for a search for biomarkers of epileptogenesis, but also a great challenge, particularly because the critical epileptogenic network change is still under dispute. There have already been several studies of major epileptogenic etiologies like traumatic brain injury that aimed to identify molecular markers in blood and cerebrospinal fluid that predict outcome, by using proteomics and metabolomics. Another question to be explored is whether a palette of molecular markers is needed, rather than a single molecule, with each marker probing a different component of epileptogenic pathology. Further, perhaps multiple biomarker platforms (e.g., imaging, proteomics, electrophysiology) should be used in combination and/or in a defined temporal sequence. Some promising candidates for detection of preclinical epileptogenesis are already emerging from imaging and blood markers, which maintains a hope that finding a clinical biomarker for epileptogenesis will be possible.

S35 Molecular Mechanisms of Neurotransmission: Coupling Exocytosis and Compensatory Endocytosis

S35-01

Molecular mechanisms of synaptic vesicle membrane retrieval and reformation

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The function of the nervous system depends on the exocytotic release of neurotransmitter from synaptic vesicles (SVs). To sustain neurotransmission, SV membranes need to be retrieved, and SVs have to be reformed locally within presynaptic nerve terminals. In spite of more than 40 years of research, the mechanisms underlying presynaptic membrane retrieval and SV recycling remain controversial. In my talk I will present our latest data on the molecular mechanisms involved in presynaptic membrane retrieval and SV reformation and present perspectives for future research.

S35-02

Modes of EXO- and endocytosis in secretory cells

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Vesicle exocytosis releases content to mediate many biological events, including synaptic transmission essential for brain functions. Following exocytosis, endocytosis is initiated to retrieve exocytosed vesicles within seconds to minutes to recycle vesicles. Four decades of studies in secretory cells reveal at least two forms of exocytosis that are followed by three forms of endocytosis. First, full-collapse fusion, involving vesicle collapse into the plasma membrane, is followed by classical endocytosis involving membrane invagination and vesicle reformation. Second, kiss-and-run may occur, which involves rapid fusion pore opening and closure. Third, bulk endocytosis, which forms endosomes much larger than a regular vesicles, may occur after intense stimulation. Here I will review our evidence supporting these different forms of exo-endocytosis at a mammalian central nerve terminal, the calyx of Held. In particular, I will focus on our recent imaging work in a secretory cell, the adrenal chromaffin cell. We found new forms of exo-endocytosis that challenges the above traditional definition of full-collapse fusion and kiss-and-run. More specifically, using confocal and STED imaging of dense-core vesicles in chromaffin cells, we found that full-collapse fusion is due to shrinking of the fusion-generated Ω-profiles, but not pore dilation of the Ω-profile. Kiss-and-run, originally defined as rapid fusion pore opening and closure within ~2 s to generate the same vesicle as the exocytosed one, is re-defined as enlargement or shrinking of the fusion-generated Ω-profile, followed by closure of the Ω-profile within 1-30 s after fusion, which may generate different sizes of vesicles rapidly or slowly. Such a fusion pore closure may also mediate bulk endocytosis, the generation of large vesicles.

S35-03

Divergent synaptic vesicle cargo retrieval during intense neuronal activity

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The accurate formation of synaptic vesicles (SVs) and incorporation of protein cargo with the correct stoichiometry is critical for the maintenance of neurotransmission. During intense neuronal activity there is a transient and acute increase in the amount of SV cargo at the plasma membrane. Little is known regarding the mechanisms underlying the retrieval of this cargo by the two SV endocytosis modes that dominate during intense stimulation, clathrin-mediated endocytosis (CME) and activity-dependent bulk endocytosis (ADBE). To address this we examined the trafficking and retrieval of an array of abundant SV cargo molecules during trains of high frequency action potentials using a series of pH-sensitive genetic reporters in primary neuronal culture. To establish the proportion of SV cargoes that were retrieved by either CME or ADBE, we intervened using a palette of pharmacological and genetic manipulations to arrest either endocytosis mode. To our surprise we found that these exogenously expressed genetic reporters displayed a selective accumulation by either CME or ADBE. Biochemical enrichment of both SVs and bulk endosomes after intense stimulation confirmed that endogenous SV cargo were also specifically accumulated in the same manner by either CME or ADBE. This reveals that these two endocytosis modes have divergent cargo selection criteria at the nerve terminal plasma membrane and that SVs derived via ADBE will have a specific molecular signature that may define their physiological function.

S35-04

Molecular connection between calcium-regulated exocytosis and compensatory endocytosis in neuroendocrine cells

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In neuroendocrine cells, release of hormones and neuropeptides occurs through calcium-regulated exocytosis of large-dense core vesicles. To allow secretory vesicle recycling and maintain a constant cell surface area, exocytosis must be followed by compensatory membrane uptake. How these cells, specialized for hormone release, coordinate exocytosis with compensatory endocytosis remains poorly understood. Here, we focused on Rho-GTPases signaling and lipid remodeling, two key processes of large dense core granule trafficking in neuroendocrine cells.

Secretory granule exocytosis is accompanied by the redistribution of phosphatidylserine (PS) leading to the disruption of plasma

membrane asymmetry. We demonstrated that PS translocation occurred at the vicinity of the secretory granule fusion sites and was dependent on Phospholipid Scramblase-1 (PLSCR-1). Remarkably, secretory granule membrane recapture after exocytosis was impaired in mouse chromaffin cells knocked out for PLSCR1 demonstrating that PLSCR1-dependent lipid rearrangement is critical for compensatory endocytosis.

Oligophrenin-1 (OPHN1), a Rho-GTPase activating protein (Rho-GAP) containing a Bin-Amphiphysin-Rvs (BAR) domain and involved in X-linked mental retardation, has been shown to control synaptic vesicle endocytosis. Using carbon fiber amperometry, we found that exocytosis is impaired at the stage of membrane fusion and that compensatory endocytosis is severely inhibited in chromaffin cells isolated from Ophn1 knockout mice. Experiments performed with ectopically expressed OPHN1 mutants indicate that OPHN1 requires its Rho-GAP domain and RhoA inactivation to control fusion pore dynamics whereas the BAR domain implicates OPHN1 in granule membrane recapture after exocytosis.

Altogether, these data demonstrate for the first time that OPHN1 and PLSCR1 are bi-functional proteins able to couple, through distinct mechanisms, exocytosis with compensatory endocytosis in adrenal chromaffin cells.

S36 Astrocyte Gliotransmission, Metabolism and Behavior

S36-01

The missing brain glutamate transporters: what we missed and how this changes our understanding of the excitable brain

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Shuttling of glutamate between astrocytes and neurons and homeostasis of glutamate are critical determinants of brain function. One prevailing assumption is that the identity of glutamate transporters that enable this shuttling has been fully established for at least a decade. Here we report the discovery and cloning of a new and functional form of a glutamate transporter called GLT1d or EAAT2d. GLT1d represents ~40% of total GLT1mRNA in adult human forebrain, is similarly abundant in rodents and is abundantly expressed at the protein level. This protein is not detected by many of the commonly used antibody tools and is potentially a major contributor to the regulation of brain excitability. We have also searched for neuronal glutamate transporter(s). The widely held view that the transporter EAAT5 is present only in the retina is incorrect; We have demonstrated that EAAT5 mRNA and protein is widely distributed in the body. In the brain we show that immunocytochemically detectable EAAT5-like protein is present in neurons and that using PCR from Exon 6 of EAAT5 onwards we can detect mRNA that is identical in sequence to retinal mRNA. We are currently evaluating whether GLT1d is also a "missing" neuronal glutamate transporter, since *in situ* hybridization studies and pharmacology studies suggest the presence of, and a role for a GLT1-like protein in neuronal glutamate uptake or release. We propose that our findings regarding GLT1d and EAAT5 will provide a clearer understanding of the roles of neurons and glia in taking up or releasing glutamate via transporters, and require a re-evaluation of our understanding of glutamate transporter expression in pathology.* Equal first author

S36-02

Metabolic regulation of vesicular glutamate release from cultured astrocytes

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Astrocytes have a prominent role in brain physiology and pathophysiology. In addition to maintaining blood flow, metabolic and ionic homeostasis, they have capability of signaling to adjacent neurons by releasing glutamate via process of regulated exocytosis. Astrocytes synthesize glutamate *de novo* owing to pyruvate entry to the citric acid cycle via pyruvate carboxylase. Pyruvate is sourced from the utilization of two metabolic fuels, glucose and lactate. Glucose can be polymerized to glycogen and stored as fuel within astrocytes and/or lysed to pyruvate, while lactate can be converted to pyruvate. To that end, we investigated the role of the above energy sources, glycogen, glucose and lactate, in exocytotic glutamate release from astrocytes. We used purified primary astrocyte cultures acutely incubated (1 h) in glucose and/or lactate-containing media. We used mechanical stimulation, known to increase intracellular calcium levels and cause exocytotic glutamate release. Using single cell fluorescence microscopy, we monitored stimulus-induced intracellular calcium responses as well as glutamate release to the extracellular space. Our data indicate that glucose, either taken-up from media or mobilized from the glycogen storage, sustained glutamate release, while the availability of lactate significantly reduced the release of glutamate from astrocytes. Based on further pharmacological manipulation, it appears that lactate caused metabolic changes consistent with an increased synthesis of fatty acids. The above metabolic and functional changes were corroborated by tandem mass spectrometry proteomics analysis which confirmed appropriate altered protein expression. These findings support the notion that the availability of energy sources and metabolic milieu play a role in glial-neuronal interactions and modulation of synaptic activity in health and disease.

S36-03

Monocarboxylate transporters and lactate receptor expression and function in the brain

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L-lactate, pyruvate, and ketone bodies like β-hydroxybutyrate and acetoacetate are monocarboxylates transported across different brain cell membranes by different monocarboxylate transporters (MCTs). L-lactate is the MCT substrate that is most abundant in the brain, and fluctuates the most in concentration. As the MCTs mediate facilitative transport, they serve to equilibrate substrate concentration across cell membranes, the concentration gradient being the driving force. This means that substrates such as L-lactate migrate from sites of production towards sites of consumption, be it between cells within an organ (e.g., between glia and neurons in the brain, or between glycolytic and oxidative fibres in skeletal muscle), or among different organs (e.g., skeletal muscle, heart, and brain) via the blood stream. The equilibrating action of MCTs also provides the basis for lactate acting as a volume transmitter that can mediate metabolic signals through the nervous tissue. The latter concept was established by the demonstration that lactate can bind to the lactate receptor GPR81 (HCA1), on brain cells and cerebral blood vessels, resulting in inhibition of adenylyl cyclase. The localisation and function of the three different MCTs (MCT1, MCT2 and MCT4) in addition to HCA1 in different brain cells will be the focus of my talk.

S36-04

Astrocytic modulation of sleep and wakefulness

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Our previous studies have demonstrated that astrocyte-derived adenosine plays important roles in the modulation of sleep homeostasis. Our results are consistent with a vesicle-mediated mechanism of ATP release that gives rise to adenosine because the astrocytic expression of dnSNARE, which is known to impair exocytosis, leads to reduced extracellular purines. Other groups have suggested that connexins may also mediate the release of adenosine. To test this possibility we have developed astrocyte specific Cx43 KO mice and studied the presence of potential sleep phenotypes. In contrast, to dnSNARE mice, Cx43 KO mice do not exhibit impaired sleep homeostasis. However, EEG/EMG studies show that these mice exhibit excessive daytime sleepiness: during the dark phase when mice are normally awake and active, Cx43 KO mice show enhanced NREM and REM sleep consistent with a narcolepsy phenotype. Using viral mediated brain region specific recombination as well as whole cell patch recordings together with pharmacological modifications we identify the locus of this narcoleptic phenotype to be in the lateral hypothalamus where the astrocytic manipulation leads to reduced excitability of orexinergic neurons. Astrocytes modulate orexinergic excitability via the lactate shuttle which is essential to sustain neuronal activity.

S37 Long-distance Signaling in Control of the Transcriptomic and Proteomic Responses to Neuronal Injury

S37-01

When synaptic proteins meet the genome -protein transport from synapse to nucleus

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NMDA receptors (NMDAR) make an essential contribution to activity-dependent gene expression. Intriguingly, NMDAR are present at both synaptic and extrasynaptic sites, and the subcellular localization of each receptor profoundly affects the nuclear response to its activation. Activation of synaptic NMDAR induces the expression of cell survival and plasticity genes, while in contrast activation of extrasynaptic NMDAR primarily drives expression of cell death genes, linking the pathway to neurodegenerative disease. An unresolved issue is, how can the distant nucleus discriminate between synaptic and extrasynaptic NMDAR-induced signals? Jacob is a synapto-nuclear protein messenger that requires the importin-mediated transport from distal dendrites for nuclear import and previous work has shown that extrasynaptic NMDAR activation drives Jacob efficiently to the nucleus, inducing sustained dephosphorylation and transcriptional inactivation of CREB. However, Jacob also translocates to the nucleus in CA1 neurons after Schaffer collateral dependent LTP but not LTD, and hence it acts as a messenger for both synaptic and extrasynaptic NMDAR. In a recent study we found that Jacob can encode in the nucleus the synaptic and extrasynaptic localization of activated NMDAR. The Janus face of Jacob is based on differential MAP-kinase activity and ERK-dependent phosphorylation of the serine 180 in protein following synaptic but not extrasynaptic activation of NMDAR. We found that the neurofilament alpha-internexin associates with Jacob in a phosphorylation dependent manner and that the trimeric complex is very stable and efficiently protected against phosphatase activity. Taken together the data indicate that long-distance signaling via Jacob require a larger signalosome-like protein complex and that Jacob following activation of synaptic but not extrasynaptic NMDAR might bring its own ERK piggyback to the nucleus. The Janus face of Jacob provides the first long-haul molecular mechanism to distinguish both NMDAR pathways in the nucleus. We speculate that Jacob operates as a mobile hub that docks NMDA-receptor derived signalosomes to nuclear target sites like CREB and potentially others.

S37-02

Activity-dependent signaling and its role in metabolic and redox homeostasis

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Activity-dependent signaling and gene regulation plays a key role in fundamental neurobiological processes such as development, plasticity, and survival. We will describe newly characterised activity-dependent transcriptional and post-translational changes which mediate important adaptive changes to neurons, such as tuning antioxidant defences and energy supply to the needs of an active neuron, and preventing mitochondrial Ca^{2+} overload. Additionally we will explain why neurons are particularly vulnerable to oxidative stress, replying on astrocytes for extrinsic support. We will also describe studies aimed at understanding the degree of rodent-human evolutionary conservation and divergence in neuronal activity-dependent gene regulation.

S37-03

C-jun n-terminal kinase regulates P75 internalization leading to retrograde axonal transport of apoptotic signalling endosomes

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Intraneuronal communication is essential for development and maintenance of neuronal circuits; the extremely polarized morphology of neurons is a challenge for intracellular communication since axons can be several other of magnitude longer that the diameter of the cell body. Given this unique morphology and the challenges it creates for intracellular communication, neurons exploit existing intracellular trafficking to move signals long distances. Significantly, impairment of endocytic trafficking of neurotrophic signals appears to underlie neurodegenerative disorders. One well-defined cell culture model of axonal to soma communication is the superior cervical ganglion (SCG) of the sympathetic nervous system. SCG neurons express two types of neurotrophin receptors, the NGF specific receptor TrkA and the p75 receptor (which binds all neurotrophins). In this work we describe for the first time that p75 was able to generate an "apoptotic signaling endosome" that is transported retrogradely to the cell bodies of neuron, resulting in their death. This process depended on the molecular motor dynein, the activity of the Rab5 GTPases (regulating early endosome sorting) and on JNK activation. Surprisingly, we found that JNK activity was required for p75 internalization. JNK has been shown to play different roles on axonal physiology including microtubule stabilization, injury signaling, axonal plasticity and axonal degeneration. Our findings point to a critical role for JNK on p75 retrograde signaling and to the existence of a competition between survival producing versus death producing signals. In essence, they point to the local activation of specific receptors in target territories, and the balance between pro-survival and pro-death signals produced there, as mediating the status of the distant neuron cell

bodies. As such, the work will be interest for the physiology of neurons and their axons, including their involvement in neuropathological conditions.

S37-04

Disruption to long-range anterograde neurotrophin signalling in alzheimer's disease

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Sleep disruption including sleep aponea, particularly during rapid eye movement (REM) sleep, is a risk factor for Alzheimer's disease. REM sleep is regulated by mesopontine tegmentum (MPT) cholinergic neurons which project their axons to the basal forebrain cholinergic neurons. Cholinergic neurons of the basal forebrain are dependent on nerve growth factor (NGF), for function and surviva and are particularly vulnerable in Alzheimer's disease, with the consequent cholinergic neurotransmitter decline affecting cognition. We have shown that lesioning MPT neurons results altered breathing patterns and in the loss of basal forebrain cholinergic neurons. We are testing the hypothesis that basal forebrain neuron death is due to activation of the neuronal death receptor p75 neurotrophin receptor ($p75^{NTR}$) in basal forebrain cholinergic neurons due to reduced anteriograde and/or retrograde supply of NGF, either produced by the MPT or due to resultant reduced activity of the cortex respecitvely, and which subsequently also results in other features of Alzheimer's disease including increased amyloid-β production, tau phosphorylation, synaptic dysfunction and cognitive decline.

S38 New Roles of Neural Glycosaminogycans (GAGs) in Development, Platicity, Regeneration and Disease

S38-01

Glypicans as novel regulators of synaptic connectivity

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Precise synaptic connectivity is essential for the proper functioning of neural circuits. We recently identified the presynaptic heparan sulfate proteoglycan (HSPG) glypican-4 (Gpc4) as a binding partner for the postsynaptic adhesion molecule LRRTM4, and showed that a trans-synaptic Gpc4-LRRTM4 interaction is required for excitatory synapse development. Whereas LRRTM4 displays a highly restricted expression pattern in the brain, Gpc4 is more widely expressed. This suggests that Gpc4 may have additional binding partners in order to regulate synapse development in other brain regions. However, little is known about the molecular mechanisms by which glypicans regulate synaptic connectivity. To address this issue, we have performed an unbiased proteomics screen to identify synaptic binding partners for the HSPG Gpc4. Using this approach, we have identified a largely uncharacterized G protein-coupled receptor (GPCR) as a potential Gpc4 interactor. Binding assays confirm that Gpc4 binds this GPCR in an HS-dependent manner. Fractionation and localization studies suggest a postsynaptic localization at excitatory synapses, and immunohistochemical analysis reveals that this GPCR is selectively expressed at a specific synapse in the hippocampus, which does not contain LRRTM4. Furthermore, we find that this GPCR can induce artificial synapse formation in cultured hippocampal neurons in an HS-dependent manner. Current experiments focus on analyzing the consequences of gain- and loss-of-function of this novel Gpc4 binding partner on synapse development and function. These findings indicate that glypicans interact with distinct postsynaptic binding partners at different synapses, and highlight the diversity of trans-synaptic interactions regulating circuit development.

S38-02

Mice lacking in an enzyme involved in chondroitin sulfate synthesis shows better recovery from spinal cord injury

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Many patients with spinal cord injuries (SCIs) suffer severe paralysis. Injured adult neurons in the mammalian CNS rarely regenerate, because some of the intracellular and cell-surface environmental factors inhibit axon regrowth. Chondroitin sulfate (CS), a glycosaminoglycan (GAG), is the most abundant and potent exogenous inhibitor of axonal regeneration and CS degradation induces some of the axonal regrowth following SCI by treatment of chondroitinase ABC (ChABC). We generated null (KO) mice of CS N-acetylgalactosaminyltransferase-1 (CSGalNAcT1), a key enzyme in CS biosynthesis. There are no major abnormalities in spinal cord of adult KO mice, and we used a compression models to induce SCI. Here, we show that KO mice recovered much faster and more completely from induced SCI than do wild-type mice and even ChABC treatment mice. Following SCI, KO mice showed smaller areas of glial scarring and exhibited many more regenerated axon terminals. Additionally, synthesis of another type of GAG, heparan sulfate (HS), was up-regulated extraordinarily at the injury sites only in KO mice. Moreover, ChABC treated mice and wild-type SCI mice were never observed the induction and up-regulation of HS. Our results indicated that CSGalNAcT1 influenced the extraordinary recovery from SCI by modulating the balance of synthesis of CS and HS. Thereafter, we tried to establish the *in vivo* knockdown (KD) system using the RNAi of CS-synthesizing enzymes with the biodegradable biomaterial. RNAi-mediated CSGalNAcT1-KD *in vivo* led to excellent recovery from SCI. This KD systems caused reduced CS synthesis, and scar sizes were significantly smaller than in ChABC treated mice. These results showed that CSGalNAcT1 is a promising best therapeutic target for treatment of the neural damage.

S38-03

Targeting the extracellular matrix to repair the damaged nervous system

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The extracellular matrix plays a central role in restricting plasticity and axon regeneration, mainly through the action of chondroitin sulphate proteoglycans (CSPGs). An important agent in removing this restriction has been the enzyme chondroitinase, which removes glycosaminoglycan (GAG) chains from CSPGs. CSPGs are upregulated in glial scar tissue around injuries to restrict axon regeneration, which can be enhanced with chondroitinase. However the positive effect of chondroitinase on functional recovery is mostly through reactivation of plasticity. Chondroitinase treatment enhances recovery from many forms of CNS damage, including spinal cord injury, where the reactivation of plasticity enables successful rehabilitation. Chondroitinase also has profound effects on memory, prolonging object memory in normal animals and restoring memory in an Alzheimer's model. CPSGs control plasticity mainly through their participation in perineuronal nets (PNNs), cartilage-like structures surrounding neurons, which appear as critical periods for plasticity close. PNNs contain inhibitory CSPGs, hyaluronan, link protein and tenascin–R, partly produced by the neurones themselves and partly by surrounding glial cells. All

neurones with PNNs express both a hyaluronan synthase enzyme and a link protein, and these are the key components that trigger the formation of the structures. Link protein knockout animals lack normal PNNs on their dendrites, and these animals retain plasticity into adulthood, and show prolongation of memory identically to animals treated with chondrotinase. The action of the CSPGs is due to their sulphated GAGs. In the CNS these bind to and localise Semaphorin3A and OTX2 to PNNs. OTX2 is involved in the maturation of inhibitory interneurons, while Semaphorin3A is an effector of the PNNs involved in control of plasticity.

S38-04

Heparan sulfate as an autism susceptibility molecule

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Heparan sulfate (HS), a class of glycosaminoglycans that exists as heparan sulfate proteoglycans, plays critical physiological roles in various cell surface signaling events. Conditional knockout (CKO) of the *Ext1* gene, which encodes a glycosyltransferase essential for HS biosynthesis, has been a powerful model to define the physiological roles of HS in mice. Using the *Ext1* CKO, we have previously shown that HS is functionally required for a number of neural developmental events, including midbrain-hindbrain patterning, cerebral neurogenesis, and multiple axon pathfinding processes. More recently, we have extended our study to the elucidation of the role of HS in synaptic transmission, cognition, and behavior. *Ext1* CKO mice targeted to postnatal excitatory neurons (*CaMKII-Cre; Ext1*$^{flox/flox}$) exhibit striking recapitulation of almost the full range of autistic symptoms, including impairments in social interaction, expression of stereotyped, repetitive behavior, and impairments in ultrasonic vocalization. This phenotype correlates with impaired activation of amygdala neurons in response to social stimulation and the reduction in the level of synaptically localized AMPA-type glutamate receptors. These results demonstrate that HS is critical for normal functioning of glutamatergic synapses, and that its deficiency mediates autism-like socio-communicative deficits in mice. The molecular mechanisms by which HS regulates glutamate receptor surface expression and the implications of these findings in autism and related mental disorders will be discussed.

S39 Chronic Pain: Lessons from Animal Models to Human Studies

S39-01

Evolutionary genome analysis reveals novel pain genes and therapeutic targets

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Worldwide, acute and chronic pain affects 20% of the adult population and represents an enormous financial and emotional burden. Interestingly many of the core genes involved in nociception are conserved from insects through to humans. Previously we used whole genome RNAi screening in the fruit fly Drosophila melanogaster to identify genes required for acute nociception. More recently, we have begun to develop tools and assays to model chronic pain in the fly. Interestingly some drugs that treat neuropathic pain in humans (e.g. anti-epileptics and anti-depressants) also have a positive effect on injured flies. Importantly, fly orthologs of known human pain genes are required for chronic pain-like responses in flies. Using a combination of genome-wide data sets for fly nociception and human chronic pain, we have pinpointed new candidate conserved pain genes/pathways, which we are currently validating functionally. Importantly, pharmacological modulation of one of these pathways can alleviate neuropathic pain in rats and these drugs may represent a new class of neuropathic painkiller with rapid translational potential.

S39-02

Neuroinflammation and cytokine dysregulation in neuropathic pain

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Injuries of peripheral nerves can cause chronic inflammation along the pain pathway involving diverse immune cell types and immune-like glial cells (e.g., astrocytes and microglia). Numerous studies show significant glial activation and infiltration of leukocytes, such as macrophages and T cells, at the site of the initial nerve lesion, at the ipsilateral dorsal root ganglia and at the dorsal horn of the spinal cord following peripheral nerve injury. Activated immune and glial cells produce and secrete cytokines and chemokines, which can be pro-inflammatory or anti-inflammatory. Dysregulation of cytokines has been implicated in a variety of painful neurological diseases and in animal models of neuropathic pain. We have previously demonstrated the contribution of pro-inflammatory cell types and their mediators to neuropathic pain and the beneficial effects of immunosuppressive T cells in controlling chronic inflammation and attenuating pain hypersensitivity. This presentation will focus on changes in cytokine profile and pain behaviours following modulation of the immune response in animal models of neuropathic pain due to peripheral nerve injury and in patients with painful peripheral neuropathy. Targeting of neuroinflammation in neuropathic pain may provide potential therapeutic opportunities.

S39-03

The pain genome-phenome project: a translational murine-human approach

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Chronic pain occurs after injury or certain diseases to the nervous system, incurring suffering to many pain patients that cannot be prevented nor cured by current treatments. Such treatments generally provide modest pain relief that is typically traded off by adverse side effects. Chronic pain is very common, afflicting about 1 in 3 adults (in the US) and 1 in 5 (in Europe). These staggering statistics make chronic pain the silent epidemic of our times. There is growing hope that accomplishments in the Pain Genome Project will identify new preventative and palliative analgesic treatment targets, which could be used to stratify patients by their genetic risk and protective factors, guiding treatment options (eg, whether to undergo surgery and if so, what type of surgery should be selected). This knowledge is additionally expected to improve our rodent models of painful neuropathies by selecting inbred strains that mimic genetic and phenomic architectures of human chronic pain, thereby facilitating new mechanistic and pharmacogenetic testing platforms of novel treatments. This presentation will provide an update on the status of pain genetics by reviewing studies that started by identifying candidate pain genes in rodent models of painful neuropathies and then showed that these genes play a role in human chronic pain, and *vice versa.* This update will additionally review studies in rodent models, exemplifying how environmental risk and protective factors can modify the inborn genetic predisposition to form individual pain trajectories, knowledge that is essential for the development of personalized pain medicine.

S39-04

Central changes in individuals with chronic neuropathic pain

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Neuropathic pain is a disease state with an enormous socioeconomic burden. Current treatment regimens for neuropathic pain are largely ineffective, which likely results from our relatively poor understanding of the underlying mechanisms responsible for neuropathic pain. Surprisingly, neuropathic pain does not increase activity in the same brain regions that are activated by acute pain. Indeed, few differences occur in on-going activity in individuals with neuropathic pain compared with controls. Although it appears neuropathic pain is not associated with overt activation of the usual pain matrix, is it associated with altered thalamocortical rhythm. Furthermore, recent human brain imaging studies report structural and activity changes in areas thought to regulate this neural rhythm. This presentation will focus on changes in structure and function in areas associated with thalamocortical rhythm in individuals with neuropathic pain and discuss these changes relative to the changes that have been reported in animal models.

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Workshops
W01 Neuroepigenetics: from Neural Development to Adult Neurogenesis

W01-01
Epigenetics in the maintenance of neural stem cells
S. Hitoshi

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During development, the cell cycle length in neural stem cells increases, which could be associated with their capabilities for self-renewal. However, the molecular mechanisms that regulate differentiation and cell cycle progression in embryonic neural stem cells remain largely unknown. We have sought factors that are involved in the cell cycle regulation and the maintenance of undifferentiated state of neural stem cells through epigenetic mechanisms. We found, for example, that upregulation of Bre1a, a histone H2B ubiquitylation factor, in neural precursor cells is required for the determination of proper timing of differentiation by downregulating the expression of Hes5, an effector gene of Notch signaling. On the other hand, the knockdown of Bre1a in neural precursor cells lengthened their cell cycle through the upregulation of $p57^{kip2}$ and downregulation of Cdk2. I would present the latest data and have discussion on them.

W01-02
Chromatin modifying enzyme histone deacetylase 3 controls the fate switch between oligodendrocytes and astrocytes
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Establishment and maintenance of oligodendrocyte identity from their progenitors ensures proper CNS myelination, however, the underlying mechanisms remain poorly understood. Here, we show that the histone deacetylase Hdac3 controls Olig2 expression and functions as a molecular switch for oligodendrocyte and astrocyte lineage determination. Hdac3 ablation in the oligodendrocyte lineage causes severe myelination defects. Genetic fate-mapping studies show that Hdac3-ablated oligodendrocyte progenitors appear to transform into astrocytes. Genome-wide occupancy analysis uncovers that Hdac3 targets lineage-specific genes and cooperates with p300 to promote oligodendrocyte lineage progression. Hdac3/p300 interaction further antagonizes astrogliogenic pathways by repressing astrocyte-enriched gene expression and astrogliogenic Stat3 activity through regulating its posttranslational modifications. Thus, our findings reveal that Hdac3 is a key priming factor for oligodendrocyte lineage commitment by establishing lineage-specific epigenetic landscape while silencing alternative astrocytic transcription profiles and Stat3 signaling, pointing to that modulation of Hdac3 activity may represent a potential approach to promote myelin repair.

W01-03
Embryonic versus adult neural stem cells
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One of the fundamental questions in understanding tissue development is how multipotent progenitors/tissue stem cells give rise to various cell types in a defined order to achieve appropriate tissue organization. Neural stem/progenitor cells (NPCs) attract much attention since these cells give rise to neuronal and glial cell types in a developmental-stage dependent manner with striking precision. We have previously shown that polycomb group (PcG) complex and high mobility group A (HMGA) proteins play pivotal roles in driving fate switches of NSCs during neocortical development. I would like to talk about how these proteins are regulated and how they control the fate of NPCs in a developmental stage-dependent manner. In contrast to embryonic NPCs, adult neural stem cells (NSCs) maintain their differentiation potentials for a long time to continue neurogenesis for life. I would also like to talk about the mechanisms underlying their long-term maintenance and the differences between embryonic NPCs and adult NSCs.

W01-04
Transgenic monkeys overexpressing MECP2 exhibit autism-like behavioral abnormalities
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Methyl-CpG binding protein 2 (MeCP2) primarily binds to methylated DNA and plays a critical role in transcriptional regulation. Mutations in *MECP2* gene are found in 90% of patients with Rett syndrome, a severe form of developmental disorders. Duplications of *MECP2*–containing loci also cause the *MECP2* duplication syndrome, which shares core symptoms with autism spectrum disorders (ASD). Although the *mecp2*-null mice recapitulate most developmental and behavioral defects found in Rett syndrome patients, it has been difficult to identify autism-like behaviors in the mouse model with MeCP2 overexpression. Here we report that lentivirus-based transgenic cynomolgus monkey (*Macaca fascicularis*) overexpressing human MeCP2 in the brain exhibited marked alterations in gene expression and mRNA splicing, and autism-like behavioral abnormalities. As compared to wild-type (WT) monkeys, MeCP2 transgenic (TG) monkeys exhibited higher frequency of repetitive circular locomotion and elevated human gaze-induced stress responses, as measured by the

threat-related anxiety and defensive (TAD) test for monkeys. The TG monkeys also showed less time spent with other monkeys within the same group (reared together for ~ 6 months). Reduced interaction time was also found for TG monkeys when two unfamiliar or familiar monkeys were paired in a single cage. Wisconsin General Test Apparatus (WGTA) revealed largely normal cognitive functions in TG monkeys, although some TG monkeys showed signs of stereotypic cognitive behavior. These results suggest the feasibility of using transgenic non-human primates for studying neural mechanisms of genetic brain disorders.

W01-05

NRSF/REST regulation of gene networks in adult neural stem cells

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Defining the transcriptional and epigenetic circuitry controlling neural stem cells is critical for harnessing the potential of adult hippocampal neurogenesis in regenerative medicine. We have systematically dissected the function and mechanisms of individual transcriptional factors that function in the stepwise progression of quiescent neural stem cells to mature dentate granule neurons in adult mammalian brain. Using conditional knockout mice to perform genetic loss-of-function and neural stem cell cultures to perform molecular and biochemical analysis, we have characterized a negative regulator of quiescent and activated stem/progenitor cells (NRSF/REST) and its co-repressors (class I histone deacetylases) to be important in maintaining the adult neural stem cell pool and preventing precocious activation of the neuronal lineage program. Furthermore, we have taken advantage of a reversible *in vitro* model of quiescence to identify the direct downstream targets of NRSF/REST involved in maintaining quiescent and proliferating stem cell populations. Our data suggest that NRSF/REST binds distinct target genes to regulate the transition from quiescent to activated progenitors, including neuronal and non-neuronal genes. Our results also provide mechanistic insight for strategies to promote the adult neural stem cell pool towards preventing age-related cognitive decline. Supported by grants from the NIH, Welch Foundation, and Texas Institute for Brain Injury and Repair.

W02 Development of Animal Models, a permanent Challenge in Neurosciences

W02-01

Neuroprotection and neuroregenerative therapy for ischemic stroke

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Neuroprotection is essential for therapy in acute stage of stroke. Both NTFs and free radical scavenger can be such neuroprotective reagents with inhibiting death signals and potentiating survival signals under cerebral ischemia. For example, topical application of GDNF greatly reduced the infarct size and brain edema after middle cerebral artery (MCA) occlusion in rats. Edaravone, a free radical scavenger, is the first clinical drug for neuroprotection in the world which has been used from 2001 in most ischemic stroke patients in Japan. Edaravone scavenges hydroxyl radicals both in hydrophilic and hydrophobic conditions, and is especially useful in thrombolytic therapy with tissue plasminogen activator (tPA). Combination therapy of Edaravone with tPA greatly increased survival of stroke animals, reduced infarct size, and inhibited molecular markers of oxidative damage in lipid, protein and DNA. Use of Edaravone greatly reduced hemorrhagic transformation accompanied by tPA treatment,and may also extend therapeutic time window with tPA therapy for more than 4.5 h in human stroke patients.

It is important for regenerative therapy that the neural stem cells which are intrinsically activated or exogenously transplanted. To support stem cell migration, an artificial scaffold can be implanted to injured brain for promoting ischemic brain repair. Addition of NTFs greatly enhanced an intrinsic migration or invasion of stem cells into the scaffold, which could provide a future regenerative potential against ischemic brain damage at chronic stage. G-CSF may promote bone marrow cell migration into ischemic brain to reduce such a damage. *In vivo* optical imaging is a recent technology to detect ischemic and other neurologic disorders without killing subjects, which make able time-dependent monitoring of the disease conditions such as MMP9 activation and macroautophagy. Transient increase of such *in vivo* optical images were detected from living mice brain after ischemic stroke. We report a cell therapy for both ischemic stroke model. As a translational stroke research, we are currently conducting a clinical trial with G-CSF for ischemic stroke patients together with Professor Shunya Takizawa at Tokai University.

W02-02

Role of granzyme-B., A cytotoxic protease in the neuronal cell death after stroke

P. P. Babu

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Infiltration of leukocytes into post-ischemic cerebrum is a well-described phenomenon in stroke injury. Because CD-8+ T-lymphocytes secrete cytotoxic proteases, including granzyme-b (Gra-b) that exacerbates post-ischemic brain damage, we investigated roles of Gra-b in the brain samples of rats and humans after stroke. After experimental stroke (MCAO) in rat brains, we observed that CTLs infiltrate the ischemic infarct within 1 h of reperfusion in rats. There was a significant increase in Gra-b levels in the ischemic region starting from 1 h until 3 day which correlated with increased levels of chemokines (IP-10/CXCL10, IL-2) and TNF-α. Further, Gra-b also showed to interact with apoptotic markers such as Bid, PARP, and caspase-3 in ischemic samples. To study the role of Gra-b in human stroke, tissue samples from post-mortem brains (both ischemic and non-ischemic) were analyzed. Results showed that ischemic brain samples contained significantly higher levels of Gra-b and interferon-gamma inducible protein-10 (IP-10/CXCL10) than non-ischemic controls. Further, cleavage of both PARP and HSP70 a hall mark for Gra-b generated signature fragments was also observed. Gra-b was also found to bind to Bid and caspase-3. Gra-b also co-localized with apoptosis in degenerating neurons. Interestingly, Gra-b inhibition protected neurons against *in vitro* neurotoxicity mediated by activated CG-SH cells and supernatants. Our findings strongly suggest that Gra-b secreted from activated CTLs might be involved in aggravating post-ischemic neuronal damage.

W02-03

Modeling Alzheimer's disease in transgenic animals

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Advances in the genetic tools available across a wide range of species have made transgenic modeling more versatile than ever before. Transgenic systems are widely used to study the cellular and molecular basis of Alzheimer`s disease (AD). While the transgenic mouse remains the most widely used model system in AD, significant efforts have been made in a variety of other species including nematodes, arthropods, fishes, rats, as well as non-human primates. These transgenic systems have enormous value for understanding the pathophysiological basis of AD and have, in some cases, been instrumental in the development of new therapeutic approaches. In this presentation, we`ll discuss the effect of aging in transgenic models of AD as an influencing factor in the phenotypic expression of a neurodegenerative process, the pros and cons of the application of the invertebrates or vertebrates models as tools for developing insight into the biological basis of the disease, the central roles of transgenic models in preclinical testing of new drugs and the outcome of these drugs in the reversion of the clinical symptoms in affected individuals

W02-04

Gene environment interactions in animal models of schizophrenia

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Schizophrenia and other psychiatric illnesses are likely caused by a combination of genetic and environmental factors, such as stress.

To model these 'two hit' gene-environment interactions, we investigated the long-term effects of corticosterone treatment (CORT) to simulate chronic stress. Brain-derived neurotrophic factor (BDNF) is involved in brain development and plasticity and is implicated in schizophrenia and depression. CORT treatment resulted in deficits in spatial memory in a Y-maze task in male BDNF heterozygous mice, but not wildtype controls (Klug *et al*., Neurobiol Dis 2012). In male maternally-separated rats, CORT induced similar deficits. In both models, there were no CORT effects in females. Male/female-specific behavioral changes in maternally-separated rats were correlated with differential effects on exon-specific BDNF expression in the dorsal hippocampus, prelimbic cortex and nucleus accumbens between the sexes (Hill *et al*., Hippocampus 2014). Moreover, these 'two hit' effects were accompanied by sex-specific alterations in regional NMDA receptor subunit expression. The striking sex differences in 'two hit' effects suggest that developmental stress effects may be modulated by estrogen as it interacts with BDNF/TrkB gene expression and signalling. Indeed, the adolescent/young adult developmental trajectory of BDNF signalling in frontal cortex and dorsal hippocampus was markedly different between male and female mice and sensitive to altered circulating levels of these sex hormones (Hill *et al*., *J. Neuroendocr.* 2012). Furthermore, in animal and human studies we observed differential effects of estrogen and testosterone in behavioral paradigms with relevance to schizophrenia, such as prepulse inhibition (PPI). We are currently investigating the 'two hit' effects of BDNF deficiency and drugs of abuse, such as cannabis and methamphetamine. Conversely, we observed that environmental interventions, such as environmental enrichment, can reverse 'two hit' effects on behaviour, suggesting avenues for early non-pharmacological treatments or prevention strategies. In conclusion, gene-environment interaction studies in animal models of schizophrenia and other psychiatric illnesses allow for detailed analysis of behavioural domains affected, molecular mechanisms involved, and potential treatments based on environmental factors or sex steroid hormones.

W03 Challenge for Central Nervous System Regeneration: A Synergistic Approach Combining Neurobiology and Biomaterial Engineering

W03-01

Bioengineered scaffolds support human embryonic stem cell-derived cortical progenitor transplants in a rodent model of stroke

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Necrotic brain injury results in primary cell death (primary insult), surrounded by a penumbra (salvagable tissue susceptible to secondary degeneration). Current therapies fail to repair the primary injury and minimally reduce secondary damage. We believe efforts to promote repair following necrotic injuries, will depend on the capacity to: (i) restore the tissue architecture; (ii) deploy replacement cells; (iii) provide a suitable environment for the integration of transplanted cells and (iv) support residual cells in the penumbra. Here, we bioengineered scaffold gels to form 'bio-bridges" across the necrotic lesion cavity that provided both physical and trophic support to newly grafted GFP+ human embryonic stem cell-derived cortical progneitors, as well as to residual host cells. Scaffolds were synthesized by the assembly of peptides for the laminin-derived epitope IKVAV - the brain's major extracellular protein. In a athymic rat model of focal ischemia (endothelin-1 delivery to the motor cortex), these scaffolds were shown to significantly improve the survival and integration of hESC-derived cortical progenitors as well as reduce secondary degeneration of the host cortex. Grafts, in the presence of IKVAV gels, showed increased functional maturity, as revealed by their electrophysiological profile, and resulted in enhance behavioural recovery, compared to cell grafts alone. While the focus of this study is on the ability to repair the injured brain following focal ischemia, these finding have broader implications for the exploitation of such conduits and stem cells for neural repair following degenerative conditions and other insults.

W03-02

Developing hydrogel scaffolds to promote spinal cord repair

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Stem cells and biomaterials were investigated for their therapeutic potential in spinal cord injury (SCI). We compared human mesenchymal stem cells (MSC) from bone marrow, a conditionally immortalized human stem cell line from fetal spinal cord (SPC-01) and human induced pluripotent stem cell-derived neural precursors (iPS-NPs) in their capacity to migrate towards lesion sites, differentiate and induce better regeneration. Spinal hemisection or balloon-induced compression lesions were used to produce acute and chronic SCIs in rat models. We studied transplanted MSCs, SPC-01 or iPS-NPs labeled in culture with iron-oxide nanoparticles for MRI tracking. Electrophysiology was used to study the properties of stem cell-derived neurons *in vitro*. Animals were tested using motor and sensory tests. We found improved function in SCI animals using all three cell types when transplanted during the first 3 weeks after injury. Various biocompatible hydrogels (degradable and nondegradable), including those based on non-woven nanofibres, have been developed to bridge tissue defects and as 3D stem cell carriers. Chronic SCI animals were implanted with a PHPMA hydrogel seeded *in vitro* with cells. Ca^{2+} imaging on single SPC-01 cells revealed voltage-activated Ca^{2+} channels typically observed in neurons. *In vivo* MRI proved that MSCs, SPC-01 or iPS-NPs migrated into the lesion and survived for several months. Implanted animals showed functional improvements 4–6 months after transplantation; MSCs rarely differentiated into neurons, while implanted SPC-01 and iNP-NPs differentiated into motoneurons. A clinical trial using autologous MSCs in SCI patients has shown improved motor and sensory scores in chronic SCI after implantation of biomaterials seeded with MSC or SPC-01. Hydrogels composed of decellularized porcine extracellular matrix (ECM) and hyaluronic acid, which facilitates constructive remodelling of various tissues, were injected acutely into the spinal cord defect and allowed to gelate *in situ.* Following implantation, we found considerable ingrowth of neurofilaments, astrocytes, new neurones and blood vessels into the biological scaffold. Therefore, modified ECM biological scaffolds are promising candidates for clinical use in chronic SCI.

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W03-03

3D Printing of layered brain-like structures

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Researchers have attempted to study the brain by modelling the architecture using two dimensional (2D) in vitro cell culturing methods. While those platforms attempt to mimic the in vivo environment, they do not truly resemble the three dimensional (3D) microstructure of neuronal tissues. Development of an accurate in vitro model of the brain remains a significant obstacle to our understanding of the functioning of the brain at the tissue or organ level. To address these obstacles, we demonstrate a new method to bioprint 3D brain-like structures consisting of discrete layers of primary neural cells encapsulated in hydrogels. Brain-like structures were constructed using a bio-ink consisting of a novel peptide-

modified biopolymer, combined with primary cortical neurons. Peptide modification of the gellan gum hydrogel was found to have a profound positive effect on primary cell proliferation and network formation. The neural cell viability combined with the support of neural network formation demonstrated the cell supportive nature of the matrix. The facile ability to form discrete cell-containing layers validates the application of this novel printing technique to form complex, layered and viable 3D cell structures. These brain-like structures offer the opportunity to reproduce more accurate 3D in vitro microstructures with applications ranging from cell behavior studies to improving our understanding of brain injuries and neurodegenerative diseases.

W03-04

Understanding self-repairing potential by biological and biomaterial engineering approaches for brain regeneration

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Once neuronal progenitor cells exit the cell cycle during development, their daughter neurons enter the post-mitotic G0 phase for terminal differentiation and lose their proliferative potential. This inability of differentiated neurons to undergo proliferation is one of the major reasons brain tissue cannot regenerate following injury.

The tumor suppressor gene, Rb and its related family members (p107 and p130) lay at the heart of the cell cycle machinery that execute cell cycle exit during development. Although the Rb family members have unique functions, redundancy among these family members is likely to mask their individual functions. Therefore, the careful genetic study such as triple knockout approach is required for elucidating Rb family roles in regulating the cell cycle of neurons.

We recently developed a technique for the conditional inactivation of all Rb family members just after cortical neurons enter the post-mitotic G0 phase, and found that once differentiating cortical neurons enter the post-mitotic G0 phase, they are prevented from undergoing cell division even after the inactivation of all Rb family members. However, Rb family inactivation in cortical progenitor cells leads to differentiating neurons that divide and proliferate, suggesting that differentiating neurons can proliferate under the specific situation. Based on these findings, we also developed a 3D proliferation culture system for differentiating neurons using porous biomaterials enriched in laminin and found that laminin-rich biomaterials can be used for brain regeneration after injury. In this symposium, I will discuss how neurons are protected from proliferation and the possibility for application combining the method of neurobiology and biomaterial engineering.

W03-05

Neurogenesis and vascularization in the damaged brain trough lactate-releasing biomimetic materials

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The CNS has limited capacity for self-repair and physical brain injuries are common causes of disability associated to extensive tissue loss. An ideal therapy requires to support neuronal and vascular growth, activate endogenous progenitors and appropriate brain developmental programs. A potential therapy for repairing the brain after an injury might also take into account that similar cues coordinate the organization of vascular and nervous systems during development.

In the developing brain lactate is a major substrate for oxidative metabolism in addition to being selectively utilized as an anabolic source for cell proliferation and differentiation. Embryonic radial glia serve as neural stem cells and as substrate for neuronal migration. They are also essential in stabilizing the nascent brain vascular network, release lactate and are retained in the adult brain of species that regenerate.

To promote endogenous central nervous system regeneration we have designed biomimetic scaffolds (L-lactate releasing poly lactic acid nanofibers, PLA fibers) that reproduce the 3D organization and supportive function of embryonic radial glia. We have demonstrated that the topology of PLA nanofibers supports neuronal migration while L-lactate acts as an alternative fuel for neurons, and is strictly required for the maintenance of neuronal progenitors. When PLA fibre scaffolds were implanted into brain cavities made in the postnatal brain, they induce robust and functional vascularization of the implant in the fibre orientation and the robust and sustained generation and survival of several types of neurons and glial cells.

Therefore, we hypothesize that by regulating biophysical and energy metabolism parameters it may be possible to reproduce some aspects of the embryonic neurovascular niches in postnatal and even adult individuals, inducing vascularization, gliogenesis, neurogenesis and synaptogenesis. Thus, leading to the functional restoration of CNS tissue lost after a lesion, without the need for exogenous cells or genetic manipulation.

W04 Microtubule-based Therapy for Alzheimer's Disease and other Tauopathies

W04-01

It cuts two ways: role of microtubule-severing proteins in microtubule loss during Alzheimer's disease

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Tauopathies are diseases of the nervous system in which tau becomes abnormally phosphorylated, thus causing it to detach from microtubules and potentially form abnormal filamentous structures. As the disease progresses, there is a steady loss of microtubule mass from the axon. Most evidence suggests that this is due to loss of tau's normal role in affecting axonal microtubules, but the situation is complex as the abnormal tau could also have toxic gain-of-function effects, and there is also a loss of microtubules from dendrites, wherein tau normally does not bind to microtubules. Alzheimer's disease (AD) is an especially complicated tauopathy, as beta amyloid can result in microtubule loss in a tau-dependent manner. While it is not the only aspect of nerve degeneration in these diseases, loss of microtubule mass from the brain is unquestionably one of the most insidious contributors. As such, staving off microtubule loss and even restoring lost microtubule mass could well be among the most effective strategies for improving and prolonging the lives of patients. Microtubule-stabilizing drugs are currently in clinical trials, after having shown promise in pre-clinical models. However, there is concern about the logic of this approach, as some data suggest that it is specifically the labile component of the neuronal microtubule mass that is lost, not the stable component. Therefore, stabilizing what is already stable is less likely to yield therapeutic success than a method that would actually restore labile microtubule mass to the neuron. Fidgetin is a microtubule-severing protein that appears to be specialized for paring back the labile domains of microtubules. Thus experimentally reducing the levels of fidgetin in neurons results in the addition of labile microtubule mass, by enabling growth of the labile domains of microtubules. Data from my research group show that fidgetin inhibition can correct for the loss of labile microtubule mass that occurs when tau is depleted from cultured neurons. We posit that inhibition of fidgetin is a more logical approach than microtubule-stabilizing drugs for treatment of AD and other tauopathies.

W04-02

Tau toxicity and rescue in cell and animal models of tau pathology

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Tau is a neuronal microtubule-associated protein. In Alzheimer Disease (AD), Tau is altered by hyperphosphorylation, mislocalization, aggregation etc. We study the physiological functions of Tau and its role in neurodegeneration in AD and other tauopathies. We are developing cell and animal models to observe the spreading of Tau pathology, the interaction with A-beta, and the effects of aggregation inhibitor compounds as a therapeutic approach. This includes transgenic mice in which Tau is expressed either in a " pro-aggregant" form, or in a non-aggregating form, which can be compared with Tau knockout mice or mice expressing wildtype forms of Tau. Aberrant mislocalization and aggregation of Tau, combined with loss of synapses and microtubules are among the hallmarks of AD. Similar features are observed in mice expressing pro-aggregant Tau, but not with anti-aggregant Tau, illustrating that the propensity for beta-structure is at the root of aggregation and pathology. Microtubules play essential roles in the maintenance of axons and dendrites as tracks for intracellular transport by motor proteins. To elucidate the cascade of events leading to microtubule breakdown we exposed mature wildtype and Tau knockout neurons to A-beta oligomers and analyzed changes in the Tau/microtubule system. Microtubule breakdown occurs in dendrites invaded by Tau and is mediated by spastin, a microtubule-severing enzyme. Spastin in turn is recruited to microtubules modified by polyglutamylation, mediated by translocation of the enzyme Tubulin-Tyrosine-Ligase-Like-6. Photoconversion of Tau labeled with Dendra2 reveals that missorted Tau in dendrites is newly synthesized and not derived from the axon. In absence of Tau (TauKO neurons), microtubules and synapses are resistant to A-beta induced toxicity. The results provide a rationale for microtubule stabilization as a therapeutic approach. - Supported by DZNE (German Center for Neurodegenerative Diseases), Max-Planck-Society, Tau Consortium.

W04-03

Targeting microtubule end binding proteins with ADNP/NAP: from autism and schizophrenia to Alzheimer's disease

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Activity-dependent neuroprotective protein (ADNP) is essential for brain formation and critical to brain function. ADNP interacts with multiple cellular targets including the SWI/SNF chromatin remodeling complex, alternative splicing and translation factors, microtubule end binding proteins (EBs) and regulators of autophagy. ADNP contains an 8 amino acid neuroprotective motif termed NAP (davunetide). In turn, NAP (NAPVSIPQ) contains the SxIP (SIP) sequence that was identified as an EB interacting motif. NAP enhances microtubule plasticity, enlists tau to microtubules (inhibiting tau pathology), increases the number of dendritic spines and provides protection against multiple toxicities associated with neurodegeneration. RNA silencing of EB1 or EB3 abolished NAP protection against zinc intoxication and EB3 silencing abolished the NAP impact of dendritic spines. Importantly, NAP enhances ADNP interaction with EB3 and with the autophagy key regulator, microtubule associated protein 1 light chain 3 (LC3B), providing protection against decreased autophagy. Decreased microtubule and

autophagy function have been associated with neurodegenerative diseases as well as with autism and schizophrenia. ADNP is mutated in autism, deregulated in schizophrenia and the protein content is significantly reduced in Alzheimer's disease patient serum. In Phase 2 clinical trials, NAP (davunetide) protected cognitive scores in patients suffering from amnestic mild cognitive impairment and activities of daily living in schizophrenia patients, paving the path to the development of microtubule-targeting neuroprotective drugs.

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W04-04

The role of cytoskeletal disruption and microtubule stabilization relative to axonal and synaptic pathology following injury and in Alzheimer's Disease

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Tau and microtubule abnormalities have been implicated in the development of neuronal pathology in Alzheimer's disease as well as following traumatic brain injury (TBI) (eg chronic traumatic encephalopathy). We have utilised both *in vitro* and *in vivo* models to study the cytoskeletal changes within axons that are affected by TBI. In both of these conditions, axonopathy is associated with the focal dissolution of microtubules and the accumulation of neurofilaments (NFs), which may precipitate further changes in tau metabolism. We have previously shown that microtubule-stabilizing drugs such as taxol protect neurons from MAP2 loss following injury, inhibits regenerative sprouting following focal axonal damage, and protects axons from fragmentation following excitotoxic injury. We have recently investigated whether a blood-brain-barrier permeable microtubule stabilizing drug, epothilone D, applied systemically to 6-month-old APP/PS1 (APP_{swe}/$PSEN1_{dE9}$) affects Abeta plaque-associated axonal and synaptic pathology. Epothilone treatment was well tolerated by APP/PS1 and wildtype mice. Epothilone treatment did not affect the plaque-associated density of neurofilament-labelled abnormal neurites, but did significantly ($p<0.05$) reduce a different subset of synaptophsyin labeled dystrophic neurites. Epothilone D may, therefore, have potential utility in reducing neuronal pathology that arises from Abeta plaque formation.

W04-05

Novel strategies to target tau and Aβ toxicity

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In Alzheimer's disease (AD) genetic and biochemical data have established a crucial role for the amyloid-β (Aβ) peptide that forms extracellular deposits known as amyloid plaques. A second key player in AD is the microtubule-associated protein Tau that forms intracellular deposits referred to as neurofibrillary tangles. Given that elevated Aβ levels in human AD brains are due to an increased production and reduced clearance of this peptide, recent therapeutic strategies either aim to reduce Aβ production by inhibition of enzymes such as γ- and β-secretase, or to assist in its clearance, pursuing active and, in particular, passive immunisation approaches. Increasingly, Tau is also recognised as an attractive target for therapeutic interventions, although it is lagging behind Aβ therapies. We will present novel approaches of reducing Aβ and tau pathology in mice, by combining therapeutic agents with novel ultrasound-based delivery methods.

Selected references:

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W05 How to Publish a Good Paper? (Quality, Reproducibility and Impact)

W05-01

What's your research story? Data quality and reliability

J. Schulz

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The recent past has seen an increase in reports about scientific fraud, authors' creation of fake user accounts to review their own papers, and a black market for authorship slots on accepted papers especially in China. All these incidences created strong awareness for the need to educate scientists about scholarly publication ethics, alongside with thorough assessment of manuscripts submitted to scientific journals to ensure data quality and adherence to Good Scientific Practice. Journals, e.g. the Journal of Neurochemistry, increasingly enforce reporting of all relevant experimental details including randomization, blinding, statistical analyses and other measures of quality. The overall aim of data reproducibility, reliability and the translation of basic research findings into the clinical practice will be discussed with particular focus on the question of why effects reported in studies do not live up to their promise in clinical trials – and what could be done about it.

W05-02

Get your message to the reviewers

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A good paper conveys its message in a comprehensible way to readers, via editors and reviewers whose duty it is to ensure quality and legibility during the peer review process. Author guidelines serve to achieve this goal, and while they differ from journal to journal, as a common denominator they should provide an overview of what standards the journal adheres to, and what is expected of authors. Since reviewers are the gateway to publication of a manuscript and therefore its widespread availability to the research community, writing a meaningful review is paramount. Various ways towards becoming a reviewer will be outlined, followed by discussion of peer review standards such as the amount and nature of additional experiments requested for acceptance of a manuscript. Everyday examples will be shared of what authors should be attentive to, about the practical screening of manuscripts for fraud (iThenticate, detection of image manipulation), guidelines for image preparation and human, animal or cell culture experimental reporting, including institutional approval of experiments and study subjects' informed consent as appropriate, and other aspects relevant to preparation of a sound manuscript. Further topics will be form and restrictions to references and manuscript length, e.g. inclusion of information into main manuscript vs. supplementary material. Workshop participants are encouraged to share their own experiences and views.

W05-03

The ethics of publishing

S. Murphy

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Preparing and submitting a manuscript for review and potential publication involves a contract between authors, editors, reviewers and the journal's publisher. Just like any contract, there is an assumption of trust in the integrity of everyone involved. As initiator, authors bear the most responsibility and guarantee both accuracy and truthfullness in their reporting. Failures are inevitably uncovered, disrupt the progress of science, and can have irreversible consequences for those concerned.

W05-04

Maximize outreach

P. D'Onghia

John Wiley & Sons, Publishing, Richmond, Australia

In scholarly publishing, there are key things that you as authors can do to create visibility of your work, broaden readership and increase the impact of your research. This session will explain, at the example of the Journal of Neurochemistry, how to optimize your paper for search-engine-findability, making your paper accessible, explaining your work, its importance and putting your research into context, enriching your work through multimedia, and promoting and sharing it through social media and public relationships (PR) to drive readership. Learn how you can contribute from the perspective of an author.

W06 Brain Environment in Motor Neuron Degeneration and Injury

W06-01

Microglial-derived factors as contributors to ALS disease progression

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Amyotrophic lateral sclerosis (ALS) is the most common adult-onset motor neuron disease and evidence from ALS mice expressing SOD1 mutations suggest that neurodegeneration is a non-cell autonomous process where microglial cells influence disease progression. However, microglial-derived neurotoxic factors still remain largely unidentified. Blocking pathways implicated in disease progression could benefit the largest ALS population. Therefore, we have studied two pathways linked to microglial activation the complement component C1q and the glutamate transporter system xc-. C1q is the initiating component of the classic complement system, produced by microglia and that we have showed expressed by motor neurons in ALS mice. We have found robust induction of complement components (C1q, C4, and C3) in spinal cords of ALS mice. To test whether this activation was a mechanistic contributor to disease, we deleted C1q in ALS mice. This led to changes in microglial morphology accompanied by enhanced loss of synaptic densities during disease. Nevertheless, onset and progression of disease were unaffected in C1q- and C3-deleted ALS mice, thus showing that classic or alternative complement pathway activation do not contribute significantly to SOD1 mutant-mediated ALS pathogenesis. With excitotoxicity being a major mechanism proposed to cause motor neuron death in ALS, our hypothesis was that excessive glutamate release by activated microglia through their system xc- (a cystine/glutamate antiporter with the specific subunit xCT) could contribute to neurodegeneration. We showed that xCT expression was enriched in microglia and absent from motor neurons. Activated microglia induced xCT expression and during disease, xCT levels were increased in ALS mice. Expression of xCT was also detectable in post-mortem tissues of ALS patients and correlated with increased inflammation. Genetic deletion of xCT in mice demonstrated that activated microglia released glutamate mainly through system xC-. Interestingly, xCT deletion also led to decreased production of specific microglial pro-inflammatory factors including NO, TNFa and IL6 indicating that xCT regulates microglial functions. In ALS mice, xCT deletion led to a slowed progressive disease phase and more surviving motor neurons, revealing system xc- inactivation, as a potential approach to slow ALS progression after onset.

W06-02

The mitochondrial dynamics after neuronal injury

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The physiological relevance of mitochondrial dynamics in damaged neurons remained to be determined. Multiple neurodegenerative studies about mitochondrial dynamics have been carried out *in vitro*, in which glial cells are missing and the environment could be far different from *in vivo*. Therefore, great controversy among studies regarding the functional significance of mitochondrial dynamics under neuronal stress remains. To address the functional consequences of mitochondrial dynamics under physiological and pathological conditions *in vivo*, we have recently established unique bacterial artificial chromosome transgenic (BAC Tg) mice, in which mitochondria are labeled with GFP and *cre* recombinase is simultaneously expressed in injury specific manner. The BAC Tg mice demonstrated that GFP-labeled shorter mitochondria were actively transported in regenerative motor axons from the earlier stage after injury and replaced pre-existing GFP-negative longer mitochondria to accelerate mitochondrial turnover and to satisfy huge energy demand, suggesting that the activity of mitochondrial fission is increased in response to nerve injury. To address the consequences of mitochondrial fission in damaged neurons, we crossed the BAC Tg mice with the dynamin-related protein 1 (Drp1) floxed mice. Drp1 is responsible for mitochondrial fission and the cross breeding successfully provided specific ablation of mitochondrial fission in injured regenerative motor neurons. The injury-inducible Drp1-deficiency in motor neurons showed tubular and elongated mitochondria at early stage after nerve injury and thereafter abnormally spherical and gigantic mitochondria. The membrane potential and axonal transportation speed of mitochondria seen in injured axons of Drp1-deficient mice became obviously worse. The highly activated microglia was accumulated in the proximity of Drp1-deficiendt injured motor neurons even at the earlier stage when elongated mitochondria still maintain the quality. The deficiency of Drp1 in injured motor neurons eventually lead to neuronal death and axonal degeneration. Collectively, mitochondrial fission could be a defensive response for injured neurons to maintain mitochondrial and neuronal integrity and to adapt to changes of intracellular and extracellular environments.

W06-03

The role of astrocyte-derived TGF-β in motor neuron disease

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Neuroinflammation, which includes both neuroprotective and neurotoxic reactions by activated glial cells and the infiltration of immune cells, is involved in the pathomechanism of neurodegenerative diseases including amyotrophic lateral sclerosis (ALS). Dominant mutations in *SOD1* (Cu/Zn superoxide dismutase) gene are the frequent causes for inherited ALS. Using mutant SOD1 transgenic mice, we and others demonstrated that astrocytes and microglia play the pivotal role in progression of "non-cell autonomous" neurodegeneration.

Among the numerous candidate molecules secreted from glial cells, we focus on the role of astrocyte-derived transforming growth factor-β1 (TGF-β1), the level of which was elevated in the peripheral blood and the cerebrospinal fluid of ALS patients. We demonstrate that astrocyte-specific overproduction of TGF-β1 in $SOD1^{G93A}$ mice accelerates later disease progression in a non-cell autonomous manner with reduced IGF-I and CD11c production in

deactivated microglia, and fewer infiltrated T cells with an IFN-γ dominant milieu. On the other hand, we also show that astrocyte-specific downregulation of mutant SOD1 in $SOD1^{G37R}$ mice slows later disease progression with lower levels of TGF-β1. Moreover, endogenous TGF-β1 levels in $SOD1^{G93A}$ mice negatively correlate with survival time. Furthermore, pharmacological administration of TGF-β signaling inhibitor after disease onset extends survival time of $SOD1^{G93A}$ mice, a finding validating our observations.

These findings indicate that astrocyte-derived TGF-β1 is a determinant of the disease progression of ALS mice by regulating the neuroprotective inflammatory response by microglia and T cells. Furthermore, targeting TGF-β in the cell-type specific manner can be a viable therapeutic candidate in ALS.

W06-04

Role of oligodendroglial dysfunction in amyotrophic lateral sclerosis (ALS)

L. Van Den Bosch

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Oligodendrocytes are well known targets for immune-mediated and infectious diseases. Healthy oligodendrocytes are also crucial for neurons and abnormal functioning of these cellscould be involved in several neurodegenerative diseases. We focused on the contribution of oligodendrocytes and their progenitor cells in the ventral grey matter of the spinal cord in amyotrophic lateral sclerosis (ALS), a neurodegenerative disease caused by the selective death of motor neurons. Degenerative changes in oligodendrocytes were abundantly present in post mortem material from ALS patients and in an ALS mouse model based on mutations in the superoxide dismutase 1 (SOD1) gene. In these mice, morphological changes in grey matter oligodendrocytes were apparent before disease onset and these changes increased as a function of disease. Ultimately, oligodendrocytes died. This loss of oligodendrocytes was compensated by increased proliferation and differentiation of oligodendrocyte precursor cells. However, these newly differentiated oligodendrocytes were dysfunctional as indicated by their reduced myelin basic protein (MBP) and monocarboxylate transporter 1 (MCT-1) expression. We conclude that oligodendroglial dysfunction may be a contributor to motor neuron degeneration in ALS and correcting the failure of oligodendrocytes to maintain MBP and MCT1 expression could be an interesting target for therapeutic intervention in ALS.

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Young Investigator Colloquia
YIC01 Circuitry, Plasticity and Development

YIC01-01

Calcium dependent regulation of actin dynamics in spines

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F-actin, representing the major cytoskeletal components of dendritic spines, is involved in structural plasticity of spines as well as protein and vesicle transport. Changes in spine morphology upon induction of long-term potentiation (LTP) or long-term depression (LTD) are based on changes in actin polymerization. Both LTP and LTD at hippocampal Schaffer collateral-CA1 synapses, require a rise in intracellular Ca^{2+} via activation of NMDARs. Pharmacological inhibition of actin polymerization blocks LTP at CA1 synapses and significantly reduces the number of synaptic AMPAR and NMDAR clusters. But what are the molecular machineries coupling local synaptic elevation of Ca^{2+} to the re-arrangement of actin cytoskeleton? We show that caldendrin, the closest homologue of calmodulin in brain is important for Ca^{2+}-dependent F-actin stabilization. Caldendrin knock-out mice show severe impairment of LTP at CA1 synapses and a decrease in the threshold for LTD induction, while no impairment of pre-synaptic function could be found. Interestingly, the application of the F-actin stabilizing drug jasplakinolide could rescue the LTP phenotye in these mice. Additionally, shRNA knockdown of caldendrin in primary neurons reduced the density and size of dendritic spines and affected dendritic morphology. These effects could also be partially rescued by pharmacological stabilization of F-actin. The mechanisms behind is based on a fact that the N-terminus of caldendrin folds back to the EF-hand domains and is released upon Ca^{2+}-binding. This unmasks its N-terminal proline-rich region which then allows binding to the SH3 domain of cortactin, releasing cortactin from its self-inhibitory state and allowing actin reorganization in spines.

YIC01-02

Regulation of AMPA receptor function by protein ubiquitination

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AMPA receptors (AMPARs) have recently been shown to undergo post-translational ubiquitination in mammalian neurons. However, the underlying molecular mechanisms are poorly understood and remain controversial. Here we report that all four AMPAR subunits (GluA1-4) are rapidly ubiquitinated upon brief application of AMPA or bicuculline in cultured neurons. This process is Ca^{2+}-dependent and requires the activity of L-type voltage-gated Ca^{2+} channels and Ca^{2+}/calmodulin-dependent kinase II. The ubiquitination of all subunits occurs exclusively on AMPARs located on the plasma membrane post-endocytosis. The sites of ubiquitination were mapped to Lys-868 in GluA1 and Lys-870/Lys-882 in GluA2 carboxy-terminals. Mutation of these lysines did not affect basal surface expression or AMPA-induced internalisation of GluA1 and GluA2 subunits. Instead, it reduced the intracellular trafficking of AMPARs to the late endosomes and thus, protein degradation. These data indicate that ubiquitination is an important regulatory signal for controlling AMPAR function, which may be crucial for synaptic plasticity.

YIC01-03

The emerging role of chondroitin sulphate proteoglycans (CSPGS) in controlling plasticity of the central nervous system

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While chondroitin sulphate proteoglycans (CSPGs) have been discovered for more than a century, the significance of CSPGs in the nervous system has only become evident in the last two decades when high concentration of CSPGs was reported in the glial scar, a structure which is highly inhibitory to the central nervous system (CNS) regeneration. Other than being a passive structural component as was first considered, CSPGs have been shown to involve in neuronal path-finding during development, containment of secondary damage and inhibition of axon regeneration after injury, and more recently, on the control of plasticity both during development and regeneration in the CNS. We have demonstrated that enzymatic degradation of chondroitin sulphate (CS) glycosaminoglycan chains on the CSPGs enhances both anatomical and functional regeneration in various spinal cord lesion models. The improved recovery is attributed to both an increase in the direct re-growth of spared neurons together with enhanced plasticity. Plasticity is a process which allows the nervous system to adapt to environmental stimuli before consolidation of its final connections. The degree of plasticity decreases during early development, concurrent with the appearance of a structure called perineuronal nets (PNNs) on the neuronal surface. PNNs are compact supramolecular complex composed of CSPGs, hyaluronan, link proteins and tenascin R. We have recently demonstrated that while the transmembrane hyaluronan synthases act as an anchor for the assembly of this complex matrix on neuronal surface, the presence of link proteins is important in maintaining the compactness of the PNN structure. Transgenic mice lacking link protein show attenuated PNNs and enhance functional recovery after injuries in two plasticity models. The results suggest that selective manipulation of molecules in the PNNs could be a potential strategy in enhancing functional

recovery after CNS injury. This talk is going to take you through the journey of CSPGs in the CNS, with evidence from our work showing the transition of concepts of CSPGS being as a regeneration blocker to a fine control on neuronal plasticity.

YIC01-04

Synaptic MMP controls structural plasticity

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Synapses are particularly prone to dynamic alterations and thus play a major role in neuronal plasticity. The ability to change synaptic connections comprises both alterations at the morphological level and changes in postsynaptic receptor composition. We report the impact of endogenous matrix metalloproteinase (MMP) activity on structural and functional plasticity in primary hippocampal cultures evoked by chemical long-term potentiation (cLTP). We used quantitative morphological analysis and showed that cLTP induces the permanent enlargement of solely subsets of small dendritic spines (i.e., short membranous protrusions that harbour primary sites of excitatory synapses) in an MMP-dependent manner. We used a superresolution microscopy approach and found that spine expansion induced by cLTP was accompanied by MMP-dependent immobilization and synaptic accumulation and the clustering of GluA1-containing AMPA receptors. Altogether, our results reveal novel molecular and cellular mechanisms of synaptic plasticity.

YIC01-05

A New Axis of Neuroprotection: Insights on How PINK1 and PKA Cooperate to Regulate Mitochondrial Function, Mitophagy, and Dendritic Outgrowth

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Neurons rely on a continuous turnover of damaged mitochondria (mitophagy) to provide the necessary healthy mitochondria required to sustain neurites and prevent the release of apoptogenic factors. To this date, little is known with regards the molecular players and signal transduction pathways that regulate mitophagy and dendritic development of neurons during physiological conditions and during oxidative stress. PTEN induced kinase 1 (PINK1) is a neuroprotective serine/threonine (ser/thr) kinase that is localized to the cytosolic and mitochondrial compartments. Protein Kinase A (PKA) is a neuroprotective ser/thr kinase that acts as the central regulator of most cyclic AMP-regulated signal transduction pathways in neurons. Like PINK1, PKA is localized to the cytosol and the mitochondrion to regulate mitochondrial function and neuronal survival. A decline in the activities of these two neuroprotective ser/thr kinases is detrimental to midbrain neurons and contributes to Parkinson's disease (PD) etiology. Over the past five years, recent experimental evidence suggests the existence of a novel neuroprotective signaling axis comprised by both PINK1 and PKA to regulate mitochondrial function, development, and survival of neurons. For the first portion of my talk, I will show experimental evidence that suggests that PINK1 and PKA regulate mitochondrial morphology and function at the outer mitochondrial membrane by suppressing the mitochondrial fission activity of dynamin related protein 1 (Drp1). Interestingly, mitochondrially-localized PKA can reverse mitochondrial dysfunction, oxidative stress, and cell death of PINK1 deficient neurons suggesting that pharmacologically upregulating PKA activity is a therapeutic strategy for reversing mitochondrial dysfunction in PD. For the second portion of my talk, I will highlight new data that shows how both neuroprotective ser/thr kinases cooperate to remodel dendrites in developing neurons. Specifically, we have found that transient expression of PINK1 and PKA promotes dendritic outgrowth by suppressing mitophagy and by increasing the anterograde velocities of mitochondria which leads to enhanced mitochondrial density at the dendrites, a cellular event that is required for supplying the necessary energy for the development of dendritic arbors in neurons. Furthermore, PKA is downstream of PINK1 in this neuroprotective signaling pathway as pharmacologically inhibiting PKA suppresses dendritic outgrowth, promotes mitochondrial fission, and increases mitochondrial turnover in PINK1 transfected neurons. Finally, I will highlight the current conceptual gaps of this neuroprotective signaling pathway that remain to be addressed and will provide a compelling rationale as to how pharmacologically enhancing PKA activity can lead to the development of new neuroprotective therapies for treating PD.

YIC02 Mechanisms of Glial Function, including Inflammation

YIC02-01

Chromatin landscape defined by repressive histone methylation during oligodendrocyte differentiation

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In many cell types, differentiation requires an interplay between extrinsic signals and transcriptional changes mediated by histone modifications. However the selection of the histone marks is time- and cell type-specific. In this study, we used rat oligodendrocyte lineage cells to characterize the genome-wide distribution of two repressive histone marks, H3K9me3 and H3K27me3. Oligodendrocytes derive from glial progenitors, which receive synaptic inputs and are characterized by electrical properties that progressively decrease during differentiation. Using genome-wide chromatin profiling of rat oligodendrocyte progenitors (OPC) and immature oligodendrocytes (iOL), we identified H3K9me3 mark as occurring at later stages of development and associated with the repression of genes related to neuronal lineage and membrane excitability. Consistent with these findings, the levels and activity of H3K9 methyltransferases (H3K9HMT) were more prominently affected by differentiating stimuli than those of H3K27HMTs. Silencing H3K9HMT, but not H3K27HMT, impaired oligodendrocyte differentiation and functionally altered the electrical properties of oligodendrocytes. Co-localization of H3K9me3 and transcription factors SOX10 or YY1, forming repressive complexes with H3K9HMTs, were detected at genes involved in neuronal lineage and excitability. These results identify repressive methylation of K9 lysine residues on histone H3 as critical for the definition of the properties of differentiated oligodendrocytes.

YIC02-02

Neuroinflammation in chronic neuropathologies: the role of the type-1 interferons

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Activation of the innate immune response occurs in both Alzheimer's disease (AD) and Parkinson's disease (PD) with studies suggesting this "neuroinflammation" contributes to the neuronal cell damage. The type-1 interferons (IFNs) are master regulators of the neuroinflammatory process, however their contribution to the progression of these neuropathologies is still largely unknown. We recently demonstrated a key role for the type-1 IFNs in mediating Abeta neurotoxicity *in vitro* and the neuroinflammatory response in the APP/PS1 mouse (Taylor *et al*., 2014). This study investigated the type-1 IFNs in PD using the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model.

C57BL/6J wildtype (WT) and IFNAR1$^{-/-}$ mice (n = 10) were administered MPTP (4 × 15 mg/kg, 2 h intervals) and brains harvested at day-1, -3 and -21 post-MPTP. Alternatively, WT mice were administered MPTP followed by an IFNAR1-blocking monoclonal antibody (MAR1) or isotype control via tail vein injection (1 mg/kg, day-1, -6, -13 post-MPTP). QPCR and western blot analysis confirmed IFNAR1$^{-/-}$ mice display reduced type-1 IFN production and signaling compared to WT mice (n = 6, $p < 0.01$). Significantly, IFNAR1$^{-/-}$ mice display reduced levels of IL-1β, TNF-α and IL-6 as measured by QPCR and reduced microglial activation (Iba-1^{+}cells) in the substantia nigra (SN) at day-3 post-MPTP. Tyrosine Hydroxylase (TH) quantification confirmed attenuated loss of SN neurons in the IFNAR1$^{-/-}$ mice (3907 ± 64 vs. 3079 ± 70, n = 10, $p < 0.001$) at day-21 post-MPTP. Blocking type-1 IFN signaling with MAR1 also resulted in reduced MPTP-induced nigral cell death (3881 ± 193 vs. 3250 ± 113, n = 10, $p < 0.05$) at day-21 with improvements in rotarod latency time (74 ± 4 vs. 66 ± 3, n = 13, $p < 0.05$).

These results confirm type-1 IFNs contribute to the neuroinflammatory response in the MPTP model of PD. Targeting type I IFN signaling may provide a novel target to reduce neuroinflammation and the progression of neuronal cell death in PD and other chronic neuropathologies.

YIC02-03

Mechanisms of STI1 protection of hippocampal neurons from adverse effects of β-amyloid oligomers

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Increased accumulation and oligomerization of β-amyloid initiates neurodegenerative cellular prion protein (PrP^C)-dependent signals in neurons. We have shown previously that PrP^C ligands γ1-laminin and stress-inducible phosphoprotein 1 (STI1) induce PrP^C-dependent neuroprotective signals, mediated by metabotropic glutamate receptors and α7 nicotinic acetylcholine receptor (α7nAChR), respectively. We tested if these two ligands interfere with PrP^C-dependent toxicity of β-amyloid oligomers (AβOs). STI1, but not γ1-laminin, largely reduced the amount of AβOs binding to PrP^C-expressing cells. Moreover, surface plasmon resonance studies

showed that STI1 competed with AβOs binding to PrP with IC_{50} of 70 nM. We tested if this competition could reduce PrP^C-dependent detrimental effects of AβOs on neurons. Addition of STI1 reversed AβO-induced synaptic loss in hippocampal neurons and also the inhibition of long-term potentiation in cultured hippocampal slices. Prolonged incubation with AβOs caused cell death in neurons, and these effects were nullified in the presence of recombinant STI1 in culture media. Endogenous levels of STI1 also influenced susceptibility of neurons to AβO toxicity. 50% reduction in STI1 expression increased neuronal cell death, while neurons overexpressing STI1 were resistant to AβO-mediated toxicity. Of importance, absence of α7nAChR compromised the survival of AβO-treated neurons and blocked the rescue by STI1. Although AβOs were ineffective to cause cell death in PrP^C-null neurons, in double-knockout neurons (α7nAChR/PrP^C) some AβO toxicity was apparent, suggesting that α7nAChRs may regulate neuronal survival *in vitro*. Our results reveal a protective role of STI1 in Alzheimer's disease which involves competition with toxic AβOs for PrP^C binding and α7nAChR-dependent neuroprotection.

YIC02-04

Behavioral transcriptomics: a molecular perspective on experience-dependent plasticity

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In the Citri Lab for Experience-Dependent Plasticity we study how the nervous system encodes experience at the molecular, synaptic and neural circuit levels. A main focus in the lab is to understand how salient experiences are encoded. Thus, we profiled transcriptional dynamics in a number of brain regions, primarily within the reward circuitry, following aversive experiences such as footshock or LiCl injections, as well as rewarding experiences, such as ingestion of palatable foods and the experience of drugs of abuse.

Recently we identified a nearly perfect correlation between robust and specific patterns of gene expression dynamics induced in a number of different brain nuclei and a variety of defined behavioral experiences. The expression dynamics differ between experiences to the extent that the recent behavioral experiences of individual mice can be inferred solely by examining transcriptional dynamics. We believe this approach, which we term "behavioral transcriptomics", provides an exciting new platform for studying experience-dependent plasticity at the molecular level, and an entry point for identifying the specific function of gene products in encoding features of behavioral experiences. Furthermore, information obtained from studying dynamic gene regulation informs us regarding the coding of features of experience, and serves as entry points to investigation of neural circuit plasticity. The function of specific gene products in the development of the response to drugs of abuse, as well as in development of preference for palatable foods is also under investigation in the lab and will be described in the talk.

Additional projects in the lab are focused on identification of potential novel components of the reward circuitry, investigation of the input-output connectivity of these brain regions, and synaptic plasticity within these brain regions following the experience of drugs of abuse.

YIC02-05

Leucine-rich repeat kinase 2 and toll-like receptor inflammatory signaling

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Missense mutations in leucine-rich repeat kinase 2 (LRRK2) are the predominant cause of autosomal-dominant familial Parkinson's disease (PD). The most common pathogenic LRRK2 mutation, G2019S, lies in the activation loop of the protein kinase domain resulting in a constitutive 2–3 fold increase in the kinase activity. As a result, substantial effort has been placed on the development of LRRK2 kinase inhibitors as potential therapeutics for LRRK2-associated PD. Additionally however, PD caused by the G2019S mutation is clinically and pathologically indistinguishable from the more common idiopathic PD. Genome-wide association studies also implicate common variations in LRRK2 with increased risk of PD. Thus, LRRK2 may play a key role in the pathogenesis of both genetic and common sporadic forms of PD.

To facilitate the translation of LRRK2 inhibitors we have developed a pharmacodynamic readout of inhibitor potency, based on the identification of two serine residues, S910 and S935, located prior to the leucine rich repeat domain of LRRK2. These residues are constitutively phosphorylated and are required for the interaction of LRRK2 with members of the 14-3-3 adaptor protein family. *In vitro* or *in vivo* treatment with LRRK2 kinase inhibitors results in a dose-dependent decrease in the phosphorylation of LRRK2. However, we have found that S910 and S935 phosphorylation can also be increased following activation of MyD88-dependent toll-like receptors (TLRs). This has potential consequences for the use of these residues as pharmacodynamic readouts, however, linking LRRK2 to inflammatory TLR signaling is exciting as inflammation has long been implicated in the pathogenesis of PD. Using LRRK2 knockout mice and selective LRRK2 inhibitors we have implicated LRRK2 in the regulation of TLR-stimulated inflammatory cytokine secretion. We have also measured inflammatory cytokines in serum and CSF ($n = 80$ in each group) from control, sporadic PD, LRRK2 G2019S-associated PD and asymptomatic G2019S mutation carriers. Results from these studies may provide insight into the pathogenic role of LRRK2 in PD.

YIC03 Behavior, Addiction and Psychobiology

YIC03-01

Synapse-to-nucleus signaling by CTBP1 regulates neuronal gene expression: implications for restorative plasticity in depression

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Persistent experience-induced changes in brain performance are connected with reconfiguration of neuronal gene expression patterns, which lead to structural and functional alterations of brain circuits. How signals about neuronal activity levels (especially in the axons and presynapses) are converted into changes of gene expression in neuronal nuclei is still poorly understood. In this talk I will present our data pointing to a novel neuronal function of C-terminal binding protein 1 (CtBP1) in the activity-induced presynapse to nucleus signaling, which controls expression of neuronal activity-regulated genes. CtBP1 is a nuclear co-repressor with distinct localization to presynaptic compartment and to nuclei in neurons. We demonstrated that synaptic retention and nuclear abundance of CtBP1 are tightly coupled and regulated by neuronal activity. The nuclear shuttling of CtBP1 requires its active exportin1-mediated nuclear export and critically influences expression of activity-regulated genes. We showed that presynaptic anchoring of CtBP1 is mediated by its direct interaction with active zone proteins Bassoon and Piccolo. This association is regulated by neuronal activity via modulation of the cellular NAD/NADH balance, restrains the availability of CtBP1 for nuclear import and thereby contributes to the regulation of activity-dependent gene expression in neurons. Finally, I will report our unpublished data showing involvement of this novel presynapse-to-nucleus pathway in the restorative plasticity induced by antidepressant ketamine in the cellular model of major depressive disorder.

YIC03-02

Endogenous CB1 allosteric enhancer balances age-related cognitive alterations

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Allosteric binding sites at cannabinoid CB1 receptors were suggested 6 years ago, after experiments with synthetic molecules. Recently, we reported an endogenous molecule (lipoxin A4, LXA4) that enhances endocannabinoid signaling through allosteric modulation of these receptors in the CNS (Pamplona *et al*. PNAS 2012). This molecule is a "first-in-class" and its discovery opens the possibility for future fine-tuning allosteric modulation of endocannabinoid signaling. Interestingly, LXA4 is also an important anti-inflammatory molecule, actually a resolvin, whose levels decrease during the aging process. Hence, we decided to investigate the potential role of LXA4 in a number of endocannabinoid-related CNS functions. Therefore, here we report that endogenous LXA4 affects mouse anxiety-like behavior and cognition in aged animals, and that absence of the LXA4-synthesizing enzyme 5-LO induces cognitive deficits in 5-LO KO mice (Leo *et al*. PLoS One 2013). Moreover, LXA4 protects mice against the cognitive deficit induced by i.c.v. injection of beta-amyloid 1–40 peptide, a hallmark of Alzheimer's Disease. As LXA4 levels decrease after 60+ years in humans, we are now pursuing the concept that LXA4 could be an important biomarker of brain vulnerability to aging-related cognitive deficit and neurodegenerative diseases in humans. This translational approach is going to be conducted over the year of 2014 in samples of over 200 patients with healthy aging, diagnosed with mild cognitive impairment or Alzheimer's Disease. LXA4 will be quantified in CSF and plasma samples, compared across these patient groups and plotted against age and cognitive performance. We expect that LXA4 may represent a novel biomarker to help identify individuals at higher risk of developing mild cognitive impairment and/or Alzheimer's disease over time, allowing pre-emptive clinical intervention.

**The abstract/talk will depict data from two recent articles published in PNAS and PlosONE along with non-published data.*

YIC03-03

Neuronal circuits in central amygdala underlying socially transferred fear

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In its simplest form empathy can be characterized as the capacity to be affected by and/or to share the emotional state of another being (emotional contagion). An animal can socially learn about potentially harmful stimuli either by observing a conspecific in danger or by interacting with a conspecific, which had experienced danger. These two ways of learning presumably involve different neuronal circuits within the amygdala, a brain structure crucial for fear learning and memory. We have compared activation of the amygdala in two rat models of emotional contagion: observational fear learning (direct danger) and social transfer of emotions (indirect danger). We observed different behaviors (passive vs. exploratory, respectively) in the observer rats and the activation of different neuronal circuits. The central amygdala was specifically activated by interaction with a conspecific, which had experienced danger. To elucidate the role of neuronal circuits in the central amygdala of the observers, we used two methods of functional mapping we have developed recently: a combination of retrograde tracing with c-Fos immunohistochemistry and transgenic rats expressing in behaviorally activated neurons a PSD-95:Venus fusion protein and injected with anterograde tracer. We have identified several brain structures in which neurons receiving projections from the central amygdala are activated during social interaction with emotionally aroused partner. All of them are rich in neurotransmitters involved in modulation of attention and learning. We also identified active projections that originate in both cortical, as well as in subcortical structures. To test whether the activated circuits are similar for the socially and non-socially induced emotions, we used double

immunodetection for a PSD-95:Venus construct and endogenous c-Fos. We show that about 70% of neurons is activated by both social interaction with fear conditioned partner and subsequent fear conditioning. These results suggest that there exists a group of neurons in the central amygdala involved in socially induced emotions that is involved in integrating information from external world and internal milieu and modulating function of other brain structures.

YIC03-04

Molecular and functional roles of nucleus accumbens circuits in motivational behaviors

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The nucleus accumbens (NAc) plays a critical role in mediating motivational behaviors. The NAc becomes dysfunctional in neuropsychiatric diseases that affect the motivation system, including drug abuse and depression. The NAc is a heterogeneous structure consisting of multiple intermixed cell subtypes. The main projection neurons in NAc are the medium spiny neurons (MSNs) that are subdivided into two subtypes based on their enrichment of dopamine receptors (D1 vs. D2) and their projections through the brain. Using a combination of optogenetic, pharmacogenetic, and genetic tools we provide new insight into the roles of the two MSN subtypes in drug abuse and depression. We find that altering activity in NAc MSN subtypes, via blue light activation of channelrhodopsin-2 (ChR2) expressing MSNs, results in opposite behavioral responses to cocaine. Similarly we can alter depressive-like outcomes to social defeat stress when manipulating activity in MSN subtypes. Chronic optogenetic activation of ChETA-A (a ChR2 variant) expressing D1-MSNs rescues depression-like outcomes to social defeat stress, while optogenetic activation of ChETA-A expressing D2-MSNs can induce depressive-like behaviors. Conversely chronic inhibition of D1-MSNs using the hM4(Gi) inhibitory designer receptor activated by a designer drug (DREADD) with its ligand clozapine-N-oxide (CNO) induces depression-like behaviors. Coupled with these functional studies we are assessing the molecular role of MSN subtypes in drug abuse and depression. We are examining the early growth response 3 (Egr3) transcriptional pathway in MSN subtypes. Using novel cell-subtype transcriptome profiling tools, we find that Egr3 expression is bi-directionally regulated in MSN subtypes after chronic cocaine: Egr3 is increased in D1-MSNs and decreased in D2-MSNs. In turn Egr3 regulates key cocaine-mediated molecules and these molecules display similar regulation, as Egr3, in MSN subtypes. We have developed novel adeno-associated viruses to over-express or knockdown Egr3 in MSN subtypes. Our results demonstrate that altering Egr3 levels in MSN subtypes leads to differential outcomes in cocaine reward and social defeat stress. Our data demonstrate distinct functional and molecular roles for two heterogeneously intermixed neuron populations in NAc in motivational behaviors.

YIC03-05

Addiction-like synaptic impairments in diet-induced obesity

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Background: There is increasing evidence that the pathological overeating which underlies some forms of obesity is compulsive in nature and therefore contains elements of an addictive disorder. However, direct physiological evidence linking obesity to synaptic plasticity akin to that occurring in addiction is lacking. We sought to establish whether the propensity to diet-induced obesity is associated with addictive-like behavior as well as synaptic impairments in the nucleus accumbens core (NAcore) considered hallmarks of addiction.

Methods: Sprague–Dawley rats were allowed free access to a palatable diet for 8 weeks then separated by weight gain into diet-induced obesity prone (OP) and resistant (OR) subgroups. Access to palatable food was then restricted to daily operant self-administration sessions using fixed (FR1, 3 and 5) and progressive ratio (PR) schedules. Subsequently, NAcore brain slices were prepared and we tested for changes in the ratio between AMPA and NMDA currents (AMPA/NMDA) and the ability to exhibit long-term depression (LTD).

Results: We found that propensity to develop diet-induced obesity is linked to deficits in the ability to induce LTD in the NAcore as well as increased potentiation at these synapses as measured by AMPA/NMDA currents. Consistent with these impairments, we observed addictive-like behavior in OP rats, including (i) heightened motivation for palatable food (ii) excessive intake and (iii) increased compulsive-like food-seeking (as measured by lever-pressing when food was unavailable).

Conclusions: Our results show overlap between the propensity for diet-induced obesity and synaptic changes associated with facets of addictive behavior, supporting partial coincident neurological underpinnings for compulsive overeating and drug addiction.

YIC04 Disease, Neurodegeneration and Therapy

YIC04-01

Metabolic deterioration underlying impaired memory performance in insulin resistance

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While the neurodegeneration process has been largely studied in diabetes, early metabolic modifications associated to such events remain to be elucidated. Aiming at identifying early biomarkers of diabetic encephalopathy, we performed a multimodal magnetic resonance study in the brain of insulin-resistant Goto-Kakizaki (GK) and Wistar rats, including T_2-weighted MRI, ^{1}H MRS and ^{13}C MRS during [1,6-^{13}C]glucose infusion. Compared to controls, GK rats displayed smaller hippocampus, cortex and whole brain, at all ages. In contrast, the ventricular volume in GK rats was larger than controls. Hippocampal and cortical neurochemical profiles were affected by insulin resistance: GK rats displayed significantly higher taurine and ascorbate, and reduced glutamine, choline, phosphorylethanolamine, *myo*-inositol, alanine, and *N*-acetylaspartylglutamate in the hippocampus; and higher taurine, and reduced glutamine, choline, phosphorylethanolamine, and aspartate in the cortex. Hippocampal-dependent spatial memory performance was impaired in GK rats, and correlated to hippocampal concentrations of ascorbate, glutamine and taurine. The ^{13}C enrichment of brain amino acids upon [1,6-^{13}C]glucose infusion revealed that insulin resistance caused mitochondrial oxidation rate to be reduced in neurons but increased in astrocytes. Additionally, both glutamatergic neurotransmission and glutamine synthesis were reduced in the brain of GK rats compared to controls. In conclusion, insulin-resistance impaired brain energy metabolism and reduced glutamate-glutamine cycle between neurons and astrocytes. This led to neurochemical alterations that were associated with the degree of brain dysfunction, namely impaired memory performance in diabetes.

YIC04-02

Rescuing tau isoforms imbalance by RNA reprogramming: towards a novel therapeutic approach for tauopathies

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Tauopathies are neurodegenerative diseases characterized by the presence of intraneuronal aggregates of the protein tau in insoluble neurofibrillary tangles (NFTs). In the normal brain, Tau is a microtubule-associated protein predominantly expressed in neurons, which participates in cellular functions such as microtubule polymerization and stabilization and axonal transport. Human Tau is encoded by the 16-exons *MAPT* gene. Alternative splicing of exons 2, 3 and 10 produces six tau isoforms in the adult brain. Particularly, the alternative splicing of exon 10 (E10) generates tau isoforms with three (3R) or four (4R) microtubule binding repeats. Tau 3R and 4R are expressed in equal amounts in the normal adult human brain. Several tauopathies are associated with mutations in the *MAPT* gene which interfere with E10 alternative splicing, disrupting the normal 3R/4R Tau isoforms balance. Correction of that imbalance might represent a plausible therapeutical approach. RNA reprogramming is a promising strategy to this end, as it does not alter the total amount and distribution of endogenous transcripts. I will summarize our achievements using RNA reprogramming to modulate Tau 4R/3R expression *in vivo*. We used a mouse model of tauopathy, carrying the human *MAPT* gene (htau mice), which displays an excess of Tau 3R over 4R, together with the presence of NFTs and neurodegeneration in the cortex and hippocampus. Concomitant cognitive impairment is also observed in aged htau mice. To restore Tau 3R/4R balance in the adult hTau mouse brain we modulated the inclusion of Tau E10, creating a chimaeric mRNA through a *trans*-splicing reaction between the endogenous tau mRNA and an exogenously delivered RNA *pre-trans*-splicing molecule (PTM). Efficient 3R to 4R isoform conversion was detected after *trans*-splicing. Recovery of behavioral and neurochemical phenotypes was observed in PTM-injected htau mice. Our results highlight the potential of RNA reprogramming to correct tau *mis*-splicing, raising promising perspectives to treat some human tauopathies.

YIC04-03

Long term *in-vivo* modelling of human ES and IPS cells for Parkinson's disease

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The development of induced pluripotent stem cells (iPS) from human patients is revolutionising neurodegenerative research. The capacity to reprogram human cells into stem cells and then differentiate them into the desired neuron type allows the effect of complex combinations of mutations/variants and genetic background on specific neuronal populations to be investigated. The major caveat of using differentiated human stem cells in the study of neurodegenerative disease is that the maturation of the neurons is prolonged. To effectively study disease processes requires non-dividing, fully mature neurons, which can be maintained over long periods of time. Maintaining neurons for such extended periods of time *in-vitro* in a biologically meaningful environment is extremely challenging, and to circumvent this issue attempts to artificially accelerate aging, or expose cultures to external stressors to accelerate the disease process have been applied. The greatest success in maturation and aging of stem cells however has been achieved through transplantation into the living adult brain. Following transplantation, stem cells (foetal, ES and iPS) have been shown to survive, mature and integrate into the adult host system over a period of months. Transplanted neurons survive for prolonged periods of time, extending to years in rodents, and

decades in humans. In order to apply this approach for the long term modelling of Parkinson's disease (PD) we have developed a model to investigate the consequence of α-synuclein transfer on foetal, ES and iPS derived neurons. Healthy neurons transplanted into a diseased host brain accumulate α-synuclein, forming aggregates in 3–6 months and allowing characterisation of the consequence of α–synuclein transfer on neuronal homeostasis and health. The ability to model the role and consequence of disease processes in long term models of PD using foetal, ES and iPS cells will improve our understanding of how the disease progresses, and potentially enable the identification and testing of therapeutics.

YIC04-04

A novel pathway by which 17β-estradiol regulates spatial memory: relevance to schizophrenia

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Approximately 75–80% of schizophrenia patients suffer from cognitive impairments and there is no current treatment that significantly improves this symptom domain. Estrogen has been shown to play a protective role in schizophrenia, and thus 17β-estradiol and selective estrogen receptor modulators (SERMs) have been targeted as a new therapeutic approach. However the mechanism as to how 17β-estradiol and SERMs exert their cognitive enhancing effects in schizophrenia remains largely unknown.

Our laboratory has demonstrated a positive influence of 17β-estradiol on spatial and recognition memory in mice which is absent under BDNF heterozygosity, suggesting that BDNF mediates the cognitive effects of estradiol.

In addition, we recently found that 17β-estradiol regulates the expression of parvalbumin (PV) in GABAergic interneurons of the hippocampus. High-frequency (30–80 Hz) gamma oscillations, which are generated by GABAergic-PV-expressing inhibitory interneurons are thought to be critically involved in higher order brain processes, including memory. Many cognitive disruptions in schizophrenia are associated with abnormal gamma oscillations and PV-expressing interneurons are impaired in schizophrenia, implying causation.

Therefore, we investigated whether 17β-estradiol regulates gamma frequency oscillations. Furthermore, we sought to determine whether the SERM, raloxifene, shows similar beneficial effects as 17β-estradiol but as a safer treatment option. Mice were thus divided into four treatment groups, sham OVX + placebo, OVX + placebo and OVX + 17β-estradiol and OVX + raloxifene and a recording electrode was surgically placed into the CA1 region of the dorsal hippocampus. Ongoing gamma frequency oscillations as well as evoked recordings during learning and memory tasks were analysed. Our preliminary data suggest a role for 17β-estradiol in regulating gamma frequency oscillations during the recall phase of the Y-maze spatial memory task.

Taken together, these studies demonstrate a novel pathway by which 17β-estradiol may regulate cognitive ability and strengthen the case for the use of estrogen-based therapies as adjunctive treatments for schizophrenia.

YIC04-05

Identification and characterization of endogenous LXR ligands in human cerebrospinal fluid; relation to motor neuron disease

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Liver X receptors are ligand-dependent nuclear receptors critical for neuronal development. Cholestenoic acids are formed as intermediates in the metabolism of cholesterol. We evaluated the cholestenoic acid profile of mammalian cerebrospinal fluid (CSF) and determined that specific cholestenoic acids activate Lxrs and increase the number of oculomotor neurons in the developing mouse. While 3β,7α-dihydroxycholest-5-en-26-oic acid (3β,7α-diHCA) promoted motor neuron survival, 3β-hydroxycholest-5-en-26-oic acid (3β-HCA) caused motor neuron cell loss. Mutations in CYP7B1 or CYP27A1, which encode enzymes involved in cholestenoic acid metabolism, result in hereditary spastic paresis type 5 (SPG5) and cerebrotendinous xanthomatosis (CTX). Analysis of CSF from patients with SPG5 revealed an excess of the toxic LXR ligand, 3β-HCA, while patients with CTX and SPG5 exhibited low levels of the survival-promoting LXR ligand 3β,7α-diHCA. Our results indicate that specific cholestenoic acids selectively work on motor neurons to regulate the balance between survival and death.

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Poster Sessions Monday/Tuesday
MTU01 Glia (Part 1)

MTU01-01

Notch signaling is regulated by IGF-1 in astrocytes

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Notch signaling pathway is crucial for cell fate specification and cell proliferation during healthy central nervous system (CNS) development. Notch is also involved in the growth of different cancer cell types, mainly under the regulation of estrogens. Notch receptors, its ligands (Jag, Dlk) and effectors (Hes) are still expressed in the adult CNS, but their roles are not fully understood yet. In recent years, it has been demonstrated that Notch pathway is involved in microglia activation against harmful stimuli.

Estradiol, progesterone and insulin-like growth factor 1 (IGF-1) are endogenous neuro-hormones that exert strong anti-inflammatory properties in the brain, including the control of reactive gliosis. Using an *in vitro* model of reactive astrogliosis induced by lipopolysaccharide (LPS) in primary cultures of cortical or hypothalamic astrocytes, we assessed the regulation of Notch signaling during astrocytic reactivity, and whether the anti-inflammatory properties of estradiol, progesterone or IGF-1 were mediated by the regulation of Notch signaling.

Notch signaling pathway was down-regulated in astrocytes after LPS stimulation by two different mechanisms: repressing Notch-1 transcription (and hence, decreasing the trans-activation of the route) and by inducing the overexpression of Jag-1 (generating cis-inhibition of Notch). These inhibitory effects of LPS on Notch pathway were observed in both cortical and hypothalamic astrocytes.

Under physiological conditions, IGF-1 increased Hes-5 transcription by inducing Notch-1 cleavage, in a MAPK pathway-dependent manner. Besides, IGF-1 completely reverted the LPS-induced downregulation of Notch pathway. However, estrogens and progesterone did not regulate Notch pathway under physiological conditions, and only reverted part of the LPS effects on Notch pathway.

Currently we are studying the involvement of Notch in cytokine synthesis, cell cycle progression and stellation, in order to decipher the exact functionality of Notch pathway during astrogliosis. All together, our findings suggest that Notch signaling pathway plays a role in astrogliosis and that it is regulated by IGF-1.

MTU01-02

Intestinal mucositis induced by 5-fluorouracil results in spinal astrocyte expression changes in rats

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Introduction: 5-Fluorouracil (5-FU; common chemotherapy drug) damages the gastrointestinal tract (intestinal mucositis) and central nervous system (CNS). Astrocytes are vital in neuronal and CNS homeostasis. Increased expression of the astrocyte marker, glial fibrillary acidic protein (GFAP), implies neuroinflammation, linked with neuropathic pain and memory impairment. We determined whether 5-FU induced astrocyte activation via immune-to-CNS signalling pathways; and secondly, if astrocyte reactivity persisted beyond intestinal injury.

Materials and methods: Female Dark Agouti rats (n = 8) were randomly allocated to saline control or 5-FU (i.p. 150 mg/kg) groups and tissues collected at injury peak (day 3) or recovery (day 5). Western Blot analysis of hippocampal and thoracic sections determined GFAP and IL-1β expression. Myeloperoxidase (MPO) assay quantified intestinal inflammation. Statistical comparisons were conducted by a two-way ANOVA with Tukey's *post hoc* test. Data expressed as mean ± SEM with $p < 0.05$ deemed statistically significant.

Results: At injury peak, the bodyweight of 5-FU treated rats was 91% that of controls (p = 0.02) and MPO activity increased by 282% in the jejunum and 213% in the ileum, compared to controls (p = 0.0007 and p = 0.0003, respectively). Interestingly, thoracic GFAP expression was significantly reduced by 28% in 5-FU treated rats at injury peak of mucositis (p = 0.04), yet normalised during the recovery phase ($p > 0.05$). IL-1β remained unchanged.

Conclusions: Down-regulation of thoracic GFAP expression is associated with astrocyte dysregulation in rats with 5-FU-induced intestinal mucositis; suggesting implications for CNS and neuronal homeostasis. Future studies will identify whether 5-FU induced glial changes may contribute to chemotherapy-related cognitive impairment.

Studies in progress: Identify microglial changes in the thoracic region of rats with 5-FU induced intestinal mucositis. Astrocyte and microglial changes will be further analysed utilizing immunohistochemistry and CLARITY staining techniques.

MTU01-03

Expression of glutamate transporter splice variants on oligodendrocytes in rat brain and in mixed glial cultures

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Excitatory amino acid transporters (EAATs)/glutamate transporters play important roles in the brain, including protecting white matter from excitotoxic injury and terminating the excitatory signal by taking up glutamate released in the synaptic cleft and converting it to glutamine for neuronal re-uptake. The expression and function of one of these transporters, GLAST, (EAAT1 in humans), is well established in astrocytes; however, while a small number of publications report GLAST expression by oligodendrocytes, others have not being able to reproduce that finding. One reason for this discrepancy could relate to more recent findings showing the existence of three splice variants of GLAST (GLAST 1a, GLAST 1b and GLAST 1c). The aim of the current study was to investigate whether all splice variants are expressed by oligodendrocytes. Antibodies were generated against the three variants, and immunohistochemical techniques were used to investigate the expression of these splice variants by oligodendrocytes and astrocytes in the brains of adult rats and in mixed glial cell cultures derived from newborn rat pups. In both the mixed glial cell cultures and in the adult brain, all three splice variants were identified in GFAP positive astrocytes, but in contrast, only GLAST 1a and 1c co-localized with PLP in oligodendrocytes. These isoforms both have a deletion of a part of the first extracellular loop of GLAST. Many of the commercially available antibodies specific for GLAST have been developed against the first 100 amino acids of GLAST, which incorporates this extracellular loop, and therefore may not recognize GLAST1a and 1c. This may account for the previously reported discrepancies in GLAST expression by oligodendrocytes. The expression of GLAST1a and 1c may be important for glutamate homeostasis by oligodendrocytes. Further studies into the functionality of these GLAST splice variants in oligodendrocytes are warranted.

MTU01-04

Modulation of spatiotemporal calcium dynamics in single astrocytes by neuronal activity

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Astrocytes produce a complex repertoire of Ca^{2+} events that coordinate their major functions. The prin- ciple of Ca^{2+} events integration in astrocytes, however, is unknown. Here we analyze whole Ca^{2+} events, which were defined as spatiotemporally interconnected transient Ca^{2+} increases. Using such analysis in single hippocampal astrocytes in culture and in slices we found that spreads and durations of Ca^{2+} events follow power law distributions, a fingerprint of scale-free systems. A mathematical model demonstrated that such Ca^{2+} dynamics can arise from intracellular inositol-3-phosphate diffusion. The power law expo- nent (α) was decreased by activation of metabotropic glutamate receptors (mGluRs) either by specific receptor agonist or by low frequency stimulation of glutamatergic fibers in hippocampal slices. Decrease in α indicated an increase in proportion of large Ca^{2+} events. Notably, mGluRs activation did not increase the frequency of whole Ca^{2+} events. This result suggests that neuronal activity does not trigger new Ca^{2+} events in astrocytes (detectable by our methods), but modulates the properties of existing ones. Thus, our results provide a new perspective on how astrocyte responds to neuronal activity by changing its Ca^{2+} dynamics, which might further affect local network by triggering release of gliotransmitters and by modulating local blood flow.

MTU01-05

Gene expression profiling of mouse astrocytes during ageing – advantages and limitations of single-cell approach

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The single-cell RT-qPCR technique brought new dimension to gene expression profiling. In contrast to bulk samples, it enables to identify rare cells or particular subpopulations within the population of studied cells and together with single-cell gene expression correlations leads to better biological interpretations. The aim of this study was to compare RT-qPCR results obtained from single-cells and bulk samples containing thousands of cells, to describe this relationship and characterize advantages and limitations of single-cell approach. For this purpose, we used FACS-sorted cortical astrocytes isolated from 1 to 22 months-old GFAP-EGFP mice. First, we described mutual dependence of these two types of results in a mathematical formulation (sigmoid function) and set the technical criteria for obtaining high quality data. We further analyzed the possibilities of single-cell data normalization according to the reference genes, which is a common practice in bulk but not in single-cell samples. We identified the most suitable reference genes (*ACTB*, *GAPDH* and *PPIA*) and the minimal amount of cells needed for such normalization. In contrast to bulk samples, results obtained from single-cells enable to calculate correlations in expression of particular genes and to perform principal component analysis which identifies potential cell subpopulations. However, the detection limit of single-cell samples does not allow analysis of genes with low expression. Having data from both, single-cell and bulk samples, provided additional information helping us to better understand changes in gene expression which we demonstrate in particular examples of genes expressed in mouse cortical astrocytes during ageing. The conclusion of our study is that the best picture of gene expression is reached in the combination of results obtained from single-cells and bulk samples. Supported by grants GACR 13-02154S and GACR P304-12-G069.

MTU01-06

Life, death, and oxidative stress: fate mapping oligodendrocyte progenitor cells following neurotrauma

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Secondary degeneration following neurotrauma is typically characterised by myelin decompaction and loss. The persistence of decompacted myelin suggests that normal maturation of oligodendrocyte progenitor cells (OPCs) is dysfunctional following injury. Additionally, OPC numbers are chronically depleted in optic nerve vulnerable to secondary degeneration (Payne *et al*., 2013). Oxidative stress is a characteristic feature of secondary degeneration that may be associated with OPC death and interfere with the differentiation of OPCs into myelinating oligodendrocytes, however, evidence in support of this hypothesis is lacking. The aim of our study is to ascertain whether OPCs vulnerable to secondary degeneration undergo oxidative damage, and relate this to their proliferation, maturation into myelinating oligodendrocytes, and/or death. Methods; a 200 μm partial transection was administered to the dorsal aspect of the optic nerve in adult female PVG rats, and the ventral optic nerve vulnerable to secondary degeneration was assessed. Intraperitoneal injection of Ethynyl deoxyuridine (EdU) was used to map the fate of OPCs proliferating within the 3 days following injury. Oxidative stress and death (TUNEL) were immunohistochemically assessed at 3, 7 and 28 days following injury. Results; the number of proliferating OPCs increased at days 3 and 7 following injury compared to controls ($p \leq 0.05$). Despite this, there was a decrease in the total number of OPCs at day 7 following injury but no significant difference in myelinating oligodendrocyte numbers at days 3 and 7 following injury, compared to controls. Preliminary findings indicate an associated increase in lipid peroxidation in OPCs ($p \leq 0.05$), regardless of proliferative state, which was not observed in myelinating oligodendrocytes. Outcomes generated from this study indicate that OPCs have a vulnerability to lipid peroxidation that is not observed in mature oligodendrocytes. In addition, the number of mature oligodendrocytes is maintained following injury.

MTU01-07

Astroglial-mediated remodeling of the interhemispheric midline underlies the formation of the corpus callosum in eutherian mammals

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The corpus callosum forms the primary connection between the cortical hemispheres in the brains of eutherian (or placental) mammals. Congenital absence (or agenesis) of this major fiber tract is a common neurodevelopmental disorder that affects neurological function, yet the etiology underlying this disorder is unknown. We identify that a key process required for midline crossing of callosal axons is the remodeling and degradation of the intervening interhemispheric fissure during development. *In vivo* gain- and loss-of-function experiments in mice show that Fgf signaling to downstream Nfi transcription factors initiates this process by promoting the transition of radial glia into multipolar astroglia. These astroglia then intercalate with one another and degrade the intervening fibroblast tissue to provide a permissive substrate for callosal axon navigation. Neuroimaging studies reveal that defects in this process are a predominant cause of human callosal agenesis. Furthermore, comparative analyses show that remodeling of the interhemispheric fissure does not occur in acallosal mammals, such as marsupials and monotremes, strongly suggesting that this glial-mediated process is associated with the phylogenetic origin of the corpus callosum.

MTU01-08

Methylphenidate regulates glutamate transporters in bergmann glial cells

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Glutamate, the main excitatory amino acid, activates a wide variety of signal transduction cascades through ionotropic and metabotropic receptors. However, recent findings suggest that glutamate transporters (EAATs), responsible for removal of the neurotransmitter from the synaptic cleft, participate in the signaling transduction pathways triggered by this excitatory amino acid. Glutamate transporters are profusely expressed in glia cells, therefore the bulk of glutamate uptake occurs in these cells. Within the cerebellar cortex, Bergmann glial cells enwrap glutamatergic synapses and participate in the recycling of their neurotransmitter through the *so-called* glutamate/glutamine shuttle. It has long been acknowledged that several neurological disorders like autism have deep relationship with the glutamatergic transmission and its regulation both in cortex and cerebellum. Methylphenidate, a drug widely used for the treatment of autism spectrum disorders, has been demonstrated to have an important effect over the glutamatergic neurotransmitter system, albeit its effects in glial cells have not been fully characterized. Using the well-established culture system of chick cerebellar Bergmann glia cells, we characterized the effects of Methylphenidate exposure in the glutamate/glutamine cycle and began the to study the signal pathway involved. We were able to demonstrate that Methylphenidate regulates glutamate uptake, increasing the number of plasma membrane glutamate transporters in a time and dose dependent manner through a mechanism dependent of protein synthesis. These results support the pivotal role of glia cells that surround glutamatergic synapses in mental health.

MTU01-09

6-sialyl-lewisc on n-glycan may be involved in microglial phagocytosis of neuron

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Glycosylation of proteins is one of the major posttranslational modifications. N-glycans harbored on membrane proteins (glycoproteins) profoundly affect the character of proteins by altering their structure or capacity to bind to other molecules. Sialic acid is an acidic monosaccharide present at the non-reducing terminal of sugar residues attached through α2,3-, α2,6- or α2,8-linkage. Sialic acid binding Ig-like lectin (Siglec) can distinguish these linkage types. Some cells interact and communicate through N-glycan-siglec interactions. Some of the siglecs are crucial for exerting brain function. We refined a method to purify and detect trace amounts of N-glycans and identified sialylated N-glycan structure, 6-sialyl-LewisC ([Galβ1,3(NeuAcα2,6)GlcNAc-]), in the mouse brain. To identify a glycoprotein that harbors 6-sialyl-LewisC, membrane proteins of mouse brain were separated by 2D-PAGE. N-glycans were extracted from CBB stained protein spots and analyzed by HPLC. Finally, we detected Calreticulin (CRT) as a candidate for a glycoprotein that harbors 6-sialyl-LewisC. CRT mRNA and protein were mainly detected in neuron using *in situ* hybridization and immunohistochemistry. Furthermore, our results of immunocytochemistry and western blot of subcellular fractionated proteins suggest CRT is accumulated not only in the endoplasmic reticulum but also in synapses. We also tried to identify siglec that recognize 6-sialyl-LewisC by using Surface Plasmon Resonance. Result of this experiment shows Siglec-h might interacts with 6-sialyl-LewisC. Cell surface CRT is known to be involved in the phagocytosis of neuron. Siglec-h is microglia specific siglec. Thus we hypothesized that 6-sialyl-LewisC harbored on CRT on neuron is recognized by siglec-h on microglia and induce microglial phagocytosis, such as synapse pruning.

MTU01-10

Crosstalk between PKC and notch pathways in bergmann glial cells

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Bergmann glial cells (BGC) are a type of specialized radial glia present in the cerebellum. Even though these cells play a major role in glutamate signaling and homeostasis, much of their biology and development remains unexplored. As part of previous studies to describe signaling events in BGCs, our group has observed morphological changes in these cells in response to 12-O-tetradecanoylphorbol-13-acetate (TPA), a diacylglycerol analogue, activator of conventional and novel protein kinase C (PKC) isoforms. In this work we attempted to characterize the phenotype acquired by BGCs in response to long-term treatment with a PKC activator. TPA-treated cells display an increased process elongation and a notable decrease in glutamate uptake activity. Changes in cell morphology were reminiscent of those induced by Notch pathway activation in other studies on BGCs. Therefore, we evaluated activation of this pathway by means of Notch promoter activity, mRNA and protein levels under control or TPA treatment. A significant increase in both promoter activity and nuclear NICD levels was found in treated cells. In order to confirm a TPA-induced Notch activation, two Notch responsive reporter vectors (pRBPJK/NICD and the Hes1 promoter) were tested. The NICD-dependent transcriptional activity increased, as expected in exposed cells. The MAPK pathway, a known activator of the Notch pathway, was evaluated as a possible link between the PKC and Notch pathways. Inhibitors for MEK1 (U0126 and PD-98059) were capable of blocking the NICD-dependent transcriptional activation. Taking into consideration that glutamate is a well-known activator for both PKC and MAPK pathways in BGCs, experiments are currently under way to evaluate the participation of this neurotransmitter as an endogenous inducer of this differentiation program.

MTU01-11

The role of vascular-glial interactions in early diabetic retinopathy

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Introduction: Diabetic retinopathy is a vision threatening complication arising from type 1 and type 2 diabetes. Hyperglycaemia triggers a cascade of vascular and glial alterations that lead to neuronal dysfunction and cell death in the eye. This work details the vascular and glial pathologies early in diabetic retinopathy.

Method: The effect of hyperglycaemia was induced in Dark Agouti rats using streptozotocin (STZ). After 4 weeks of insulin controlled diabetes blood flow kinetics were assessed by sodium fluorescein video angiography. Video sequences were image stabilised and analysed using a custom program that quantified fluorescein flow kinetics for arterioles, capillaries and venules. Blood vessel diameters were measured from fundus images. Microglia (Iba1), astrocyte (GFAP/EAAT4), and pericyte (NG2) association with each vessel type (IB4) was quantified using immunohistochemistry.

Results: In STZ diabetic rats, arterioles showed a pan-retinal reduction in fill and drain rates (fill: -21.9%; 95% CI = −6.8% to −36.9%; drain: −22.8% [−7.3% to −38.0%]). STZ capillaries also filled (−18.1% [−5.2% to −30.9%]) and drained more slowly (−18.2% [−0.59% -to −35.8%]) compared with controls. Venular fill rates were decreased in the inferior (−16.9% [−5.4% to −28.2%]), superior (−13.8% [−1.8% to −26.4%]) and temporal (−15.5% [−1.9% to −31.1%]) quadrants, however the drain rate was only affected temporally (−15.2%; [−1.32% to −29.34%]). There was no alteration in arteriole, or venule diameters. Anatomical data showed variable glial interaction with the retinal vasculature.

Conclusion: Alterations in fluorescein dynamics, which signal abnormal blood flow, as a result of hyperglycaemia primarily affect retinal arteriole and capillary beds. These vessels also show differential interactions with retinal glial cells. Abnormal retinal glial cells and blood vessel interactions are associated with blood flow abnormalities in this early stage of diabetic retinopathy.

MTU01-12

Reversible control of pain by optogenetic stimulation of spinal astrocytes

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Although glial activation and neuroinflammation are closely involved in pathological pain, the precise mechanism of glia-mediated pain modulation is not clear. Here, we investigated whether astrocytes can evoke pain hypersensitivities by using optogenetic technique in the spinal cord for the first time. Adenovirus containing channelrhodopsin-2 (ChR2) under the control of GFAP promoter was injected intrathecally. Blue laser stimulation delivered through the optic fiber in the cannula implanted on the lumbar vertebra produced mechanical and thermal hypersensitivities. Optic stimulation of spinal astrocytes induced allodynia and hyperalgesia in a reversible and time-dependent manner, definitively demonstrating the causal relationship between spinal astrocyte activation and central sensitization. Optically induced pain responses were accompanied by reactive astrocytes and microglia, phosphorylation of MAPKs, enhanced c-Fos immunoreactivity, and upregulation of pro-inflammatory cytokines and chemokines in the lumbar spinal cord ipsilateral side. Moreover, optic stimulation of ChR2-expressing astrocytes in culture recapitulated some of these phenotypes including ATP release. Taken together, our results based on the optogenetic manipulation of astrocytes in the spinal cord suggest that the central sensitization and pathological pain responses are primarily governed by spinal astrocytes. These findings may deepen our understanding of the role of astrocytes in pain pathogenesis and may lead to a novel therapeutic strategy for the management of chronic pain via astrocytic modulation.

MTU01-13

Glial microvesicles transfer MIR-146A to neurons and modulate synaptotagmini translation

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Beyond the classical secretory mechanism through which glial cells influence brain activity, astrocytes and microglia, release circular membrane fragments, the extracellular vesicles (EVs). EVs contain several components of the donor cell (RNAs/proteins/lipids) and transfer their cargo to recipient cells, functioning as an efficient intercellular delivery mechanism. Aim of this study was to investigate whether glial cells may regulate neuronal gene expressions through EV secretion. Using miRNA real-time-PCR panels, we identified a set of miRNAs differentially expressed in EVs produced by pro-inflammatory compared to pro-regenerative microglia. Among them we found a glia-enriched microRNA, the miR-146a, which is altered in brain disorders and targets neuron-specific genes. To investigate possible glia-to-neuron shuttling of miR-146a, we performed a *Renilla*/Luciferase-based assay transfecting hippocampal neurons with a miR-146a-specific sensor, and exposing them to glial-EVs for 24 h. Neuron exposure to glial EVs caused an increase in neuronal miR-146a levels, with a consequent decrease in the immunoreactivity of a validated miR-146a target, the synaptic vesicle protein synaptotagminI. Transfection of donor glial cells with an anti-miR-146a inhibitor or clocking phosphatidyl-serine residues on glial EVs, a determinant for EV recognition on neurons, resulted in unchanged mir-146a concentration in target neurons. Taken together, these data show that glia-derived EVs transfer biologically active mir-146a to neurons, highlighting the capability of glia to modulate neuronal gene expression. To investigate how the transfer of miRNA cargo takes place, we combined optical manipulation with live imaging. EVs positioned on the cell body make a quite stable interaction with neurons, remaining attached to the neuronal surface up to 1 h. Together with confocal analysis of fixed neurons exposed to EVs for different time points, this observation rules out the possibility that EVs undergo rapid internalization or full fusion with cell membrane. Further investigation is ongoing to clarify whether EVs can open a transient pore to transfer their cargo to neurons.

MTU01-14

Oligodendrocyte macrostructure in CNS white matter – elucidating the mechanisms underlying linear array formation

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Linear arrays of oligodendrocytes, which align with the axis of nerve fibre tracts, are a prominent feature of white matter in various species, such as rodents, marmosets and humans. However, the mechanisms responsible for array formation in health and disease are poorly understood.

Aims: We investigated the cellular dynamics underlying the formation of linear arrays in mice both during early postnatal development and in the course of remyelination in the adult.

Methods: C57BL/6 mice of various postnatal ages were submitted to pulse-chase labelling using thymidine analogues. Sections of corpus callosi were labelled with cell-type specific markers to define the cellular composition and differentiation events during linear array formation. To assess the clonal relationship between cells within linear arrays during remyelination, transgenic H253 mice, in which the beta-galactosidase gene is localised to one copy of the X chromosome, were submitted to the cuprizone model of de- and remyelination.

Results: Linear arrays were comprised primarily of oligodendroglial cells at all time-points assessed. Array formation occurred principally after postnatal day 7, reflected by significant labelling of constituent cells by thymidine analogues delivered at this time-point. Cuprizone challenge in adult mice abolished linear arrays but these were largely regenerated within 7 weeks after cuprizone withdrawal. Comparable patterns of cellular X-gal labelling within linear arrays were evident in control and cuprizone-challenged mice at recovery.

Conclusions: The time-course of linear array formation and oligodendroglial differentiation closely mirrors the timing of

myelination within the corpus callosum. Our data suggest a model of linear array formation in which both *in situ* proliferation and random alignment of oligodendroglial cells contribute, a developmental mechanism that is reinstated during remyelination.

MTU01-15

Sim super-resolution microscopy to define the location and relationship of SVCT2 with synaptic proteins in cortical neurons

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We have used spectral confocal microscopy and super-resolution microscopy to analyze cellular differentiation patterns and synaptic connectivity in lentiviral transduced neurons overexpressing the ascorbic acid transporter, SVCT2. Three Dimensional Structured Illumination Microscopy (3D-SIM) projects a structured light pattern onto the sample. The illumination pattern interacts with the fluorescent probes in the sample to generate interference patterns known as moiré fringes. Using this information, super-resolution images with double the lateral and axial resolution are obtained. 3D-SIM techniques work with traditional fluorescent proteins and dyes commonly used in fluorescent imaging. The vitamin C transporter was tagged with YFP and packaged into lentiviral particles that efficiently transduce neurons. Additionally, transporter functionality was evaluated with radiolabeled ^{14}C-L-ascorbic acid. Spectral-confocal microscopy analysis confirmed neuronal arborization induced by SVCT2 overexpression, a typical neuronal maturation phenotype. In a second experimental approach, we analyzed transporter clustering with 3D-SIM in specific areas of the neuronal cell membrane and the induced cellular projections, with a resolution above 120 nm in the XY plane and 250 nm in the Z plane. SVCT2 was organized forming a linear signal in the cell membrane or forming bigger size structures that allow proposing transporter clustering. SVCT2 positive areas were additionally identified with specific markers to dendrites (MAP2), axons (MAP1B) and dendrite spine-like projections that contact presynaptic terminals positive for presynaptic (Piccolo and vGlut) and postsynaptic (PSD-95) markers. 3D-SIM microscopy allowed us, for the first time, to define the distribution of this transporter in different domains of the neuronal cell membrane.

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MTU01-16

Control of neurogenic vs astrogliogenic fate in a restricted spinal cord progenitor domain

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Studies of the last years have unravelled the high heterogeneity in morphology and function of astrocytes in central nervous system. However, there is still an incomplete understanding about the cellular and molecular control of their development. By using mouse genetics, we studied a small group of ventral progenitors of the embryonic spinal cord -called p0-, which expresses the transcription factor Dbx1. After the production of the motor-related V0 Interneurons (V0-IN), this progenitor pool is later committed to generate a group of glial cells that we identify as astrocytes and named as "vA0" (ventral astrocytes from p0 domain). vA0 precursors begin to leave the ventricular zone at E14.5 and follow a stereotyped radial migratory pathway, probably through nuclear translocation, to populate a defined region of the lateral spinal cord. Mosaic fluorescent labelling showed that vA0 population is composed by both protoplasmic and fibrous astrocytes, demonstrating that a single progenitor domain produces astrocyte with different morphological features. We found that Dbx1 controls specification of vA0 astrocytes, as in its absence vA0 is augmented at the expense of V0-INs, which were born earlier. Notch signalling plays key roles in binary fate-cell decisions and glial determination. We evaluated if this pathway modulates early decision between producing V0 neurons and vA0 astrocytes, and whether differential Notch activity could be involved in Dbx1 function. Presenilin1 mutants, that have severely attenuated Notch signalling, exhibited diminished p0-derived glial cells, while V0-INs numbers were increased. Impairing Notch pathway with Ly411575 only at neurogenic stages, but not later, showed similar results. Additionally, gliogenic precursors are increased in Dbx1 mutants and are reduced in Psen1 mutants after the neurogenic stage. This prompted us to analyze key players of Notch signalling pathways in Dbx1 wt and mutant neural tubes before the initiation of gliogenesis. Surprisingly, while wild type p0 domain expresses Delta1 ligand, this domain is converted into Jagged1 positive in the absence of Dbx1. Our results suggest that the type of Notch ligand directed by Dbx1 transcription factor controls the differentiation of p0 precursors by biasing neurogenic vs astrogliogenic fate.

MTU01-17

The importance of astrocytes and the blood-brain barrier for central cardiovascular control

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Background: Astrocytes play a number of important roles within the central nervous system (CNS). Astrocytes make direct connections with both the cerebral vasculature and neurons. Evidence shows that this juxtaposition is key to regulating the blood-brain barrier (BBB) integrity and function. Recent studies have shown that the BBB becomes disrupted in hypertensive rats. This results in circulating peptides such as angiotensin II, which is normally excluded from the CNS, to access brain areas critical for blood pressure control. The association between astrocytes, the BBB and hypertension is still poorly understood.

Aim: To determine the importance of astrocytes in maintaining BBB integrity and central cardiovascular homeostasis.

Methods: Male Sprague-Dawley rats (250–400 g) were anaesthetised (pentobarbitone, 60 mg/kg i.p.), placed into a stereotaxic frame and nanoinjected with sodium fluoroacetate, a specific gliotoxin that ablates astrocytes, into the paraventricular nucleus of the hypothalamus (PVN). Blood pressure and heart rate were continuously recorded throughout. Evan's blue dye (4 mL/kg) was then infused intravenously following microinjections to assess BBB integrity. Changes in baroreceptor reflex function were also studied following fluoroacetate administration.

Results: There was a significant increase in mean arterial pressure (23.1 ± 5.8% increase from baseline, $p < 0.001$) and heart rate (5.0 ± 1.2% increase, $p < 0.001$) 2-h following nanoinjections into the PVN. There was also a significant increase in the mean density of Evan's blue dye within the PVN (17.38 ± 5.8 AU, $p < 0.05$). We also found a significant decrease in the range of the baroreceptor reflex curve (17.47 ± 2.8 bpm, $p < 0.01$).

Conclusion: Our results are the first, to our knowledge, to show a functional link between astrocytes, BBB disruption and central regulation of cardiovascular function. Loss of astrocytes within the PVN led to impaired cardiovascular function. Given these findings, further investigation of BBB function and astrocytes may lead to novel therapeutic strategies to treat cardiovascular diseases.

MTU01-18

The immunomodulatory properties of skin-derived schwann cells: implications for cell transplant therapy in nerve injury

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Previous studies have shown beneficial effects of skin-derived precursor Schwann cell (SKPSC) therapy in PNS injuries, with one of the hypothesized beneficial mechanisms being their regulation of macrophages. In the present study, we investigated the immunomodulatory properties of adult SKPSCs (ASKPSCs) and their complex inflammatory effects in nerve injury. ASKPSCs expressed a battery of cytokines, including INFγ, IL1β and most-abundantly IL6. When in the presence of conditioned media from ASKPSCs, macrophages secreted over 1000 pg/ml of TNFα. Following the transplantation of ASKPSCs into injured nerves, we observed a SKPSC density-dependent increase in macrophages and decrease in myelin debris. To determine the role of IL6, in the context of proinflammation, macrophage cultures were primed with IL6, LPS/INFγ/IL1β or LPS/INFγ/IL1β+IL6. We observed a 301% and 212% increase in the number of $iNOS^+$ proinflammatory macrophages in LPS/INFγ/IL1β+IL6 and LPS/INFγ/IL1β conditions respectively, whereas there was no effect on cultures that received IL6 alone. In contrast to cultured neurons treated with conditioned media from IL6-alone-primed macrophages, neurons treated with conditioned media from LPS/INFγ/IL1β+IL6 and LPS/INFγ/IL1β macrophages were subject to a reduction in axonal outgrowth. Importantly, the combined therapy of anti-IL6 antibody and ASKPSC transplant following sciatic nerve injury resulted in a 2-fold increase in gastrocnemius CMAP amplitudes compared to IgG+ASKPSC treatment. Interestingly, this treatment paradigm did not alter macrophage numbers or debris clearance but instead reduced iNOS expression. Understanding the mechanisms underlying potentially harmful aspects of SKPSC therapy and developing approaches to subdue these responses is an important next-step for the development of Schwann cell therapies.

MTU01-19

GABA transporters are regulated by P2Y1 receptor through a calcium signalling-dependent mechanism in rat cortical astrocytes

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Astrocytes express purinergic (P2) receptors, involved in astrocytic communication through increases in $[Ca^{2+}]_i$. P2Y receptors regulate cytoplasmic Ca^{2+} levels through PLC-PKC pathway (Abbracchio *et al*. Pharmacol Rev. 58:281–341, 2006). Since P2Y receptors operate through PLC activation and GABA transporters (GAT) activity is modulated by the PLC transducing system (Cristóvão-Ferreira *et al*. J Neurochem. 109:336–347, 2009), we hypothesized that ATP-induced Ca^{2+} signalling would influence GABA transport in astrocytes.

To access this possibility we tested the influence of P2Y receptors upon GABA transport into astrocytes. Primary cortical astroglial-enriched cultures express functional P2Y receptors, as evaluated through Ca^{2+} imaging, being $P2Y_1$ the predominant P2Y receptor. ATP (100 μM, 1 min) caused an inhibition of GABA transport through either GAT-1 or GAT-3, by decreasing the V_{max} value. This inhibitory effect was mimicked by a specific agonist for $P2Y_{1,12,13}$ receptor (2-MeSADP, 100 μM). The effect of 2-MeSADP was completely blocked by P2 (PPADS, 30 μM) and $P2Y_1$ selective (MRS2179, 30 μM) receptor antagonists, as well as by PLC inhibitor (U73122, 3 μM). In astrocytes where intracellular calcium has been chelated (BAPTA-AM, 20 μM) or with calcium stores depleted (CPA, 10 μM), 2-MeSADP failed to inhibit GABA transport. Also the inhibition of NCX activity (KB-R7943, 30 μM) blocks $P2Y_1$-mediated GAT inhibition, suggesting that increased NCX activity is the link between GAT inhibition and $P2Y_1$-induced Ca^{2+} increase.

In conclusion, $P2Y_1$ receptors in astrocytes inhibit GABA transport through a mechanism dependent of $P2Y_1$-mediated calcium signalling.

MTU01-20

Astrocytic chondroitin sulfate proteoglycans in brain injury and in glutamate uptake function

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OASIS, (Old Astrocyte Specifically Induced Substance; also referred to as CREB3L1) can play important roles in the endoplasmic reticulum (ER) stress response and ER protein quality control in particular tissues or cells. In the injured brain, OASIS is specifically expressed in reactive astrocytes in the region surrounding an injured area and activates the transcription of target genes such as Bip. We focus on the potential role of OASIS in chondroitin sulfate proteoglycan (CSPG) production in the adult mouse cerebral cortex. CS-C immunoreactivity, which represents chondroitin sulfate moieties, was significantly attenuated in the stab-injured cortices of OASIS-knockout mice compared to those of wild type mice. The levels of chondroitin 6-O-sulfotransferase 1 (C6ST1, one of the major enzymes involved in sulfation of CSPGs) mRNA and protein increased after cortical stab injury of wild type, but not of OASIS-knockout, mice. A C-terminal deletion mutant OASIS overexpressed in rat C6 glioma cells increased C6ST1 transcription by

interacting with the first intron region. These results suggest that OASIS regulates the transcription of C6ST1 and thereby promotes CSPG sulfation in astrocytes. Through these mechanisms, OASIS may modulate axonal regeneration in the injured cerebral cortex.

Chondroitin sulfate chains of tenascinR (TNR), which were recognized by the CS56 antibody, constituted a patchy extracellular matrix in the adult mouse cortex. The TNR-immunoreactive structures uncover a subpopulation of cortical astrocytes and delineate astrocytic territories. In this study, we examined the molecular functions of TNR in cultured astrocytes. Astrocytes can modulate synaptic activity by releasing gliotransmitters and uptaking glutamate. Glutamate uptake activity was decreased and the expression level of GLAST but not GLT-1 was significantly lower in the TNR-knocked-down astrocytes than in the control astrocytes. TNR modulates GLAST expression and is thereby involved in synaptic transmission.

MTU01-21

Analysis of myosin superfamily in mature cultured oligodendrocytes and in cuprizone-treated de- and remyelination model mice

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Myelin is a unique multilamellar structure and crucial for normal neuronal function. In the CNS, oligodendrocytes (OLs) extend several processes and generate myelin around axons. The membrane transport system precisely regulated by motor proteins is required for myelin formation and maintenance. Unconventional myosin Va (Myo5a) has been reported as a motor protein, controlling morphology of OLs and myelination. In the transcriptome database of OLs, another unconventional myosin VI (Myo6) mRNA has been found in O4-positive differentiated OLs. Recently, we revealed that the other unconventional myosin Id (Myo1d) is expressed in mature OLs (Yamazaki, R., *et al.*, 2014) and is required for myelin-like-membrane formation *in vitro*. However, functional relations of these three unconventional myosins expressed in mature OLs are still unclear. In this study, to examine the role of myosin superfamily in myelination by OLs, we performed immunofluorescence staining using culture OLs and cuprizone (CPZ)-tereated de- and remyelination model mice. First, we proved the expression of Myo6 in O4-positive differentiated OLs at protein level by anti-Myo6 antibody. In the comparison of the distributions of three myosins in cultured mature OLs, Myo6-positive signals were detected in MBP-positive myelin-like membrane sheets, Myo5a signals were distributed in main thicker processes, whereas Myo1d was enriched in the leading edge of myelin-like membrane sheets. These results suggest that three myosins may have different roles in mature OLs. In the corpus callosum of non-treated control mice of CPZ experiment, while Myo5a and Myo1d were detected on myelinated fibers, Myo6-positive signals were found in CC1-positive mature oligodendrocyte cell body. In the demyelinated corpus callosum after 5 weeks of CPZ exposure, Myo5a and Myo1d signals were much decreased and Myo6 signals were increased in the activated astrocytes. These changes were fairly recovered to the pretreatment state during remyelination processes. Thus, these myosins may be involved in myelin formation and remyelination by the different ways.

Reference:

Yamazaki, R., *et al.,* Unconventional Myosin ID is Expressed in Myelinating Oligodendrocytes. J Neurosci Res: 92 (10): 1286–1294, 2014.

MTU01-22

HF-RTMS treatment ameliorates acute cuprizone- induced demyelination and behavioral deficits

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Schizophrenia is a complex and severe chronic brain disorder resulting in impaired social function and disruption of the perception of reality. Recent research found that using rTMS as a tool for the treatment of schizophrenia was effective in reducing auditory hallucinations, negative symptoms and working memory problems. The exact mechanism of rTMS remains unclear. Studies have reported abnormalities in the white matter of schizophrenic brains, suggesting the involvement of OL/myelin in the etiopathology of schizophrenia. Indirect evidence suggests rTMS treatment might influence the OL/myelin in brain; no direct research has reported direct effects of rTMS on OL/myelin in schizophrenia. To address this issue, we used animal models of "acute demyelination" and "chronic demyelination" by administration of CPZ. High frequency rTMS (HFrTMS) was used to treat animals after CPZ exposure began. Behavioral tests, histological staining and western blotting were used to evaluate the efficacy of HFrTMS. HFrTMS was found to reverse CPZinduced behavioral alterations in acute but not chronic demyelination. In acute demyelination, HFrTMS alleviated CPZinduced brain demyelination, increased oligodendrocyte progenitor cells in demyelinated sites and decreased astrogliosis in hippocampus. HFrTMS had no effect on the accumulation of activated microglia in demyelinated sites.

MTU02 Gene Regulation and Genetics

MTU02-01

The effect of allopregnanolone infusion on $GABA_A$ receptor subunits mrna expression in the prefrontal cortex of rats

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Allopregnanolone is a neurosteroid capable of producing an antidepressant-like effect in rats. It is believed that this effect is due to its interaction with the $GABA_A$ receptors ($GABA_A$-R), in which allopregnanolone plays a role as a positive modulator. However, allopregnanolone influence on $GABA_A$-R subunits mRNA expression in specific brain regions is still not completely elucidated, as well as its asymmetrical role. In this work, we evaluated the effect of bilateral infusion of three doses of allopregnanolone (low: 1.25 μg/rat; intermediate: 2.5 μg/rat; and high: 5 μg/rat) in the prefrontal cortex of Wistar male rats on the mRNA expression of $GABA_A$-R subunits γ2 and δ in both hemispheres of the same region infused using the real-time quantitative PCR technique (endogenous control genes: β-actin and GAPDH). As results, there was no difference between hemispheres on the δ subunit mRNA expression ($p = 0.971$), but the high dose of allopregnananolone was able to increase its mRNA expression when compared to controls ($p = 0.001$) and the low dose ($p = 0.004$). In the γ2 subunit, the right hemisphere had a higher mRNA expression than the left hemisphere in the controls ($p = 0.007$) and in the low dose ($p = 0.011$). The γ2 mRNA expression was increased only in the left hemisphere by the high dose when compared to controls ($p = 0.013$) and the low dose ($p = 0.007$), while in the right hemisphere there was no significant change. These results indicate that allopregnanolone is capable of increasing the mRNA expression of δ and γ2, the latter being specifically on the left hemisphere, normalizing an asymmetry found in animals with no treatment.

MTU02-02

Positive association of MAOA and VMAT2 with autism spectrum disorder (ASD) in the indian population

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Platelet hyperserotonemia is the most replicated endophenotype of ASD, which is a group of heritable, male predominant and childhood onset behaviorally-defined neurodevelopmental disorder. Accumulating evidences from neurochemical, pharmacological and behavioral studies provide indications of involvement of serotonergic system genes in ASD pathophysiology. Therefore this study was designed to investigate the association of two serotonergic genes, vesicular monoamine transporter (*VMAT2*) and monoamine oxidase A (*MAOA*) genes with ASD in the Indian population. The present study was conducted in 449 subjects including 227 controls and 222 cases using two markers [rs363272 A/G at intron13, rs14240 C/T at 3'UTR] of *VMAT2* and one marker of *MAOA* [rs3027407 A/G at 3'UTR]. Analysis of genotypic distribution revealed that all the three markers conformed to HWE, except for rs14240 in the cases. Case-control association analysis revealed significant male-specific genotypic effect for the *VMAT2* marker, rs14240 ($p = 0.003$) and female-specific allelic effect for the *MAOA* marker, rs3027407 ($p = 0.018$). Low LD strength was noted for the marker pairs of *VMAT2*. Gene interaction studies revealed highest single gene effect by the *MAOA* marker. The interaction pattern in total subjects indicated redundant interaction of the two *VMAT2* markers with *MAOA* marker in ASD. However in the case of females, such a pattern was significantly evident only for the interaction between the markers of *MAOA* and *VMAT2* (rs14240). On the other hand, in the male cases the *VMAT2* markers showed synergestic effect, where combined genetic effect of rs14240 and rs363272 was more than the singular effect in ASD. The results of the present study indicates that markers of *MAOA* and *VMAT2* alone and in combination is associated with ASD in the Indian population and there is a distinct gender-specific influence in the distribution of variant alleles in the disease condition.

MTU02-03

Hyperacute changes in patterns of MRNA expression in the blood of rats over time after middle cerebral artery occlusion

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Introduction: Stroke is one of the leading causes of death in industrialized countries and the major cause of disability. Thrombolysis with recombinant tissue plasminogen activator (rt-PA) given within the first 4.5 h of symptoms onset is the most powerful treatment for acute ischemic stroke (IS). Unfortunately, thrombolysis is disappointingly underused mainly because of diagnosis uncertainty and unknown onset of stroke. Specific acute stroke biomarkers acting as a stroke clock would be extremely valuable in clinical practice and help to increase the number of stroke patients that can beneficiate from thrombolysis.

Methods: Blood samples were collected from 8 male spontaneously hypertensive rats at 0, 1, 2, 3, 6 and 24 h post stroke induction by middle cerebral artery occlusion. RNA was extracted from whole blood stabilized in PAXgene tubes and mRNA expression was detected by oligonucleotide Affymetrix microarray.

Results: Using a pairwise comparison model, 2045 genes were identified to vary significantly over time ($p \leq 10^{-7}$). Some of the

top 20 most changed genes are already known to be relevant to the ischemic stroke physiopathology (e.g. Il-1R, Nos2, Prok2). Cluster analysis showed multiple stereotyped and time dependent profiles of gene expression. Direction and rate of change of expression for some profiles varied dramatically during these 24 h. Profiles with potential clinical utility including hyper acute or acute transient upregulation (with expression peaking from 2 to 6 h after stroke and normalisation by 24 h) were identified. Combining different genes profiles (e.g. Myo1E, Mrga10 and Prok2) will allow the construction of a tool capable of determining the stroke onset time.

Conclusion: Gene expression profiles vary acutely in the blood of rats after stroke and have the potential to allow the construction of a stroke clock and to have an impact on IS treatment decision making.

MTU02-04

Gender specific distribution of thyroid stimulating hormone receptor gene variants in subjects with down syndrome

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Both intellectual disability and thyroid disorder are of frequent occurrence in subjects with Down syndrome (DS). The thyroid gland plays a crucial role in the maintenance of normal growth, brain development, neuronal activity, intelligence, behavioral activities etc. Binding of the thyroid stimulating hormone (TSH) to the TSH receptor (TSHR) activates a series of downstream pathways controlling development and function of the thyroid gland. TSHR is a G-protein coupled seven-transmembrane receptor encoded by the human chromosome 14. To find out the importance of functional *TSHR* gene variants in the etiology of DS, we investigated 12 SNPs in 129 families with DS proband and 170 ethnically matched control individuals. Informed written consent was obtained from the participants and peripheral blood was collected for genomic DNA isolation. Genotyping was done by DNA sequencing. Case-control analysis showed a significant difference in genotype frequency for rs2075178 ($\chi^2 = 8.660$; $p = 0.013$). Stratification based on gender revealed a statistically significant difference for allelic (rs2075178 $\chi^2 = 6.130$; $p = 0.013$ & rs2075179 $\chi^2 = 6.370$; $p = 0.012$) as well as genotypic frequencies (rs2075178 $\chi^2 = 13.700$; $p = 0.001$ & rs2075179 $\chi^2 = 12.100$; $p = 0.002$) for male probands as compared to the male controls. Family-based TDT analysis showed biased paternal transmission of rs2075178 'A' allele (LRS = 3.962; $p = 0.046$) and rs2075179 'T' allele (LRS = 5.062; $p = 0.024$) to the male probands. We may infer that the TSHR may play a vital role in the etiology of DS, especially in the male subjects, which is contributed paternally.

MTU02-05

Utility and challenges of whole-exome sequencing in the hunt for neurodegeneration genes

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Frontotemporal dementia (FTD) is a neurodegenerative disorder that presents with behavioural changes and/or language deficits and shows clinical and pathological overlap with motor neuron disease (MND). 50–70% of FTD cases with a positive family history do not have mutations in known genes, indicating that additional FTD genes remain to be identified. We performed whole-exome sequencing (WES) of 16 FTD probands with a family history of dementia and/or MND and no mutations in known dementia/MND genes and examined possible strategies for prioritising disease variants. Strategy 1 identified genes with likely pathogenic variants in multiple probands ($n = 59$). Strategy 2 prioritised genes in biological pathways relevant to known FTD/MND genes (cell death, lysosome function/autophagy, RNA binding, ubiquitin system: $n = 183$). We performed *in silico* coexpression analysis with 20 known FTD or MND genes in human brain using BrainGEP [Oliver *et al.* (2014) PLoS ONE 9:e102079]. 10/52 Strategy 1 and 78/178 Strategy 2 genes showed significant coexpression with ≥ 1 known FTD/MND gene, including *PPA1*, a phosphate metabolism gene that regulates neurite outgrowth and coexpresses strongly with *SOD1* ($R = 0.748$) and *SQSTM1* ($R = 0.614$). Genic intolerance (GI) scores were also examined to identify genes in our dataset where variation is more likely to influence disease [Petrovski *et al* (2013) PLoS Genet 9:e1003709]. Examination of coexpression patterns and GI scores led to prioritisation of 52 genes from our combined strategies for further analysis. 2/16 patients harboured a missense variant (Pro34Ser) in *CHCHD10*, a mitochondrial gene that has recently been implicated in FTD and MND. Pro34Ser has previously been reported as pathogenic, however our observations (non-segregation with disease, presence in non-demented elderly controls and in a patient with another established pathogenic mutation) indicate that this variant is not causative of disease. In summary, our complementary strategies have identified several highly plausible candidate FTD genes for downstream analysis. We also caution that the apparent absence or low frequency of any putative disease-causing variant in public WES databases should not be solely relied upon to deduce its pathogenicity.

MTU02-06

Gene profiling in different stages of Alzheimer's disease: a genome-wide study

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Alzheimer's Disease (AD), the most common form of age-related dementia, is a progressive, neurodegenerative disease characterized

clinically by a gradual cognitive decline including loss of memory, orientation and reasoning and pathologically characterized by neurofibrillary tangles and amyloid plaques in the brain. There is currently no simple definite test to diagnose AD. Hence there is a considerable interest in reliable early detection. Despite recent progress in genetic research, gene testing is currently not sufficiently reliable for most individuals and has limited utility for predictive purposes. We here examined gene expression profiles and microRNA patterns using the Affymetrix Human Transcriptome array 2.0 in saliva in patients across several stages of AD (mild, moderate and severe) compared to a healthy control group matched for gender and age. AD patients (with diagnosis established according to NINCDS-ADRDA criteria) were classified into mild (n = 8), moderate (n = 7) and severe (n = 7) stages of AD dependent on their clinical profiles and cognitive performance. The control group (n = 19) consisted of individuals with no family history of AD. Bioinformatics analysis revealed several pathways significantly expressed in AD patients compared to their controls (GeneGo software), including the transcription and chromatin pathways (p = 0.03). We are currently confirming these differential gene expression profiling's for histones, histone deacetylases and acetyl transferases by qPCR.

This is the first study examining a complete genetic profile specific at each stage of AD in saliva samples using a whole genome expression array. This work will allow identification of the disease mechanisms, as yet not clearly understood, and the novel genes associated with disease progression, which could be potentially used as novel biomarkers. Altogether, this study may lead to the establishment of specific diagnosis profiles and to new avenues of therapy for this devastating disease.

MTU02-07

Role of GNAS imprinted gene in neurodevelopment, sleep and cognition

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Recent evidence has pointed out that imprinted genes, which exert fundamental roles in embryonic development, are also important in the regulation of sleep and cognitive processes in adulthood. Moreover, sleep abnormalities and cognitive defects are hallmarks of a number of neurodevelopmental disorders (e.g., autism, Down syndrome, attention deficit disorder, Prader-Willi syndrome (PWS) and Angelmann syndrome (AS)), suggesting that neurodevelopment is pivotal for the formation of sleep and for cognition. However, the link between early developmental mechanisms and sleep-wake regulatory processes remains largely unknown. Recently, our group has demonstrated that loss of imprinting in the *GNAS* locus dramatically affects sleep physiology and hippocampus-dependent cognitive functions in mice. *GNAS* main product, Gαs, is an ubiquitously expressed G protein α-subunit, which is essential for the action of many hormones, neurotransmitters and autocrine/paracrine factors, by stimulating the generation of intracellular cAMP, a second messenger indispensable to memory consolidation during sleep. In order to shed light on the precise function of Gαs in neural development and sleep formation, we tested the functional role of Gαs expression during hippocampus development. To this aim, we knocked down Gαs hippocampal levels in mice during development by 3-electrode intrauterine electroporation. Our results indicate that Gαs is involved in pyramidal neurons morphology and migration during neurodevelopment. Currently, we are in the process of analyzing whether the effects of Gαs on neurodevelopment are related to Gαs function over sleep physiology, circadian rhythms and cognition in adult mice. This work will provide valuable new insights into the link between genomic imprinting, neurodevelopment and sleep.

MTU02-08

Serotonin transporter gene (SLC6A4) has modulatory role on the expression of autistic phenotypes

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As platelet hyperserotonemia is observed as a heritable biomarker for autism spectrum disorders (ASD) in a subset of patients, who respond effectively to selective serotonin reuptake inhibitors, serotonergic dysfunction is postulated as a risk factor for the disorder. Maintenance of serotonin level in the platelets and the synapses is performed by serotonin transporter, which is coded by *SLC6A4* gene. It is a quantitative trait locus for blood 5-HT levels and hence considered as potential susceptibility gene for ASD. Inconsistent results on association studies in *SLC6A4* made it difficult to accurately identify genetic variants conferring ASD susceptibility. Therefore, this study investigates association of *SLC6A4* markers with ASD phenotypes and platelet serotonin content. Study involves analysis of six polymorphisms in 478 subjects comprising of 225 cases and 253 controls from West Bengal. Platelet 5-HT content in autistic probands was significantly higher than controls, where C/C genotype of rs6354 was correlated with low 5-HT content. Positive association of *SLC6A4* markers, rs6354, rs4583306, rs140701 and rs7499014 and its various biomarker haplotypes with ASD was also observed. C and T alleles of rs6354 and rs4583306 respectively was significantly over-represented in the mild to moderate autistic group than in the severe group. C/C genotype of rs6354 is significantly associated with low severity of specific behavioural attributes such as activity level and adaptation to change, which are responsible for emotional reactivity and social communication respectively. Bioinformatic analysis shows rs6354 as exonic splicing Enhancer/Silencer site, where A allele causes deletion of target sites for CCAAT/enhancer-binding protein alpha (C/EBPα) belonging to C/EBP family, which are enriched in neurons playing pivotal roles in transcriptional programs underlying complex brain functions, learning and memory. Overall results suggest that *SLC6A4* is likely to be associated with the display of various autistic phenotypes.

MTU02-09

The role of mirnas in the regulation of α-synuclein expression

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Chronic alcohol abuse results in alterations in gene expression in brain regions susceptible to the neurotoxic effects of alcohol. α-Synuclein exists in a number of different splice-variants and its expression is influenced by genetic factors and microRNAs. Here we investigate the influence of ethanol on the expression of the variants in human alcoholic brain and an ethanol exposure cell-culture model. We also examine miRNA-mediated regulation of the variants and the influence of genetic variation on α-synuclein expression. Real-time PCR was used to measure α-synuclein splice-variant mRNA (*SNCA*-140, *SNCA*-112 and *SNCA*-115) levels. Comparisons were made between chronic and chronic-intermittent treatment with and without a withdrawal period to determine if the variants are differentially expressed in response to ethanol. Two transfection methods were used to investigate the miRNA-mediated regulation of α-synuclein. Several genetic variants were genotyped in a Caucasian population consisting of 125 controls and 115 alcoholics. A significant decrease in expression levels of the *SNCA*-140 and *SNCA*-112 variants was observed in the prefrontal cortex of cirrhotic alcoholics compared with controls ($p < 0.001$, $n = 12$; $p = 0.048$, $n = 12$). An increase in expression of the *SNCA*-115 variant was seen in cirrhotic alcoholics compared to controls ($p = 0.008$, $n = 12$). Similarly, results show that the expression of *SNCA*-140 and *SNCA*-112 was down-regulated following chronic ethanol exposure in HEK293T cells with ($p < 0.001$, $n = 16$) and without withdrawal ($p < 0.001$, $n = 8$), whereas the expression of *SNCA*-115 was up-regulated ($p < 0.001$, $n = 16$ and $p < 0.001$, $n = 8$). These findings suggest that ethanol may alter the expression of the α-synuclein variants differently. MiRNAs may regulate the expression of these variants and could be responsible for many of the gene expression changes that occur in the brain of chronic alcoholics.

MTU02-10

Identification and neural circuit analysis of SPEXIN neuropeptides in zebrafish

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Neuropeptide is a small protein-like molecule that plays an important role in physiological functions such as sleep, learning, memory, food intake and regulation of body temperature. In addition, abnormality of neuropeptides could contribute to brain dysfunctions. Spexin (SPX) is a recently identified novel neuropeptide which has the functions in reproduction, feeding control, cardiovascular, renal function and nociception. In this study, To further investigate the physiological function of SPX *in vivo* in zebrafish, we first identified expression of spx and their putative receptor, galanin receptor2 (galr2), in the developing and adult zebrafish brain using whole-mount *in situ* RNA hybridization and RT-PCR. In addition, we generated and analyzed reporter transgenic zebrafish which express fluorescent protein under the control of spx and galr2 promoter, to reveal the SPX-GALR2 neural network *in vivo*. Zebrafish has two spexin genes (spx1 and spx2) and their putative receptor, galanin receptor type II genes (galr2a and galr2b) which are very similar to other species. To analyze the *in vivo* functions of spx, we generated GAL4/UAS mediated inducible transgenic and spx1/spx2/galr2a/galr2b gene knockout zebrafish. These model will be useful for the analysis of *in vivo* functions of SPX-GALR2 system.

MTU02-11

Genome-wide association study identifies the SLC2A14 gene on chromosome 12P13 as a trans-acting locus for methylation of MAPT gene

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Aberrant DNA methylation of the microtubule-associated protein tau (*MAPT*) gene has been associated with increased risk of the tauopathy, progressive supranuclear palsy and the movement disorder, Parkinson's disease. We performed a genome-wide association study (GWAS) of 1032 leukocyte samples from two cognitively healthy cohorts to identify trans-acting loci capable of influencing *MAPT* methylation. We confirmed the cis-acting effect of the *MAPT* H1/H2 haplotype on *MAPT* promoter methylation (minimum $p = 1.215 \times 10–46$ for rs147908125). We identified a second genome-wide significant peak (minimum $p = 1.845 \times 10\text{-}8$ for rs12312185) at the Solute Carrier Family 2 Member 14 (SLC2A14) gene on chromosome 12p13. The polymorphism rs10845990 ($p = 1.942 \times 10\text{-}8$), a quantitative trait locus for Alzheimer's disease pathology, is in absolute linkage disequilibrium with rs12312185. We replicated the effect of rs10845990 on *MAPT* methylation in three out of four additional cohorts ($p < 0.05$ after adjustment for multiple testing) derived from leukocyte and brain tissue samples. The rs10845990-G allele was associated with increased *MAPT* methylation. Overexpression of *SLC2A14 in vitro* led to an increase in *MAPT* expression with concomitant decrease in

MAPT methylation. Overlap analyses of genes from top-associated polymorphisms of this study and published GWASs confirmed a significant enrichment of trans-acting loci identified in this study that are also likely to have a role in tau-mediated neurodegeneration and regulation of DNA methylation in brain tissue. This study highlights a potentially novel mechanism for the regulation of *MAPT* methylation levels across multiple tissue types.

MTU02-12

Experience-dependent regulation of PIRNA activity in the adult mouse hippocampus

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Piwi-interacting RNAs (piRNAs) are a unique class of small regulatory RNAs, which modulate gene expression via RNA interference and other epigenetic mechanisms. The primary role of the piRNA pathway is to maintain control of transposon expression during spermatogenesis in mammals, and more broadly throughout germ cell and stem cell lineages in other species. However, despite an increasing number of studies demonstrating their expression beyond gametes, relatively little is known about their function in the mammalian brain. Using small RNA sequencing on samples derived from adult mice, we have identified a number of piRNAs that are expressed in the hippocampus. Importantly, several of these piRNAs are responsive to behavioral experience. Additionally, we have also detected low-level expression of the Piwi-like proteins Piwil1 and Piwil2, the latter of which exhibits activity-dependent regulation in cultured mouse neurons and is also responsive in the adult mouse hippocampus following behavioral training. These preliminary findings suggest a potential role for the piRNA pathway in regulating experience dependent neuronal transposon activity and gene expression in the adult brain. We are currently performing experiments using adenovirus-mediated knockdown of Piwil1 and Piwil2 in the mouse hippocampus to evaluate the role of the piRNA pathway in learning and memory. Further characterisation of this pathway may uncover new insights into activity-dependent gene regulation in the mammalian brain.

MTU02-13

Expression of 14-3-3 transcript isoforms in response to ethanol exposure and their regulation by miRNAs

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The 14-3-3 proteins are a family of highly conserved molecular chaperones with over 200 known binding partners. Through these protein-protein interactions, 14-3-3s are involved in the regulation of metabolism, stress response, protein trafficking, cell-cycle control, signal transduction, transcription, apoptosis and neurotransmission. 14-3-3 proteins have also been implicated in the pathophysiology of neurodegenerative disorders including Alzheimer disease and Parkinson disease. Recent studies have also shown that14-3-3s are differentially expressed in the frontal cortex of human alcoholics suggesting a potential role in the pathophysiology of alcohol misuse. Here we measured the expression of 14-3-3 transcripts in HEK293T cells in response to chronic ethanol treatment. Five of the seven transcripts were significantly down-regulated following chronic exposure to ethanol for a 5 day period (14-3-3β: $F_{4,\ 47} = 44.01$, $p < 0.001$; 14-3-3γ: $F_{4,\ 47} = 61.04$, $p < 0.001$; 14-3-3ζ: $F_{4,\ 47} = 61.11$, $p < 0.001$; 14-3-3ε: $F_{4,\ 47} = 370.49$; $p < 0.001$; 14-3-3θ: $F_{4,47} = 46.02$; $p < 0.001$) with these changes persisting even after withdrawal from ethanol treatment. One transcript, 14-3-3σ, was significantly up-regulated following chronic ethanol exposure ($F_{4,\ 47} = 7.54$: $p < 0.005$) and 14-3-3η showed no differences in expression in the same treatment model. The pattern of expression changes is similar to those seen in the frontal cortex of human alcoholics. To investigate the role of miRNAs in mediating the expression changes we measured the expression of the 14-3-3 transcripts following transfection with miR-203, miR-144 and miR-7 mimics. Although these miRNAs had predicted target sites in the 3'untranslated region of each 14-3-3 isoform, only miR-203 resulted in a down-regulation of 14-3-3θ transcript. This suggests that the change in expression of the 14-3-3 transcripts following ethanol exposure does not occur via a miRNA-mediated mechanism.

MTU02-14

Dysregulation of SRY in the male brain: a genetic basis for sex-biased neurological disorders

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Sex differences in the dopamine (DA) pathway are likely to underlie male susceptibility to neurological disorders such as Parkinson's disease (PD) and Attention Deficit Hyperactivity Disorder (ADHD). Aside from sex hormones, emerging evidence has revealed that sex-chromosome genes also contribute to the sex differences in the DA pathway. The Y-chromosome gene, *SRY (Sex determining region Y)*, is an ideal candidate to study, as it is expressed in male DA neurons, and regulates DA biosynthesis in males. Our aim was to determine the regulators and down-stream targets of *SRY* in male DA neurons *in vitro* and *in vivo*. Using the human male dopaminergic cell line, M17, time-course studies revealed that *SRY* mRNA expression was significantly increased at 1 h, 6 h and 12 h following DA treatment. The increase in *SRY* expression at 6 h and 12 h was associated with significant increases in *GADD45γ* (a marker of DNA damage) mRNA expression, whilst there were no significant changes in *GADD45γ* mRNA expression at 1 h post DA treatment. Similarly, nigral *SRY* and *GADD45γ* expression was significantly increased at 7 days following injection with the DA toxins 6-hydroxydopamine or rotenone in male rats, indicating that *SRY* is regulated by either a physiological DA receptor-dependent mechanism or a pathophysiological *GADD45γ*-dependent mechanism in male DA cells. Reducing *SRY* expression in M17 cells and in the substantia nigra of male rats, reduced expression of DA machinery genes, such as *TH, dopa decarboxylase*, *monoamine oxidase A*, and *dopamine D2 receptor*, suggesting that *SRY* positively regulates the nigrostriatal DA pathway in healthy

males. In conclusion, our results indicate a positive feedback mechanism between *SRY* and the DA system in males, and disruption of this regulation may underlie the male-bias in disorders, such as PD and ADHD.

MTU02-15

A pilot study on the eastern indian ADHD probands to explore role of CDK 5 in the disease etiology

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Background: Attention Deficit Hyperactivity Disorder (ADHD), a common childhood onset neurobehavioral disorder, is speculated to be caused due to dysfunctions in different neurotransmitters. Apart from the major features of inattention and hyperactivity/impulsivity, ADHD probands frequently suffer from co-morbid Learning Difficulty (LD) & Mood Disorder (MD). Cyclin Dependent Kinase 5 (CDK5) is a master protein controlling neural development, synaptic transmission, post-receptor modulation and synaptic clearance and thus may have a role to play in ADHD.

Purpose: We investigated the role of three CDK5 functional polymorphisms in the etiology of ADHD, with and without co-morbid LD & MD.

Method: Recruitment of families with ADHD probands and ethnically matched controls was performed based on the DSM-IV criteria. Peripheral blood was collected after obtaining informed written consent for participation. Genomic DNA isolated was used for PCR based amplification followed by either restriction fragment length polymorphism or DNA sequence analysis of target sites. Data obtained was analyzed for association with the disorder by population- as well as family-based methods.

Result: Statistically significant association of genotypes harbouring rs2069456 "C" allele was found to be associated with ADHD+LD, while rs2069459 "G" allele showed association with ADHD+MD. Significant over transmission of rs2069456 "A" allele was also noticed.

Conclusion: The present study for the first time indicated association of CDK5 gene variants with the disorder, especially ADHD+MD and ADHD+LD in the studied population.

MTU02-16

Dissociable roles of GADD45A/B in the rat perirhinal cortex and hippocampus for object memory: different forms of dna methylation?

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DNA methylation and demethylation are necessary for long-term memory in various brain regions, including the hippocampus (HPC); however, the role of these epigenetic mechanisms in perirhinal cortex (PRh), a structure critical for object memory, has not been characterized. Using siRNAs, we have assessed the effects of selective DNA methyltransferase (DNMT) and growth arrest and DNA damage-inducible 45 (GADD45) inhibition in the HPC and PRh on object-in-place memory; this task requires the HPC to processes the spatial location of objects and PRh to process object identity. We also measured learning-induced changes in DNMT and GADD45 mRNA expression following OiP learning. Our results demonstrate a double dissociation between the necessity of *de novo* (DNMT3a, DNMT3b) and maintenance (DNMT1) methyltransferases in the HPC and PRh, respectively. Specifically, DNMT3a and DNMT3b mRNA levels were up-regulated in the dentate gyrus (DG) of the HPC following learning, and only DNMT3a siRNA impaired long-term object-in-place memory; conversely, in PRh, DNMT1 was up-regulated following learning, and only DNMT1 siRNA impaired long-term memory. The involvement of GADD45a and GADD45b was also found to be dissociable in the HPC and PRh, as GADD45b was up-regulated in the DG following learning and only GADD45b siRNA impaired long-term OiP memory; in contrast, expression of GADD45a and GADD45b mRNA was enhanced in PRh following learning, and only GADD45a siRNA impaired long-term memory. Collectively, these findings indicate that different forms of DNA methylation, and possibly DNA demethylation, are required for different mnemonic processes (spatial vs object identification) in different brain regions. We are currently investigating the possibility that the role of DNMT1 in PRh is to re-methylate following DNA demethylation-induced DNA damage and repair, and that this is modulated by GADD45a.

MTU02-17

Monoamine oxidase B (MAOB) gene polymorphisms modulate symptom severity of autism spectrum disorder and platelet serotonin level

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Autism spectrum disorders (ASDs) are a group of behaviorally defined neurodevelopmental disorders. With the finding of elevated blood serotonin (5-HT) level in ASD children, disturbances in serotonergic neurotransmission have been suggested as biological substrates of ASD. Level of 5-HT is maintained by the concerted action of various serotonergic system genes. Based on this, *MAOB* encoding MAOB enzyme, an outer mitochondrial membrane protein responsible for the oxidative deamination of 5-HT, is selected as a potential susceptibility gene for this disorder. Additionally, the gender bias in ASD also projects it as a candidate because it is located in the X-chromosome. In the present study, we focus on the association of *MAOB* with ASD and the platelet serotonin level using population based approach. Eight markers were genotyped for 439 subjects including 203 cases and 236 controls. Case-control analysis revealed that rs2283728 (C/T at intron 12; p: 0.007), rs2283727 (C/A at intron 12; p: 0.011) and rs3027440 (T/C at 3'UTR; p: 0.007) showed biased distribution of alleles among the cases and controls, of which rs2283727 displayed female-specific effect. LD analysis indicated decay of LD at rs1799836 and the HAPMAP database on YRI population reveals a recombination spot

at rs3027442, which is only 357 bp from the rs1799836. Haplotype analysis using bimarker haplotypes also demonstrated biased distribution with gender-specific differential distribution. Quantitative trait analysis revealed correlation of A allele of rs1799836 and T allele of rs6324 with increased platelet serotonin level and severity of ASD as per CARS score respectively. Results of the present study suggest positive association of the gene with ASD and with severity of the behavioral phenotype by modulating the platelet serotonin level.

MTU02-18

Exploring the role of histone demethylase, UTX, in mediating sex differences in fear-related learning and memory

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It is well established the there are significant sex differences in physiology and behaviour. For example, we have recently shown that female mice are resistant to fear extinction (Baker-Andresen *et al.*, 2013), which may be function of prolonged activity in the prelimbic prefrontal cortex (PL), an area of the brain that is known to mediate the expression of fear (Fenton *et al*., 2014). Sexually dimorphic behaviours are mainly attributed to the action of steroid hormones (Becker *et al*., 2005). However, not all sex differences can be attributed to sex-specific hormones (Arnold *et al*., 2004). Non-hormonal sources may include X-linked escape from inactivation genes such as the epigenetic modifier, Utx, where it is most highly expressed in the female brain. Given the important role of histone modification and DNA methylation in learning and memory, we hypothesized that sexually dimorphic Utx expression, and its effect on the epigenetic regulation of gene expression, may be a critical mechanism driving sex differences in the expression of learned fear. We are currently testing this hypothesis by infusing Utx overexpression and knockdown constructs into the PL of male and female mice and examining its influence on the acquisition and extinction of cued fear. These findings will provide important new insight by providing an epigenetic basis for sex differences in behavioural adaptation.

MTU02-19

Key role for microRNA-223 in optic nerve regeneration in zebrafish

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Zebrafish have the ability to regenerate damaged parts of their central nervous system and regain functionality. However, the molecular and cellular mechanisms involved remain unclear. Here we test the hypothesis that gene regulatory mechanisms, specifically microRNAs, are instrumental in orchestrating regenerative responses in zebrafish. We used microarrays to analyse and integrate the mRNA and microRNA (miR) expression profiles of zebrafish retina after optic nerve crush to identify potential regulatory mechanisms that underpin central nerve regeneration. We used bioinformatic analysis to identify candidate miRs and selected miR-223 for further analysis. We validated predicted gene targets of miR-223 using RT-qPCR and luciferase assays. We also injected miR-223 inhibitor (i-miR), scrambled control, or vehicle into the zebrafish eye immediately following an optic nerve crush and assessed the impact on retinal ganglion cell axon regeneration. Expression of miR-223 and gene targets was analysed post injury and the amount of RGC axon regeneration (distance and density) determined immunohistochemically at three time points (1, 2, 7 days post-injury; $n = 3$ per time point). qPCR revealed the miR-223 inhibitor to be target-specific. Injection of the inhibitor reduced the distance reached by regenerating RGC axons at 7 days post crush. The density of RGC axons in the optic tract was also reduced compared to controls. Our results identified a key role for miR-223 in zebrafish regeneration, providing a framework for future studies in which to investigate not only the cellular processes required for CNS regeneration, but also how these mechanisms might be regulated to promote successful repair and return of function in the injured mammalian brain.

MTU02-20

"Genotype-first" approaches on a curious case of idiopathic progressive cognitive decline

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Background: In developing countries, many cases with rare neurological diseases remain undiagnosed due to limited diagnostic experience. We encountered a case in China where two siblings both began to develop idiopathic progressive cognitive decline starting from age six, and were suspected to have an undiagnosed neurological disease.

Methods: Initial clinical assessments included review of medical history, comprehensive physical examination, genetic testing for metabolic diseases, blood tests and brain imaging. We performed exome sequencing with Agilent SureSelect exon capture and Illumina HiSeq2000 platform, followed by variant annotation and selection of rare, shared mutations that fit a recessive model of inheritance. To assess functional impacts of candidate variants,

we performed extensive biochemical tests in blood and urine, and examined their possible roles by protein structure modeling.

Results: Exome sequencing identified NAGLU as the most likely candidate gene with compound heterozygous mutations (chr17:40695717C > T and chr17:40693129A > G in hg19 coordinate), which were documented to be pathogenic. Sanger sequencing confirmed the recessive patterns of inheritance, leading to a genetic diagnosis of Sanfilippo syndrome (mucopolysaccharidosis IIIB). Biochemical tests confirmed the complete loss of activity of alpha-N-acetylglucosaminidase (encoded by NAGLU) in blood, as well as significantly elevated dermatan sulfate and heparan sulfate in urine. Structure modeling revealed the mechanism on how the two variants affect protein structural stability.

Conclusions: Successful diagnosis of a rare genetic disorder with an atypical phenotypic presentation confirmed that such "genotype-first" approaches can particularly succeed in areas of the world with insufficient medical genetics expertise and with cost-prohibitive in-depth phenotyping.

MTU02-21

Regulation and role of MIR-34 family in neuronal differentiation and apoptosis

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MicroRNAs (miRNAs) are small regulatory RNA molecules, which control protein synthesis by binding to 3'-UTR region in sequence specific manner. By carrying out global miRNA profiling of nerve growth factor (NGF) differentiated PC12 cells, we have identified dramatic up-regulation in miR-34 family during formation of mature neurons from PC12 cells. However, over-expression of miR-34 did not induced differentiation in PC12 cells. We further studied the role of miR-34 family in differentiating PC12 cells and found its direct role in inducing apoptosis. Over-expression of miR-34 induced apoptosis in PC12 cells. Exposure of cypermethrin, a neurotoxic pesticide also induced apoptosis and expression of miR-34 miRNAs in PC12 cells. Interestingly, we have found role of p53 in induction of miR-34 genes. When PC12 cells were exposed with CP-31398 (a p53 stabliser), expression of miR-34a increased dramatically. *In silico* studies have shown that promoter region of miR-34a have sites for p53 binding. In conclusion our studies have shown that miR-34 family regulates apoptosis in differentiating PC12 cells.

MTU02-22

Monoamine oxidase a gene (MAOA) polymorphisms reveal male-specific effect on specific ASD phenotypes

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Autism spectrum disorders are childhood onset neurodevelopmental disorders, which are characterized by impairments in social communication and behavior. Pharmacological, biochemical, neuroanatomical and behavioral studies suggest that serotonin modulates behavioral phenotypes. Abnormalities in serotoninergic function is thought to play a major role in ASD pathophysiology. Monoamine oxidases, encoded by two X-chromosomal genes *MAOA* and *MAOB* regulate the serotonergic function by the degradation of serotonin. Some genetic and knockout studies have revealed positive evidences for involvement of *MAOA* with ASD. Based on these information in the present study, *MAOA* is selected as a potential susceptibility gene for ASD. Therefore the objective of this study is to investigate association of *MAOA* with autism using population-based case-control approach. Genetic correlation of *MAOA* markers with the severity of specific behavioral traits as scored by CARS (Childhood Autism Rating Scale) has been examined using quantitative trait (QT) analysis. Genotyping analysis was performed for eight markers in 437 subjects (227 controls & 210 ASD) by PCR followed by RFLP, electrophoresis and/or DNA sequencing. A significant overrepresentation of 'T' allele of rs6323 was observed in ASD, which was significant only in male cases (Total subjects: p-value: 0.002; males: p-value: 0.016). Bimarker haplotypes involving rs6323 only showed this effect and was even more significant. Interestingly, QT analysis for 30 bp VNTR showed a similar male specific association with the severity of two behavioral attributes such as relation to people ($p = 0.027$) and adaption to change ($p = 0.008$). Significant genetic effect of bimarker haplotypes involving VNTR polymorphism was observed for two additional phenotypes, nonverbal communication and general impression. Results of this phenotype-genotype correlation study indicates that *MAOA* displays gender-specific genetic effect in ASD, very specifically in specific behavioral phenotypes.

MTU02-23

Genetic knockdown of GADD45G inhibits fear memory

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Fear learning is an evolutionarily conserved behavioural adaptation that is essential for survival. However, the same neural mechanisms that promote this beneficial response to threat can also lead both to pathological memories of adverse events, and to the development of post-traumatic stress disorder (PTSD) and phobia. Dynamic epigenetic mechanisms, including DNA modification, regulate animal behavior and memory. Recent discoveries aim to the growth arrest and DNA damage inducible 45 (Gadd45) family in activity-dependent demethylation in the adult brain; however, its role in learning and memory has yet to be fully elucidated. There are three members in the Gadd45 family: Gadd45a, Gadd45b and Gadd45 g. Previous studies focused on Gadd45b and showed that Gadd45b is involved in contextual fear memory in hippocampus. Here we found cued fear memory-associated changes in the expression of Gadd45b, not Gadd45a and Gadd45 g in the medial prefrontal cortex. We directly injected shRNA lenti-virus into the medial prefrontal cortex (mPFC) and found that only knockdown of Gadd45 g, but not Gadd45a and Gadd45b, led to significant impairments in fear memory. Therefore, Gadd45 g may be a novel therapeutic target for the cognitive deficits associated with many neurological, and psychiatric disorders.

MTU02-24

Activity-dependent RNA methylation in learning and memory

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Methylation of adenosine residue or N^6-methyladenosine (m^6A) is the most prevalent internal modification on eukaryotic RNA. m^6A is catalysed by an RNA methyltransferase complex and is reversed by the m^6A demethylating enzyme, such as FTO. In brain, the level of m^6A is developmentally upregulated and peaks in adulthood, suggestive of its significant roles in adult brain function and plasticity. To date, the function of m^6A in the mammalian brain is unknown.

Using an antibody-based m^6A capture technique followed by high throughput RNA sequencing (MeRIP-seq), we showed for the first time that m^6A transcriptomic landscape was dynamically regulated in the mouse prefrontal cortex following behavioural training. A general increase in m^6A stoichiometry at specific mRNA loci was induced by novel context exposure and more intensely, by associative fear conditioning paradigm. The distribution of m^6A along mRNA was highly enriched around the stop codon. Neuronal depolarization resulted in similar upregulation of locus-specific m^6A. In probing the functional impact of m^6A on RNA, we promoted demethylation through FTO overexpression. This led to increased stability of m^6A target mRNA.

Finally, the impact of modulating m^6A on learning was tested *in vivo*. Since increase in m^6A stoichiometry manifested following context- and fear conditioning, we assessed the impact of pre-amplifying m^6A levels on learning and memory through FTO knockdown. Basal anxiety levels were not affected in FTO knockdown mice; however, cued fear memory performance was significantly enhanced 24 h post-fear conditioning training suggesting increased memory consolidation in this mice. Taken together, these findings provide the first demonstration of m^6A role in learning and memory.

MTU02-25

Discrimination of genetic determinants contributing to fear responsiveness and fear memory

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It is becoming clear that genetic variation plays a major role in the way we respond to stressful and fearful stimuli, and how we remember traumatic events. However, few genes have actually been identified that contribute to variations in these traits. Two mouse strains, C57Bl/6J (B6) and DBA/2J (D2), show variation in stress responsiveness and fear memory and we have used both conventional genetic analysis of these strains and congenic strains to fine map the loci for stress responsiveness. We have identified two closely linked loci on chromosome 12, *Str1* and *Str2*, linked to stress responsiveness. Each of these loci have been mapped to a very small region containing less than 20 genes. Analysis of the genes within the *Str1* locus reveals two possible candidate genes which show significant differences in coding sequence between the high and low stress strains. In addition, the *Str2* interval contains only 2 genes of which only one, *Mdga2,* is expressed in the mouse brain. This makes *Mdga2* a prime candidate for involvement in stress responsiveness. We have also found significant differences in fear learning between B6, D2, Str1 and Str2 mice, indicating that these regions are not only involved in stress responsiveness but also in the storage of fear memories. We have thus gained insight into the multigenic determinants that underlie differences in stress responsiveness and fear memory.

MTU03 Neuroinflammation

MTU03-01

Harnessing the immunomodulation milieu in the quest for neuroprotection in stroke: a systems biology approach

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A perplexing problem for neuro-restoration is the role of immune-dynamic/inflammatory milieu, and it spatiotemporal change across the centrifugal gradient along the umbra-penumbra-oedema tissue zones. Using a systems biology methodology, we aim to explore the dose-time orchestration of therapeutic agents to marshal a favourable outcome. Utilising experimental immune-fluorescence recordings of cerebral stroke, as time elapses, we obtain a quantitative relationship between the relevant parameters: (i) inflammation (microglial activation: $ED1^+$ cells) vis-à-vis (ii) neurogenesis ($BrdU/NeuN^+$ cells), at 0.25, 1.0, and 4.0 week time-horizon. After linearization of the double-log map, we obtain a Michaelis-Menten relationship therein. Then, with the help of immune-histochemical observation under the action of pharmacologiacal agents, we analyse the neurogenesis enhancement of anti-inflammatory immune-modulative drug, minocycline, whence a Hill relationship results, with maximum neurogenesis occurring with 45 mg/kg/day minocycline for 1 week, whilst the full neurogenesis duration persists biphasically till 4 months. We formulate the quantitative dynamics of microglial activation vis-à-vis neurogenesis response. Based on immune-histochemical findings, we delineate the possibility of optimizing the therapeutic enhancement of neurogenesis. We apply Chopp-Ruth's rodent-to-human extrapolation principle of comparative pharmacology, whereby the iso-effect dose in humans would be 400 mg/day minocycline for 6 months. This dose is found to be tolerated toxicologically. Based on this finding, the critical shortcomings of current stroke trials is evident. The customary, clinical trials for stroke have been using 200 mg/day minocycline for 5 days, and the results have been much ambiguous. Thus the conventional immune-modulative trials for stroke have used substantially lower pharmacological dose/duration than is necessary, and this elucidates why such stroke trials are unsuccessful in phase-III. In contrast, the high dosages that we have inferred (400 mg/day for 6 months) as are well used for tissue-remodelling/cytogenesis, in pulmonary and dermatological conditions clinically, without toxicity. Paraphrasing, immunomodulation in stroke is crucial, but needs quantitatively-optimized protracted substantial dosing of minocycline.

MTU03-02

Regulation of the inflammatory cell response after traumatic brain injury by suppressor of cytokine signalling 2 (SOCS2)

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Suppressor of cytokine signalling 2 (SOCS2), a negative regulator of the JAK-STAT pathway, is expressed at the highest levels in the hippocampal CA3 region and dentate gyrus in the adult brain. Our lab previously showed that SOCS2 overexpressing (SOCS2Tg) mice have increased survival of newly born adult hippocampal neurons. We hypothesised that SOCS2Tg mice may also display enhanced newborn neuron survival following traumatic brain injury (TBI) compared to wildtype mice. Therefore, SOCS2Tg mice and littermate wildtype controls were subjected to a mild or moderate controlled cortical impact (CCI) or sham surgery. Mice were pulsed with EdU beginning immediately after injury once per day for 7 days to label cells proliferating in response to the injury. Motor function of moderately injured mice was tested at 2, 7 and 33 days post-injury. All brains were collected 35 days post injury to examine newborn neuron survival, glial and inflammatory cell responses. Newborn $EdU^+/NeuN^+$ neurons were identified in injured animals of both genotypes following moderate but not mild CCI, suggesting that unlike the hippocampus SOCS2 does not regulate adult SVZ neurogenesis. SOCS2Tg mice showed functional improvement at 7 days compared to wildtype mice, with a smaller lesion size. Further, there was a two fold increase in the total number of EdU^+ cells in the injured cortex of SOCS2Tg animals compared to wildtype. $CD11b^+$ microglia/macrophages were the major contributors to this increase. $EdU^+/Olig2^+$ cells were also present and their number was significantly, but not differentially, increased post-TBI in both genotypes. These results suggest a role for SOCS2 in modulating the inflammatory cell response after TBI. Further studies are underway to gain insight into wether SOCS2 overexpression is skewing the inflammatory response towards an anti-inflammatory direction, as suggested by the tissue sparing and functional improvement.

MTU03-03

Antagonism of pacap or microglia function worsens the cardiovascular consequences of kainic acid induced seizures in rats

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Seizures are accompanied with cardiovascular changes which are a major cause of sudden unexpected death in epilepsy (SUDEP). Seizures activate inflammatory responses in the cardiovascular nuclei of the medulla oblongata and increases neuronal excitability. Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide with autocrine and paracrine neuroprotective properties. Microglia are key players in inflammatory responses in the CNS. We sought to determine if PACAP and microglia mitigate the adverse effects of seizure on cardiovascular function in a rat model of temporal lobe epilepsy. Kainic acid (KA)-induced seizures increased splanchnic sympathetic nerve activity (SNA) by 97%, accompanied by increase in heart rate (HR) but not blood pressure

(BP). Intrathecal infusion of the PACAP antagonist, PACAP(6-38) or the microglia antagonists, minocycline and doxycycline, augmented sympathetic responses to KA-induced seizures. PACAP(6-38) caused a 161% increase while minocycline and doxycycline caused a 225% and 215% increase, respectively. In intrathecal PACAP antagonist treated rats, both BP and HR increased, whereas following treatment with microglial antagonists, only BP, was significantly increased compared to control. Our findings support the idea that PACAP and its action on microglia at the level of the spinal cord, elicit cardioprotective effects during seizure. However intrathecal PACAP did not show additive effects suggesting that the agonist effect was at maximum. The protective effect of microglia may occur by adoption of a M2 phenotype and expression of factors such as TGF-β and IL-10 that promote neuronal quiescence. In summary, therapeutic interventions targeting PACAP and microglia could be a promising strategy for preventing SUDEP.

MTU03-04

Reduction in brain hemispheric swelling in TREM2-deficient mice following traumatic brain injury

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Annually, 1.7 million people report traumatic brain injuries (TBI) in the United States. The neurological consequences of TBI are diverse, but include an increased risk for Alzheimer's disease (AD). Microglia are brain-specific macrophages whose primary function is to continually monitor the cerebral microenvironment. They are distinguished from other macrophage populations by their 20-fold higher levels of the Triggering Receptor Expressed on Myeloid Cells-2 (TREM2).Following TBI, microglial expression of TREM2 increases ~10-fold.Three observations highlight the importance of TREM2-dependent microglial functions. (i) Humans completely lacking a functional TREM2 develop early-onset cognitive dementia. (ii) Humans carrying a heterozygous mutation in the ligand-binding pocket of TREM2 have a 3-fold higher risk of developing AD. (iii) TREM2 knock-out (KO) mice have fewer excitatory [AO1] neurons in the hippocampus. We hypothesized that mice lacking the anti-inflammatory TREM2 receptor would suffer from increased hemispheric swelling following TBI due to increased inflammatory response. In contrast, we found that TREM2-deficienct mice exhibit a 90% reduction in injuried hemispheric swelling 7 days following TBI, compared to WT mice. To determine the role of astrogliosis and microgliosis in the response to TBI we quantified immunoreactive astrocytes and microglia in brain tissues 7 days post-TBI from WT and TREM2KO mice. The area encompassed by astrogliosis within the injuried hemisphere of TREM2KO mice was decreased 20%[AO2] compared to WT littermates[AO3]. In aggregate with our previous findings, our data suggest that a preconditioned, pro-inflammatory state[AO4] will reduce some of the deleterious clinical outcomes associated with TBI. Strikingly, these data suggest that the role of TREM2 as a strictly anti-inflammatory receptor needs to be re-evaluated, acknowledging its role in neuroprotective functions following TBI.

MTU03-05

Astrocytic ECGF1/TP and VEGF-a drive blood-brain barrier opening in inflammatory CNS lesions

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In inflammatory CNS conditions such as multiple sclerosis (MS), breakdown of the blood-brain barrier (BBB) is a key event in lesion pathogenesis, predisposing to edema, excitotoxicity, and ingress of plasma proteins and inflammatory cells. Recently, we showed that reactive astrocytes drive BBB opening, via production of vascular endothelial growth factor-A (VEGF-A). Here, we now identify endothelial cell growth factor-1/thymidine phosphorylase (ECGF1/TP) as a second key astrocyte-derived permeability factor, which interacts with VEGF-A to induce BBB disruption. The two are co-induced NFκB-dependently in human astrocytes by the cytokine interleukin-1β (IL-1β), and inactivation of *Vegf-a in vivo* potentiates Ecgf1/Tp induction. In human CNS microvascular endothelial cells (CMVEC), VEGF-A and the ECGF1/TP product DDR cooperatively repress tight junction proteins, driving permeability. Notably, this response represents part of a wider pattern of endothelial plasticity: DDR and VEGF-A produce transcriptional programs encompassing angiogenic and permeability genes, and together regulate a third unique cohort. Functionally, each promotes proliferation and viability, and they cooperatively drive motility and angiogenesis. Importantly, introduction of either into mouse cortex promotes BBB breakdown, and together they induce severe barrier disruption. In the MS model EAE, Ecgf1/Tp and Vegf-a co-localize to reactive astrocytes, and correlate with BBB permeability. Critically, blockade of either reduces neurologic deficit, BBB disruption and pathology, and inhibiting both in combination enhances tissue preservation. Suggesting importance in human disease, Ecgf1/Tp and Vegf-a both localize to reactive astrocytes in MS lesion samples. Collectively, these data identify ECGF1/TP as an astrocyte-derived permeability factor, and suggest ECGF1/TP and VEGF-A together promote BBB breakdown.

MTU03-06

Deletion of IFNAR1 in APPSWE/PS1ΔE9 mice results in increased cognitive function and changed microglial phenotype

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Introduction: Neuroinflammation has been implicated in Alzheimer's disease (AD) pathology. Type-1 interferons (IFNs) are master regulators of the innate immune response; their role in AD progression is unclear. We have previously demonstrated that type-1 IFN production/signalling is upregulated in human AD brains and $APP_{SWE}/PS1_{\Delta E9}$ mice, and that primary cultured $IFNAR1^{-/-}$

neurons are protected from Aβ1-42 toxicity (Taylor *et al.*, 2014). To characterise the role of type-1 IFNs in progression of AD we generated 9-month old $APP_{SWE}/PS1_{\Delta E9}$ x $IFNAR1^{-/-}$ mice, lacking IFNAR1 and the resultant type-1 IFN signalling, and assessed the severity of disease.

Methods and results: Spatial learning and memory deficits were rescued in $APP_{SWE}/PS1_{\Delta E9}$ x $IFNAR1^{-/-}$ mice as assessed by the Morris water maze, showing reduced escape latency and path length and greater trial success compared to $APP_{SWE}/PS1_{\Delta E9}$ mice ($n = 14$–18, $p < 0.05$). Immunohistochemistry of serially sectioned $APP_{SWE}/PS1_{\Delta E9}$ x $IFNAR1^{-/-}$ mice revealed little change in amyloid plaque load, but western blot densitometry revealed a decrease in soluble monomeric Aβ compared to $APP_{SWE}/PS1_{\Delta E9}$ mice ($n = 6$, $p < 0.05$). QPCR identified attenuated IFNα, IRF7 and TNFα expression in $APP_{SWE}/PS1_{\Delta E9}$ x $IFNAR1^{-/-}$ mice and western blot confirmed down-regulated Stat-3 phosphorylation compared to $APP_{SWE}/PS1_{\Delta E9}$ mice ($n = 4$–6, $p < 0.05$). Interestingly, immunohistochemistry $APP_{SWE}/PS1_{\Delta E9}$ x $IFNAR1^{-/-}$ mice revealed enhanced cortical astrogliosis (GFAP) surrounding Aβ deposition; microgliosis (IBA-1), in these same mice, was attenuated, not ablated ($n = 9$, $p < 0.05$). Microglia can polarise to an M1 (neurodegenerative) or an M2 (neuroprotective) phenotype in response to inflammatory stimuli. QPCR identified elevations in M1 markers iNOS, CD11b, CD32 and CD33 in $APP_{SWE}/PS1_{\Delta E9}$ mice and in comparison M2 markers YM1, ARG1 and TREM2 were up-regulated in $APP_{SWE}/PS1_{\Delta E9}$ x $IFNAR1^{-/-}$ mice ($n = 6$, $p < 0.05$).

Conclusions: These results confirm that type-1 IFNs contribute to the inflammatory pathology of $APP_{SWE}/PS1_{\Delta E9}$. Removing type-1 IFN signalling attenuates pro-inflammatory cytokine secretion, alters cortical gliosis, triggers a neuro-protective M2-like microglial polarisation and rescues cognitive deficits.

MTU03-08

An enriched environment reduces inflammation, increases BDNF and modifies microRNA levels in the hypothalamus of obese mice

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The hypothalamus is the structure that regulates energy balance. Recent studies have shown that obesity induces a low grade inflammatory state characterized by the activation of the JNK and IKK pathways in the hypothalamus, which impairs both insulin and leptin signaling. Accordingly, the inhibition of JNK1 or IKK beta in hypothalamic neurons leads to a reduction in food intake and weight gain, and increases insulin sensitivity. Interestingly, an enriched environment paradigm prevents the development of obesity by increasing BDNF levels in the hypothalamus. Nonetheless, it is not known whether this effect involves inhibition of the JNK and IKK pathways nor whether an enriched environment is capable of reestablishing hypothalamic function in obese mice that already present alterations in the glucose metabolism.

We found that an enriched environment decreases basal glucose levels, glucose intolerance and insulin resistance; it also decreased the activation of both JNK and IKK pathways and restored c-Fos and BDNF protein levels in the hypothalamus of obese mice. Using high throughput sequencing we found that both a high fat diet and an enriched environment alter the expression of microRNAs in the hypothalamus. Among these microRNAs, we identified that miR-182 is overexpressed in the hypothalamus of obese mice and is downregulated by an enriched environment. *In vitro* experiments showed that miR-182 targets BDNF. Together, our results suggest a molecular mechanism in which an enriched environment can alter gene expression and the activation of different signaling pathways to ameliorate the detrimental effects of obesity.

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MTU03-09

Mice experimentally infected with schistosoma mansoni exhibit molecular markers of idiopathic neurodegenerative diseases

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Introduction: Schistosomiasis is a parasitosis affecting approximately 200 million people worldwide and characterized by high rates of morbidity. Acute infection of CNS is rare and causes seizures, delirium, increased intracranial pressure and ataxia. However, chronic schistosomiasis is known to impair the ability of infected people to perform daily tasks, which has been traditionally attributed to the development of severe damage to liver and urinary bladder. Since schistosomiasis is endemic to areas presenting low economic development and poor access to healthcare services, the possibility that the behavioral and cognitive impairments observed in the prevalent non-neurological disease are related to biochemical changes in the CNS is largely neglected.

Objective: To investigate the occurrence of biochemical markers associated to inflammation and neurodegeneration in the brain of BALB/c mice subjected to a model of chronic infection with *S. mansoni*.

Results: Three- to five-days old mice were infected with *S. mansoni* cercariae and the brain cortex was removed 60 days after infection for analysis. Increases in phosphorylated tau (ser202), phosphorylated GSK-3α/β (ser21/ser9), β-amyloid (1-42) and α-synuclein were observed; IL-1β, nNOS and GFAP levels were also increased, while TNF-α did not change. Non-enzymatic antioxidant potential, nitrotyrosine levels, protein carbonylation and reduced sulfhydryl content were all altered in infected animals. Daily systemic injection of the antioxidants N-acetylcysteine or Trolox for 5 days before analysis inhibited the changes in all parameters, except β-amyloid.

Conclusion: BALB/c mice infected with *S. mansoni* present increased biochemical markers commonly associated to neurodegenerative diseases. The effect of antioxidants suggest that oxidative stress play an important role in the modulation of the modifications taking place in the brain of infected animals.

MTU03-10

Microglial WNT signaling inhibition promotes microglia activation and oligodendrocyte maturation blockade

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Neuroinflammation is a key pathomechanism in numerous brain diseases, including encephalopathy of prematurity resulting from premature birth, which is the commonest cause of disability in children under 5 years. Microglia (MG) and macrophages (Mφ) modulate neuroinflammation and are capable of acquiring diverse phenotypes involved in the cytotoxic response, immune regulation, and injury resolution.

To model the systemic inflammation observed in premature infants and that is associated with poor neurodevelopmental outcomes, we have developed a mouse model. We induce a chronic systemic inflammatory response by intraperitoneal administration of IL-1β during a period corresponding to 28–34 weeks of human pregnancy (P1–P5). This model mirrors the main anomalies observed in premature infants: MG/Mφ activation, arrested oligodendrocyte maturation leading to myelination defects, cognitive deficits, and MRI diffusion anomalies.

Our goal was to characterize the molecular mechanisms controlling the MG/Mφ phenotypic profile in this model, allowing the potential identification of novel targets for neuroprotection.

In response to IL-1β, brain MG/Mφ exhibited an immediate and sustained over time pro-inflammatory phenotype while a transient phenotype compatible with injury resolution was observed with a significant delay. Transcriptome analysis of MG/Mφ sorted from newborn brains revealed the down-regulation of multiple members of the Wnt pathway in activated cells. In primary cultures of MG, genetic or pharmacological inhibition of the Wnt pathway was sufficient to promote a pro-inflammatory phenotype while activation of the Wnt pathway prevented IL-1β-induced MG activation. *In vivo* MG/Mφ-specific deletion of β-catenin (main effector of the canonical Wnt pathway) induced MG/Mφ activation, oligodendrocyte maturation arrest and myelin defect. Experiments currently underway *in vivo* target MG/Mφ with nanoparticles carrying a plasmid expressing a constitutively active form of β-catenin to ascertain if this can reduce the IL-1β-induced brain anomalies. Modulating Wnt signaling in brain MG/Mφ may be a promising therapy for neuroinflammation in preterm infants and, potentially, in other brain disorders with neuroinflammation.

MTU03-11

The effects of chronic neuroinflammation on the cholinergic system contributes to cognitive decline and motor function loss

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Inflammation in the central nervous system and disruption of its immune privilege are major contributors to the pathogenesis of multiple neurodegenerative disorders, including Alzheimer's (AD), Parkinson's disease (PD), Creutzfeldt-Jakob disease (CJD), multiple sclerosis (MS), traumatic brain injury and more. Neuroinflammation and the loss cholinergic input from the basal forebrain to the hippocampus and the cortex have both been intensely associated with the development of dementia, and particularly AD, however the specific link between neuroinflammation and cholinergic cell death, and the interaction of cholinergic neurons and activated glia cells, is still an unexposed area. Interleukin-6 (IL6), among other proinflammatory cytokines such as IL-1 and TNF-α, is a common early inflammatory marker in AD and PD. Over the past decade, it has been shown that neuroinflammation plays a role in the development of neuronal degeneration, and as such, is a promising therapeutic target. In this study we used heterozygous transgenic mouse (GFAP-IL6, which express pro-inflammatory IL-6 in astrocytes under the control of the glial fibrillary acidic protein promoter; causing low level, chronic inflammation in the brain. It is known that homozygotes GFAP-IL6 mice exhibit neuroinflammation and a degenerative disorder associated with several deficits such as ataxia, seizures. In this study, we have used heterozygous GFAP-IL6 mice to study motor-coordination, anxiety and cognitive performance together with immunohistochemistry. At 6 months of age, GFAP-IL6 mice showed decreased performance on rotarod, elevated narrow beam walk, hind limb clasping and ledge tests as well as in novel object recognition memory, indicating deficits in fine motor coordination. The behavioral changes were accompanied by increased microglia numbers and activation throughout the brain, and compromized cholinergic arborization in the hippocampus. These results confirm that the GFAP-IL6 transgenic mouse displays subtle deficits in fine coordination and memory already at 6 months of age. This mouse model may suitable to test anti-inflammatory medications for the treatment of AD and PD.

MTU03-12

Andrographolide attenuates lipopolysaccharide-induced chemokine upregulation: implication for anti-neuroinflammation therapy

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Andrographolide is a bioactive molecule isolated from *Andrographis paniculata* with anticancer and anti-inflammatory activities. Here, we studied the effects of andrographolide on chemokine expression following lipopolysaccharide (LPS) treatment. In primary astrocytes, andrographolide reverses LPS-induced activation of NF-κB/JNK and upregulation of chemokines in the CCL, CXCL and CX3CL families. Similarly, chemokine levels are attenuated in the cortex of LPS-treated mice with andrographolide co-treatment. Our data suggest that andrographolide should be further studied as a potential therapeutic for CNS diseases characterized by astrocyte-mediated neuroinflammatory processes.

MTU03-13

Functionalised self-assembling nanofibrous peptide hydrogel influences astrocyte phenotype *in vitro*

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After traumatic brain injury (TBI), the astrocytic response is essential to repair: demarcating the lesion site; preventing secondary degeneration; and arresting growth in the acute phase[1]. This response is detrimental if persistent, as chemical and physical cues from the 'reactive' astrocytes prevent functional recovery. Therefore, an important therapeutic target to address TBI is to control the phenotype change of astrocytes after injury, from 'reactive' to 'active'. Currently, 2D cell culture systems used to study cell behaviour do not recapitulate the 3D *in vivo* environment, limiting the translation of findings to *in vivo* experiments. We have shown that astrocytes cultured on nanofibres adopt a cytotrophic phenotype [2], however nanofibres have limited potential for *in vivo* deployment for TBI as they don't effectively fill a void. Therefore, we have investigated the use of a self-assembling peptide (SAP) hydrogel, Fmoc-DIKVAVl, as a 3D *in vitro* model that can be deployed *in vivo*. Fmoc-DIKVAV presents the laminin sequence IKVAV, self-assembles under physiological conditions and has a nanofibrous network[3]. We have functionalised FMOC-DIKVAV with an anti-inflammatory molecule and have shown that providing astrocytes with biologically relevant cues and a 3D environment induces a cytotrophic phenotype. This system could be implemented *in vivo* to induce a phenotype change in astrocytes after injury, and encourage functional repair after injury.

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MTU03-14

TREM2 deficient microglia display decreased phagocytosis without altering tam receptor expression after systemic inflammation

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Microglia are the resident macrophages of the central nervous system (CNS). As such, they act as the primary phagocytes in the CNS during normal development and tissue homeostasis. However, monocyte derived macrophages also contribute to phagocytosis in response to CNS injury or infection. Recently, a single amino acid mutations in the microglial expressed molecule TREM2 was found to correlate with a 3-fold increased risk of Alzheimer's disease. We hypothesize that increased risk is due in part to decreased phagocytosis function of microglia and macrophages. In this study we have analyzed wild-type (WT) and TREM2 deficient (TREM2KO) microglia and macrophages phagocytosis of bacteria to get a more global understanding on TREM2 mutations effects on processes such as synaptic pruning or initiating a pathogen defense rather than just focusing on amyloid phagocytosis. A question that is of particular interest to us is: "Are TREM2 mutations relevant only in the context of neurodegenerative diseases, or is it contributing to a life long alterations in responses that becomes more visible with age?" We find that microglia acutely isolated from healthy adult brains display barely detectable levels of phagocytosis. By contrast, only a subset of microglia and macrophages isolated from mice 24 h post-challenge with intraperitoneal (IP) LPS display robust phagocytosis. Cells from TREM2KO mice show a decreased phagocytosis following systemic challenge as compared to the WT. In both WT and TREM2KO mice, macrophages displayed a greater rate of phagocytosis. Tyro3, Axl and Mer comprise the TAM family of receptor tyrosine kinases that promote phagocytosis and suppression of pro-inflammatory innate immune responses by myeloid cells. Using flow cytometry, we find that microglia express all three members of the TAM family although at a lower level than observed on CNS-infiltrating macrophages. Only Tyro3 expression was increased in response to IP LPS expression. Surprisingly,TREM2 deficiency had no effect on baseline or LPS inducible TAM expression on adult microglia or CNS-infiltrating macrophages.

MTU03-15

Neuroimmune changes in chemotherapy-induced peripheral neuropathy

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Chemotherapy-induced peripheral neuropathy (CIPN) is associated with neuropathic pain and can severely affect cancer patients undergoing treatment with chemotherapy drugs, such as oxaliplatin, a platinum-based anti-neoplastic agent. The mechanisms underlying CIPN remain unclear and there are no effective treatments to alleviate neuropathic pain in cancer chemotherapy patients. Here, we used an animal model of oxaliplatin-induced peripheral neuropathy to examine cellular changes in the nervous and immune systems. We treated C57BL/6 male mice with oxaliplatin (20 mg/kg cumulative dose administered over 4 alternating days) or saline (vehicle control), and measured changes in mechanical and cold allodynia and locomotion. We then examined cellular changes in the lumbar spinal cord and dorsal root ganglion (DRG) using immunohistochemistry and changes in the inguinal lymph nodes, blood, and spleen using flow cytometry. Finally, we conditionally depleted regulatory T (Treg)-cells using transgenic DEREG mice following treatment with oxaliplatin and examined changes in pain hypersensitivity. Oxaliplatin induced significant mechanical and cold allodynia, peaking at day 13 and 16 following drug treatment, and reduced average speed of movement in male mice. There was a significant increase in astrocyte activation and loss of non-peptidergic C-fibre central terminals in the spinal cord dorsal horn, as well as increased neuronal injury in the DRG on day 13 following oxaliplatin treatment. In addition, oxaliplatin caused an increase in CD4 + and CD8 + T cells in the blood and a reduction in Treg cells in the lymph nodes. Treg cell depletion in oxaliplatin-treated DEREG mice caused prolonged

mechanical allodynia compared to oxaliplatin-treated wildtype mice. These results indicate a neuroimmune cross-talk during CIPN, and may aid in the discovery of novel therapeutic targets.

MTU03-16

Peripheral immune complement activation in neurodegenerative disease

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There is mounting evidence that the peripheral immune system interacts with the degenerating central nervous system (CNS), and influences disease outcome. Activation of the complement system, a key branch of innate immunity, is also a hallmark of neurodegenerative pathologies. We previously demonstrated that the inhibition of a key complement activation fragment, C5a, improves symptoms and extends survival in murine models of amyotrophic lateral sclerosis (ALS), Alzheimer's disease and Huntington's disease. In the current study, we aimed to assess the involvement of peripheral complement activation in neurodegenerative diseased patients. 54 ALS and 49 healthy control patients were recruited from the Royal Brisbane and Women's Hospital Motor Neuron Disease multidisciplinary clinic, and peripheral blood sampled. Levels of the complement activation markers C5a and the terminal component C5b-9 were assessed using Cytometric Bead Array and ELISA. We found that the levels of C5a and C5b-9 were significantly elevated in ALS patients' plasma compared to healthy controls. C5a was also significantly elevated within leukocytes from ALS patients suggesting heightened C5a-C5a receptor (C5aR) interaction. Our preliminary studies also show that, parallel to what happens in the CNS, C5aR expression is upregulated in circulating leukocytes, and specifically in monocytes, in ALS patients. Overall, our data demonstrate specific activation of the terminal complement activation pathway in ALS patients, and an upregulation of C5aR in their circulating immune cells. This suggests a potential for these peripheral immune cells to respond in a pathogenic manner when interacting with the degenerating CNS. We are now extending this work into other neurodegenerative diseases such as Huntington's disease and Friedreich's ataxia, and establishing a bio-bank of serum, plasma and white blood cell samples from patients and healthy volunteers.

MTU03-17

Neuroinflammmation in experimental progressive hydrocephalus

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Neuroinflammation is a prominent feature in hydrocephalus and plays an important role in its pathophysiology. The indices of neuroinflammation have been found both preceding and as a consequence of ventricular enlargement. An understanding of its progression is important to identify potential time points for therapeutic intervention, especially when surgical treatment may be unavailable or contraindicated. The present study, which is still ongoing, was performed to analyze glial cell activation and cytokine expression in an experimental model of hydrocephalus during the progression of ventricular enlargement. Twelve albino Wistar rats aged 3 weeks underwent induction of hydrocephalus by injection of aluminium trisilicate (kaolin) into the cisterna magna. They were sacrificed (4 each) 1, 4 and 8 weeks later, respectively, with age-matched controls that received sham injection. Brain sections were processed with single, double and triple immunohistochemistry for the visualization of astrocytes (with GFAP antibodies), microglia (with OX-42 antibodies), the pro-inflammatory cytokine interleukin (IL)-1beta, and neurons (with NeuN antibodies). Stereological cell counts and densitometric analysis were carried out. A marked increase in astrocyte density and immunosignal intensity were observed in the hydrocephalic brains. Microglia activation and a non-significant decrease in neuronal number were also observed in the hydrocephalic brains. Triple immunofluorescence showed expression of IL-1beta in astrocytes, but not in microglia in the hydrocephalic brains. These findings indicate that neuroinflammation occurs early in hydrocephalus, astrocytes are the main source of IL-1beta in this condition, and that neuronal population is not significantly altered early in the disease. Supported by ISN-CAEN.

MTU03-18

Neurotoxic profiles of vanadium when administered at the onset of myelination in rats: the protective role of vitamin E

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Vanadium has been reported to cause oxidative stress and subsequent neurotoxicity. In this study, we exposed neonatal rats during myelination to vanadium and examined the protective effect of Vitamin E to any the resulting neurotoxic effects. Four groups of neonatal rats and their dams were used;

Four groups, Control, Vanadium treated, Vanadium+ Vitamin E and Vitamin E alone was used. The pups were subjected to

behavioural tests prior to sacrifice on PND22 (Open field test, negative geotaxis test and hanging wire test), after which the brains were removed, weighed and subjected to histological examination. Haematoxylin & Eosin (H & E), Cresyl violet stain and Immunohistochemical staining procedures were carried out. Behavioural tests also showed reduced upper body strength and heightened anxiety behaviour which was protected by administration of Vit E, the hanging wire test showed significant difference in Gp B and C ***($p < 0.001$). H & E revealed apoptotic neurons of the medulla in Vanadium exposed rats, Cresyl violet stain showed depletion of the external granule layer of the cerebellum which was confirmed as neural stem cells by Nestin immunohistochemistry. Further "immune" assays revealed glial activation (GFAP stain), demyelination (CNPase) and activated microglia (Iba1) due to vanadium which were ameliorated by the administration of Vit E to the dams. The pups whose dams were administered with Vit E alone showed signs of cellular degeneration which might be due to peroxidative effects of tocopherol in the absence of an oxidative stress condition. Our study shows in conclusion that Vit E administration via lactation can ameliorate neuropathological changes in rats exposed to Vanadium at the onset and peak of myelination process.

MTU03-19

Effects of IL-35 gene therapy on neuroinflammation and neuropathic pain following peripheral nerve injury

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Peripheral nerve injury often results in neuropathic pain involving immune and inflammatory responses. While pro-inflammatory cytokines have been shown to play a critical role in promoting peripheral and central sensitisation and contributing to pain hypersensitivity, anti-inflammatory cytokines have been implicated in inhibition of neuropathic pain. Interleukin (IL)-35 is a novel anti-inflammatory cytokine produced by regulatory T and B cells, and is known to supress inflammation. Here, we investigated the effects of IL-35 gene therapy on neuroinflammation and neuropathic pain in mice with peripheral nerve injury. Following intrathecal delivery (days 1 and 4) of the pVAX–IL-35 DNA construct, the successful production of IL-35 protein in the lumbar spinal cord was confirmed by ELISA in naïve C57BL6/J mice at day 5 ($n = 3–4$). We then treated mice following chronic constriction injury (CCI) of the sciatic nerve (Day 0) with either pVAX-IL-35 plasmid or pVAX control at days 1 and 4 post-CCI. We examined spinal glial cell reactivity by immunohistochemistry, prevalence of systemic regulatory T and B cells by flow cytometry ($n = 3–5$), and mechanical and thermal pain hypersensitivity in the hindpaws ($n = 6$) of nerve-injured mice. We found that IL-35 concentration was significantly increased ($p < 0.001$) in the spinal cord of pVAX-IL-35-injected mice relative to control mice. Compared to CCI-mice treated with control plasmid, microglial activation was significantly inhibited in CCI-mice treated with pVAX-IL-35. There was no difference in the prevalence of regulatory T cells and B cells between the groups. Furthermore, all nerve-injured mice developed mechanical and thermal pain hypersensitivity in the ipsilateral hindpaw, but there was no difference between IL-35-treated and control mice. Our results show that although intrathecal administration of pVAX-IL-35 was effective in IL-35 gene expression in the spinal cord and significantly attenuated spinal gliosis, there were no significant effects on pain hypersensitivity. These findings suggest that IL-35 is unlikely to play a key role in inhibition of neuropathic pain.

MTU03-20

Astrocyte-targeted IL10 production modifies expression of TREM2 in activated microglia after perforant pathway transection

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One of the endogenous mechanisms regulating inflammatory cell activation following brain injury is the expression of modulator and/or inhibitor membrane receptors.Recent studies demonstrate that regulatory receptor TREM2-mediated phagocytic function of microglia/macrophages (MM) is required for debris clearance and maintenance of Central Nervous System (CNS) tissue homeostasis. TREM2 needs an adaptor protein DAP12 to initiate the intracellular signalling cascade via an ITAM domain and tyrosine kinases. The factors that control the expression of TREM2 after CNS injury are still unclear, although the influence of the microenvironment, especially the local production of cytokines may play a key role. Hence, the objective of this study was to characterize the effects of local production of the anti-inflammatory cytokine IL-10 on TREM2 expression using an axonal anterograde degeneration model. For this purpose, unilateral perforant pathway transection (PPT) was performed in transgenic mice with astrocyte-targeted production of IL-10 (GFAP-IL10Tg) and their corresponding wild types (WT) littermates. At 2, 3, 7 and 14 days post-lesion (dpl) animals were perfused with 4% of paraformaldehyde and brains processed for immunohistochemistry against TREM2 and DAP12. Our results showed a basal low expression of TREM2 in GFAP-IL10Tg animal in contrast to WT where TREM2 was mostly absent. An increase in TREM2 + CD68 + phagocytizing MM was noted in the ipsilateral hemisphere of the GFAP-IL10Tg animal especially in the molecular layer of the dennervated dentate gyrus and was always higher than that of its respective WT. Almost all TREM2 + cells colocalized with DAP12 in both WT and GFAP-IL10Tg animals at all time-point studied. In conclusion, local production of IL-10 by astrocytes in the CNS may modify the microglial response associated with PPT by modulating endogenous regulatory receptors as TREM2. Future studies are concentrated on evaluating whether the differences of TREM2 expression affects axonal sprouting. Supported by Ministry of Science and Innovation (BFU2011-27400, BFU2014-55459).

MTU03-21

Pathologic T cell cytokines have both beneficial and deleterious effects on oligodendrocyte lineage cells

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The acute multiple sclerosis (MS) lesion is a highly inflammatory environment characterized by demyelination and oligodendrocyte

loss. A clear pathologic role for inflammatory T cell subsets (Th1 and Th17) and cytokines (IFNγ, IL-17, GM-CSF) in MS and the animal model EAE have been demonstrated. Evidence for direct cytokine-induced effects on oligodendrocyte progenitor cells (OPCs) that undertake the reparative process of remyelination has been less forthcoming. We first investigated cytokine receptor expression in isolated mouse OPCs by RT-PCR. Then OPCs were stimulated with IFNγ, IL-17, or GM-CSF and their viability, proliferation, and maturation were assessed by histological and molecular techniques in culture. The prototypical Th1 cytokine IFNγ had a deleterious effect directly inducing cell death in pure cultures of OPCs. Surprisingly the more recently identified Th17 cytokines IL-17 and GM-CSF had mixed effects. IL-17 inhibited OPC proliferation in vitro with no loss in cell viability. Rather IL-17 stimulated OPC maturation indicated by increased expression of mature myelin proteins. IL-17 also increased the myelin-specific protein, proteolipid protein (PLP), in an ex vivo cerebellar slice culture assay. Conversely GM-CSF kept OPCs in a quiescent state, inhibiting OPC proliferation and differentiation in vitro. IL-17 and GM-CSF induced unique chemokine and receptor expression changes suggesting varied roles in OPC migration as well. These results suggest that inflammatory cytokines despite contributing to aberrant immune function in the lesion can have different effects on remyelination and repair. A full characterization of pro- and anti-inflammatory cytokine effects on oligodendrocytes and remyelination may provide clues for more targeted therapeutic strategies for MS.

MTU03-22

The complement receptor C5AR controls acute inflammation and astrogliosis following spinal cord injury

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This study investigated the role of the complement activation fragment C5a in secondary pathology following contusive spinal cord injury (SCI). *C5ar*$^{-/-}$ mice, which lack the signalling receptor for C5a, displayed signs of improved locomotor recovery and reduced inflammation during the first week of SCI compared to wild type mice. Intriguingly, the early signs of improved recovery in *C5ar*$^{-/-}$ mice deteriorated from day 14 post-SCI onward, with absence of C5aR ultimately leading to poorer functional recovery, larger lesion volumes, reduced myelin content and more widespread inflammation at 35 days post-SCI. Pharmacological blockade of C5aR with a selective antagonist (C5aR-A) during the first 7 days post-SCI improved recovery compared to vehicle-treated mice, and this phenotype was sustained up to 35 days after injury. Consistent with observations made in *C5ar*$^{-/-}$ mice, these improvements were, however, lost if C5aR-A administration was continued into the more chronic phase of SCI. Signalling through the C5a-C5aR axis thus appears injurious in the acute period but serves a protective and/or reparative role in the post-acute phase of SCI. Further experiments in bone marrow chimeric mice suggested that the dual and opposing roles of C5aR on SCI outcomes primarily relate to its expression on CNS-resident cells and not infiltrating leukocytes. Additional *in vivo* and *in vitro* studies provided direct evidence that C5aR signalling is required during the post-acute phase for astrocyte hyperplasia/hypertrophy and glial scar formation. Collectively, these findings highlight the complexity of the inflammatory response to SCI and emphasise the importance of optimizing the timing of therapeutic interventions.

MTU03-23

Seizure susceptibility after traumatic injury to the pediatric brain

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The occurrence of post-traumatic epilepsy is particularly high after injury at a young age, and has been associated with poorer functional outcomes, suggesting that the developing brain may show elevated vulnerability to post-traumatic epileptogenesis. However, most existing models of post-traumatic epileptogenesis focus on injury to the adult brain. Here, we have investigated seizure susceptibility in mice after injury at postnatal day 21, using a well-characterized model approximating a toddler-aged child. Within weeks, brain-injured mice showed a pronounced seizure response to the convulsant pentylenetetrazol (PTZ) compared to sham-operated controls. A heightened seizure response to PTZ was still apparent by 3 months post-injury (adulthood), and associated with the pathological presence of abnormal mossy fiber sprouting in the ipsilateral hippocampus. The inflammatory mediator interleukin (IL)-1β has been implicated in processes of neurodegeneration and epilepsy, and a robust elevation of both the ligand and key signaling receptor (IL-1R1) were detected after injury at postnatal day 21. Acute post-injury treatment with a recombinant IL-1R antagonist was found to reduce seizure susceptibility after both moderate and severe brain injuries, and was associated with a reduction in reactive gliosis but no preservation of hippocampal granule cells or interneurons. These results provide evidence of persistent hyperexcitability in the immature injured brain, and implicate reactive glial cells in this process.

MTU03-24

Comparing innate immune cell and progenitor cell responses 24 h after spinal cord injury in neonates, juvenile and adult rats

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Spinal Cord Injury (SCI) is a complex pathology with a high impact. There exists a trend for a better functional recovery in younger patients, compared to adults, which is also reported for animal studies. The reasons for this and its potential impacts are yet to be elucidated. A current candidate being explored for regenerative cell therapies are endogenous neural progenitor cells (eNPC),

located in the ependymal layer of the spinal cord central canal, that have been shown to respond to traumatic spinal cord injury. Inflammation has been shown to play a significant role in central nervous system (CNS) pathologies and SCI is no exception. Using a mild contusion injury model from a NYU impactor adult (9 week), juvenile (5 week) and infant (P7) Sprague-Dawley rats were compared at 24 hrs after injury (n = 38). The lesion area, extent of haemorrhage and the prevalence of swollen axons were measured and compared between groups. The innate cells in the inflammatory response were examined using neutrophil counts and ED1/IBA1 double labelling for microglia/macrophages. Glial fibrillary acidic protein (GFAP) immunohistochemistry was used to compare the astrocytic response and Nestin was used for eNPC. This study found a decreased inflammatory response in the infant group, compared to the mature animals, in terms of neutrophil infiltration and microglial activation. After SCI in all 3 ages there was an obvious increase from the control levels of Nestin staining in the ependymal layer of the central canal, with long basal processes extending into the parenchyma. There was also a uniform increase in the astrocytic density at the lesion site; in all 3 groups however there was no significant difference between age groups in nestin activation and GFAP response at 24 hrs. These results point to significant differences in the inflammatory response between infants and adults, which may contribute to the observed better recovery in young patients rather than an increased activation of eNPC, or increased cell replacement.

MTU03-25

A novel 3-(4,5-diphenyl-L,3-oxazol-2-YL)propanal oxime compound is a potent transient receptor potential ankyrin 1 and vanilloid 1

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Transient Receptor Potential Ankyrin 1 and Vanilloid 1 (TRPA1, TRPV1) ion channels expressed on nociceptive primary sensory neurones are important regulators of pain and inflammation. TRPA1 is activated by several inflammatory mediators including formaldehyde and methylglyoxal that are products of the semicarbazide-sensitive amine-oxidase enzyme (SSAO). The 3-(4,5-Diphenyl-l,3-oxazol-2-yl)propanal oxime or SZV-1287 is a newly developed SSAO inhibitor which is described to inhibit both acute and chronic inflammatory processes. Since its chemical structure is similar to other oxime derivatives which were described as TRPA1 antagonists, we investigated the effects of SZV-1287 on the TRP receptor activation of trigeminal ganglion neurones and nerve terminals.

Calcium influx in response to the TRPA1 agonist allyl-isothiocyanate (200 μM) and the TRPV1 stimulator capsaicin (330 nM) in rat trigeminal neurones was measured by microfluorimetry. Calcitonin gene-related peptide (CGRP) release as the indicator of 100 μM allyl-isothiocyanate- or 100 nM capsaicin-induced peripheral sensory nerve terminal activation was measured by radioimmunoassay.

SZV-1287 (100, 500 and 1000 nM) exerted a concentration-dependent significant inhibition on both allyl-isothiocyanate- and capsaicin-evoked calcium influx in trigeminal neurones. It also significantly inhibited the TRPA1, but not the TRPV1 activation-induced CGRP release from the peripheral sensory nerve endings in a concentration-dependent manner. In contrast, the reference SSAO inhibitor LJP 1207 with a different structure had no effect on TRPA1 or TRPV1 activation in either model system.

This is the first evidence that our novel oxime compound SZV-1287 originally developed as a SSAO inhibitor has a potent antagonistic action on TRPA1 and TRPV1 ion channels on the cell bodies and peripheral terminals of primary sensory neurones.

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MTU03-26

Changes of electrophysiology and pathology of optic nerves after visual prosthesis implantation

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Many studies has shown that optic visual prosthesis (OVP) can bring a hope of visual restoration for age-related macular degeneration patients. But the electrophysiology and pathology of electrode implantation remain to be evaluated. Here we reported that the changes of retinal electrophysiology and pathology after OVP were implanted to rabbit optic nerves. Briefly, New Zealand male rabbits were anaesthetized with isoflurane and were detected by an electroretinogram(ERG) system under photopic or scotopic condition. After the first detection of ERG, rabbits were performed implantation of OVP on the optic nerve. The homemade prosthesis electrode is composed of 3 paralleled platinum-iridium alloy electrodes pieced a C-shape silicone tube. After optic nerve was exposed, C-shape silicone tube with was surrounded the optic nerve at 2 mm away from the eyeball and the electrodes was pressed into the optic nerve by pushing the end of electrodes out of silicone tube. The rabbits were detected by ERG with a 3 days interval after surgery respectively. The results show that there were no significant differences in both A and B wave duration and amplitude before and after optic prosthesis implantation of photopic and scotopic adaption. Immunostaining results showed that the number of Iba1 (microglia marker) and GFAP (astrocyte marker) positive cells of optic nerves with an optic prosthesis increased at 2w and recovered at 4w, while the density of myelin PLP (myelin marker) positive fibers increased at 2w and 4w. These results suggest that visual prosthesis implantation induced a transient inflammation of optic nerve but did not change in retinal electrophysiology.

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MTU04 Molecular Mechanism of Parkinson's Disease

MTU04-01

Carrier mediated delivery system bearing dopamine for effective management of Parkinsonism

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Delivery of drug and sustaining it in effective concentration in brain is challenging due to blood brain barrier (BBB). In the present investigation, amino acid coupled liposomes bearing dopamine-HCl were prepared to deliver drug to the brain utilizing receptor-mediated transcytosis for effective management of parkinsonism. L-lysine stearylamine conjugate (LSC) was synthesized & LSC coupled liposomes bearing dopamine HCl was prepared by lipid cast film method. Formulations were analyzed for average vesicle size, drug entrapment, *in-vitro* drug release and *in-vivo* efficacy of the formulations was assessed by measuring the reduction in the degree of drug induced catatonia in albino rats. Average particle size was found in the range of 1.92-0.80 mm. There was increase in the size for coupled liposomes due to the inclusion of LSC in liposomal bilayers. The percent encapsulation efficiency decreased from $46.82 \pm 2.17\%$ in uncoupled to $38.13 \pm 1.18\%$ in coupled liposomes. The *in-vitro* drug release after 24 h was $58.9 \pm 2.94\%$ with uncoupled while the coupled liposomes showed $43.7 \pm 2.18\%$ drug release. The lower value for coupled formulation could be due to the retardation of drug release caused due to the incorporation of LSC in the liposomal bilayers, which enhanced the structural integrity of the bilayer. *In-vivo* study reveals that the animals receiving uncoupled liposomes showed partial reduction and animals that received coupled liposomes showed almost complete reduction in catatonia. Fluoresence study clearly indicates the uptake of 6-CF in blood vessels and accumulated in brain. This could be due to enhanced uptake of Lysine coupled liposomes through amino acid transporters present at BBB surface.

MTU04-02

Ferritin dysfunction and iron dyshomeostasis in Parkinson's disease: investigations in caenorhabditis elegans

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Parkinson's disease is the second most common neurodegenerative disease. The disorder is characterized by progressive motor impairment attributed to gradual loss of dopaminergic neurons in the *substantia nigra pars compacta*. Generally, the *substantia nigra* has the highest distribution of iron in the brain, presumably because iron is an essential co-factor for the dopamine synthesis enzyme, tyrosine hydroxylase. However, in Parkinson's disease the iron levels are more elevated in this region. This iron dyshomeostasis is thought to contribute to neuronal death via generation of damaging free radicals. We speculate that the imbalance stems from dysfunctions in the system that regulates iron. One key player in iron regulation is ferritin, the major iron storage protein. Ferritin has been implicated in some diseases, such as neuroferritinopathy and Restless Leg Syndrome, which present with a neurological component and iron dyshomeostasis. Therefore we hypothesize that ferritin dysfunction may play a significant role in the iron imbalance observed in Parkinson's disease. To determine whether ferritin is essential for dopaminergic neuronal health, we generated a *Caenorhabditis elegans* ferritin knockout strain expressing green fluorescent protein in dopaminergic neurons. Over time, the neurons were scored for age-related neuronal abnormalities, which included axonal/dendritic blebbing. Our results show that knocking out ferritin accelerates formation of these age-related neuronal structures, suggesting that ferritin is crucial for maintenance of dopaminergic neurons. It is likely that in Parkinson's disease, the ability of ferritin to regulate iron is disrupted. We propose that ferritin dysfunction may arise from either perturbed ferritin levels, altered iron loading capacity and/or compromised structural integrity. This disruption causes build-up of iron in the *substantia nigra* leading to dopaminergic loss. Our current follow-up studies include investigating the impact of ferritin overexpression on survival and dopaminergic neuronal health. The strains that we have generated offer great models that we can also use for drug discovery to rescue the iron accumulation in Parkinson's disease and other iron-associated diseases.

MTU04-03

Defining how dopamine modulates α-synuclein oligomerisation

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The pathology of Parkinson's disease is characterized by intracellular inclusions called Lewy bodies and Lewy neurites which are composed of fibrillized a-synuclein protein. Another prominent feature of Parkinson's disease is the loss of dopaminergic neurons. We have shown that dopamine can inhibit the aggregation of a-synuclein into fibrils and instead promotes a-synuclein to form SDS-resistant soluble oligomers that are non-amyloidogenic (i.e. are Thioflavin T (ThT) negative). We have investigated the molecular basis for this interaction between the neurotransmitter dopamine and a-synuclein and found that part of its mode of action is to oxidise the methionine residues in a-synuclein. a-synuclein is typically characterised as a natively unfolded monomer that can adopt tertiary structures under certain conditions. But studies on "native" a-synuclein isolated from mammalian cell lines, brain tissue or red blood cells suggest it is present as a stable tetramer that is resistant to aggregation, and that the destabilization of this tetramer is what leads to fibrillization and progression of disease (Bartels Nature 2011). This native a-synuclein does not aggregate into ThT positive aggregates in comparison to recombinant a-synuclein that readily undergoes aggregation. However, other data suggests that brain derived a-synuclein is predominantly monomer and prone to

aggregation in comparison to the red blood cell a-synuclein which was found to be tetrameric (Burre Nature 2013). We are studying two characteristics of native a-synuclein: (i) Can adding pre-formed a-synuclein seeds to native a-synuclein promote its aggregation into fibrils? As is the case for recombinant a-synuclein (ii) Does dopamine promote the oligomerisation of native a-synuclein into SDS-resistant soluble oligomers? And does this involve dopamine mediated oxidation of native a-synuclein?

MTU04-04

Throwing light on neurodegeneration; small angle X-ray scattering studies on protein misfolding at the australian synchrotron

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It is widely understood that inherently disordered proteins (IDPs) of neuropathological significance, such as α-synuclein (α-syn) and the β-amyloid peptide (Aβ) are not truly random. Rather, their energy landscapes may contain several low entropy regions. Tantalising clues to the existence of these have been found as long range interactions by molecular dynamics studies, NMR, and FRET, while conformational heterogeneity in α-syn has been demonstrated by AFM. Small angle X-ray scattering (SAXS) with ensemble optimisation modelling (EOM) takes the search for these regions and their relevance further. This approach is based on the generation of a large pool (typically 10 000) of theoretical structures derived with side-chain interaction constraints from the sequence of the protein. The theoretical X-ray scattering profiles calculated from these structures are then matched for fit against the experimental scattering profile to create an ensemble of best fit structures. A wide distribution of radius of gyration (R_g) and size (D_{max}) would indicate a truly random distribution of structures and a multi-modal distribution would indicate the existence of a number of distinct conformers. Using this technique we have already shown conformational heterogeneity in Aβ and α-syn. In the former the conformer distribution varies with the solution environment of the peptide and is readily reduced to a defined structure by the addition of Cu^{2+} or compounds such as Clioquinol. With α-syn the distribution is dramatically changed by point mutations, suggesting that oligomers and fibrils arise from two distinct conformer pools. These results may pave the way for therapeutic intervention by manipulating these distributions. Here we shall describe recent studies on combining SAXS-EOM with dipolar residual couplings of α-syn variants selected from the literature for their behaviour in a range of *in vivo* systems.

MTU04-05

Reduced subventricular zone neurogenesis in Parkinson's disease is associated with increased phosphorylated α-synuclein

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Restorative therapies for Parkinson's disease (PD) based on stem cell technologies are currently under intense investigation. Endogenous neurogenesis in the PD brain, however, is significantly reduced, suggesting that the environment of the PD brain is inconsistent with the normal birth, maturation or survival of new neurons. The effectiveness of restorative treatments may thus be compromised by a non-supportive cellular environment in the PD brain. Data from both animal models and PD patients implicate two key Parkinson-associated proteins, α-synuclein and leucine-rich repeat kinase 2 (LRRK2), with reduced neurogenesis in this disorder. In this work we employed immunoblotting to investigate expression of α-synuclein and LKKR2, their phosphorylated forms, and levels of protein markers of neurogenesis in the dentate gyrus and subventricular zone in 10 cases of PD compared with 9 healthy age-matched controls. In the subventricular zone, α-synuclein, but not LRRK2, was significantly decreased by 69% in the PD brain, while levels of the phosphorylated form of α-synuclein were increased 9-fold ($p = 0.02$ and $p = 0.05$ respectively). This was associated with a 56% decrease in levels of the early neuronal marker neuron-specific class III β-tubulin (TUJ-1, $p = 0.04$). In the dentate gyrus, levels of phosphorylated, but not total, α-synuclein were increased 12-fold ($p = 0.05$), while phosphorylated, but not total, levels of LRRK2 were increased 1.6-fold ($p = 0.03$). Levels of the marker proteins for proliferative cells, glial fibrillary acidic protein-δ, proliferating cell nuclear antigen and TUJ-1 quantified by immunoblotting were unchanged in the dentate gyrus in the Parkinson's disease, compared with age-matched controls. These data suggest that the phosphorylated (active) forms of the Parkinson-associated proteins α-synuclein and LRRK2 may play a role in the modulation of neurogenesis in the human brain. Further they support the hypothesis that neurogenesis in the subventricular zone is reduced in the Parkinson's disease brain.

MTU04-06

Fatty acid-binding protein 3 (FABP3) is critical for alpha-synuclein oligomerization in Parkinson disease

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Fatty acid-binding protein 3 (FABP3) participates in the uptake, intracellular metabolism and transport of long-chain polyunsaturated fatty acids (LCPUFAs) such as arachidonic acid (AA). We previously reported that FABP3 is highly expressed in acetylcholine interneurons and glutamatergic terminals in the striatum. FABP3 binds to the intracellular third loop of dopamine D_2 long receptor (D_2LR) and is critical for D_2LR functions to regulate extrapyramidal motor function in mice (J Neurosci. 2010;30:3146–3155). We here found that FABP3 is expressed in the dopamine neurons in the

substantia nigra in mouse brain and binds to α-synuclein (J Biol Chem. 2014;289:18957–18965). Since α-synuclein oligomerization triggers Lewy body formation in Parkinson disease (PD) and an excessive AA intake is risk factor for Parkinson disease patients, we addressed the mechanism underlying AA-induced α-synuclein oligomerization through FABP3. Incubation with AA promoted the α-synuclein oligomerization *in vitro*. MPP^+ treatment triggered α-synuclein oligomerization in cultured PC12 cells. FABP3 overexpression with AA treatment further aggravated the MPP^+-induced α-synuclein oligomerization and promoted cell death in PC12 cells. FABP3 mutant lacking fatty-acid binding site failed to aggravate the AA-induced α-synuclein oligomerization. We also confirmed that compounds which block the interaction between FABP3 and α-synuclein inhibit DA neuron degeneration in MPTP-treated mice, thereby improving the dysfunction of motor coordination and cognition in MPTP-treated mice. Taken together, FABP3 with AA aggravates the α-synuclein oligomerization under oxidative stress in DA neurons and FABP3 is novel target for PD therapeutics.

MTU04-07

Intrastriatal injection of alpha-synuclein fibrils induces olfactory deficits in mice

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Alpha-synuclein (α-syn) localises mainly in the presynaptic terminals of neurons where it is plays a role in neurotransmitter release. Mutations in the *SCNA* gene encoding α-syn cause familial Parkinson's disease (PD), and α-syn is the primary constituent of Lewy bodies, the defining neuropathology of PD, which follows a defined pattern of spread with disease progression. Recently, investigations in to mechanisms underlying the spread of α-syn pathology have focused on rodent models where short fibrils of α-syn are injected into various brain regions and pathology and functional deficits assessed. Since α-syn-positive structures occur early in the PD olfactory bulb, and olfactory dysfunction is a common, pre-motor symptom of PD patients, we investigated olfaction in the intrastriatal α-syn fibril injection mouse model. Every 30 days after injection, mice performed a wire pull up task as an indicator of motor function, and an odour detection test where mice investigated pairs of cartridges loaded with a damper soaked with vehicle or a novel odour (essential oils) at increasing concentrations. Ability to pull up on the wire and to detect an odour at $1{:}10^6$ were impaired in α-syn injected mice from about 90 days after injection onward. At day 180, olfactory bulbs were assessed for synaptic markers by immunoblot. Expression of AMPA GluR1, GluR2/3/4, synaptophysin, PSD95 and the NMDAR1 subunit were significantly elevated in the olfactory bulbs of α-syn-injected mice relative to vehicle-injected mice, indicting altered synaptic function. This is consistent with literature reporting increased basal synaptic transmission through NMDA receptor activation and enhanced contribution of calcium-permeable AMPA receptors in α-syn oligomer-treated slices, and impaired olfaction and increased neurogenesis of short-lived neurons in A30P α-syn overexpressing mice. In summary, α-syn injection induced neuropathological changes distant from the striatum in mice, supporting the hypothesis that spread of α-syn-induced pathology may induce synaptic alterations contributing to the early non-motor symptoms of PD.

MTU04-08

PINK1 phosphorylated ubiquitin is a parkin activator

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PINK1 and *PARKIN* are causal genes for hereditary Parkinsonism. PINK1 is a Ser/Thr kinase that specifically localizes on depolarized mitochondria, whereas Parkin is an E3 ubiquitin ligase that catalyzes ubiquitin transfer to mitochondrial substrates. PINK1 acts as an upstream factor for Parkin, and is essential for activation of the latent E3 activity of Parkin and for recruiting Parkin onto depolarized mitochondria. Recently, mechanistic insights into PINK1/Parkin-mediated mitochondrial quality control have been revealed, and PINK1-dependent phosphorylation of Parkin has been reported. However, PINK1 function was not bypassed by phosphomimetic Parkin mutation, and how PINK1 accelerates the E3 activity of Parkin on damaged mitochondria is still obscure. Here we report that ubiquitin is the genuine substrate of PINK1. Ser65 of ubiquitin was phosphorylated by PINK1 *in vitro* and in cells, and a Ser65 phosphopeptide derived from endogenous ubiquitin was only detected in cells in the presence of PINK1 and following a decrease in mitochondrial membrane potential. Surprisingly, phosphomimetic ubiquitin makes PINK1 dispensable for activation of phosphomimetic Parkin mutant in cells, and phosphorylated recombinant ubiquitin activated phosphomimetic Parkin *in vitro*. The phosphorylation-dependent interaction between ubiquitin and Parkin suggests that phosphorylated ubiquitin unlocks autoinhibition for the catalytic cysteine. Our results show that PINK1-dependent phosphorylation of both Parkin and ubiquitin are sufficient for full activation of Parkin E3 activity, and thus phosphorylated ubiquitin is a Parkin activator.

MTU04-09

Increased TLR2 expression on Parkinson's disease neurons

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Inflammation contributes to the pathogenesis of Parkinson's disease, however, little is known about the inflammatory pathways involved. Toll-like receptors are an established family of pattern recognition receptors well known for regulating inflammation. We

therefore examined the expression of toll-like receptors in pathologically affected brain tissue from Parkinson's disease patients ($N = 17$) compared to unaffected controls ($N = 10$), obtained following approvals from the Sydney Brain Bank. Following protein extraction, Western blotting found an increased expression of TLR2. Immunohistochemical analysis with double fluorescence labeling in fixed tissue sections revealed that TLR2 was expressed by microglial cells, but also significantly upregulated on neurons in pathologically affected areas of Parkinson's disease brain. Increased neuronal TLR2 expression was highly associated with increased pathological accumulation of alpha-synuclein, and TLR2 staining was present in the majority of Lewy bodies. These results suggest a potential role for TLR2 in the pathogenesis of Parkinson's disease, and that TLR2 signaling in the disease state may not be confined to microglia.

MTU04-10

Genetic basis for male susceptibility to Parkinson's disease

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Whilst the cause of dopamine cell loss in Parkinson's disease (PD) is unknown, it is clear that the male-sex is a strong risk factor. The incidence and prevalence of PD is 2-fold higher in males, and disease progression more rapid in males than females. Aside from the protective actions of sex hormones, growing evidence suggests that sex-specific genes contribute to this male-bias in PD. We previously showed that the male-sex determining gene, SRY, co-localises with male dopamine neurons, where it regulates dopamine biosynthesis and motor function in males. Here, we investigated the regulation and function of nigral SRY in (i) normal and (ii) toxin (6-OHDA or rotenone) lesioned hemiparkinsonian rats. We assessed the effect of reducing nigral SRY levels, via repeated injection of SRY antisense oligonucleotide into the rat substantia nigra (SNc), on motor and dopaminergic function. In normal male rats, SRY antisense treatment significantly reduced motor function in the cylinder and rotarod tests (−21% and −24%), which was associated with a reduction in nigral TH mRNA and striatal dopamine content (−45% and −32%), compared to the sense-treated group. In contrast, SRY antisense treatment in female rats did not affect motor function. In toxin-injected male rats, nigral SRY mRNA was significantly up-regulated (+310 and 210% compared to vehicle control) at 7 days following 6-OHDA or rotenone injection. Remarkably, SRY antisense treatment significantly attenuated 6-OHDA and rotenone-induced motor deficit and dopamine cell loss in male rats, compared to the sense-control group, indicating a detrimental role for SRY in the injured male SNc. These data indicate a double-edged role for SRY in normal and injured dopamine neurons in the male SNc and that inhibition of SRY may be a novel therapeutic target for PD in males.

MTU04-11

PARK9 deficiency mediated behavioural and movement disorders in mice

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PARK9 (ATP13A2) is identified as a lysosomal integral membrane protein enriched in the brain. Mutations of *PARK9* are associated with early-onset Parkinsonism and dementia (Kufor-Rakeb syndrome, KRS). However, the function of PARK9 in regulating neural activity remains unclear including specific trans-membrane transport of different trace metals, alpha-synuclein and lipids. To investigate the roles of PARK9 in brain functions and aetiology of Parkinson's disease (PD), we sought to inactivate the *PARK9* gene in mice. Knockout of PARK9 resulted in discernible behavioural phenotypes characteristic of obsessive compulsive-like abnormality. While female PARK9 KO mice displayed excessive grooming, male PARK9 KO mice showed enhanced mating activity, in association with high levels of anxiety. We will present further evidence and discuss the molecular basis and regulations of the phenotypes by genetic factors and gene mutations in PD.

MTU04-12

LRRK2 regulates microglial α-synuclein clearance through the endocytosis pathway

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Introduction: α-Synuclein (αSYN) is the molecule responsible for Parkinson's disease (PD). Excess amounts of αSYN are toxic to neurons, finally resulting in PD. Leucine-rich repeat kinase 2 (LRRK2) is another molecule associated with PD. While LRRK2 is known to be expressed in microglia, its true function remains to be elucidated.

Aims: To examine whether microglial LRRK2 has a hitherto unrecognized functional role, we analyzed LRRK2-knockout (KO) microglia treated with αSYN.

Methods: Primary microglia isolated from LRRK2-KO and wild-type (WT) mice were treated with αSYN, and then analyzed by Western blotting, immunostaining, and ELISA.

Results: We investigated αSYN clearance by microglia isolated from LRRK2-KO mice and found that αSYN was taken up in larger amounts and cleared from the supernatant more effectively than from microglia isolated from WT mice. This higher clearance ability of LRRK2-KO microglia was thought to be ascribable to an increase

of Rab5-positive endosomes, but not Rab7- or Rab11-positive endosomes. Increased coordination between Rab5 and dynamin 1 was also observed in LRRK2-KO microglia. We also analyzed TLR4-mediated TNF-α production by LRRK2-KO microglia upon stimulation with αSYN and found that this was decreased in comparison with WT microglia.

Conclusion: Our research has revealed that LRRK2 negatively regulates the clearance of αSYN through down-regulation of the endocytosis pathway, and positively regulates the production of αSYN-induced TNF-α production. Our findings reveal a new functional role of LRRK2 in microglia and offer a new insight into the mechanism of PD pathogenesis.

MTU04-13

Impact of MPP^+ on mitochondria and on DAT and SERT internalisation in MESC derived dopaminergic and serotonergic neurons

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Altered mitochondrial function and dynamics seem to play an important role in the development and evolution of different neurodegenerative diseases due to the numerous important cellular functions that these organelles play, such as the regulation of calcium homeostasis, the production of energy as adenosine triphosphate (ATP), or the control of programmed cell death. Mitochondrial dysfunction has been linked with Parkinson's disease, a neuronal disorder characterized by loss of dopaminergic neurons in the *substancia nigra* of the brain, as a result of the incapability to provide energy to the cells and to enable synaptic communication. This project attempts to follow the mechanisms of MPP^+ poisoning (an inhibitor of complex I of the mitochondrial electron transport chain) in living, pharmacologically defined dopaminergic and serotonergic neurons derived from mice. To assess the aim of the study two different approaches are followed: first, we studied the effect of MPP^+ on mitochondrial heterogeneity and function in dopaminergic and serotonergic neurons differentiated from mouse embryonic stem cells (mES). Stem cell-derived serotonergic and dopaminergic neurons exhibited a dose-dependent toxic response to MPP^+ treatment, which elicited nuclear translocation of AIF. Results shown here indicate an increase in round shaped mitochondria in soma as well as in axon when serotonergic cells were treated with sub-lethal concentrations of MPP^+. The same tendency is observed in dopaminergic neurons. This data suggest an enhanced fission process caused by MPP^+ and would be in accordance with previous findings. In addition, the impact of MPP^+ toxicity on the regulation of cell surface dopamine (DAT) and serotonin (SERT) transporter expression is discussed.

MTU04-14

Inflammation and dopamine synthesis in neurodegeneration

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Inflammation has been known to occur during neurodegenerative process and could be a critical element in the development of the disease. A single peripheral injection of lipopolysaccharide (LPS) caused a slow progressive loss of dopaminergic neurons in the substantia nigra (SN), but not in the ventral tegmental area (VTA). An understanding of why dopaminergic neurons and in particular the cells of SN should be specifically targeted have been lacking. The aim of this study was to investigate inflammation and dopamine synthesis in specific subset of dopaminergic neurons in an inflammatory animal model of Parkinson's disease. Simultaneous measurements of cytokines levels and tyrosine hydroxylase regulation (TH phosphorylation, TH activity and TH protein) in brain (SN and VTA) tissue extracts from LPS-peripheral injected rats were compared to saline at different time points. We found significant increases in IL-1α, IL-1β, IL-6, IL-10, IL-12(p70), IFN-γ and TNF-α levels in SN 1 day after LPS injection and all had returned to basal levels by 1 week. GM-CSF levels were found increased in SN 6 months after LPS injection. In contrast, all measured cytokines were unchanged in VTA between 1 day and 6 months after LPS injection. We found significant increases in TH phosphorylation and TH activity levels in VTA 1 day after LPS injection and both had returned to basal levels by 1 week. In SN there was a similar increase in TH phosphorylation and TH activity at 1 day but TH activity remained increased up to 1 month after LPS injection. This was despite there being that no increased TH phosphorylation in SN at the later times. We found significant increases in TH protein levels in SN up to 1 month after LPS injection but no changes in VTA. TH protein level in SN was significantly decreased 3 months after LPS injection. This indicates that there are substantial differences in the effect of LPS injection on inflammation and dopamine synthesis in SN and VTA.

MTU04-15

Hereditary parkinsonism-associated mutations in ATP13A2 (PARK9) cause glycolytic dysfunction

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Kufor-Rakeb syndrome (KRS) is an autosomal-recessive, juvenile-onset, levodopa-responsive Parkinson's disease (PD). Mutations in *ATP13A2* (*PARK9*), a gene encoding a lysosomal P5-type ATPase, have been identified as a genetic cause of KRS. Although the physiological function of ATP13A2 remains largely unknown, ATP13A2 is predicted by sequence homology to be a cation transporter, with several metal ions having been associated with ATP13A2. Previously, we reported KRS patients carrying compound heterozygous ATP13A2 mutations (p.Leu1059Arg/p.Leu1085TrpfsX4) and showed that cell lines derived from these patients had a complete loss of ATP13A2 protein due to proteasomal degradation and manifested with mitochondrial dysfunction.

Recently, we and others identified abnormal Zn^{2+} metabolism in KRS patient-derived cell lines, implicating ATP13A2 as a Zn^{2+} transporter, and demonstrated its pathogenic involvement in mitochondrial dysfunction. In addition to mitochondrial energy production, the glycolytic pathway also plays a crucial role in cellular bioenergetics by providing pyruvate for mitochondrial ATP production. Interestingly, increased intracellular Zn^{2+} has been shown to inhibit glycolytic function. These findings prompted us to hypothesise that glycolytic function may be impaired in KRS and we tested this hypothesis using KRS patient-derived cells by measuring extracellular acidification rate and biochemical parameters of glycolysis, in addition to assessing the efficacy of pyruvate supplementation in promoting mitochondrial ATP production and cell viability. KRS patient cells showed a marked decrease in the maximal glycolytic activity as well as pyruvate/lactate production and the NAD^+/NADH ratio, indicating glycolytic dysfunction in KRS patient cells. Glycolytic dysfunction was aggravated by treatments that increased intracellular Zn^{2+} concentrations, while ATP13A2 overexpression and pyruvate supplementation completely blocked the Zn^{2+}-mediated toxicity. Furthermore, addition of pyruvate significantly increased mitochondrial ATP production and abolished Zn^{2+} -induced toxicity on mitochondria, indicating that glycolytic dysfunction contributes to the pathogenesis of KRS by further impairing mitochondrial function. Taken together, our data indicate that human ATP13A2 deficiency results in Zn^{2+} dyshomeostasis and impaired cellular bioenergetics, providing a valuable insight into the molecular mechanisms, with ATP13A2 as a molecular link, underlying these two established aetiological factors for PD.

MTU04-16

The effect of TLR agonists on LRRK2 S910/S935 phosphorylation in human peripheral blood mononuclear cells

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Missense mutations in leucine-rich repeat kinase 2 (LRRK2) are the major cause of autosomal-dominantly inherited Parkinson's disease (PD). The most common pathogenic LRRK2 mutation increases the enzymes catalytic kinase activity, resulting in much industry investment in the development of LRRK2 kinase inhibitors as potential PD therapeutics. The standard readout of LRRK2 inhibitor potency in cell and animal models involves measuring the phosphorylation levels of LRRK2 on serines 910 and 935. Constitutive phosphorylation on these residues is decreased in a dose-dependent manner with LRRK2 kinase inhibition. Recently we found that phosphorylation levels on LRRK2 S910/S935 could also be increased when mouse macrophage cells are treated with certain agonists of the inflammatory toll-like receptor (TLR) pathway. In order to know if this is also the case in humans, we have performed the same set of experiments on human immune cells. The data again showed that TLR agonists blunt LRRK2 kinase inhibitor-induced dephosphorylation of S910/S935 in a dose-dependent manner. This suggests that inflammation or activation of TLR pathways has the potential to complicate the use of these phosphorylation sites as a readout of the potency of LRRK2 kinase inhibitors *in vivo*. Indeed, it remains unknown if these phosphorylation sites behave differently in patients with PD, where LRRK2 and inflammatory pathways are thought to be pathologically involved. Thus, in a final set of experiments we will study immune cells from PD patients treated *ex vivo* with LRRK2 kinase inhibitors. These studies will determine whether phosphorylation of LRRK2 S910/S935 is similarly reduced with inhibitors in PD patients compared to controls, and thus, whether these phosphorylation sites can be used as peripheral pharmacodynamic biomarkers for LRRK2 inhibitors in patient trials.

MTU04-17

Effects of mesenchymal stem cell differentiation induced by linear micro and nano-topology and the associated MIRNA profiling

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Precursor cell differentiation of Dopamine (DA) neural is the most feasible way for Parkinson`s disease (PD) treatment, and Mesenchymal Stem Cells (MSC) have potentiality into DA neuron differentiation which was influenced by many factors. Physics factor was focused on the MSC proliferation and differentiation. We used processing technique of micro and nano-meter topology to get 0.7 and 2 μm substrates with linear pattern (LMP) for MSC culturing. We found that LMP depressed adult neural stem cells proliferation when compared to non-patterned substrates. Thus, this finding has led us to the rational for the proposed research, which will validate LMP and relative miRNA could regulate MSC into DA differentiation. The aims of study are: (i) Improve the LMP substrates and choose optimal substrates to increase MSC into DA differentiation; (ii) Microarray and real-time PCR were used to select relative miRNAs about differentiation of MSC. Then cells were infected with lentivirus-miRNAs, for investigating the molecular mechanism of MSC into DA differentiation by miRNAs; (iii) Analyze the therapeutic effect with DA induced by LMP and relative miRNAs in PD animal model. The data showed that LMP could increase the differentiation of MSC to DA. There are up-regulated miRNAs and down-regulated miRNAs checked in MSC cultured on LMP.

Taken together, successful completion of these studies will therefore form a basis for algorithms to help elucidate mechanism of MSC regulating DA neuron differentiation by nano-substracts and miRNA, to provide the strategies aimed at laying the foundation for further PD clinical studies.

MTU04-18

The interaction of MIRO1 and α-synuclein in mitophagy

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Mitophagy is a selective degradation of mitochondria that prevents the accumulation of damaged organelles. Mitochondrial impairments are central to Parkinson's disease (PD) pathogenesis. Mutations of α-synuclein are linked to familial PD. α-synuclein has been shown to bind to the mitochondrial membrane, and increased membrane-bound α-synuclein in PD contributes to the functional

disturbance of mitochondria. Overexpression of A53T-mutated α-synuclein has been shown to induce mitophagy *in vitro* and *in vivo*. Mutations in the PINK1 and PARK2 genes are associated with PD, and corresponding proteins Pink1 and Parkin are crucial in the mitophagy. Miro1 is an outer mitochondrial membrane protein involved in mitochondrial trafficking. It is degraded shortly after mitochondrial damage in a Pink1 and Parkin-dependent mitophagic pathway. The aim of this study was to elucidate the involvement of Miro1 in α-synuclein-induced mitophagy. We have found that α-synuclein is one component of the Miro1 interactome. Moreover, co-expression of Miro1 restored mitochondrial length and density in primary neuronal culture overexpressing A53T-mutated α-synuclein. Miro1 overexpression did not change the basal mitophagy, but decreased significantly α-synuclein-induced mitochondrial removal. Together, our results suggest that Miro1 and α-synuclein may interact in the mitophagic pathway.

MTU04-19

Cytotoxicity of low-dose dopamine is mediated by α-synuclein induced mitochondrial dysfunction in shsy5y cells

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In catecholaminergic neural cell lines or primary culture of mesencephalic neurons, toxins like MPTP, rotenone, paraquat, 6-hydroxydopamine cause dose-dependent cell death through multiple mechanisms which are extensively studied to understand the neurodegeneration of Parkinson's disease (PD). However, these toxins are not endogenously present and thus cannot explain the genesis of clinical cases of sporadic PD, and hence the use of dopamine (DA) as a neurotoxin in a cell based model is physiologically more relevant. The cytotoxic effects of dopamine (DA) in several catecholaminergic cell lines such as PC 12 and SHSY5Y have been demonstrated using a very high non-physiological concentration of DA. In contrast, we have shown here that exposure to low concentrations of DA (10 μM) for 96 h leads to apoptotic cell death and mitochondrial dysfunction in SHSY5Y cells as well as an accumulation of α-synuclein. These effects of DA are abolished by both the reactive oxygen species (ROS) scavenger N-acetyl cysteine (2.5 mM) and NF-κB inhibitor SN50 (20 μM) implying a role of redox signalling. Cyclosporin A which blocks mitochondrial permeability transition pore and mitochondrial depolarization also prevents cell death under our experimental conditions. More interestingly, the DA cytotoxicity is prevented by knocking down α-synuclein gene by specific siRNA. In separate experiments, it has been observed that α-synuclein causes mitochondrial membrane depolarization during *in vitro* incubation which can be blocked by cyclosporine. Our results tend to suggest that α-synuclein mediated mitochondrial depolarization triggers apoptotic death of SHSY5Y cells under low DA exposure.

MTU04-20

Effect of TLR2 and TLR3 activation on SH-SY5Y neuronal-like cells: potential implications for Parkinson's disease

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Parkinson's disease (PD) is a debilitating multifaceted neurodegenerative disorder. In some instances, PD can be caused by genetic mutations with evidence from these cases suggesting key roles for inflammation, mitochondrial dysfunction and autophagy dysfunction in the onset of PD. Inflammation, mitochondrial function and autophagy can all be regulated by innate immune toll-like receptors (TLRs). TLR activation is commonly observed in microglial cells however, interestingly, TLR2 and TLR3 are also expressed on neurons. It is largely unknown how TLR activation effects inflammation, mitochondrial function and autophagy in neuronal cells but this may be important as TLR2 is increased on neurons in PD brain. Using multiplex inflammatory cytokine ELISAs we have found that TLR2 and TLR3 agonists indeed induce an inflammatory response in neurons, leading to the secretion of a number of pro-inflammatory cytokines and microglial recruiting chemokines. TLR2 activation also resulted in the production of reactive oxygen species whilst both TLR agonists promoted autophagy. These results suggest that neurons themselves may contribute to the toxic inflammatory milieu found in PD brain.

MTU04-21

Sodium butyrate, a histone deacetylase inhibitor, attenuates 6-hydroxydopamine induced hemi-Parkinsonism in rats

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Parkinson's disease (PD) is the second most common neurodegenerative disorder. Recent studies have investigated the involvement of epigenetic modifications in PD. An imbalance between the acetylation and deacetylation of the histone proteins has been reported in PD. This acetylation and deacetylation regulates the expression of various neuroprotective genes. Histone deacetylase (HDAC) inhibitors have been reported to be beneficial in cognitive and motor deficit states. The present study was designed to investigate the effect of sodium butyrate, a HDAC inhibitor in 6-hydroxydopamine (6-OHDA) – induced experimental PD like symptoms in rats. To produce motor deficit, 6-OHDA was administered unilaterally in the medial forebrain bundle. 3 weeks after 6-OHDA administration, the rats were challenged with apomorphine. Following this, the animals were treated with sodium butyrate (150 and 300 mg/kg i.p) once daily for 14 days. Movement abnormalities were assessed by battery of behavioral tests. Moreover, levels of oxidative stress markers, neuroinflammation and dopamine were measured in striatal brain homogenate. Further, to explore the molecular mechanism, we measured the level of global H3 histone acetylation and brain derived neurotrophic factor (BDNF). 6-OHDA administration results in increased contralateral rotations after apomorphine challenge. Also, 6-OHDA administration results in motor deficits along with significant reduction in striatal dopamine level. Moreover, 6-OHDA treated rats showed

elevated oxidative stress and neuroinflammatory markers. Treatment with sodium butyrate results in significant attenuation of motor deficits and increased striatal dopamine level. Moreover, sodium butyrate treatment attenuated the oxidative stress and neuroinflammatory markers. These effects occur concurrently with increased global H3 histone acetylation and BDNF levels. Thus, the observed results of the present study are indicative for the therapeutic potential of HDAC inhibitors in PD.

MTU04-23

Investigation of the co-function of parkin and PACRG

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Mutations in parkin (*PARK2*) result in early-onset Parkinson's disease (PD). *PARK2* shares a bidirectional promoter with Parkin Co-Regulated (*PACRG*). Bidirectionally regulated genes have been shown to function in common biological pathways. Parkin is an ubiquitin ligase that mediates protein and organelle turnover via the proteasome and autophagy systems. PACRG is a highly conserved protein of unknown function that has been associated with cilia, aggresome formation and autophagy in response to proteasomal impairment. *We hypothesise that the function of PACRG is coupled to parkin by its co-regulation through the shared bidirectional promoter.*

Evolutionary analysis determined that the *PARK2-PACRG* bidirectional promoter originated from an exon gain during the chordate-vertebrate transition, and that a number of motifs within the promoter are highly conserved in descendants. Furthermore, the very large genomic span of both genes originated simultaneously with the establishment of the bidirectional promotor. Relative expression of *PARK2* and *PACRG* was determined in an array of human tissues, including 22 unique brain regions. *PARK2* and *PACRG* were found to be convergently expressed in the brain tissues only. Collectively, the data suggests that parkin and PACRG function together in the mammalian brain.

To investigate the co-function of parkin and PACRG *in vivo*, two novel mouse models were developed: a *Pacrg* knockout and a *Park2-Pacrg* knockout. At 12 months of age, neuropathological examination suggested there was no gross abnormality of the dopaminergic system in the substantia nigra and no significant change in the number of dopaminergic neurons in either knockout model. However, in the single *Pacrg* knockout there was a trend of a ~15% reduction in the number of dopaminergic neurons ($P = 0.07$). Given that PD is an age dependent syndrome, behavioural, pathological and neurochemical analysis of an increased number of mice at older time points is ongoing to fully characterise the phenotype of these models.

MTU04-24

A novel proteinaceous aggregate associated with neuronal loss in Parkinson's disease

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Movement symptoms characterising Parkinson's disease (PD) arise from the progressive and selective death of vulnerable dopaminergic neurons within the substantia nigra. Protein aggregation is believed to represent a key component in the neurodegenerative cascade, representing a significant target for the development of disease-modifying therapies aimed at attenuating nigral degeneration. Abnormal aggregation of α-synuclein protein in PD is linked to neuronal loss in this disorder; however the widespread end-stage distribution of α-synuclein pathology exceeds the more limited pattern of neuronal death. Our group has previously shown that the activity of the key neuronal antioxidant, superoxide dismutase 1 (SOD1), is altered in PD in a regional pattern reflecting neuronal loss. Employing immunofluorescence studies in post-mortem human brain tissue we have recently identified a novel SOD1-immunopositive proteinaceous aggregate in the PD brain, the distribution of which more closely corresponds to the pattern of neuronal loss in PD than that of α-synuclein pathology. An increase in high-molecular weight SOD1 protein in immunoblots, and a positive isoelectric point shift in SOD1, in nigral tissue from the PD brain compared with healthy age-matched controls are also indicative of conformational, oxidative, or post-translational modifications in SOD1 in the vulnerable substantia nigra in the PD brain. Aggregation of SOD1 protein is strongly linked to motor neuron death in amyotrophic lateral sclerosis, thus we hypothesise that aggregation of SOD1 may also be involved in neuronal death in PD. The identification of a novel vulnerability-specific pathological phenomenon in the PD brain may provide a new target for disease-modifying therapies for PD.

MTU04-25

Influence of sumoylation inhibitors on a Parkinson's disease cell model

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An array of cellular responses attempt to defend cells from the formation of protein aggregates in neurodegenerative diseases, including Parkinson's disease (PD), by removing or detoxifying the aggregated proteins. SUMO-1 participates in the autophagy response to protein aggregates via SUMOylation of lysosomal Hsp90. In this study, the influence of two specific chemical inhibitors of the SUMOylation E1 enzyme, ginkgolic acid and

anacardic acid, on a cellular model of PD was determined. Depolarization of SH-SY5Y neuroblastoma cells with 50 mM KCL was used to induce calcium influx and α-synuclein aggregation analogous to that observed in PD. Parallel studies were conducted on a cellular model of Huntington's disease. Immunofluorescence revealed a decrease in the frequency of α-synuclein aggregates under both ginkgolic acid ($p < 0.01$) and anacardic acid ($p < 0.1$) treatments at 10 μM. It also revealed that there was a significant reduction of punctate co-localization of SUMO-1 and lysosomal marker, Cathepsin D, under ginkgolic acid ($p < 0.001$) and anacardic acid ($p < 0.01$) treatments at 10 μM. Furthermore, there was significant up-regulation of LC3 ($p < 0.05$), marking autophagosomes, under ginkgolic acid treatments from 3 to 10 μM and under anacardic acid treatments from 6 to 10 μM. The findings suggest that inhibition of the SUMOylation pathway disrupts SUMO-1 co-localization with lysosomes. The increased levels of LC3-positive autophagosomes and decline in the frequency of protein aggregates in cell culture suggest that macroautophagy may have a key role in mediating protein aggregate clearance in response to SUMO-1 inhibition. On-going studies are analyzing Hsp90-SUMOylation and its relation to autophagy under SUMO-1 inhibition.

MTU04-26

The interplay between Purinergic signaling and the toxicity of extracellular alpha-synuclein

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Extracellular ATP plays an important role in neurotransmission and neuromodulation processes that are mediated by the activation of cell surface receptors including ligand-gated ion channels (P2X) and G-protein coupled (P2Y) receptor subtypes. Recently, extracellular ATP and specific purinergic receptor subtypes have been shown to be involved in neurodegenerative disorders including Parkinson's and Alzheimer's diseases. However, till now the mechanisms underlying the disturbances in ATP-mediated neurotransmission are not well established. Our previous results indicated that α-synuclein (ASN), a presynaptic protein, which under pathological conditions is deposited in neurons in the form of Lewy bodies, plays an important role in cell death pathway. It was suggested that ASN liberated from damaged cells into extracellular space could be involved in progression of neurodegeneration. In this study we postulate that extracellular ASN may alter ATP-dependent signaling and that P2 purinergic receptors and ATP are involved in the neurotoxicity induced by this protein. The experiments performed in human SH-SY5Y neuroblastoma cells, differentiated with the all-trans retinoic acid (ATRA), showed that ASN (10 μM) evoked cell death about 50% and promoted the extracellular release of ATP, causing the increase in Ca^{2+} influx. This effect was partially eliminated by the P2X receptors antagonist, PPADS (200 μM). Moreover, this antagonist prevented the cytotoxic effect evoked by extracellular ASN as well as ATP. Interestingly, the elevated cytosolic Ca^{2+} concentration, induced by the activation of P2 receptors, resulted in the significant increase of intracellular ASN. Further investigation revealed that the intracellular accumulation of ASN is a result of its decreased release caused by ATP-treatment. Taken together, our results indicated the interplay between purinergic signaling and the toxicity of extracellular ASN. These findings provide new insight into our knowledge of the role of purinergic neurotransmission in Parkinson's disease and may be helpful in identifying new therapeutic targets for this disease.

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MTU04-27

Identification of RAB39B mutations in early-onset Parkinson's disease

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Recent advances in understanding the aetiology of Parkinson's disease (PD) have been driven by the identification of causative familial mutations. However, only ~10% of patients have a defined genetic aetiology, suggesting that variants in additional genes remain to be identified.

We studied an Australian kindred with 3 males displaying early-onset Parkinsonism with intellectual disability. Genetic analysis identified a ~45 kb deletion in the affected males resulting in the complete loss of *RAB39B*. Subsequent analysis of an independent Wisconsin kindred displaying a similar phenotype identified a missense mutation in *RAB39B* (c.503C>A [p.Thr168Lys]). *In silico* and *in vitro* studies demonstrated that the missense mutation resulted in protein destabilisation and loss of function.

The role of Rab39b was investigated *in vitro* utilising shRNA mediated knockdown in cultured mouse hippocampal neurons. Reduction of Rab39b levels by ~50% correlated with a ~50% reduction in α-synuclein levels. Similarly, the density of dendritic α-synuclein immunoreactive puncta was significantly reduced compared to the scramble control (0.52 ± 0.03 vs. 0.76 ± 0.03, mean±SEM, $p \leq 0.0005$) suggesting a role of Rab39b in α-synuclein homeostasis.

Post mortem examination demonstrated typical PD neuropathology, including loss of dopaminergic neurons in the substantia nigra and classic lewy pathology. Several non-conventional features were also observed including cortical Lewy bodies, axonal spheroids, iron accumulation and tau immunoreactivity. To further investigate the *in vivo* pathogenesis we have used CRISPR/CAS9 genome editing to generate a *Rab39b* knockout mouse. Ongoing biochemical and neuropathological studies are expected to provide insight into mechanisms underlying RAB39B-mediated neurodegenerative disease.

Overall, we have demonstrated that loss of *RAB39B* causes early-onset PD and dysregulation of α-synuclein homeostasis. This implicates RAB39B in the pathogenesis of PD and potentially other neurodegenerative disorders.

MTU05 Neurological Dysfunction

MTU05-01

Characterisation of the AMPA and kainate receptor in the hypoxic ischaemic piglet

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Severe perinatal asphyxia occurs in 4/1000 births. Combined hypoxic and ischemic (HI) injury is one of the main causes of the neurodevelopmental sequelae observed. In research to date, it has been shown that during the insult there is an excessive production of the excitatory neurotransmitter, glutamate. Glutamate receptors, AMPA/Kainate, are concentrated in the brain regions susceptible to HI neuronal injury. Activation of these receptors *in vitro*, results in neuronal death. Studies *in vivo* show that systemic administration of AMPA/Kainate antagonists prevent injury. This investigation characterised the AMPA and Kainate receptor subunits (GluR 1, 2, 5, 6 and KA 1, 2) in the neonatal pig brain. Piglets (< 24 h old, $n = 45$) were anaesthetised, ventilated and subjected to 30 min HI insult. Subsequently piglets were recovered for 72 h. Following euthanasia, brain tissue was collected and stored at −80°C. The binding profile and expression of AMPA and Kainate receptor subunits was measured using tritiated-ligand binding and western blot techniques. Changes in the binding were observed between both anaesthetised control and HI piglets. Similarly, western blots demonstrated variances in the expression of receptor subunits in HI piglets when compared to controls. When collated, the loss in AMPA and Kainate receptor subunits alters the profile of the receptors in the HI brain. This has implications for the treatment of HI insults in the human infant: whereby excitotoxic injury can be reduced, at least in part, by protecting AMPA/Kainate receptor function.

MTU05-02

Effect of angiotensin-receptor-blocker in mouse model of chronic fatigue syndrome

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Chronic Fatigue Syndrome (CFS) is post-infectious syndrome that results in a dramatic decline in physical activity and stamina. The present study aimed to explore the role of angiotensin-receptors in chronic fatigue syndrome. Swiss albino mice (either sex; 6–8 weeks; 25–30 g) were used in this study. The experimental protocol was approved by institutional Animal Ethics Committee. Candesartan (Angiotensin-receptor blocker) was administered daily to separate groups of mice in two doses (1 and 2 mg/kg; i.p.) for 21 successive days. CFS was induced in mice by administration of Lipopolysaccharide (1 mg/kg; i.p.) followed by exposing the mice to swimming for a period of 10 min daily for 21 successive days. After 21 days, behaviour assessment of each mouse was done using elevated plus maze (for memory), elevated zero maze (for anxiety), open field (for ambulation) and tail-immersion test (for stress induced hyperalgesia). After behavioural evaluation, the blood glucose, brain TBARS and GSH levels were measured. Administration of candesartan significantly ($p < 0.05$) reduced the immobility time of mice during their forced swim session as compared to control group. Further, administration of candesartan to mice not only prevented memory impairment, but also exerted anxiolytic activity in mice as compared to CFS controls. Candesartan treated mice showed a significant ($p < 0.05$) increase in GSH and decrease in brain TBARS. Thus, angiotensin-receptor blockers may prove to be a useful remedy for the management of chronic fatigue syndrome.

MTU05-03

Obesity or excess energy intake is not necessary for diet-induced cognitive deficits: the role of omega-6, saturated fat and sugar

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High energy diets impair cognition however, the rapidity and time-course of these memory deficits and the relative contributions of fat and sugar remain unclear. We have previously shown that rats exposed to a cafeteria diet (± 10% sucrose solution), or regular chow supplemented with 10% sucrose showed impaired hippocampal-dependent place, but not perirhinal-dependent object recognition memory after 5, 11 and 20 days exposure. The deficits significantly correlated with hippocampal inflammation while neurotrophic markers were unaffected (Beilharz, Maniam, & Morris, 2014). To determine whether inflammation was present when the deficits first emerged, a new cohort ($n = 12$/group) was culled after the first memory test. We again found impaired place, but not object, recognition in the high energy groups after 8 days. The rats consuming chow plus sucrose had 25% higher hippocampal TNF-α mRNA expression than the cafeteria rats and the memory deficits were correlated with higher IL-1β mRNA. Hypothalamic inflammatory and neurotrophic markers were unchanged. Next, to separate the effects of fat and sugar, a second cohort was fed novel in-house diets of comparable energy density varying only in their fat and sugar content ($n = 12$/group). Rats fed a high saturated fat or sugar diet showed similar place deficits after 8 days, while rats fed a polyunsaturated fat diet high in omega-6 had improved place recognition (Place ratios- Control 0.66; Saturated fat 0.54; Sugar 0.54; Polyunsaturated fat 0.75). All groups showed similar object memory. The diets did not affect hippocampal inflammation but in the hypothalamus NfκBia and TLR4 mRNA were down-regulated in the fat and sugar groups indicating dampened inflammation. Together these results show that hippocampal-dependent memory is particularly sensitive to dietary manipulations. Spatial deficits occurred prior to body or fat mass differences and were specific to the dietary macronutrient composition. The rapid inflammatory changes observed appear to be driven by the liquid sugar component of the diet.

MTU05-04

Molecular changes within the ageing inner ear

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Dizziness, vertigo and loss of balance are common in the elderly and can result in quality of life loss and fall-related hospitalisations. Impairment of the inner ear vestibular organs is thought to be a contributing factor to these conditions. Inner ear, vestibular hair cells are sensory cells that detect head motion through precise regulation of ionic fluxes across cell membranes. During ageing, vestibular hair cells do not decline in number until decades after functional impairment. This has led us to suggest other more complex molecular mechanisms underlie vestibular dysfunction. However, nothing is known about the effects of ageing on the molecular profile of vestibular organs, so we have initially taken a discovery based genomics approach to identify potential mechanisms for more targeted analyses. We conducted a genome-wide gene expression analysis of vestibular organs from both rats and mice, comparing young and old. We discovered age-related changes in expression for genes involved in candidate mechanisms, such as mechanotransduction, ion homeostasis, and mitochondrial energy production, all of which have the potential to perturb vestibular function. A number of genes found to be differentially expressed based on our microarray expression analysis, have been confirmed by quantitative Polymerase Chain Reaction (qPCR). For example, the gene for the cystic fibrosis transmembrane conductance regulator (CFTR), a chloride channel, was found to be upregulated in both the vestibular organs and cochlea of old animals. We have also confirmed this channel has a functional role in vestibular hair cells of young animals. This CFTR finding is novel and indicates the channel may play an important role in the inner ear hair cells of both young and old animals, including humans. In addition to CFTR, our data indicate mechanotransduction and mitochondrial processes may contribute to vestibular dysfunction with age. These results are the first of their kind and open up potentially new avenues of research into ageing and the vestibular balance system.

MTU05-05

Tricaprin - a new anticonvulsant with multiple mechanisms of actions

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The ketogenic diet reduces seizures in children with epilepsy. Because the diet is too restrictive to follow for most people, medium chain triglycerides, which can increase blood ketone levels, have been investigated as more manageable alternatives. We found that feeding 35% of calories as tricaprin, the triglyceride of capric acid (C10), was anticonvulsant in two acute mouse seizure models, namely the fluorothyl and the 6 Hz model. There were no significant increases in ketone levels. However, we found several protective mechanisms that are likely to be involved in the anticonvulsant effects. The plasma of the tricaprin-fed mice showed an increased antioxidant capacity, similar to the effects found with the well-known antioxidant sulforaphane, indicating that capric acid is an antioxidant. Also, after tricaprin feeding mRNA levels of several antioxidant genes were upregulated in the hippocampus, namely Heme oxygenase I (Hmox1) and Fork-head box O1 (FoxO1), a transcription factor which can regulate Hmox1, superoxide dismutase and catalase. In cultured astrocytes, 200 μM capric acid (a concentration that has been achieved in the plasma of children fed medium chain triglycerides) increased basal respiration and ATP turnover, indicating that it is an alternative fuel used by astrocytes. In summary, antioxidant mechanisms as well as the provision of an alternative fuel source may contribute the anticonvulsant effects of tricaprin.

MTU05-06

Effects of prolonged diazepam administration on hippocampal EEG and histology in a mouse model of temporal lobe epilepsy

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Mesial temporal lobe epilepsy (MTLE) is highly resistant to treatment by current anti-epilpetic drugs. Characteristic of MTLE is a prolonged seizure-free period that occurs between the initial insult (status epilepticus, stroke, trauma) and the onset of intractable, spontaneous, recurrent, focal seizures. Epileptogenic processes likely occur during the seizure-free period and targetting these may be able to reduce the subsequent appearance of intractable seizures. This study investigated the effect of administering diazepam during the putative epileptogenic period in a mouse model of MTLE. Twenty-one mice were stereotaxically injected with kainic acid into the right hippocampus (50 nL, 20 mM; AP −1.8, ML −2.0, DV −2.0) and subsequently underwent status epilepticus (SE) for 3 h (terminated with diazepam, 5 mg/kg i.p.). Over the next 5 days, 12 mice were injected twice daily with diazepam (10 mg/kg i.p.), the remaining 9 injected with saline carrier. All mice had electrodes implanted into the left and right hippocampus (AP −1.8, ML ±2.0, DV −2.0) with 2 h EEG recordings taken 4, 5 and 6 weeks post SE. Brains were harvested, Nissl stained, and analysed histologically following the week 6 EEG. The EEG seizure phenotype was significantly improved in diazepam treated mice (12.71 ± 4.77 seizures/h, $n = 12$) as compared to the saline controls (36.14 ± 3.83 seizures/h, $n = 9$, $p < 0.05$). Interestingly in the diazepam treated cohort, 7/12 mice had fewer than 4 seizures/h, the remaining 5/12 being similar to control, suggesting groups of responders and non-responders. All mice with high seizure frequencies displayed obvious hippocampal sclerosis and dentate gyrus dispersion whereas mice with low seizure frequencies did not have obvious histological abnormalities. Overall our results demonstrated that diazepam administration during the epileptogenic period mitigated subsequent seizures in some mice suggesting that reduced GABAergic signalling may be a factor in epileptogenesis.

MTU05-07

Aquaporin expression correlates with astrocytoma grade and microvascularity

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Background: The poor prognosis of malignant primary brain tumours and the limited gains in median survival with orthodox chemotherapies has stimulated intense interest in novel molecular interventions targeted against specific tumour mechanisms. Numerous lines of evidence now suggest that aquaporins (AQPs), transmembrane water channels, may participate in multiple fundamental processes in brain cancer pathophysiology, including angiogenesis and cell migration. The present study sought to characterise the expression of AQP1 and AQP4 in human brain tumours.

Methods: Human archival astrocytoma specimens of grades II ($n = 9$), III ($n = 6$), and IV ($n = 11$) and normal brain ($n = 3$) were used. Standard streptavidin-biotin immunohistochemistry was applied to stain for AQP1, AQP4, and the endothelial marker CD34. To investigate associations between AQPs and angiogenesis, the AQP expression profile was correlated against microvascular density in each tumour specimen.

Results: Increased staining was observed for AQP1 and AQP4 in all tumour samples, with statistical significance versus our limited number of normal brain samples obtained in grade IV for AQP1 ($p < 0.01$), and in grades III and IV for AQP4 ($p < 0.05$, $p < 0.01$ respectively). Significance was also obtained between high-grade and low-grade lesions for both AQPs ($p < 0.05$), suggesting an association between AQP expression and tumour grade. Significant positive correlations were observed between microvascular density and both AQP1 ($R^2 = 0.87$) and AQP4 ($R^2 = 0.76$).

Interpretation: These results suggest that AQPs may facilitate the progression of malignant gliomas, and support their role in angiogenesis. Further studies using quantitative protocols such as ELISA would corroborate these findings.

MTU05-08

Rauwolfia vomitoria and gongronema latifolium extracts combination protects young rats' dentate nucleus

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Rauwolfia vomitoria (*RV*) and *Gongronema latifolium* (*GL*) are two herbs with medicinal properties used independent of each other. *RV* acts as a sedative and is use locally in psychiatry management, but adverse effects have also been ascribed to it. There is little or no reported adverse effect of *GL*, an antioxidant-rich herb. Hence, this study was to investigate the potency of the combined herbs on the dentate nucleus of Wistar rat. 24 young Wistar rats of average body weight, 130 g were grouped equally into 4 groups of six animals each. Group 1 animals were designated the control and administered a placebo, while groups 2–4 were designated the test groups and were respectively administered either 200 mg/kg body weight of *RV* or *GL*, or their combination for 14 days. On day 15, the animals were anaesthetized with ketamine hydrochloride (i.p.) and sacrificed. Their brains were preserved by transcardial perfusion using 10 % buffered formalin. The brains were extracted and the dentate gyrus excised and routinely processed for histomorphology and immunoreactivity. Histomorphology results of the dentate nucleus showed pyknosis and karyorrhexis of the granular cells in the *RV* group. These features were not found in the *GL* group, and were reduced in the *RV+GL* compared with the control group. GFAP expression was up-regulated in the *RV* group, but unaltered in the *GL* group, while being slightly up-regulated in the *RV+GL* group. Neuron enolase 2 expression was down-regulated in the *RV* and *GL* group but slightly up-regulated in the RV+GL group compared with control. In conclusion, *RV* and *GL* combination protects the brain from *RV* induced dentate nucleus injury and may be considered as an adjuvant for *RV* treatment.

MTU05-09

In vivo electrophysiological characterisation of mouse lumbosacral dorsal horn neurons receiving visceral inputs

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Chronic abdominal pain is a common symptom of Inflammatory Bowel Disease (IBD) that often persists in the absence of active inflammation. While the mechanisms responsible for pain are unknown, preclinical evidence suggests plasticity within the spinal cord dorsal horn (DH) is involved. We therefore developed an *in vivo* preparation to study the properties of DH neurons that receive inputs from the colon. Mice (C57Bl/6J, male, ~P35) were anaesthetised (isoflurane, 1–3%) and the L6-S1 spinal cord segments were exposed. Whole-cell patch-clamp recordings were made from laminae I-II DH neurons. We tested whether neurons received colonic inputs via noxious colorectal distension (CRD, 80 mmHg) and then assessed neuron intrinsic and synaptic properties. Recordings were obtained from 80 DH neurons, 10 of which responded to noxious CRD. Action potential discharge was observed in response to CRD in 3/10 neurons, while the remaining 7 neurons responded with subthreshold depolarisation or hyperpolarisation. Most CRD-responsive neurons (80%) also had a cutaneous receptive field compared with < 50% of CRD-nonresponsive neurons. Several differences were observed in the intrinsic and synaptic properties of CRD-responsive and -nonresponsive neurons. CRD-responsive neurons had a hyperpolarised resting membrane potential, larger rheobase currents, and reduced levels of excitatory drive. In summary, we have identified differences in the properties of neurons that receive visceral input in naïve mice. Our preparation, which allows *in vivo* patch-clamp recording, will permit future detailed analysis of the mechanisms that determine DH neuron excitability in inflammatory conditions that produce abdominal pain.

MTU05-10

Zinc-dependent regulation of synapse function in autism spectrum disorders

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Autism Spectrum Disorders (ASD) are a set of neurodevelopmental disorders characterised by impaired communication, social behaviour, learning deficits and repetitive behaviour. The behavioural dysfunctions that occur with ASD are thought to be causally linked to changes that converge at synapses. ASD has a strong genetic component where many of the mutated genes, such as the Shank family of proteins, encode proteins localised to excitatory glutamatergic synapses. Previously we have shown that Shank3 can signal across the synapse to regulate both pre- and postsynaptic function, and that ASD-associated mutations in Shank3 weaken excitatory glutamatergic synaptic transmission and prevent this trans-synaptic effect. As zinc plays a modulatory role in synaptic transmission and plasticity at glutamatergic synapses and also recruits Shank3 to synapses, we hypothesised that zinc could regulate these Shank3-dependent effects on synaptic function. ASD-associated Shank3 mutations were expressed in primary hippocampal neurons and the effects of zinc on glutamatergic synapse function were measured by whole cell patch clamp electrophysiology. Our results show that zinc treatment can rescue synaptic transmission in neurons expressing specific Shank3 mutations. Furthermore, we utilised a Shank3 ASD mutant mouse model to perform behavioural and electrophysiology studies. We hypothesised that zinc-dependent regulation of Shank proteins will normalise synaptic and circuit function to rescue normal behaviour in ASD. Our results show that dietary zinc supplementation has a rescuing effect on repetitive and anxiety type behaviours. Together these data reveal zinc-dependent regulation of Shank proteins in ASD.

MTU05-11

First identification of a human mutation in synaptotagmin1 reveals perturbation of synaptic vesicle cycling

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The integral synaptic vesicle (SV) protein synaptotagmin-1 (SYT1) is the calcium sensor responsible for mediating fast synchronous neurotransmitter release, and additionally regulates the kinetics of endocytosis. Despite the key role for SYT1 in presynaptic function, there has been no confirmation to date that this gene and the molecular events it controls are essential for human neurodevelopment, because no individual with a pathogenic *SYT1* variant has been identified. We describe the first known human condition associated with a rare variant in *SYT1*. Clinical features are an early-onset dyskinetic movement disorder, severe motor delay, and profound cognitive impairment. Structural MRI is normal but EEG shows extensive neurophysiological disturbance. Trio analysis of whole exome sequence revealed a *de novo* missense variant (I368T) in *SYT1*. Rat SYT1 containing the equivalent human variant was expressed in mouse primary hippocampal cultures to determine the impact of this mutation on SV recycling dynamics. SYT1 localised to nerve terminals correctly and was expressed in approximately equal proportions to wild-type endogenous protein. Mutant SYT1 slowed SV fusion kinetics, in agreement with the published role for I368T in calcium-dependent membrane penetration. Interestingly, expression of this SYT1 variant also altered the kinetics of SV endocytosis. The clinical features, electrophysiological phenotype and *in vitro* neuronal phenotype associated with this dominant negative variant in *SYT1* highlight presynaptic mechanisms essential for human motor control and cognitive development.

MTU05-12

Effects of the KCC2 membrane transporter on neuronal excitability and seizure models in the hippocampal slice preparation

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Neuronal excitability and brain function depends on a critical balance between excitation and inhibition. Efficacious $GABA_A$-receptor-mediated inhibition relies on robust K^+ Cl^- co-transporter (KCC2) activity to maintain Cl^- homeostasis, particularly during periods of intense activity. The aim of this study is to investigate whether enhancing the expression of functional KCC2 *in vivo* modifies the neuronal excitability and GABA inhibition subsequently assayed *in vitro*. CA1 population field potentials in response to stimulation of Schaffer collateral afferents were recorded in 350–400 μm hippocampal slices isolated from transgenic mice in which forebrain-restricted expression of KCC2 could be upregulated by withdrawal of doxycycline from the diet. The baseline characteristics of evoked responses were similar between control and KCC2 upregulated mice, with no difference in the stimulus strength-response relationship (0–30 V) or paired-pulse ratio (30–100 ms). At an inter-stimulus interval of 50 ms, the paired pulse ratio in control slices was 1.14 ± 0.04 which was similar to the ratio of 1.09 ± 0.01 in KCC2 upregulated slices ($n = 6$–7). The application of a stimulus train (100 Hz, 1 s) at 10 min intervals resulted in the appearance of afterdischarges (ADs) that stabilise in number after 1 h. The mean number of ADs observed in control mice was 15.0 ± 1.2, and this was significantly decreased to 1.6 ± 0.8 in KCC2 upregulated mice ($p = 0.016$, $n = 3$). Similarly, perfusion of zero Mg^{2+} solutions resulted in the appearance of recurrent short discharges within 15–30 min. There appeared to be a modest decrease in the frequency of these spontaneous potentials in KCC2 upregulated slices when compared to control (0.16 ± 0.03 vs. 0.24 ± 0.02 Hz; $p < 0.05$; $n = 3$). Our data suggest that increasing KCC2 has little effects on basal synaptic transmission in hippocampal slices but reduces the propensity of neurons to develop into a hyperexcitable state, possibly by maintaining more efficacious GABAergic inhibition.

MTU05-13

SEZ6 binds the analgesic target α2δ and contributes to neuropathic pain plasticity

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Nerve damage or disease can trigger persistent pain, referred to as neuropathic pain, where normally non-noxious stimuli are perceived as painful (allodynia and hyperalgesia). The accompanying sensitization of primary afferent synapses in the spinal cord dorsal horn can be inhibited by the neuropathic analgesics gabapentin and pregabalin acting at their receptor, $\alpha_2\delta$. Along with the trafficking of voltage-gated calcium channel pore subunits and enhancement of excitatory neurotransmitter release probability, $\alpha_2\delta$ appears to act as an adhesion molecule in synaptogenesis. Previously, we linked the seizure-related protein Sez6 to excitatory synapse development. We now present evidence for the involvement of Sez6 in structural and functional plasticity in a mouse model of neuropathic pain: (i) co-immunoprecipitation of Sez6 and $\alpha_2\delta$ and co-expression in a sub-population of sensory dorsal root ganglion neurons; (ii) a functional role of the Sez6-$\alpha_2\delta$ complex in excitatory synapse formation *in vitro* that is blocked by gabapentin; (iii) attenuated heat hyperalgesia in Sez6 knockout (KO) mice after a peripheral nerve injury (chronic constriction injury of the sciatic nerve, or CCI); iv) reduced excitatory neurotransmission after agonist-induced transient receptor potential vanilloid 1 (TrpV1) activation in Sez6 KO CCI spinal cord slices compared to wild-type CCI controls; v) a Sez6- and CCI-dependent increase in dendritic spine density on pyramidal neurons in the medial pre-frontal cortex, a region involved in affective aspects of neuropathic pain. In conclusion, Sez6 promotes excitatory synapse formation via $\alpha_2\delta$ while a lack of Sez6 alleviates neuropathic pain states, including excitatory synapse structural and functional plasticity, along the central "pain" neuraxis.

MTU05-14

N-acetylaspartate: genomics, phenomics and metabonomics

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N-acetylaspartate (NAA) is the second most abundant metabolite in the CNS, and is used as a marker for neuronal health in brain imaging, yet its functional role remains elusive. NAA is believed to be the major source of free acetate utilised by oligodendrocytes for lipid synthesis, suggesting a role in myelination. Moreover, NAA is used to produce the neurotransmitter and most abundant CNS dipetide N-acetylaspartylglutamate (NAAG). In humans, the lack or accumulation of NAA is not tolerated well and coincides with distinct CNS pathologies. The lack of the NAA-degrading enzyme aspartoacylase (ASPA) causes the neurodegenerative condition Canavan Disease (CD), characterized by severe psychomotor deficits, CNS vacuolization and growth retardation.

Here we aimed to model the neurological consequences of genetically altered NAA metabolism in novel mouse models. NAA is produced by the synthesising enzyme N-acetyl transferase 8-like (NAT8 l). A transgenic mouse line for neuronal over expression of Nat8 l was produced by pronuclear injection of a Thy1-Nat8 l mini gene cassette. This mouse model and Nat8 l-knockout mice were characterized and examined for phenotypic abnormalities. As expected, the mutant lines showed either absence or elevated Nat8 l mRNA, NAA and NAAG. However, brain morphology and myelin appeared normal and we did not detect any changes in the mRNA levels of enzymes involved in NAA metabolism. However, over expression of Nat8 l caused reduced body weight similar to CD indicating that this aspect of the complex CD pathology is caused by NAA toxicity rather than the loss of ASPA function. Our findings confirm that Nat8 l is the sole NAA-synthesising enzyme and suggests NAA as the sole NAAG precursor. However, NAA is not vital for myelin lipid synthesis nor normal neuronal function.

MTU05-15

The effects of fluoride and arsenic exposure on the cholinergic-nitrergic system, cognitive functions and inflammatory markers

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Fluoride and arsenic are nurodevelopmental toxicants causing disabilities, in children worldwide. The present study was conducted to explore their effects on cholinergic-nitrergic system, cognitive functions and inflammatory markers in rat brain after sub-chronic exposure to fluoride, arsenic, fluoride and arsenic combination. Oxidative stress parameters, histopathological changes were studied. Wistar rats were assigned to four groups. Controls drank tap water, the three exposure groups drank water with sodium fluoride (10 mg/L), sodium arsenite (08 mg/L), sodium fluoride (10 mg/L) and sodium arsenite (08 mg/L) combination for 3 months. Spatial learning and memory was measured in Morris water maze. The level of nitric oxide synthase (NOS), acetylcholine esterase (AChE), proinflammatory cytokine, tumor necrosis factor (TNF)-α, interleukin 6 (IL-6) was detected respectively. Our results showed inhibition of SOD, GSH, GPx, CAT activities and a stimulation of MDA levels in fluoride, arsenic alone or combination treated groups, indicating oxidative stress in the brain. Microscopic examinations revealed neurodegenerative characteristics and necrotic changes in neurons with combined exposure of fluoride and arsenic, when compared with other groups, in hippocampus and cortex regions of the rat brain. Compared with controls, learning and memory ability declined in rats that were exposed to fluoride and arsenic alone and combined. Combined fluoride and arsenic exposure had a more

pronounced effect on spatial learning and memory compared with arsenic and fluoride exposure alone. The level of TNF-α increased and IL-6 decreased significantly in rats exposed to fluoride and arsenic. Interestingly, fluoride and arsenic combination decreased AChE and increased NOS expression in the hippocampus, suggesting a synergistic effect of fluoride and arsenic. These data indicate that fluoride and arsenic, either alone or combined, can decrease learning and memory ability in rats. The mechanism may be associated with changes of cholinergic-nitrergic system and enhanced oxidative stress, pathological changes and inflammation in the brain.

MTU05-16

Homeostatic intrinsic plasticity in cerebellar purkinje cells requires agonist-independent action of group 1 metabotropic glutamat

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Homeostatic plasticity, non-hebbian form of neural plasticity, is triggered to stabilize neural network when long-lasting changes of neural activity occurs. Group 1 metabotropic glutamate receptor (mGluR1) can monitor neuronal activity which results in neural plasticity. Here, we show that agonist-independent action of mGluR1 induces homeostatic intrinsic plasticity via upregulation of the hyperpolarization-activated current (I_H). First we observed that activity-deprivation by chronic treatment of tetrodotoxin (TTX) for 2 days decreased evoked firing rates from cerebellar Purkinje cells (PCs). Interestingly, co-treatment of mGluR inverse-agonist under activity-deprived condition, not neutral antagonist, caused recovery from downregulation of excitability, suggesting that agonist-independent action of mGluR1 was involved in homeostatic intrinsic plasticity. Next, we confirmed attenuation of neuronal input resistance by analyzing active properties in control and deprived neurons. After activity-deprivation, I_H components, including voltage sag and current density, was elevated in that we concluded that upregulated I_H results in lowered excitability. Indeed, homeostatic upregulation of I_H was also rescued by blockade of constitutive mGluR activity with suppressed network activity. Together with our data, this study strongly suggests that mGluR1 is a key player for homeostatic control via modulating I_H.

MTU05-17

Oxidative stress in dementia patients - study using dROMs and bap test

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Purpose: The oxidative stress and biological antioxidant potential of dementia patients were measured using a free radical elective evaluator (FREE).

Subjects: The subjects of this study consisted of 48 untreated dementia patients examined at the Department of Geriatric Medicine of the Hachioji Medical Center (19 men, 29 women, average age: 79.6 years).

Methods: All subjects underwent an ADAS-Jcog assessment, oxidative stress was assessed by measuring serum dROM levels (oxidative stress), BAP (biological antioxidant potential) and BAP/dROM ratio (latent antioxidant potential) using FREE, and correlations between ADAS scores and oxidative stress indicators were tested. Degree of dementia was determined by using ADAS scores to categorize the subjects into three groups consisting of a mild group (ADAS score: 0–9 (13 subjects)), moderate group (ADAS score: 10–19 (26 subjects)) and advanced group (ADAS score: 20 or higher (9 subjects), and analyzing variance for dROM levels, BAP and BAP/d-ROM ratio among each group.

Results: The average ADAS-Jcog score for all subject patients was 15.1. The average values for d-ROM levels, BAP and BAP/d-ROM ratio were 441.8, 2497.1 and 6.06, respectively, and when compared with each of their reference values, d-ROM levels indicated severe oxidative stress while BAP values indicated appropriate biological antioxidant potential. There was no correlation obtained between ADAS scores and d-ROM values, and a negative correlation was observed with BAP values ($r = 0.313$, $p = 0.03$). An analysis of variance among the three groups yielded a relationship such that BAP/d-ROM ratio was significantly higher in the advanced group in a comparison between the mild group and advanced group ($p = 0.048$).

Discussion: Elevated d-ROM levels and normal BAP values suggest an increase in oxidative stress accompanying chronic inflammation. A correlation between this finding and the chronic inflammation theory associated with DAT is extremely interesting.

MTU05-18

Developing a new model of spontaneous intracerebral hemorrhage in rats

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Background: Intracerebral haemorrhage (ICH) accounts for ~15% of all strokes. It has a high mortality rate and an effective treatment is yet to be found. This may be explained by failure of current animal models of spontaneous ICH to closely mimic the human condition.

Aim: To induce spontaneous ICH in rats using a model of acute-on-chronic hypertension.

Methods: Male rats (16–32 weeks) received L-NAME (100 mg/kg/day) via drinking water and systolic blood pressure (SBP) was measured daily by tailcuff. Osmotic-minipumps containing Angiotensin II (AngII, 1 μg/kg/min) were implanted in inducing chronic hypertension over 7 days. Bolus injections of AngII (500 μg/kg, s.c., twice daily) were administered from day 8–28 inducing peaks in SBP. Animals were sacrificed upon signs of stroke or at day 28 and presence of ICH assessed macroscopically and quantified using image analysis software (Image J).

Results: Chronic AngII infusion significantly increased mean SBP in young (control:141.96 ± 30.99 mmHg; AngII:160.50 ± 27.94 mmHg, $t(56) = 6.293$, $p < 0.0001$) and aged (control: 141.96 ± 30.99 mmHg; AngII:181.26 ± 25.25 mmHg, $t(56) =$

2.011, $p = 0.049$) animals. No difference was found in the degree of SBP increase in young and aged AngII treated animals ($t(56) = 1.615$, $p = 0.112$). Bolus AngII injections lead to further increases in SBP between 212–248 mmHg, peaking 3 h post-injection, with no apparent effect of age. Bolus injections of saline in control animals did not affect SBP. No control rats ($n = 16$) showed signs of ICH. Bleeds were detected in 75% of young (1.04 ± 0.37 μL, $n = 8$) and 69% of aged treated rats (1.6 ± 0.93 μL, $n = 13$).

Conclusion: Acute-on-chronic hypertension successfully induces ICH in both young and aged rats. This approach has several advantages over existing models of ICH.

MTU05-19

Nociceptin inhibits neuronal excitability and epileptiform activity in the entorhinal cortex

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Recurrent uncontrolled seizures or epilepsy is a common neurological disorder that is characterized by excessive excitation of many brain regions including the entorhinal cortex (EC) and hippocampus. Nociceptin (NOP) or orphanin FQ is an endogenous opioid-like peptide that selectively activates the opioid receptor-like (ORL-1) receptors without effects on the traditional mu, delta and kappa opioid receptors. Whereas the EC expresses high densities of NOP receptors, the functions of NOP in the EC have not been determined. We examined the effects of NOP on neuronal excitability and epileptiform activity induced by application of picrotoxin and by deprivation of extracellular Mg^{2+} in entorhinal slices. Our results demonstrate that NOP exerted robust inhibition of epileptiform activity via activation of ORL-1 receptors. NOP did not alter synaptic transmission mediated by glutamate or GABA, but significantly inhibited the action potential firing frequency recorded from the entorhinal neurons by hyperpolarizing these neurons. NOP-induced hyperpolarization was mediated by activation of a K^+ conductance and inhibition of a cation channel. Intracellular molecules including cAMP and protein kinase A are not involved in NOP-mediated hyperpolarization, suggesting a direct G protein coupling. Our results provide a novel cellular and molecular mechanism underlying NOP-mediated depression of epileptiform activity.

MTU05-20

Role of intersectin-1 in metal ion dyshomeostasis, cognition and motor function in down syndrome

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Objectives: All individuals with Down syndrome (DS) have partial or whole extra chromosome 21. Intersectin-1 (*ITSN1*) is located on chromosome 21 and up-regulated in DS. It has two isoforms; long (expressed in neurons of the brain) and short (expressed in all the tissues). This study characterizes genetically modified mice to provide a better understanding of the biological changes due to absence or overexpression of ITSN1.

Methods: Mice where the long isoform of ITSN1 is knocked out (LKO) and mice where both short and long isoforms are over-expressed (Tg) are used for this study. We investigated behaviour (the Morris water maze), brain activity (long term potentiation-LTP), motor function (rotarod and locomotor tests), cell signalling (western blotting), metals in the brain (mass spectrometry) and brain weight.

Results: For $n \geq 6$ per genotype in each case: for LKO, we found significant decreases in brain weight, long term and spatial memory, LTP, cell signalling activity (Mitogen-activated protein kinase (MAP kinase) and AKT), and a significant increase in the cortical levels of iron, zinc and copper. The Tg mice showed no difference in long term and spatial memory, LTP or cell signalling activity but motor function was affected as evidenced by a significant reduction in the number of movements in the open field. Consistent with the altered motor activity, cerebellar iron, zinc and copper levels were significantly decreased.

Conclusion: These data show firstly that over-expression of ITSN1 disrupts metal ion homeostasis in the cerebellum which may explain the motor dysfunction observed, and secondly that the ITSN1 long isoform may have an important role in cognition and metal ion homeostasis in the cortex.

MTU05-21

Characterising tachykinin NK-1 receptor and caveolin-1 expression in cerebral metastases

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Purpose: A key event in the development of brain metastasis is the migration of malignant cancer cells through the specialized blood-brain barrier. Both primary and secondary metastatic brain tumours have been linked to an impairment of the barrier, however the mechanisms underpinning this process and the developing malignancy are undefined. The neuropeptide Substance P (SP) and its tachykinin NK-1 receptor (NK-1R) have been acknowledged to play a central role in cancer biology through the promotion of tumour cell proliferation, angiogenesis and migration. Similarly, the endothelial caveolae scaffolding protein Caveolin-1 (Cav-1) known to house NK-1R, and importantly has an identified role in cancer progression. The current study aimed to characterise expression of NK-1, and Cav-1 from cerebral tumours arising from four primary sites; lung, breast, colon and malignant melanoma and elucidate their potential involvement in tumour progression.

Methods: Immunohistochemical stains were performed on archived specimens of cerebral neoplastic surgical biopsies from 41 patients with an established diagnosis of metastatic melanoma ($n = 11$), lung carcinoma ($n = 12$), breast carcinoma ($n = 8$), or colorectal carcinoma ($n = 10$). Two independent investigators evaluated immunoreactivity for,NK1-R and Cav-1.

Results: Investigation of metastatic invasion patterns demonstrated considerable variability. Caveolin-1 was well conserved on

cerebral blood vessels both intra and extratumourally across cancer types, specifically intense membranous staining was apparent on melanoma tumour cells suggesting a unique role in CNS metastatic melanoma invasion. NK-1R immunoreactivity was observed in tumour cells with greatest expression in breast tumours. Whilst still abundant in remaining tumour types, it was to a lesser degree. Therefore tumour cell proliferation may be the primary role of NK-1R in metastatic invasion.

Conclusion: The incongruent expression of cav-1 and NK-1R suggest separate yet, prominent roles in tumour progression.

MTU05-22

Striatal deficits underlie conflicted patterns of action selection in ageing

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For our goal-directed action to remain adaptive, we must constantly acquire new strategies to accommodate environmental changes, a process that largely depends on striatal function. However, it has long been known that, during memory updating, new and old learning can actively interfere, resulting in conflict and, eventually, forgetting. Although this type of memory interference is a cognitive shortcoming that is particularly common in the elderly, the neural correlates underlying these behavioural deficits are largely unknown. Here, we sought to address the hypothesis that an age-related decline of striatal function affects the capacity of older mice to flexibly adapt their actions to changing conditions in the environment. Using sophisticated behavioural paradigms in rodents, we found that aged mice were able to encode initial goal-directed learning but failed to adjust this learning when conditions were altered, leading to conflict in action selection and reduced adaptive capacity. Using large-scale functional profiling of neurons, we observed that this deficit was accompanied by reduced cellular activity in the striatal cholinergic system, and that it specifically correlated with defective activation of striatal output neurons, in line with recently proposed models for the flexible control of action. These results demonstrate for the first time an age-related memory interference effect in associative learning, and suggest that such cognitive shortcoming during the integration of goal-directed memories can be particularly detrimental for the flexible adaptation of older individuals to changing environments.

MTU05-23

Neonatal seizures are independently associated with loss of $GABA_A$ a3 protein expression in the hypoxic ischaemic piglet

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Background: Seizures are a common manifestation of hypoxic-ischaemic brain injury in the neonate. Current anticonvulsant drug treatments for neonatal seizures that target the $GABA_A$ receptor ($GABA_AR$) are largely ineffective. In adults following cerebral ischaemia, changes in $GABA_AR$ subunit protein expression have been reported. However, detailed investigation of $GABA_AR$ subunit protein expression after neonatal hypoxia-ischaemia (HI) is unknown. Using our pig model of HI and subsequent spontaneous neonatal seizures, we investigated changes in protein expression of the three predominant α-subunits of the $GABA_AR$; α_1, α_2 and α_3.

Method: Anaesthetised, ventilated newborn pigs (< 24 h old) were subjected to 30 min HI and subsequently recovered to 24 or 72 h. Amplitude-integrated electroencephalography was used to monitor brain activity and identify seizure activity. Brain tissue was collected post-mortem and $GABA_AR$ α-subunit protein expression was analysed by western blot and immunohistochemical techniques.

Results: $GABA_AR$ α_1 and α_3 protein expression was significantly reduced in animals that developed spontaneous seizures; HI animals that did not develop seizures did not exhibit the same reduced expression. Multivariable analysis, controlling for HI severity and brain histopathology (neuronal loss) revealed that neonatal seizures after HI were independently associated with a significant reduction in α_3 expression. Immunohistochemistry analysis revealed altered α_1 and α_3 expression in seizure animals.

Discussion: A loss of α-subunit expression after HI-induced neonatal seizures may potentially change $GABA_AR$ binding efficacy. Alterations to $GABA_AR$ subunit proteins are known to influence receptor pharmacology. Thus, these findings may explain, at least in part, the ineffectiveness of current GABA anticonvulsant drugs in the neonatal brain.

MTU05-24

Cytoarchitectural and morphometric analyses of the lateral prefrontal cortex of rats administered with nicotine during gestation

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The rate of consumption of nicotine-containing substances is still high in the developing world despite the knowledge of its deleterious effects on the body. While some authors consider nicotine replacement therapy an alternative to tobacco smoking in pregnancy, others have questioned its effectiveness and safety. The current study was designed to determine some of the structural and molecular abnormalities in the lateral prefrontal cortex (LPFC) that underlie the neurological dysfunctions associated with prenatal nicotine at different stages of gestation in Wistar rats. Based on the average twenty-one gestational days of rats, pregnant Wistar rats were randomly divided into three groups after determination of their oestrous cycle: group A (days 1-7), group B (days 8-14), and group C (days 15-21); grouping also represented the time of treatment of the animals. Each group has a control, low dose nicotine-treated and high dose nicotine-treated subgroup. The animals received treatment intraperitoneally for five consecutive days within each group. At postnatal day 15, the pups were anaesthesised with IM ketamine and transcardially perfused. LPFC was processed using cresyl fast violet for Nissl bodies, fluorescence immunohistochemistry for the expression of choline acetyltransferase (ChAT) and myelin basic protein (MBP), while morphometric studies were carried out for brain cells and the data were statistically analysed. Findings revealed significant low birth weight in nicotine-exposed pups, with catch-up growth in pups exposed within the first 7 days, while minimal increase in brain weight was observed across the groups; neuronal

and oligodendroglial counts decreased, but astrocytic count increased; progressive cellular degeneration, nuclear disintegration, reduction in cell sizes and Nissl bodies depletion occurred; and, there was marked reduction in the expression of MBP and ChAT. Conclusively, administration of nicotine at any gestational age is detrimental to neurodevelopment and could result in long-term neurologic and cholinergic dysfunctions, similar to those seen in offspring of smoking mothers. However, therapeutic measures that target ChAT or MBP activity may be useful in managing some of the postnatal neurologic effects.

MTU05-25

Regeneration of sensory but not motor axons following visceral nerve injury

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Following peripheral nerve injury, restoration of function occurs via regeneration of injured axons or compensatory growth of spared collateral axons. Injury to visceral nerves controlling urogenital organs is a common consequence of pelvic surgery, however their capacity to reinnervate target tissue is poorly understood. To determine if and how visceral sensory and motor connections are re-established, a novel surgical model that unilaterally transects nerves innervating the bladder was performed in adult male Wistar rats (n = 4–6/group). Bladder-projecting motor and sensory neurons in pelvic ganglia and lumbosacral dorsal root ganglia were identified by applying retrograde tracers to the bladder ipsi- and contralateral to injury, thereby allowing distinction between projection pathways. Neuronal phenotypes were distinguished immunohistochemically. In naive animals, most sensory and motor neurons projected ipsilaterally, while ~20% projected contralaterally and ~5% bilaterally. Following unilateral visceral nerve transection, injured axons of motor neurons were unable to regenerate by 4 weeks. In contrast, at this time-point injured sensory neurons of all phenotypes regrew axons to reform a plexus within the detrusor and suburothelial tissues of the bladder wall. The responses of sensory and motor neurons to injury were also indicated by the upregulation of activating transcription factor-3, which was sustained in motor neurons but transient in sensory neurons. Neuronal death was not detected in any ganglia, suggesting axotomy had little effect on the survival of injured bladder neurons. Uninjured motor and sensory neurons in contralateral ganglia extended axons into denervated tissue, as indicated by an increase in bilateral innervation. This evidence suggests a second mechanism i.e. compensatory growth, by which neural function is restored to the bladder. In conclusion, only axons of visceral sensory neurons are able to regenerate, while both visceral components undergo a second mechanism of repair in which uninjured collateral axons undergo spontaneous growth, and replace lost terminal fields. For full functional recovery to be achieved, understanding environmental and cellular mechanisms that inhibit axonal regeneration of autonomic neurons is needed.

MTU05-26

Investigation of the amyloid precursor protein derivative APP96-110 as a novel therapeutic agent following traumatic brain injury

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Following traumatic brain injury (TBI), neurological damage is ongoing through a deleterious cascade of physiological events. However, no effective drugs exist to limit this. Recently, the amyloid precursor protein (APP) derivative APP96-110, a peptide corresponding to amino acid residues 96-110, has shown encouraging neuroprotective properties following TBI. However, the efficacy of intravenous APP96-110 has not yet been studied. As such, this study examined the efficacy of a single intravenous dose of 0.005–0.5 mg/kg APP96-110 at both 30 min and 5 h following moderate-severe diffuse impact-acceleration injury. Male Sprague-Dawley rats were assessed daily for 7 days on the rotarod to examine motor outcome. Immunohistochemistry was also undertaken at 3 days post-TBI to assess the degree of axonal injury (AI) with APP and neuroinflammation using GFAP and Iba1 stains. Rats treated with APP96-110 at 30 min post-TBI demonstrated considerable improvement in motor outcome (n = 7–9), indicating that intravenous APP96-110 acutely after injury is efficacious. Histological examination of brain tissue showed that treatment with APP96-110 was not only able to significantly reduce the degree of AI in the corpus callosum at 3 days post-TBI, compared to vehicle treated rats ($p < 0.05$, n = 5), but also significantly reduce the extent of neuroinflammation in the same region ($p < 0.001$, n = 5), demonstrating significant anti-inflammatory effects. Furthermore, rats treated with APP96-110 at 5 hours following injury demonstrated significant improvements in motor outcome over 7 days (n = 7–8), compared to vehicle treated rats ($p < 0.0001$–0.05, n = 8). Therefore, given that APP96-110 can attenuate neurological deficits, AI and neuroinflammation, and importantly, is able to be administered intravenously up to 5 h post-TBI, this study identifies APP96-110 as a novel and clinically relevant therapeutic option for TBI.

MTU05-27

Sertraline (antidepressant) inhibits 4-AP-induced ionic channel activation more efficiently than several anti-epileptic drugs

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Several neurological diseases including epilepsy can implicate ion channel dysfunctions. In line, ion channels are targets of various antiepileptic drugs (AEDs). In cerebral presynaptic nerve endings, Na^+ and Ca^{2+} channels are particularly abundant as they control the release of neurotransmitters, including the release of the main

excitatory amino acid neurotransmitter, Glu. In previous studies we found that the anti-depressant sertraline inhibits presynaptic Na^+ channels and Glu release *in vitro*, as well as seizures induced by the convulsing agent 4-aminopyridine (4-AP) *in vivo*. Taking into account that depression is among the most common psychiatric disorders in epileptic patients, in the present study the effectiveness of sertraline to inhibit the Na^+ dependent increase in Ca^{2+} induced by 4-AP was compared with the effect of the new anti-seizure drug, vinpocetine, and with the effect of several classic AEDs in hippocampal isolated nerve endings. Results show that the rise in Ca^{2+} induced by 4-AP, which depends on external Na^+ and is tetrodotoxin sensitive, is completely prevented by sertraline and by vinpocetine, both at concentrations 10 times lower than the AEDs: carbamazepine, phenytoin, lamotrigine and oxcarbazepine. In contrast, the increase in Ca^{2+} induced by 4-AP, as well as other Na^+ or Ca^{2+} mediated responses induced by depolarization, including Glu release, were unchanged by the AEDs: valproic acid, topiramate and levetiracetam. Present data indicate: 1. the high effectiveness of sertraline as an anti-seizure drug is due to its' effect on cerebral presynaptic ionic channels permeability; 2. that 4-AP is a powerful experimental tool to investigate the anti-seizure mechanism of action of a specific AED involving a direct decrease of cerebral ion channels permeability.

MTU05-28

Dopamine transporter deficiency syndrome: new clinical findings and disease modelling in zebrafish

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Dopamine transporter deficiency syndrome (DTDS) is caused by mutations in *SLC6A3*, encoding the human dopamine transporter (hDAT). DTDS associated mutations result in diminished dopamine/Na^+ binding affinity, reduced cell-surface expression or a loss of post-translational glycosylation, leading to loss of function of the dopamine transporter. Patients classically present with infantile-onset progressive parkinsonism-dystonia. As the disease progresses, patients develop hypokinesia with parkinsonian features, and in the absence of disease modifying therapies, many DTDS patients die in adolescence. In this study, we identified two new unrelated DTDS patients, born to non-consanguineous parents, who presented with irritability, the typical movement phenotype and elevated homovanillic acid (HVA)/5-Hydroxyindoleacetic acid (5-HIAA) ratios. *SLC6A3* mutations were identified in both patients (G433R and H547 fs in patient 1, G386W in patient 2). Detailed *in vitro* functional investigation revealed that the two novel missense mutations led to loss of function of the human dopamine transporter due to decreased binding affinity of dopamine to hDAT. Surprisingly, and differing from previously reported DTDS cases, glycosylation of hDAT was not affected in these mutations. This demonstrates the further complex multi-faceted pathogenicity of *SLC6A3* mutations in DTDS. In addition, we have generated a stable zebrafish DAT mutant using CRISPR/cas9 genome editing technology. Originally utilized by bacteria to mediate defense against viruses and other foreign nucleic acid, CRISPR/Cas9 systems can be engineered to cleave double stranded DNA *in vitro*, specific to regions of interest. Using CRISPR/cas9 to mutate *slc6a3* in zebrafish provides a robust disease model to study DTDS, and also constitutes a new high-throughput assay to identify possible novel therapeutic molecules for treating this devastating disorder.

MTU05-29

Phospho- and ubiquitinated-proteomics of aging mice brain by iTRAQ-based quantitative analysis

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Aging is thought as the main risk to develop neurodegenerative disorders and dementia. To elucidate the mechanism of protein modification in the normal aging brain, we evaluated the change of phosopho- and ubiquitinated-proteins.

Coritices of C57BL/6 mice at 3 and 21 months of age were isolated and sonicated. Phospho-proteins and ubiquitinated-proteins were enriched by using PhosPro or Ubiquitinated protein enrichment kit. After phospho-proteins and ubiquitinated-proteins were labeled by iTRAQ, labeled peptides were analyzed by MALDI-TOF MS/MS. There were 328 phospho-proteins and approximately 200 ubiquitinated-proteins to be identified in mice cortex. Fifteen phospho-proteins and seven ubiquitinated-proteins were significantly changed in 21 months mice cortex as compared with 3 months mice cortex. In particular, six phospho-proteins, calreticulin, myelin-associated oligodendrocyte basic protein, myelin proteolipid protein, calnexin, glycogen phosphorylase and hemoglobin subunit beta-1 were increased and nine phospho-proteins, creatine kinase B-type, complexin-2, neurogranin, S-phase kinase-associated protein 1, acyl carrier protein, myristoylated alanine-rich C-kinase substrate, gamma-enolase, serine/threonine kinase 6 and reticulocalbin-2 were decreased in 21 months mice cortex. These findings indicate that aging alters the levels of phospho- and ubiquitinated-proteins proteins in the cerebral cortex. We propose that changed post-transcriptional modifications might play an important role in developing neurodegenerative disorders and dementia.

MTU05-30

Stromal interaction molecule 1 (STIM1) is required for correct nervous system development

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Stromal interaction molecule 1 (STIM1) is a calcium sensory protein located within the endoplasmic reticulum (ER) membrane. When the ER is depleted of calcium, STIM1 activates calcium entry across the plasma membrane to potentiate calcium signaling and to refill depleted stores, in a process termed store-operated calcium

entry. Store-operated calcium entry regulates a range of cellular processes, including immune cell activation and endothelial cell migration. However the role of store-operated calcium entry within the nervous system is less well understood. We are interested in determining the role of STIM1 during nervous system development. The wiring of the nervous system requires precise guidance of axons through the nervous system to find and connect with their signaling targets. We previously demonstrated that expression of STIM1 is necessary for correct axon guidance *in vitro.* As axon guidance is vital for establishing the functional circuitry of the nervous system, we hypothesise that expression of STIM1 is required for normal nervous system development. To investigate the importance of STIM1 expression for nervous system development we employed zebrafish (*Danio rerio*) as an experimental model and observed the effect of reducing STIM1 expression on axon pathfinding. We demonstrate that STIM1 is ubiquitously expressed within the nervous system throughout embryonic development. Crucially, primary motor neurons within the spinal cord express STIM1 concurrently with the process of axon pathfinding. Preliminary data suggests that reducing STIM1 expression throughout development results in perturbed axon pathfinding by spinal cord primary motor neurons. Mean axon length at 24 h post fertilisation and the angle of axon trajectory upon exiting the spinal cord are both significantly reduced in STIM1 morphants compared with sibling controls. These data suggest that expression of STIM1 and, hence, store-operated calcium entry, is required for the correct axon pathfinding *in vivo*. Our results demonstrate that expression of STIM1 is necessary for correct nervous system development.

MTU05-31

The effect of an organophosphorus agent on human neuroblastoma cell line SK-N-SH

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Twenty years have passed since a horrifying terrorist attack with sarin gas (isopropyl methyl phosphonofluoridate) at Tokyo. There are many people that have been plagued by severe sequelae and neurological symptoms. As acute toxicity, sarin is known to act as an acetylcholinesterase inhibitor, but the mechanism of action of the chronic toxicity is not known. We synthesized a sarin-like organophosphorus compond; bis (isopropyl methyl) phosphonate (BIMP). In this study, the neuronal toxicity and its mechanism of BIMP on human neuroblastoma cell line SK-N-SH and differentiated SK-N-SH were examined. To differentiate SK-N-SH into neuronal cells, cells were grown in MEMα including 5% FCS together with 40 μM retinoic acid, NGF and BDNF on polylysine coated plates and the medium were replaced every 3 days for 8–30 days. For estimating the toxicity of BIMP, cell viability was analyzed using the conventional MTT reduction assay after incubation for 24 h. To evaluate the effect of BIMP on wound healing process. scratch assay was done using SK-N-SH and differentiated SK-N-SH. When the stresses such as toxicity or oxidation is applied to the cells, the endoplasmic reticulum stress causes and increases abnormal proteins. We investigated the effect of BIMP on the endoplasmic reticulum stress. To estimate the change of the endoplasmic reticulum stress expression, major ER chaperon, BiP was measured using Real-time PCR in combination with TaqMan probe. BIMP inhibited the cell proliferation of SK-N-SH and the IC50 value of inhibition was 44 μM on MTT assay. BIMP showed the inhibitory effect of wound healing on SK-N-SH and differentiated SK-N-SH using a scratch assay. BiP increased after incubation of BIMP for 3 h. These results suggested that BIMP induced the chronic neural toxicity by the mechanism related to endoplasmic reticulum stress.

MTU05-32

Balance hypothesis of behavioral characteristics and urinary monoamine metabolites in neurodevelopmental disorders

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Although several studies have investigated levels of dopamine (DA), norepinephrine (NA), serotonin (5-HT), and their metabolite excretion in people suffering from attention deficit hyperactivity disorder (ADHD) and in ADHD animal models, the results have been contradictory. The amount of the metabolite 3-Methoxy-4-hydroxyphenylglycol (MHPG) excreted in the urine of ADHD children has been reported to be less than that of a control group of children. Additionally, levels of the metabolites 5-hydroxyindoleacetic acid (5-HIAA) and Homovanillic acid (HVA) were shown to be lower in human cerebrospinal fluid (CFS) of ADHD patients. In contrast, others have reported that levels of urinary NA and MHPG in ADHD adults showed no differences from control subjects and that ADHD children had higher HVA concentration in the CFS than controls did. On the other hand, in autistic spectrum disorders (ASD), it has been reported the concentration of monoamines and its metabolites were increased. However, hyperactivity-impulsivity in overlap between ADHD and ASD are closely linked.

Therefore, here, we compared the levels of urinary MHPG, HVA, and 5-HIAA, in ADHD, ASD, the overlap between ASD and ADHD and controls. Furthermore, we classified participants based on the characteristics of their conditions into the following groups: careless dominant type, hyperactivity-impulsivity dominant type, and ASD dominant type. We then investigated the relationship between these types and the levels of monoamine metabolites. We found that a careless tendency rises when MHPG and 5-HIAA are high and HVA is low, hyperactivity-impulsivity becomes high, when HVA is high and MHPG is low, and a tendency for ASD rises when MHPG and HVA are high, and 5-HIAA is low.

These results suggest that characteristics of these deficits are expressed by the relative balances of these molecules, rather than by any specific individual monoamine. Moreover, urine samples used in this study are likely to be an important biomarker as a non-invasive method of examining the brain monoamine dynamics.

MTU05-33

Modulator of apoptosis 1 (MOAP-1) may influence behavior

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Modulator of apoptosis 1 (MOAP-1) plays a key role in mediating Bax function. MOAP-1 protein is found to be highly enriched in brain but its function is unknown. As MOAP-1 knockout (MOAP-$1^{-/-}$) mice do not show any obvious phenotype, we investigate whether these mice show any behavioral differences from wildtype (MOAP-$1^{+/+}$) controls. In the present study, MOAP-1 knockout mice were used to investigate the influence of MOAP-1 on depression using the forced swim test (FST) and tail suspension test (TST), and social dominance using the tube-dominance test. In the FST, immobilization time was significantly higher in young (3–6 months) and middle-aged (10–14 months) groups. While in the TST, higher immobilization time was observed only in the middle-aged group. In contrast, the aged (22–26 months) group did not show any difference between wildtype and MOAP-1 knockout mice. These results suggested that MOAP-1 knockout mice exhibit a depressive trait that diminished with age. In contrast, knockout mice were found to be socially dominant over the wildtype mice in all age groups. This is consistent with our observation that the frequency of fights occurring among the knockout mice were higher than that of the control wildtype mice. The social dominance may be related to their tendency to show aggression. Further studies will be performed to support the present observations.

MTU05-34

Tau reduction prevents neurodegeneration in a mice model of traumatic brain injury

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Objective: Targeting phosphorylated tau by pharmaceutical treatment has shown promise as a potential treatment strategy for diseases with secondary neurodegenerative features such as traumatic brain injury. We wanted to determine whether genetic ablation of tau may also be of benefit in traumatic brain injury.

Methods: We treated tau-knockout mice and aged-matched wild-type (WT) mice with lateral fluid percussion injury (LFPI) or sham injury. After a 3-month recovery period, in-vivo MRI imaging was used to assess brain structural damage. A battery of behaviour tests was employed to evaluate the cognitive and psychological function. Western-blot analyses were performed to assess levels of hyper-phosphorylated tau and related pathologies.

Results: TBI in WT mice led to significant brain atrophy and behavioral impairments, however, these were prevented in tau-KO mice. Tau dysregulation, which causes a reduction in synaptic protein levels, may be responsible for the cognitive decline observed in TBI mice. Concomitantly, we demonstrated the finding that depletion of endogenous tau might mitigate synaptic deficits induced in TBI mice.

Conclusion: Tau reduction may be of therapeutic benefit in experimental TBI and represents a potential therapeutic target for patients with traumatic brain injury.

MTU06 Cholinergic Transmission

MTU06-01

The role of P75NTR in cholinergic basal forebrain structure and function

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The role of the p75 neurotrophin receptor ($p75^{NTR}$) in adult cholinergic basal forebrain (cBF) neurons is unclear due to conflicting results from previous studies and to limitations of existing $p75^{NTR}$-knock-out mouse models. In the present study we used a novel conditional knock-out line (ChAT-cre $p75^{in/in}$) to assess the role of $p75^{NTR}$ in the cBF by eliminating $p75^{NTR}$ in choline acetyl-transferase-expressing cells. We show that the absence of $p75^{NTR}$ results in a lasting increase in cBF cell number, cell size, and cholinergic innervation to the cortex. Analysis of adult ChAT-cre $p75^{in/in}$ mice revealed that mutant animals show a similar loss of cBF neurons with age to that observed in wild-type animals, indicating that $p75^{NTR}$ does not play a significant role in mediating this age-related decline in cBF neuronal number. However, the increased cholinergic axonal innervation of the cortex, but not the hippocampus, corresponded to alterations in idiothetic but not allothetic navigation as well as dysfunction in fear extinction consolidation. These findings support a role for $p75^{NTR}$-mediated regulation of cholinergic-dependent cognitive function, and suggest that the variability in previous reports of cBF neuron number may stem from limited spatial and temporal control of $p75^{NTR}$ expression in existing knock-out models.

MTU06-02

Nuclear organization and morphology of the sleep related nuclei in the brain of the arabian oryx, oryx leucoryx

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Arabian oryx, *Oryx leucoryx*, are members of the superorder Cetartiodactyla and belong within the family Bovidae. They are native to the Arabian Desert and are currently listed as "vulnerable" on the IUCN red data list. The current study describes the nuclear organization and neuronal morphology of the systems involved in the generation and control of the sleep-wake cycle in the Arabian oryx. The systems examined included the cholinergic, catecholaminergic, serotonergic and orexinergic as well as the GABAergic neurons associated with these nuclei. The aim of the study was to identify these neural systems and to compare the results to that reported for other members of Cetartiodactyla and mammals in general. The majority of the neuronal systems examined followed the typical mammalian organizational plan; however, some differences were observed: (1) the neuronal morphology of the cholinergic laterodorsal tegmental (LDT) and pedunculopontine tegmental (PPT) nuclei as well as the parvocellular subdivision of the orexinergic main cluster exhibited Cetartiodactyl-specific features; (2) the dorsal division of the catecholaminergic anterior hypothalamic group (A15d), which has not been reported in any member of the Cetartiodactyla studied to date, was present in the brain of the Arabian oryx; and (3) the catecholaminergic tuberal cell group (A12) was notably more expansive than previously seen in any other mammal. The A12 nucleus has been associated functionally to osmoregulation in other mammals, and thus its expansion could potentially be a species specific feature of the Arabian oryx given their native desert environment and the need for water conservation.

MTU06-03

Effects of postnatal nicotine exposure on nicotine acetylcholine receptors in the piglet brainstem

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Introduction: During infancy, exposure to nicotine via inhalation of cigarette smoke is known to affect brain function including IQ, cognition, hearing as well as cardiorespiratory and sleep and arousal control. It is also a contributing risk factor for Sudden Infant Death Syndrome (SIDS). The brainstem is a site containing important nuclei involved in cardiorespiration, sleep and arousal.

Aim & Methods: By utilising a piglet model subjected to early postnatal nicotine exposure, and employing immunohistochemistry, this study determines the protein expression of 6 subunits of nicotine acetylcholine receptors (nAchR) in 7 regions of the brainstem medulla: nucleus of the solitary tract (NTS), hypoglossal nucleus (XII), dorsal motor nucleus of the vagus (DMNV), cuneate nucleus (CUN), nucleus of the spinal trigeminal tract (NSTT), inferior olivary nucleus (ION) and vestibular nucleus (VEST), and compare expression levels between control piglets ($n = 12$) and nicotine treated piglets ($n = 12$).

Results: Compared to controls, the nicotine treated piglets had significantly increased expression of: $\alpha 2$ in the NTS, VEST and NSTT, $\alpha 3$ in VEST and XII, $\alpha 4$ in NSTT ($p < 0.05$), but decreased expression of $\alpha 4$ in VEST, DMNV, and $\beta 1$ in NTS, XII and DMNV ($p < 0.05$). As the functions of these subunits contribute to the maintenance of excitation and inhibition ($\alpha 2$), synaptic transmission ($\alpha 3$), antinociception ($\alpha 4$), and excitatory effects ($\beta 1$), an abnormal expression of these subunits may consequently result in physiological abnormalities related to these specific brainstem regions affected including DMNV (lung and heart control), NTS (cardiac control) and VEST (balance and orientation).

MTU06-04

Hippocampal and striatal histomorphology, ache and neurogenic markers activities following repeated administration of nicotine

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Nicotine has shown potential therapeutic value for neurodegenerative diseases via activation of nicotinic acetylcholine receptors. However, there are concerns of possible neurotoxicity. This study evaluated histological changes in hippocampus and striatum following chronic administration of varying doses of nicotine. Also, the study investigated the effects of nicotine on acetylcholinesterase (AChE) activities. Furthermore, the study assessed the effect of nicotine on activities of certain neurogenic markers; protein Ki-67 (Ki67), glial fibrillary acidic protein (GFAP) and neuron specific enolase (NSE) in the dentate gyrus (DG). Adult male Wistar rats were given daily subcutaneous injections of nicotine at doses of 0.25, 2 or 4 mg/kg body weight for 28 days. Analysis of H&E stained sections revealed significant increase in percentage of neurons showing degenerating features in 2 and 4 mg/kg treatment groups. However, there was significant decrease in AChE activities in the hippocampus and striatum following 2 and 4 mg/kg nicotine but not at 0.25 mg/kg compared to control. Nicotine treatment at 0.25 and 4 mg/kg significantly decreased immunoreactivity of Ki67 and NSE in the DG compared to control. Contrastingly, 2 mg/kg nicotine did not alter Ki67 immunoreactivity but rather produced significant increase in NSE immunoreactivity. In conclusion, this study shows that high doses of nicotine could induce neurodegenerative changes in hippocampus and striatum. Interestingly though, the study shows that nicotine at such high doses may inhibit AChE activities in the brain, thereby having a direct or indirect influence on prevention of central acetylcholine degradation. Also, this study suggests that nicotine effects on neurogenesis may include improvement of maturation of adult born neurons in the DG, but this may occur only at certain doses administered within specific time in the process of neurogenesis.

MTU06-05

The blood-brain barrier choline transporter

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Choline is one of the essential nutrients for all cells, and is needed for the synthesis of the major membrane phospholipids phosphatidylcholine and sphingomyelin. Furthermore, choline provides methyl groups for methionine and S-adenosylmethionine synthesis, which serves as a substrate for DNA and histone methyltransferases, and is thus required for the establishment and maintenance of the epigenome. In the brain, choline plays an additional role as a precursor for the synthesis of the neurotransmitter acetylcholine. Because the brain has only limited capacity to synthesize choline *de novo*, most central nervous system choline is derived from the systemic circulation. Therefore, transport of choline from blood to brain through the blood-brain barrier is a physiologically important process. In this study, we examined the molecular and functional characterization of choline transport into cultured human brain microvascular endothelial cells (hBMECs). Choline uptake into hBMECs showed independence on extracellular Na^+. Choline uptake was saturable, and mediated by a single transport system. Choline uptake was significantly decreased by acidification of the extracellular medium and by membrane depolarization. Choline uptake was inhibited by choline, acetylcholine and the choline analogue hemicholinium-3. Various organic cations also interacted with the Na^+-independent choline transport system. Choline transporter-like protein 1 (CTL1) and CTL2 mRNA were highly expressed, while mRNA for high-affinity choline transporter 1 (CHT1) and organic cation transporters (OCTs) were not expressed in hBVECs. CTL1 protein was recognized in both plasma membrane and intracellular compartments. On the other hand, CTL2 protein was localized in mitochondria. We conclude that hBVECs express an intermediate-affinity Na^+-independent choline transport system. This system seems to occur through a CTL1 and is responsible for the uptake of extracellular choline and organic cations in these cells. CTL2 participates in choline transport in mitochondria, and may be the major site for the control of choline oxidation.

MTU06-06

Classical and atypical agonists activate M1 muscarinic acetylcholine receptors through common mechanisms

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Xanomeline and N-desmethylclozapine (NDMC) are atypical M_1 functionally preferring agonists and represent promising prototype of a new class of selective agonists suitable for therapeutic use. The molecular mechanism determining their functional selectivity is still unclear. To elucidate their unique pharmacological profile we mutated key amino acids of the human variant of the M_1 muscarinic receptor involved in ligand binding (D105, D99), receptor activation switch (D71), and receptor/G-protein interaction (D122, R123). We compared the effects of these mutations on two atypical (xanomeline and NDMC) and two classical non-selective orthosteric agonists (carbachol and oxotremorine). Mutations of aspartate in the orthosteric binding site (D105) decreased both affinity and potency of all tested agonists at M_1 receptor. The effect of these mutations on affinity and potency was greatest for xanomeline and smallest for oxotremorine. Mutations of D105 also decreased affinity and but not potency of wash-resistant xanomeline. Mutation of residues (D71N and R123N) completely abolished functional response to both classical and atypical agonists. Our data show that both classical and atypical agonists activate M_1 receptors via the same molecular switch D71 in the second transmembrane helix and interact with G-protein α-subunit by the same way (via R123). Our data demonstrate key role of the orthosteric binding site in binding of and activation by both classical and atypical agonists. The principal difference

between classical and atypical agonists is thus the way they interact with the orthosteric binding site.

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MTU06-07

ALPHA7 nicotinic acetylcholine receptor-deficient mice exhibit sustained attention impairments that are reversed by ABT-418

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Disruptions of executive function, including attentional deficits, are a hallmark of a number of neurodegenerative and neuropsychiatric diseases. In schizophrenia for example, deficits in attention are one of the core negative symptoms of the disease. One of the most profound molecular changes in schizophrenia is the reduced expression of the a7 nicotinic acetylcholine receptor (a7nAChR) encoded by the *CHRNA7* gene. Acetylcholine (ACh) in the prefrontal cortex (PFC) plays a central role in regulating attentive behaviour. However, the role of a7nAChRs in attention is contentious and exactly how distinct cholinergic receptors contribute to attention performance is still unclear. Here we tested CHRNA7$^{-/-}$ mice using a touchscreen based 5-choice serial reaction time task (5-CSRT) to evaluate attentional performance. Compared to wild-type controls, CHRNA7$^{-/-}$ mice displayed a deficit in their ability to sustain attention. Administration of a7nAChR agonists improved attentional performance of wild-type mice in the 5-CSRT at low doses, but at higher doses had deleterious effects on their performance. None of the effects of a7nAChR agonists were observed in CHRNA7$^{-/-}$ mice. When treated with a b2nAChR agonist (ABT-418), the sustained attention deficits of the CHRNA7$^{-/-}$ mice were rescued, whereas the effect in wild-type mice was to improve attentional performance. Activation of a7nAChR increased cFos expression and ERK1/2 phosphorylation levels in the PFC of wild-type mice, whereas these effects were absent in CHRNA7$^{-/-}$ mice. Interestingly, CHRNA7$^{-/-}$ mice treated with a b2nAChR agonist showed marked increases in PFC cFos and ERK1/2 activation. These results suggest that a7nAChRs may contribute to attentional performance, but activation of b2nAChRs can bypass a7nAChR attentional deficits. Our experiments reveal an intricate relationship between distinct nicotinic receptors to regulate attentional performance and provide the basis of targeting b2nAChRs pharmacologically to decrease attentional deficits due to a7nAChR dysfunction.

MTU06-08

Effect of carbachol stimulation on medial prefrontal cortex network activity in acute slices from neuropathic and control mice

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Besides its prominent role in psychiatric diseases like major depression or schizophrenia, the medial prefrontal cortex (mPFC) has also been suggested to be important for the modulation of pain processing. Since the cholinergic system is strongly associated with brain and mood disorders, and neuronal processing in mPFC is altered in animal models of neuropathic pain, we investigated the effect of cholinergic stimulation of mPFC on neuronal activity in acute slices obtained from mice treated with spared nerve injury (SNI) and untreated controls.

Using *in vitro* 59 channel multielectrode arrays we recorded the effect of carbachol (CCh) stimulation on spontaneous action potential firing in acute brain slices of male C57BL/6J mice. After 5 minutes of baseline recording, slices were stimulated with 100 μM CCh for 5 min, followed by a washout phase of either 1, 10 or 20 min.

We found that 100 μM CCh induced firing of neurons in all recorded mPFC regions (i.e., prelimbic, infralimbic and anterior cingulate cortices). The overall amount of electrodes recording action potentials was increased from 6% before CCh stimulation to 45% after stimulation (n = 123). The effect of CCh stimulation ceased within 2 min after start of the stimulation and strong tachyphylaxis was observed upon repetitive applications.

A possible modulation of cholinergic signaling following SNI treatment is currently being analyzed.

MTU06-09

Microrna discriminators of brain-intestinal TLR9-cholinergic communication

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Brain-intestinal communication notably reacts to stressful, cholinergic and innate immune signals by changes in microRNAs (miRNA), small ~22–25 nucleotides long RNA suppressors of entire biological pathways. However, whether and how stress-mediated signals modify the intestinal cholinergic and innate immune pathways via miRNA regulators remained unexplored. Here, we report changes in several miRNA regulators of cholinergic genes (CholinomiRs) and correspondingly modified cholinesterase activities in intestine, splenocytes and the circulation of mice exposed to canonical and alternative oligonucleotide activators or blockers of Toll-Like Receptor 9 (TLR9). Stressful intraperitoneal injection of saline, anti-inflammatory TLR9 agonists or inflammation-activating TLR9 oligonucleotides all increased the expression of the acetylcholinesterase (AChE)-targeting miR-132. In comparison, anti-inflammatory TLR9 activation, but not stressful insults, elevated intestinal miR-129-5p, miR-186 and miR-200c, all predictably targeting both AChE and the homologous enzyme butyrylcholinesterase. Cultured immune cells, murine splenocytes and human mononuclear cells all reacted to anti-inflammatory TLR9 activation oligonucleotides by reduced AChE activity and nitric oxide, reflecting suppressed inflammatory reactions. This response was preventable by the TLR9 blocking oligonucleotides, suggesting joint TLR9-cholinergic control over inflammatory insults. The differential reaction of CholinomiRs to distinct TLR9 challenges indicates upstream co-regulation of the alternative anti-inflammatory NFκB pathway and cholinergic signaling by miRNAs. However, many CholinomiRs are primate-specific (Nadorp and Soreq, 2014) and single nucleotide polymorphisms (SNP) interrupting their functions may have detrimental consequences. For example, SNP-mediated interference with AChE suppression by the primate-specific miR-

608 associates with elevated AChE activity, blood pressure and inflammation (Hanin *et al*., 2014). To study primate-specific CholinomiRs in this cholinergic-intestinal communication, we engineered a humanized transgenic mouse line carrying the primate-specific CholinomiR hsa-miR-608. These mice show a similar expression pattern of hsa-miR-608 to the expression pattern in humans, including intestinal tissues, which constitutes a primate-reminiscent research system. Our findings highlight robust miRNA-mediated TLR9-cholinergic interaction and suggest studying TLR9 oligonucleotide potentiation of miRNA regulation, enhancing cholinergic signaling and promoting the resolution of inflammation for manipulating bowel diseases.

MTU06-10

Cholinergic mechanisms of meningeal nociception

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Parasympathetic innervation of meninges and the ability of carbamoylcholine, an agonist of acetylcholine receptors (AChRs), to induce migraine-like headache suggests an involvement of cholinergic mechanisms in migraine pathophysiology. However, the neurochemical mechanisms of cholinergic regulation of peripheral nociception in meninges are almost unknown. In the current project, using *ex vivo* rat hemiskull preparation with intact dura mater and preserved meningeal innervation we studied an action of cholinergic agents on peripheral nociceptive firing recorded by local microelectrodes. We found that 50 μM algogen carbamoylcholine significantly increased a number of nociceptive spikes recorded from a meningeal trigeminal nerve branch. This effect was sensitive to a muscarinic antagonist atropine (10 μM) suggesting an involvement of metabotropic AChRs in pro-nociceptive action of carbamoylcholine. However, nociceptive firing was also increased by 100 μM nicotine implying contribution of nicotinic ligand-gated receptors. Consistent with this, the action of nicotine was prevented by a nicotinic antagonist d-tubocurarine (50 μM) but was insensitive to TRPA1 antagonist HC-300033 (25 μM). To test a role of endogenous acetylcholine in regulation of meningeal nociceptive firing we applied the classical inhibitor of acetylcholinesterase – 12.5 μM neostigmine to *ex vivo* rat hemiskull preparation. Whereas the inhibition of acetylcholinesterase by neostigmine did not change the firing *per se*, a significant neostigmine-induced increase in nociceptive activity was observed after sensitization of meninges with the migraine mediator CGRP (calcitonin gene-related peptide). Performing calcium imaging on rat trigeminal ganglion cell culture we found that both nicotine and carbamoylcholine can induce $[Ca^{2+}]_i$ transients in trigeminal ganglion cells. Notably, nicotine mostly activated trigeminal ganglion neurons whereas carbamoylcholine massively induced $[Ca^{2+}]_i$ rise in satellite cells. Thus, peripheral nociceptive neurons in meninges express pro-nociceptive nicotinic and muscarinic AChRs which could represent a potential target for novel therapeutic interventions in migraine.

MTU06-11

ECTO-NTPdases, the ATP metabolizing enzymes, are critical determinants of cholinergic inhibition by adenosine in the human urinary

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Adenosine receptors are present in the human bladder where they can modulate filling sensations and urine voiding. Differences in the distribution of ecto-NTPDases and in the kinetics of ATP metabolism/adenosine formation between luminal and serosal sides of the urothelium may explain the dominant role of the nucleoside in suburothelium and detrusor smooth muscle layers. The catabolism of ATP (30 μM) was faster in the serosal side ($t½$ 26 ± 3 min, n = 3) compared to the luminal side ($t½$ 35 ± 3 min, n = 4) of the human urothelium. Higher amounts of ADP compared to AMP were detected following ATP (30 μM) incubation of urothelial strips with the luminal side facing up. This pattern suggests a dominant involvement of NTPDase2, which is a preferential nucleoside triphosphatase hydrolyzing ADP 10–15 times less efficiently than ATP with minimal AMP accumulation. AMP (30 μM) was dephosphorylated into adenosine more rapidly in the serosal side (67 ± 13 min, n = 5) than in the luminal side (117 ± 32 min, n = 3) of the urothelium. Immunoreactivity against NTPDase1 and NTPDase2 spans all layers of the human urothelium, while NTPDase3 stains only the terminally differentiated superficial umbrella cells. In the human detrusor, adenosine (100 μM) and its analogues, NECA (1 μM) and R-PIA (0.3 μM, selective A_1 receptor agonist), inhibit [^{3}H]ACh release from stimulated cholinergic nerves, when these drugs were used in concentrations unable to cause relaxation of myogenic contractions induced by acetylcholine (10 μM). Data suggest that adenosine formation from the extracellular catabolism of adenine nucleotides via distinct NTPDase subtypes is positioned to favour a dominant inhibitory role of the nucleoside on VAChT-positive cholinergic nerve afferents, which might contribute to control bladder overactivity. Support *FCT (FEDER funding, PTDC/SAU-OSM/104369/2008, PEst-OE/SAU/UI215/2014). IS is in receipt of a PhD fellowship by FCT (SFRH/BD/88855/2012).*

MTU06-12

Decreased nicotinic acetylcholine receptor expression in the developing piglet hippocampus after postnatal nicotine exposure

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Introduction: Postnatal exposure to cigarette smoke is a major risk factor for the Sudden Infant Death Syndrome (SIDS). Nicotine

is the major neurotoxic agent present in cigarettes and induces its action(s) by binding and activating the nicotinic acetylcholine receptors (nAchR) of which there are a total of 16 mammalian subunits.

Aim & Methods: Using a piglet model of early postnatal nicotine exposure, and applying immunohistochemistry, this study aimed to determine normal protein expression of 8 subunits (α2, α3, α4, α7, α9, β1, β2, δ) of the nAchR in 5 regions of the hippocampus : CA1, CA2, CA3, CA4 and the Dentate Gyrus (DG) of the control group (n = 14), and compared expression in the nicotine exposed piglets (n = 14).

Results: Compared to controls, the nicotine exposed group had significantly decreased α3 expression in the CA1 region (p = 0.02), β1 expression in the CA1 & CA2 regions ($p < 0.001$) and β2 in the CA3 (p = 0.04). Given the role of these subunits in synaptic transmission (α3), excitatory effects (β1) and neuronal survival (β2) these findings suggest that postnatal nicotine exposure may affect these physiological functions via the abnormal regulation of protein expression of these subtypes.

MTU07 Synaptic Transmission

MTU07-01

The direct action of cannabidiol at GABA-A receptors

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Introduction: Cannabidiol (CBD) is the major non-psychoactive component of cannabis. It possesses anti-epileptic, anxiolytic and anti-psychotic properties in humans, which could suggest GABAergic involvement. Endogenous and synthetic cannabinoids act at various GABA-A receptors, including the major central endocannabinoid, 2-arachidonylglycerol (2AG). CBD is currently being trialled in the treatment of pediatric epilepsy, however the actions of the phyto-cannabinoids have not been investigated at these inhibitory receptors. Here we present a study on the direct actions of cannabidiol (CBD) at specific GABA-A receptor combinations.

Objectives: Assessing the direct activation and modulation of CBD and 2AG upon GABA-A receptors. Specifically, to contrast the selectivity of these compounds between alpha and beta subunits of synaptic GABA-A receptors; i.e. α1-6β2γ2L and α2β1-3γ2L subunit containing GABA-A receptors.

Methods: Recombinant DNA techniques and *two-electrode voltage clamp electrophysiology* of receptors expressed in *Xenopus laevis* oocytes.

Results: CBD and 2AG modulate GABA, with CBD the more efficacious. There is selectivity for the alpha2 subunit with a greater than fourfold modulation of GABA EC5 with 100 μM CBD. At 10 μM CBD, the modulation of the GABA EC5 upon α2β2γ2L receptors was approximately twice that of other alpha subunit receptor combinations (i.e. 241% vs 160–180%, $p < 0.05$, $n = 6$).

In terms of beta subunit selectivity, modulatory activity was abolished when beta1 was introduced. At 10 μM CBD, the modulation of a GABA EC5 upon α2β2/3γ2L receptors was approximately the same (i.e. 241–246%) however upon α2β1γ2L was 0%.

CBD and 2AG were weak partial agonists at α1-5β2γ2L & α2β1-3γ2L GABA-A receptors. A compelling direct activation was observed at α6β2γ2L receptors, where CBD was 20% as efficacious as GABA.

Discussion: CBD directly activates and modulates GABA when applied upon specific synaptic GABA-A receptor combinations. CBD modulates these receptors more efficaciously than 2AG. In addition, selectivity for the alpha 2 and beta 2/3 subunits was discovered for synaptic GABA-A receptor combinations which may account for some of the intriguing central effects seen with CBD.

MTU07-02

Glutamine transport mechanisms in perisynaptic astrocytes

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Astrocytes adjacent to synapses are thought to release glutamine, which is subsequently sequestered by presynaptic terminals and used as a precursor for the neurotransmitters glutamate and GABA. Astrocytes in cell culture express a number of different amino acid transporters, which could mediate this release of glutamine. These include the system N transporters SNAT3 and SNAT5, the system A transporters SNAT1 and SNAT2, the system ASC transporter ASCT2, the system L transporters LAT1 and LAT2, and the system y^+L transporter y^+LAT2. By using combined pH imaging and whole-cell patch-clamp of astrocytes in acutely isolated brain slices we have investigated the nature of the glutamine release mechanism *in situ*. Astrocytes were recorded in brainstem slices from rats aged 10 to 15 days old. Application of 10 mM glutamine resulted in an inward current (-20.3 ± 2.2 pA; $n = 30$), which was inhibited by 20 mM methylaminoisobutyric acid (MeAIB; -1.3 ± 1.0 pA; $n = 4$; $P < 0.05$) and by removal of external Na^+ (0.44 ± 1 pA; $n = 6$; $P < 0.001$). Glutamine also caused an alkalinisation, which was still present when external Na^+ was replaced with Li^+, or when MeAIB was applied the pH change was inhibited by 20 mM histidine and not by leucine. Additionally, unlike glutamine, serine did not cause a significant pH change. These data identify at least two distinct glutamine transport systems within astrocytes recorded *in situ*. Properties of the glutamine-induced membrane currents indicate expression of system A while pH imaging reveals a second non-electrogenic glutamine transport system with properties identifying it as system N. Furthermore, the lack of effect of serine suggests that the SNAT3 not the SNAT5 isoform predominates. As SNAT3 is known to be readily reversible under physiological conditions and we propose that it is responsible for the astrocytic glutamine release, which mediates the recycling of glutamate and GABA at synapses.

MTU07-03

Molecular determinants in two loops of the glutamate transporter 1 involved in transporter reorganization

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The excitatory amino acid transporters (EAATs) constitute the major mechanism of removing extracellular glutamate in the central nervous system (CNS), and are thus involved in modulating glutamatergic signaling [1]. While significant progress in understanding the mechanism of transport has been made, the specifics of the molecular determinants of several steps of the translocation of substrate are still elusive. Structural insight into the outward- and inward facing conformations of Glt_{Ph} shows two loops connecting a static trimerization domain and a dynamic transport domain [2, 3]. The two loops, intracellular loop 1 (ICL1) and extracellular loop 3 (ECL3), show very different arrangements in the two different confirmations, unwinding from one alpha helix and rewinding onto another to accommodate the movement between the two domains of the transporter. Based on these Glt_{Ph} structures, we have probed the homologous loops in the glutamate transporter 1 (GLT-1), the rodent ortholog of human EAAT2, by semi-random mutagenesis and assayed the effect using [^{3}H]-D-Asp uptake in HEK293T cells.

Preliminary data hints at some residues that perturb enzymatic function. This is true both for residues that are conserved across subtypes, such as G117, as well as residues that are unique for subtype 2, such as S106. These findings suggest residues that are important for the reorganization of the transporter between inward and outward facing during the translocation process, and which will be investigated further.

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MTU07-04

Polysialic acid controls neuronal activity in the nucleus of the solitary tract influencing the tonic and reflex control of blood pressure

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The adult nucleus of the solitary tract (nTS), a key site for the tonic and reflex control of blood pressure highly expresses the extracellular sugar –polysialic acid (PSA). PSA in higher brain regions appears to alter neuronal signalling however its role in the nTS is unknown. We investigated the role of PSA in neuronal signalling in the nTS at the cellular and systems levels. Whole-cell recordings of second order neurons, identified by electrical stimulation of the solitary tract, were obtained from brain slices perfused with the PSA cleaving enzyme endo NF. Endo NF treatment decreased evoked EPSCs (eEPSC) amplitudes (216.4 ± 54.2 vs. 469.0 ± 86.1pA; $n = 5$; $p < 0.01$) and increased latency of the eEPSC (4.36 ± 0.7 vs. 3.48 ± 0.7 ms; $n = 5$; $p < 0.05$) when compared to control. Decreases in spontaneous EPSCs (sEPSC) amplitude and frequency were also observed. These changes were blocked by pre-infusion of the trkB receptor antagonist, K252a. Microinjection of another sialic acid cleaving enzyme, neuraminidase (NEU), into the extent of the NTS of urethane-anaesthetised male Sprague-Dawley rats caused brief hypertension, tachycardia and sympathoexcitation. Sympathetic baro-reflexes were also attenuated. In contrast, microinjection of enzymes that cleave different sugar residues had no effect. These results demonstrate PSA alters signalling in the nTS decreasing primary afferent glutamatergic excitatory transmission and appears to mediate this effect via BDNF. PSA may act as a scaffold for BDNF, influencing its release onto trkB receptors within the nTS.

MTU07-05

Paraventricular nucleus of the hypothalamus afferents excite second order nucleus of the solitary tract neurons in mice

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Neurons in the nucleus of the solitary tract (NTS) receive sensory afferent signals from internal organs and substantial input from the paraventricular nucleus of the hypothalamus (PVN). These neurons also express the angiotensin type 1A receptor ($AT_{1A}R$). We hypothesised that PVN inputs excite $AT_{1A}R$-expressing NTS neurons that also receive direct viscerosensory input. To test this we employed optogenetics in combination with *in vitro* slice electrophysiology. An adeno-associated virus expressing channel rhodopsin 2 under control of a ubiquitous promoter (CAG) was microinjected into the PVN of transgenic mice expressing a fluorescent reporter (GFP) in $AT_{1A}R$-expressing neurons. Twelve weeks later 250 μm horizontal brain stem slices were prepared and superfused with aCSF. Stimulation of the solitary tract (ST) evoked low jitter, excitatory post-synaptic currents (EPSCs) that identified second order NTS neurons. Optical activation of PVN afferents (450 nm, 10 mW, 10 ms) evoked inhibitory or EPSCs. Of 39 NTS neurons recorded (15 GFP positive), LED evoked EPSCs were observed in 1 GFP negative neuron (amp = −9 pA, latency = 11 ms, jitter = 1276 μs) and 1 GFP positive neuron (amp = −38 pA, latency = 13 ms, jitter = 1071 μs). An LED evoked inhibitory post synaptic current (Amp = 68 pA, Latency = 62 ms, jitter = 17 720 μs) was observed in 1 GFP positive NTS neuron which displayed high jitter ST evoked EPSPs (jitter = 390 μs). Our results show that PVN afferents can directly excite some $AT_{1A}R$ expressing 2nd order NTS neurons. Future work will determine the conditions in which this connection is activated *in vivo* and whether this modulates autonomic reflexes.

MTU07-06

Analysing dendritic NMDA spikes from synchronous and asynchronous multi-site synaptic activation

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Dendritic integration of multiple synaptic inputs along basal dendrites of L5 cortical pyramidal neurons generates NMDA spikes and eventually triggers somatic action potentials. These inputs can originate from multiple correlated pre-synaptic neurons causing synchronous or asynchronous synaptic release of neurotransmitters. Here, we investigate this phenomenon using a custom-built holographic two-photon (2P) microscope, where we perform simultaneous and sequential multi-site uncaging pertaining to synchronous and asynchronous inputs, respectively.

Methods: Parasagittal cortical slices (300 μm) were prepared from Wistar rats (P15–19) and perfused with ACSF. We filled the neurons through the recording electrode with standard intracellular solution and 200 μM of Alexa-488. A 3D image of the neuron was rendered to map the basal dendrites. Simultaneous multi-site uncaging (~2 ms dwell-time) was done using holographic projection

of multiple foci, while sequential uncaging was achieved by swiping the laser focus spanning 30 μm along the dendrite with ~1 ms dwell-time per spine. Recordings of NMDA spikes were done in whole-cell current-clamp. NMDA spikes, characterised by a significant depolarisation of local membrane potential and half-width duration > 100 ms, were generated via 2P uncaging of MNI-caged-glutamate along the basal dendrites > 100 μm away from the soma. To confirm the NMDA spikes, we applied APV to block and consequent washout re-establishes the spikes.

Results: Sequential uncaging of glutamate onto basal dendrites reliably produces NMDA spikes with typical peak EPSP amplitude of 7.8 ± 2.5 mV and rise-time of 46.6 ± 5.1 ms. We compared this result with sub-threshold EPSP following simultaneous uncaging of glutamate. For simultaneous uncaging, the rising phase of the EPSP, extrapolated to a typical NMDA peak amplitude of 7.8 mV, was 22.3 ± 5.6 ms, which is two times faster than sequential activation. Moreover, the EPSP from simultaneous uncaging has a shorter latency (~1 ms) compared to sequential uncaging (> 10 ms). Our results show differences in temporal dynamics and kinetics of dendritic integration between synchronously and asynchronously activated synaptic inputs on basal dendrites of L5 pyramidal neurons.

MTU07-07

Phosphorylation of synaptic vesicle protein 2A at THR84 controls the specific retrieval of synaptotagmin-1

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Synaptic vesicle protein 2A (SV2A) has proposed roles in both SV trafficking and neurotransmitter release, however little is known regarding how SV2A function is regulated. We demonstrate that phosphorylation of SV2A by Casein kinase 1 family members triggers binding to the C2B domain of human synaptotagmin-1 (SYT1), the calcium sensor for fast synchronous neurotransmitter release. Phosphorylation of Thr84 on SV2A is essential for this interaction. SV2A phosphorylated on Thr84 binds to a pocket on the surface of the SYT1 C2B domain formed by three conserved Lys residues. Phosphorylation of SV2A at Thr84 had no effect on its own targeting to SVs or retrieval kinetics during endocytosis; however, it did control the retrieval of SYT1. Mutant SYT1 that cannot bind SV2A showed increased plasma membrane localisation and altered retrieval during endocytosis when expressed in primary hippocampal neuronal cultures. Importantly, knockdown of SV2A, or rescue with a phosphorylation-null Thr84 SV2A mutant, also caused increased surface stranding of SYT1 and specifically altered SYT1 retrieval during endocytosis. We propose that SV2A is a phospho-dependent chaperone required for the specific retrieval of SYT1 during SV endocytosis.

MTU07-08

Effects of gamma subunit mutations on mobility and clustering of GABA-A receptors studied by super-resolution microscopy

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$GABA_A$ receptors are ligand-gated ion channels that mediate fast ionotropic neurotransmission throughout the central and peripheral nervous system. They consist of five subunits arranged around central pore. Mutations in these subunits are linked to central nervous system diseases including epilepsy, schizophrenia and insomnia. However, little is known about how these mutations affect ligand affinity, channel activation, receptor surface expression, mobility and clustering. Here we focus on the $GABA_A$ receptor containing $\alpha 2$, β_2 and γ_2 subunits in order to examine how γ_2 subunit mutations, which modulate physiological properties of the channel, affect receptor surface expression, mobility and clustering.

We first express the $GABA_AR$ subunits of interest together with neuroligin 2 in HEK293 cells, and co-culture these on a bed of GABAergic neurons to induce the formation of synapses from neurons onto HEK293 cells. The synapses formed exhibit functional characteristics of real synapses, including spontaneous and evoked synaptic currents and synaptic vesicle recycling. We then employ patch-clamp electrophysiology to quantify the effects of mutations on synaptic current rise times, peak magnitudes and decay times. To determine the effect of mutations on the surface distribution, synaptic clustering and mobility of $GABA_ARs$, we tag the γ_2 subunit with either Super-Ecliptic pHluorin or mEos2 fluorescent proteins and use Single Particle Tracking (SPT) and Photo-activated Localisation Microscopy (PALM) to record movement of single ion channels and morphing of their clusters in live cells. These single molecule imaging approaches make it possible to measure the size and shape of these structures with high precision and spatial resolution of 40 nm. Our initial assessments show that for example $\alpha_2\beta_2\gamma_{2L}$(T350L) $GABA_A$ channels express in a cell membrane and form clusters with diameter very similar in size to $\alpha_2\beta_2\gamma_{2Lwt}$ channels, but they move with different speeds. We expect that such a behaviour will affect the $\alpha_2\beta_2\gamma_{2L}$(T350L) $GABA_A$ propensity to cluster at synapses which will additionally modulate recorded synaptic currents.

MTU07-09

Identifying the GABA-A receptor ivermectin binding site

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$GABA_A$ receptors are the major inhibitory neurotransmitter receptors in the mammalian brain. They consist of five subunits that arrange together to form an ion channel. We utilized the X-ray structure of the homomeric *C. elegans* glutamate-gated chloride channel (GluCl), as a structural model to study the binding and modulatory properties of ivermectin (IVM) at alpha1 beta2 gamma2L $GABA_A$ receptors. IVM is a macrocyclic lactone that is used as an antiparasitic agent in humans and animals. IVM binds in the transmembrane domain of the receptor at subunit interfaces. We performed a series of experiments aimed at understanding the mechanism of ivermectin modulation using site-directed mutagen-

esis of the equivalent GluCl IVM binding pockets followed by patch clamp electrophysiology. By the respective phenylalanine mutation we isolated the GABA alpha1 A293 and gamma2 S301 residues located at the 3rd transmembrane domain (M3) as key elements promoting the access of IVM in the binding pockets conformed by the interfaces alpha-beta and beta-gamma, in a similar way as it was showed in the homomeric Glycine receptor mutant alpha1 A288G, achieved in correlation to the equivalent position occupied by G281 (M3-Gly) in the GluCl structure. We conclude the alpha-beta and gamma-beta, but not beta-alpha interfaces are important for the IVM access into the $GABA_A$ receptor, since a methionine (M285) naturally block the M3-Gly position in the beta subunit.

MTU07-11

Towards a detailed description of AP180 assembly domain-protein interactions in clathrin mediated ENDOCYTOSIS6

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Clathrin assembly protein AP180 is a key component of the clathrin mediated endocytosis protein machinery, which enables synaptic vesicle biogenesis. As such, it is present in purified clathrin coated vesicles at the equal highest concentration, with adapter complex AP2. AP180 knockdown studies have shown that AP180 controls synaptic vesicle size and shape. And this property has been shown to be mediated by the AP180 C-terminal assembly domain (AD). However, detail on the mechanisms of AD-protein interactions are lacking. Within the AD sequence, a central acidic clathrin and adapter protein (CLAP) binding sub-domain contains all of the known short binding motifs for clathrin and AP2. The role of the remaining ~16 kDa C-terminal basic sequence (ADΔCLAP) has not been clear. Most of the known phosphorylation sites are also in the CLAP domain, but their function is not clear. We now show that specific sequences in the ADΔCLAP are essential for efficient clathrin binding. We have also determined the effect of AP180 phosphorylation on binding to the major AP180 binding partners, AP2 and clathrin. These observations update and add important details to the long-standing model of AP180-protein interactions during clathrin mediated endocytosis.

MTU07-12

Intrinsic circuitry of the lateral central amygdala

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The amygdala is a region of the brain that is responsible for the processing and memory of emotions. Understanding the neural circuitry of this area is essential to grasping how it achieves this function and may aid in developing treatments for a range of anxiety disorders. Two main subregions of the amygdala, the basal and lateral nuclei (BLA), and the central nucleus (CeA), play key roles in the acquisition and expression of emotions respectively. While the BLA has been extensively studied, the CeA has only recently gained interest, and as such, its intrinsic circuitry is under intense study. Cells in the CeA are mainly inhibitory GABAergic (gamma-aminobutyric acid) cells and single unit recordings of the activity of specific cell populations suggest that these cells form strong local inhibitory connections. These local connections are central to the proposed circuitry underlying the behavioural roles of the CeA. However, our understanding of these local connections at the cellular level is in its infancy; the strength and physiological role of individual connections are largely unknown. We have made dual whole-cell recordings in the lateral CeA to determine the physiological properties and strength of local inhibitory connections. Current-clamp recordings showed three types of discharge properties: regular spiking (RS), late firing (LF) and fast spiking (FS). In paired recordings, 32 out of 94 ($\approx$ 34%) were connected, of which 29 connected pairs were unidirectional and three were bidirectional. Connections were on average 22 $\pm$ 5 pA when voltage-clamped at -40 mV, and were inhibited by picrotoxin, indicating that these were indeed GABAergic connections. In these pairs, presynaptic cells were RS (47%), LF (35%) or FS (18%) whereas postsynaptic cells were mostly LF (70%). Finally, the inhibitory connection was sufficient to halt firing in the postsynaptic cell. These results confirm that cells in the lateral portion of CeA form local inhibitory connections, which are capable of silencing the postsynaptic cell.

MTU07-13

Targeting changes in inhibitory signalling in chronic pain

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Chronic pain can be difficult to manage with current therapeutics. A large body of evidence in animal models and humans suggests that chronic pain involves persistent pathological adaptations. These include changes to excitatory synaptic plasticity and inhibitory neurotransmission. Some of these adaptations in neuropathic pain are potential new therapeutic targets. Much of the fast inhibitory neurotransmission in the spinal cord is mediated by glycine which, when impaired, results in the pathological symptoms of neuropathic pain. In this study we used a partial sciatic nerve ligation (PNL) model of chronic pain in adult rats. Inhibitory synaptic currents were measured in whole-cell voltage-clamp from laminae II cells. Tungsten electrodes placed in the inner laminae were used to elicit eIPSCs. We have found that glycinergic neurotransmission is reduced, or occasionally abolished, in a subset of neurons in the superficial laminae of the dorsal horn of animals with neuropathic pain, whilst GABAergic neurotransmission remains the same. In this study we investigated structural and cellular differences in inhibitory synaptic connections to identify the basis for changes in neuropathic pain. Lastly we assess the effects of GlyT2 inhibitors on spinal signalling in the neuropathic pain model. These inhibitors increase glycinergic neurotransmission by prolonging the synaptic current. Our results suggest that inhibition of GlyT2 may be another potential target for neuropathic pain.

MTU07-14

Synthesising and characterising new ivermectin analogues targeting ligand-gated chloride channels

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Purpose: The membrane-bound ligand gated ion channels or cys-loop receptors mediate transmission of ionic current across cell membranes, an important process for numerous physiological responses e.g., muscle contraction, electrolyte secretion and synaptic transmission. The members of this family include eukaryotic nAChRs, $GABA_A$Rs, GlyRs and 5-HT_3 receptors and prokaryotic GluClRs. Ivermectin, a mocrolcyclic lactone (ML) is a well-tolerated anthelmintic drug that activates nematode GluClRs and mammalian GlyRs and $GABA_A$Rs. Although crystal structure of ivermectin docked to the *C. elegans* α-GluClR clearly defines the orientation in its site, the molecular interactions that mediate ivermectin binding are still unanswered. Understanding the molecular interactions, structure-activity relationships (SAR) and subtype-specific effects of MLs will define novel therapeutic pharmacophores that may be useful as improved therapeutics for such neurological disorders as inflammatory pain, epilepsy, spasticity and tinnitus. Here we aim to synthesise a range of ML analogues as pharmacological probes for investigating binding interactions and SARs.

Methods: 22 newly synthesised MLs were screened against α1 GlyR expressed in HEK293 cells using an anion-sensitive yellow fluorescent protein assay. The potencies of active analogues were quantitated at α1 GlyR by patch-clamp electrophysiology.

Results: SAR studies revealed that 7-OH, 5-OH, 2-unsaturated alicyclic moiety were very crucial to retain the ivermectin activity on α1 GlyR whereas removal of sugar part at C14 did not affect activity significantly. Removal of 7-OH or conversion of alicyclic to aromatic abolished the effect completely and any change in 5-OH made the molecules less potent or lost the activity.

Conclusion: The early data elucidate the structural mechanisms by which ivermectin binds to GlyRs and perhaps other chloride channels. We are currently synthesising more ivermectin analogues. Together with mutant-cycle analysis, these findings will improve our understanding of a poorly understood therapeutic pharmacophore and provide structural information relevant to the design of new high affinity, subtype-specific GlyR- or $GABA_A$R-targeted therapies.

MTU07-15

Fluoxetine induces restoration of plasticity in the adult vestibular system

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Antidepressant fluoxetine is known to restore neuronal plasticity in the adult visual cortex by modulating GABA level. We hypothesize that fluoxetine also reinstates plasticity in the adult vestibular nucleus (VN) by altering GABAergic transmission. We previously showed that derangement of spatial navigation in adult rats was observed with neonatal blockade of $GABA_A$ receptor ($GABA_A$R) in the VN but not with blockade applied after P14. In the present study, we demonstrated that deficits in spatial navigation could still be induced in adult rats that received both bicuculline treatment in the VN and oral administration of fluoxetine from P21 onwards. This implies that fluoxetine restores the plasticity observed in critical period during which bicuculline induces deficits in spatial navigation. With western blot analysis, we found that oral administration of fluoxetine during P21–28 significantly reduced protein expression of potassium-chloride co-transporter (KCC2) in the VN. Since KCC2 is responsible for the extrusion of chloride ions, lower level of KCC2 expression indicates less chloride ions flowing through $GABA_A$R, causing a reduction in the impact of inhibitory GABAergic transmission. The latter is corroborated with the observation that the proportion of VN neurons exhibiting $GABA_A$R-mediated long-term depression (LTD) was significantly increased from 34.6% in controls to 50% in P28 rats pre-treated with fluoxetine during P21–28. These results imply that fluoxetine induces a reduction in GABAergic transmission, thus contributing to the restoration of the plasticity observed in critical period. [Supported by HKU 761812M].

MTU07-16

Glutamate receptor expression and stability is regulated via TSG101-dependent lysosomal degradation in neurons

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The processes that regulate protein degradation in neurons are of fundamental importance for normal cell function. Perturbations of degradative systems and the resulting protein aggregations are implicated in a wide range of neuropathological conditions including Alzheimer's, Huntington's and Parkinson's diseases. A prominent feature of these neurodegenerative disorders is the accumulation of proteins that can overwhelm various pathways. Membrane protein degradation occurs via their sorting into intralumenal vesicles of late endosomes and subsequent fusion with lysosomes, targets these proteins to degradation. This initial sorting event is primarily mediated by three large protein complexes termed the endosomal sorting complex required for transport (ESCRT)-I, -II and -III. These complexes act sequentially in the sorting of mono-ubiquitinated membrane proteins including neurotransmitter receptors into subdomains on the endosomal surface prior to invagination and lumenal vesicle formation. TSG101 (tumour susceptibility gene), is a central component of ESCRT-I that binds ubiquitinated cargo proteins prior to their passage to ESCRT-II and -III, and subsequent degradation. Previously we have shown that TSG101 is expressed in rat brain and involved in neurotransmitter receptor degradation in HEK cells. This study used both biochemical and imaging approaches to show that TSG101 protein affects the sorting and expression of glutamate receptors in dispersed cultured neurons. Specifically GluA1, GluA2, GluK2 and mGluR5 receptor expression was increased when TSG101 expression is inhibited using Lenti-virus mediated knock-down. On the other hand TSG101 overexpression using Sindbis virus reduced the level of receptor expression. From the results it can be concluded that TSG101 and the ESCRT system is a general mechanism for receptor degradation in neurons and plays an important role in determining the

availability of neurotransmitter receptors. This has implications in synapse formation, maturation, stability and plasticity, which in turn dictate the connectivity and network in the central nervous system.

MTU07-17

Structural and energetic pathways in glycine receptor activation

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Pentameric ligand-gated ion channels (pLGICs), such as the glycine receptor (Gly-R) mediate fast chemo-electrical transduction in the nervous system. Naturally occurring mutations to the Gly-R can give rise to rare movements disorders, such as hyperekplexia. These point mutations most commonly occur in the α1 subunit of α1β heteromeric Gly-Rs, and profoundly affect the activation mechanism of the receptor. We investigated three, spatially clustered, hyperekplexia-causing mutations in α1 homo- and α1β heteromeric Gly-Rs using single channel current recordings. All three of these residues are located in the signal transduction zone of pLGICs. The charge of residues at the positions 19' and 24' were systematically altered on a background of the α1Q−26'E mutation. Examining the conductance and durations of single channel activations (clusters) monitored functional changes in the mutated receptors. These parameters were among those used to determine interaction coupling-energies, which we correlated with structural displacements associated with receptor activation, as measured in pLGIC crystal structures. The α1Q−26'E mutation alone reduced single channel conductance and enhanced cluster durations. Strong coupling energy was determined between α1Q−26'E and α1R19' across the subunit interface suggesting this interaction is vital for receptor activation. Cross-linking experiments demonstrated that the segment containing the 24' residue is highly mobile, but a lack coupling between α1−26'and α124' implies that 24' mutations disrupt activation via other pathways. Reciprocal mutations in the β subunit show a similar lack of energetic coupling between α1−26' and suggests that the β subunit remains relatively static during receptor activation. The functional effects of α1Q−26'E on α1β receptors, however, are not absent, suggesting the presence of at least one α1−α1 interface per heteromeric pentamer. The changes in coupling energy between α1−26' and α119' correspond to local structural displacements revealed by crystal structures of pLGICs in putative conducting and non-conducting states, implying that it comprises a key energetic pathway in activating Gly-Rs and other pLGICs.

MTU07-18

Measuring KCC2 function in brain slices using BCECF imaging

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KCC2 is a Cl^--extruding neuron-specific K^+–Cl^--cotransporter important for maintaining Cl-homeostasis in the brain and for robust $GABA_A$-receptor mediated hyperpolarization. To investigate the effects of increasing KCC2 function of neuronal inhibition we are utilising a conditional tetracycline-dependent KCC2 transgenic mouse in which KCC2 mRNA and protein expression is increased following withdrawal of doxycycline (DOX) from the diet. The aim of this study was to determine if increased KCC2 expression results in increased functional KCC2-mediated transport. KCC2 can also transport NH_4^+ into cells enabling KCC2 transport to be measured by changes in fluorescence of the pH- sensitive dye, BCECF-AM. Using high concentrations of BCECF (50–150 μM for 40–50 min) we were able to achieve robust loading of pyramidal neurons in hippocampal slices of young adult mice (2–3 months old). Bath application of NH_4Cl (10 mM) resulted in a passive alkalinisation (via NH_3 diffusion) followed in some slices by a KCC2-mediated acidification. In slices from homozygous and DOX-on control mice, the alkalinisation peaked about 1–2 min after application of NH_4Cl (at a fluorescence intensity of 103.6 ± 1.8 of control values, $n = 16$ neurons from 3 different slices) and was followed by a very modest acidification (to 89.2 ± 2.9,). Re-application in the presence of the KCC2 blocker furosemide (0.5 mM, note that all experiments were done in the presence of bumetanide 10 μM) only marginally affected this acidification. In Dox-off mice, the initial alkalinisation was seemingly reduced (100.7 ± 0.8 of control fluorescence at 1–2 min, $n = 31$ neurons from 4 different slices) compared to control mice, but the subsequent acidification was larger and significantly greater compared to control mice (73.2 ± 2.9 of control, $p < 0.05$). Furthermore, this acidification was significantly inhibited by furosemide (89.9 ± 1.9). Our results indicate that increasing KCC2 expression and protein levels in the transgenic mice results in increased functional KCC2 membrane transport.

MTU07-19

Impact of APP and APLP2 deletion on the hippocampal PAZ proteome

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The amyloid precursor protein (APP) belongs to a conserved gene family that comprises in mammals the amyloid precursor like proteins 1 and 2 (APLP1 and APLP2). All APP family members are constituents of the presynaptic active zone (PAZ) but their physiological functions within the CNS is still unknown. Interestingly, double knockout mice, lacking APP and APLP2 die shortly after birth. To circumvent lethality, the NexCreAPP/APLP2 mouse line (NexCre-cDKO) was recently designed allowing the analysis of the APP/APLP2 deletion in hippocampal neurons at any age. Quantitative proteomic analysis of the immunopurified presynaptic active zone proteins revealed significant changes in the abundance of hippocampal PAZ constituents in adult mice. NexCre-cDKO mice display changes in abundance for proteins (calmodulin, neuromodulin, neurogranin) involved in the regulation and maintenance of Ca^{2+}-homeostasis. Since Ca^{2+}-signaling and Ca^{2+}-homeostasis are essential for synaptic plasticity, the dysregulation of calcium proteins in NexCre-cDKO mice may be in line with the reported impairments in LTP associated with deficits in learning and

memory. Furthermore, the reported deficits in LTP can be connected to impairments in synaptic transmission. Neurotransmitter release requires functional fusion machinery, comprising the core constituents of the SNARE complex: synaptobrevin2/VAMP2, syntaxin-1 and SNAP25. In this study, quantitative proteomic analysis revealed that these proteins are unaltered. However, α-synuclein, an important mediator of coordinated neurotransmitter release was severely decreased. These results strikingly indicate a dysregulation of presynaptic active zone proteins involved in synaptic transmission and plasticity. Whereas these alterations tend to be downregulated in adult APP single knockout mice, they become obvious in the NexCre-cDKO pointing to a compensatory effect of APLP2. In summary, quantitative proteomic analysis of APP mutant mice provide an important tool for unravelling the impact of APP on dynamic synaptic structure and function.

MTU07-20

Synthesis of methyl palmitate in the rat superior cervical ganglion

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Cerebral blood vessels receive dense sympathetic innervation originating in the superior cervical ganglion (SCG). Palmitic acid methyl ester (PAME) or methyl palmitate, a saturated fatty acid, is released from the preganglionic neurons of the SCG upon depolarization by field electrical stimulation or nicotine. The released PAME has been shown to play a role in modulating ganglionic transmission, and possibly cerebral sympathetic neuronal activity and the cerebral vascular function. The synthetic pathway of PMAE in the SCG, however, remained undetermined. The isolated SCGs of the Spraque Dawley rats were incubated in neurobasal medium (37° C) containing stable isotope ^{13}C-palmitic acid (^{13}C-PA). After incubation in different time periods, the mediums were collected and ^{13}C-PAME analyzed by GC/MS. In mediums which contained ^{13}C-PA without the SCG, no ^{13}C-PAME in the incubation mediums was detected. In the incubation mediums containing the SCG and ^{13}C-PA, significant concentrations of ^{13}C-PAME in an incubation time-dependent manner were found, indicating synthesis and release of PAME in the SCG neurons. ^{13}C-PAME concentrations in the mediums were significantly decreased by co-incubation with S-adenosyl homocysteine (100 mM) which is an endogenous feedback inhibitor of catechol-O-methyltransferase (COMT). Similar inhibition was found by tolcapone (10 nM) which is a nitro catechol type inhibitor of COMT. Moreover, ^{13}C-PAME concentrations in incubation mediums were reduced by ML-9 (30 mM), a myosin light chain kinase (MLCK) inhibitor. These results suggest that PAME is synthesized from PA catalyzed by COMT in cerebral sympathetic ganglionic neurons.

MTU07-21

Subunit-specific modulation of glycine receptors by ginkgolic acid

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Ginkgolic acid, a constituent of *Ginkgo biloba* lipophilic extract, has been shown to have anti-tumoral activity, ability to inhibit HIV protease and SUMOylation. However, its effect on receptor operated channels was not studied. Using whole-cell configurations of patch-clamp recordings, we analysed the effects of ginkgolic acid (100 nM–25 μM) on different subunits (α1, α2 and αH3-long) of the glycine receptor (GlyR) and on $GABA_A$Rs expressed in cultured CHO cells. Pre-treatment of homomeric α1 GlyR with ginkgolic acid (25 μM; 20–40 s) caused an increase in the amplitude of currents induced by sub-saturating glycine concentrations and a decrease in the mean EC_{50} for glycine from 36 ± 3 μM (control) to 22 ± 1.4 μM (during ginkgolic acid application). Similar potentiation was observed for heteromeric α1/β GlyR. In contrast, ginkgolic acid reversibly inhibited currents mediated by homomeric α2 or heteromeric α2/β receptors. The effects on α3-long GlyR and $GABA_A$R were negligible. The kinetics of α1 GlyR potentiation development was slow, on a time scale of minutes, while the inhibitory effect of ginkgolic acid on α2 GlyRs was much faster and developed in less than 30 sec, suggesting different mechanisms of action. Mutation of three residues (T59A/A261G/A303S) in the α2 subunit converted the inhibitory action of ginkgolic acid into potentiation, which developed slowly, similar to that for the α1GlyR. Our results suggest that (i) ginkgolic acid selectively enhances the function α1 GlyRs and attenuates the function of α2 GlyRs; (ii) mutation of α2 subunit converts effect of ginkgolic acid from inhibition to potentiation.

MTU07-22

Transport rates of a glutamate transporter homologue are influenced by the lipid bilayer

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The aspartate transporter from *Pyroccocus horikoshii* (Glt_{Ph}) is a model for the structure of the SLC1 family of amino acid transporters. Crystal structures of Glt_{Ph} provide insight into mechanisms of ion-coupling and substrate transport; however structures have been solved in the absence of a lipid bilayer so provide limited information regarding interactions that occur between the protein and lipids of the membrane. Here, we investigate the effect of the lipid environment on aspartate transport by reconstituting Glt_{Ph} into liposomes of defined lipid composition where the primary lipid is phosphatidylethanolamine (PE) or its *methyl* derivatives. We show the rate of aspartate transport and the transmembrane orientation of Glt_{Ph} is influenced by the primary lipid in the liposomes. In PE liposomes, we observe the highest transport rate and show that 85% of the transporters are orientated in the right-side out, while in *tri*-methyl PE liposomes 50% of transporters are right-side out and we observe four-*fold* reduction in transport rate. Differences in orientation can only partially explain the lipid composition effect on transport rate. Crystal structures of

Glt_{Ph} reveal a tyrosine residue (Y33) that we propose interacts with lipid head-groups during the transport cycle. Using site-directed mutagenesis, we propose a cation-π interaction between Y33 and the lipid head-groups can influence conformational flexibility of the trimerization domain and thus, the rate of transport. These results provide a specific example of how interactions between membrane lipids and membrane-bound proteins can influence function, and highlight the importance of the role of the membrane in transporter function.

MTU07-23

Role of bassoon in the regulation of synaptic vesicle pool size

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The active zone (AZ) is a region of the presynaptic bouton where neurotransmitter release takes place, characterized by the presence of an electron-dense structure called the cytomatrix at the active zone (CAZ). This cytoskeletal matrix is composed of multidomain scaffold proteins (RIM, RIM-BP, Munc-13, CAST/ELKS, Liprins α, Piccolo and Bassoon), which act as functional and structural organizers of the release sites and regulators of synaptic vesicle exocytosis. The role of Bassoon (Bsn) in presynaptic function has been investigated in several studies, which suggest that this protein does not have an essential role in synapse formation but rather contributes to the plasticity of neurotransmitter release. These studies showed that in conventional and ribbon synapses Bassoon is involved in the reloading of vesicles and the regulation of RRP size, but the molecular mechanisms underlying this process are still not well understood. In order to clarify the mechanism of this Bassoon function, we characterized presynaptic composition and function in primary hippocampal neurons derived from mice lacking Bsn expression. We observed decrease in the synaptic abundance of most CAZ proteins in Bsn lacking synapses and in line with our previous studies, defects in synaptic vesicle release. To dissect this phenotype we used the synaptophysin-pHluorin-based reporter technique allowing analysis of synaptic vesicle pools size and of release characteristics. We found that ready-releasable pool (RRP) and recycling pool (RP) were reduced and proportion of resting vesicles that do not participate in release was increased in the absence of Bsn. Using phamacological intervention we identified involvement of CDK5/calcineurin B and PKA-dependent signaling in this process. Together our study provides new inside into mechanistical understanding of Bassoon function at presynapse.

MTU07-24

Analysis of the localization of glutamate receptors in X11/X11 l double-deficient mice

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X11/Mint1 (Munc18-1 interacting protein 1), X11-like (X11L/Mint2), and X11-like 2 (X11L2/Mint3) are adaptor proteins whose are highly conserved evolutionarily. The X11 family proteins comprise a PTB (Phosphotyrosine binding) domain and two PDZ (PSD95/discs large/ZO-1) domains in their carboxyl terminal half along with a poorly conserved N-terminal region. Among X11s, X11 is predominantly and X11L is specifically expressed in brain. X11s regulate localization of NMDA receptor and AMPA receptor *in vitro* or in *C. elegans* [review in 1, 2, 3]. Therefore, these previous reports indicate that X11 and X11L play an important role in the postsynaptic function, but little is known for their exact roles in the postsynapse *in vivo*. Therefore, we analyzed X11/X11L double-deficient mice (X11/X11L DKO) to evaluate the role of X11 and X11L in postsynapse. First, I measured amount of NMDA receptor and AMPA receptor in cortex and hippocampus, but no remarkable differences are observed. Second, I measured amount of NMDA receptor and AMPA receptor in PSD fraction, but significant changes of receptor amounts between DKO and wild-type mice was not detected. Finally, I labeled cell surface proteins and quantified the receptor numbers in acute hippocampal slice. The amounts of NMDA receptor on cell surface were significantly changed in DKO mice. These data suggest that both X11 and X11L play an important role in the regulation of glutamate receptors localization of dendrites. Neural adaptor proteins play an important role in the molecular mechanisms to regulating glutamate receptor activity, which works in memory and learning.

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MTU07-25

Calcium independent and voltage dependent exocytosis in mouse chromaffin cells

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It is well known that a cytosolic Ca^{+2} elevation is the fundamental trigger of transmitters release in neurons and neuroendocrine cells. However, in the last 12 years some investigators proposed an additional mechanism of neurotransmitter/hormone secretion that is Ca^{+2}-independent and is related to membrane potential changes. We recently noted that a brief depolatization resembling an action potential applied on mouse chromaffin cells in conditions of complete inhibition of Ca^{+2} currents (ICa^{+2}) induced a moderate exocytotic process. To study more systematically this phenomenon we applied square depolarizations (from −80 to +10 mV) of variable duration in presence of (i) 0 mM external Ca^{+2} or (ii) 5 mM Ca^{+2} + 100 μM Cd^{+2}. In both conditions we measured an increase in capacitance that saturated at 17 ± 2 and 14 ± 1 fF respectively (~12 vesicles) at 100 ms pulse duration. To buffer any possible contaminant Ca^{+2} that might enter to the cell or any release from internal stores we made experiments in 0 extracellular Ca^{+2} and 4 mM intracellular BAPTA, obtaining again a significant exocytosis (14 ± 2 fF) in response to 100 ms depolarizations. The application of the calcium release blocker 2-APB and a pretreatment with the SERCA inhibitor thapsigargin were both unable to block this exocytosis process (15 ± 2fF and 14 ± 2 fF, respectively). Moreover, this Ca^{+2}-independent exocytosis process followed a sigmoid dependence with membrane potential, reaching the 50% of the saturating value at approximately −30 mV. When this vesicle

pool was completely depleted by application of a 100 ms depolarization, it recovered with a time constant of 1.04 ± 0.18. In agreement with this result, synchronous exocytosis did not decrease noticeably at low frequency stimulation (0.2–0.5 Hz), but the application of trains at higher frequencies (2–5 Hz) induced a pronounced decrease in this parameter. Additionally, we show data that suggest that the recovery after depletion of this Ca^{+2}-independent vesicular pool is linked to a dynamin dependent fast endocytic process. These results suggest the existence of a Ca^{+2} independent, but membrane potential dependent, mechanism of secretion in chromaffin cells that would be relevant at low frequencies.

MTU07-26

Neuroligin-3 controls excitatory synaptic transmission onto hippocampal parvalbumin interneurons and mediates fear extinction

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Neuroligins (NLs) are a family of 4 postsynaptic proteins (NL1-4) that interact with presynaptic neurexins. This trans-synaptic interaction between neurexins and neuroligins has been implicated in the formation, specification, maintenance and activity-dependent strengthening of synapses in various brain regions. Disruption of either the presynaptic neurexins or postsynaptic NLs has been implicated in autism spectrum disorders and schizophrenia. Parvalbumin (PV) interneurons have been implicated in cognition, learning and memory, as well as in the pathophysiology of various psychiatric disorders. However, little is known about the molecular mechanisms that facilitate synaptic transmission onto these cells.

To study the putative role of NL3 at synapses onto PV interneurons, we made a conditional knockout mouse for NL3 (*NL3* $^{fl/fl}$), and crossed it to a mouse line expressing cre under the parvalbumin (PV-cre) promoter. Whole cell recordings were made from PV cells in the stratum-oriens of the CA1 region. In the $NL3^{Pvcre-/-}$ mice, a decrease in the paired pulse facilitation onto PV cells was observed, suggesting an increase in release probability. We have identified that this change in pre-synaptic release probability is caused by a lack of Group-III mGluRs at the presynaptic. In addition to the presynaptic effects of NL3 knockout in PV-cre neurons, an ~50% decrease in synaptic NMDAR content was observed. We also identified that the loss of NL3 on PV interneurons renders these mice incapable of acquiring extinction memories while the initial contextual fear learning memory is unaltered. This diminished cognitive ability is also seen in patients with schizophrenia as well as autism spectrum disorders. These results demonstrate that NL3 plays an important role in specifying the properties of synaptic transmission at excitatory synapses onto PV interneurons, and also defines a role for PV interneurons in behavioral plasticity.

MTU07-27

Depolarization-dependent syndapin i phosphorylation in nerve terminals

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Syndapin I is a synaptically enriched member of the F-BAR (FCH-BIN amphiphysin RVS) family of proteins. It consists of two functional domains; an N-terminal F-BAR, which can bind to and deform phospholipid membranes, and a C-terminal src homology 3 (SH3). Syndapin I is an important regulator of activity-dependent bulk endocytosis (ADBE) of synaptic vesicles (SV). It is an *in vitro* phospho-protein, and we have identified that syndapin I is phosphorylated in rat brain nerve terminals and total brain. Syndapin I was dephosphorylated when nerve terminals were depolarized with 40 mM KCl, and the dephosphorylation was blocked with the calcineurin inhibitor, cyclosporin A. Therefore, syndapin I is a calcineurin substrate and a new member of the dephosphin family of proteins. Six *in vivo* phosphorylation sites were identified and sequenced using tandem mass spectrometry: Ser-76, Thr-181, Ser-343, Ser-345, Ser-346, and Ser-358. Quantitative mass spectrometry iTRAQ data on syndapin I phosphorylation indicates that only Ser-358 phosphorylation changes with KCl-dependent depolarization of the nerve terminals, while phosphorylation of the four sites, Thr-181, Ser-343, Ser-345, Ser-346, did not change. This suggests phosphorylation/dephosphorylation of syndapin could be a key signalling mechanism for controlling the function of syndapin I in ADBE, important for synaptic transmission.

MTU07-28

Light induced fos expression in GABA and TPH cells in the dorsal raphe nuclei of mongolian gerbil

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Purpose: A subset of Y-like retinal ganglion cells could innervate dorsal raphe nuclei (DRN) of Mongolian gerbil. DRN-projecting ON Y-cells out number DRN-projecting OFF Y-cells four to one and selective activation DRN-projecting OFF Y-cells increases DRN 5-HT levels. In this study, we hypothesize that DRN projecting ON Y-cells innervate DRN GABA cells and DRN-projecting OFF Y- cells synapse on DRN TPH cells.

Method: Brain slices in the following groups were conducted dual staining of c-Fos/GABA and c-Fos/TPH immunofluorescence: 1. Dark and Light 2. Saline-dark and Saline-light 3.MNU-dark and MNU-light.

Results: In normal animals, the over whelming majority of Fos-expressing cells in DRN are either GABA or TPH neurons under both light (95%) or dark (88%) conditions with most Fos+ cells being GABA neurons (≈80%). Light treatment induced a far greater increase in the number of Fos+ GABA vs. Fos+ TPH cells. After

MNU treatment, Fos+ neurons in DRN increased dramatically and most of the Fos+ cells being TPH neurons.

Conclusion: Since most DRN GABA cells are interneurons that inhibit DRN 5-HT cells, under dark conditions the relatively high level of Fos expression in GABA neurons may account for the paucity of Fos+ 5-HT cells noted in this study. Light-treatment produced a large increase in the number Fos+ GABA cells but an even greater relative increase in Fos+ DRN TPH cells. Thus the majority of ON Y-cell input to the DRN is likely to be to GABA cells as hypothesized, the effect of the input of these cells may be offset somewhat by the direct OFF Y-cell innervation of TPH cells. After MNU treatment, activity of ON Y-cell will be in a silencing state and OFF Y-cell will fire with high frequency, and this leads to the increased Fos expression in TPH cells.

MTU07-29

Testing the effectiveness of an ivermecting activated human silencing receptor

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The use of silencing receptors as a tool to control neuronal excitability in a defined neuronal population has allowed researchers to investigate the relationship between electrical activity and behaviours. Many human neurological diseases are caused by dysfunction in neuronal excitability. The development of tools to target and modulate neuronal excitability in known populations of neurons could prove valuable in treating neurological diseases like motor neuron disease, which is characterized by progressive and selective loss of motor neurons due to persistent depolarization. Our current study tests the effectiveness of a minimally mutated human α1 glycine receptor chloride channel (F207A/A288G-GyR) in silencing neurons and controlling animal behaviour. Using whole cell patch-clamp electrophysiology in mice expressing the F207A/A288G-GyR we show that ivermectin application induces chloride currents and reduces firing frequency in cultured neurons as well as in neurons in brain slices. Behavioural studies in mice expressing F207A/A288G-GyR unilaterally in the striatum show that ivermectin treatment can reversibly induce rotation behaviour. Our silencing receptor is an ideal candidate for use in human clinical trials as the receptor is of human origin and activated by the FDA approved drug ivermectin. Currently we are testing the ability of the receptor to rescue the neurons involved in motor neuron disease in a SOD1/ChatCre mouse line.

MTU07-30

A unique western blot method to measure the number of glutamate receptor subunits

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Physiological functions of glutamate receptor channels vary depending on the composition of their subunits, therefore, the measurement of their quantitative amounts in various brain regions is important. A western blot is a useful method to measure the same molecules from different samples, but cannot compare molecules from different species, because each antibody against each molecule has its own titer. To clarify the concentration of all GluR subunits in mouse brain, we first determined subunit-specific antibody titers using unique chimeric subunits composed of an N-terminal portion of GluA1 or GluA2 and a C-terminal portion of three AMPA receptor subunits, five NMDA receptor subunits, five kainite receptor (KAR) subunits and delta subunits. We determined each subunit concentration relative to GluA1 and/or GluA2 using these values. Since no high titer of anti-GluK1 antibody was obtained, GluK1 concentration was not analyzed. Next, to calculate an absolute molecular number of each subunit, we generated standard molecular materials. His-tagged N-terminal portions of GluA1 (19~537aa: 59010.17 Mw) and GluA2 (22~524aa: 58763.01 Mw) were produced by pCold GST vector (Takara Bio) and E.coli Rosetta2 (Novagen) at 15°C. These proteins were dissolved with 6 M guanidine-HCl and purified with Ni-agarose (Ni-NTA; QIAGEN) affinity chromatography. The purity of the resultant His-GluA1 and His-GluA2 proteins, which was measured by SDS-PAGE staining, western blot and slot blot, was 0.82 and 0.85, respectively. These standard proteins were used to determine the molecular number of each glutamate receptor subunit in various brain regions and subcellular fractions.

MTU07-31

A novel murine mouse model for hyperekplexia

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The dysfunction of glycinergic neurotransmission is the underlying mechanism in the neuromotor disorder hyperekplexia, which is a hypertonic movement disorder with seizures caused by sudden tactile or auditory stimuli.

Mutations in the human *GLRA1* gene are the most common cause for hyperekplexia. Since mice, carrying a mutation in glycine receptor subunit genes, result in a similar neuromotor phenotype compared to humans, mouse models are excellent tools. GL*RA1* encodes for the glycine receptor α1 subunit (GlyRα1), which forms

together with the GlyRβ subunit the adult heteromeric receptor complex enabling fast inhibitory neurotransmission.

Here, we characterize a novel spontaneous hyperekplexia mouse model "*shaky*". The underlying mutation is a point mutation in loop F of the N-terminal portion of the GlyRα1 subunit. Homozygous mutant mice develop a startle phenotype.

Behavioral studies showed a decreased ability of homozygous shaky mice to gain weight after postnatal day p14, including an increase in symptoms. Treatment with clonazepam leads to a decrease in symptoms for a short period of time. This indicates that glycinergic neurotransmission is affected.

First *in vitro* experiments on cell culture basis with and without the GlyRβ subunit gave no hints for the molecular mechanism. The membrane localization and the ion channel function were not affected. Studies on mixed spinal cord neuronal cultures of *shaky* mice showed that the expression level of the GlyRα1 at the neuronal cell surface was indistinguishable from wild type. Thus, the biogenesis of the GlyRa1 seems not to be disturbed.

These *in vitro* results are not able to explain the *in vivo* situation with death during the first postnatal weeks (week 3–6). These results were rather unexpected and not observed for any other point mutation analyzed in other mouse models for hyperekplexia. We conclude that the disturbances are not at the level of expression, and hypothesize that the mutation affect most probably synaptic localization and/or synaptic anchoring.

MTU07-32

Morphological and biochemical analyses of PSD-core structure of type i excitatory synapses

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Postsynaptic density (PSD) is a specialized cytoskeleton, localizing immediately underneath the postsynaptic membrane and play important roles in signal processing upon receiving neurotransmitter and in generation of synaptic plasticity. In the previous study, we analyzed systematically purification process of PSD and postsynaptic membrane rafts (PSRs) from rat forebrain SPM after treatment with three different detergents, Triton X-100, n-octyl ß-D-glucoside and 3-([3-Cholamidopropyl]dimethylammonio)-2-hydroxy-1-propanesulfonate (CHAPSO) at varied concentrations, and found clear difference in the separation of subsynaptic structures among these detergents (Zhao et al., J. Neurochemistry, 2014, 131: 147–162). We also reported in the paper several novel subsynaptic fractions different from PSDs or detergent-resistant membrane. We report here analyses of one of the novel fractions, which is highly enriched in actin. The fraction contained mainly mesh-like structure, which resembled previously reported "PSD-lattice" structure. The presence of PSD-derived similar structures was suggested in other conditions, such as extreme solubilization of PSDs, detergent-insoluble PSDs prepared from brains rapidly frozen with liquid nitrogen after dissection of the brains, and immature PSDs prepared from forebrains of 7-day-old rats. The 2nd condition produces lean PSD (Suzuki et al., 1994, 63: 1529–1537). Thus, it was suggested that the mesh-like structure is a core which is buried inside the PSD and associated with a number of PSD molecules. The similar structure was present even at an early stage of PSD construction, when PSD scaffolding proteins, such as PSD-95, were not expressed profoundly. Molecular composition and organization of this PSD mesh-like structure was investigated by Western blotting and mass spectrometry.

MTU07-33

Changes in odour coding across the input layer of the mouse piriform cortex *in vivo*

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The anterior piriform cortex (aPC) is a relatively simple paleocortical structure dedicated to processing odour information. The aPC is highly laminar, with its main input layer (layer 2) containing two distinct populations of glutamatergic neurons: semilunar (SL) cells in layer 2a and superficial pyramidal (SP) cells in layer 2b. However, little is known about how odour information is represented in neuronal populations in different sublayers of layer 2. Here, we simultaneously measured the activity of up to 200 neurons in different planes across layer 2 of the aPC in anesthetised mice *in vivo*. We used 2-photon microscopy and functional calcium imaging, employing the calcium indicator dye Cal-520, or the genetically encoded calcium sensor GCaMP6s. With the higher signal-to-noise ratio of Cal-520 and GCaMP6s, we detected spontaneous activity in the somata of SL and SP cells, as well as in their dendrites in layer 1. Presentation of a palette of seven structurally-distinctive odorants excited up to 15% of neurons in an ensemble pattern that was unique for each odorant. On average, a given SL or SP cell responded to 1.1 of seven odorants (SL vs. SP, $p = 0.35$, *t*-test). However, the distribution of the number of odours that each cell responded to was 3.3 times more positively skewed in SP cells than in SL cells (skew: SL, 1.6 ± 0.2; SP, 5.3 ± 0.7; mean ± SEM, $p < 0.001$, *t*-test, $n = 5$ mice), indicating that responsive SP cells tend to be excited by a larger number of odorants. These results indicate that SL and SP cells are spontaneously active in the mouse aPC and may employ distinctive codes for representing odours *in vivo*.

MTU07-34

Vesicular release of ATP from dopaminergic neurons in the retina and brain

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In 2008, the first and so far only, vesicular nucleotide transporter (VNUT) was identified as the mediator of active accumulation of ATP into vesicles for exocytotic release. However, the cell types that express this vesicular ATP transporter in the retina and brain remain unknown. A novel polyclonal antibody directed against VNUT was developed in conjunction with Merck-Millipore. Antibody specificity for VNUT was confirmed using Western blot on purified murine VNUT peptide, retina and brain samples. Using fluorescence immunohistochemistry and confocal microscopy, VNUT labelling was found to colocalize with tyrosine hydroxylase (TH) positive, dopaminergic cells (DA) in the mouse retina and brain. In the mouse brain, DA neurons of the substantia nigra and ventral tegmental area (VTA) co-expressed VNUT and TH, but VNUT expression by extranigral non-DA neurons was also observed. In the retina, VNUT labeling was found to colocalize solely with TH-positive DA interplexiform cells (DA-IPCs). Further investigation by three-dimensional reconstructions of double-labeled retinal flatmounts revealed that VNUT-positive DA-IPC distal processes in the outer retina were in close contact with horizontal

cell processes and cone photoreceptor terminals, which express P2 purinergic-receptors. A functional assay, whereby dissociated retinal neurons were co-labelled with VNUT antibody and loaded with fluorescent ATP (Mant-ATP) and DA (FFN102) and observed in real time using time lapse confocal imaging, was performed *in vitro* (X63 objective, X7 zoom, 1400 Hz). Mant-ATP and FFN102 puncta were found to co-localise in VNUT positive neurons. Intracellular Mant-ATP fluorescence was reduced in response to application of a depolarising high potassium (55 mM) physiological saline and this response was blocked in the presence of cadmium, suggesting ATP release was occurring via calcium dependent, exocytosis. Taken together, these findings indicate that DA neurons may co-release ATP via exocytosis in the mouse retina and brain. In the retina, the release of ATP by DA-IPCs might play an important role in modulating outer retinal processing, specifically horizontal cell and photoreceptor activity.

MTU07-35

The influence of proline residues within the TM3-4 loop of the human glycine receptor on channel functionality

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Hyperekplexia is a rare human neuromotor disorder characterized by exaggerated startle reflexes and loss of postural control. It is caused by mutations in postsynaptic glycine receptors (GlyR), its associated anchoring proteins or presynaptic glycine transporter. Most of the known mutations accumulate within the first or second transmembrane domains and result either in altered chloride permeability or disturbed membrane surface expression. Here, we characterize a novel mutation P366L located within the intracellular loop connecting transmembrane domains 3 and 4 (TM3-4 loop). Using transfected HEK293 cells stained for the human α1 subunit, we demonstrate that the hyperekplexia-like symptoms observed in a patient suffering from this mutation are not due to disturbances in receptor biogenesis. Furthermore, expression in *Xenopus* oocytes and fluorescence labeling of extracellular residues N203 and R271 in addition to P366L indicate an unaltered channel gating behaviour. Instead, patch-clamp recordings revealed a significant reduction in glycine-induced maximum inward currents. P366L is localized in a proline-rich stretch in the TM3-4 loop suggested to generate a poly-proline helix. Functional analysis of constructs generated to gradually destroy this possible secondary structure show that the motif ^{365}PPPAPSKSP influences desensitization properties of the channel. Together these results provide novel evidences for intracellular subdomains in the TM3-4 loop modulating desensitization in addition to already described conformational changes in extracellular regions mediating channel closure.

MTU07-36

Control of autophagosome axonal retrograde flux by presynaptic activity unveiled using botulinum neurotoxin type-A

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Botulinum neurotoxin type-A (BoNT/A) is a highly potent neurotoxin that elicits flaccid paralysis by enzymatic cleavage of the exocytic machinery component SNAP25 in motor nerve terminals. However, recent evidence suggests that the neurotoxic activity of BoNT/A is not restricted to the periphery but also reaches the central nervous system following retrograde axonal transport. As BoNT/A is internalized in recycling synaptic vesicles, it is unclear which compartment facilitates this transport. Using live-cell confocal and single-molecule imaging of rat hippocampal neurons cultured in microfluidic devices, we show that the activity-dependent uptake of the binding domain of the BoNT/A heavy chain (BoNT/A-Hc) is followed by a delayed increase in retrograde axonal transport of BoNT/A-Hc carriers. Consistent with a role of presynaptic activity in initiating transport of the active toxin, activity-dependent uptake of BoNT/A in the terminal led to a significant increase in SNAP25 cleavage detected in the soma chamber when compared with non-stimulated neurons. Surprisingly, most endocytosed BoNT/A-Hc was incorporated into LC3-positive autophagosomes generated in the nerve terminals, which then underwent retrograde transport to the cell soma, where they fused with lysosomes, both *in vitro* and *in vivo*. Blocking autophagosome formation or acidification with wortmannin or bafilomycin A_1 respectively inhibited the activity-dependent retrograde trafficking of BoNT/A-Hc. Our data demonstrate that both the presynaptic formation of autophagosome and the initiation of their retrograde trafficking are tightly regulated by presynaptic activity.

MTU07-37

Effects of components of sake on GLUN1/GLUN2A and GLUN1/GLUN2B subtypes of NMDA receptor

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NMDA (*N*-methyl-D-aspartic acid) -type glutamate receptors (NMDA receptors) are widely distributed in the central nervous system and play critical roles in synaptic plasticity and excitotoxicity. Dysfunctions of NMDA receptors are involved in several central nervous system disorders, including stroke, chronic pain and schizophrenia. There are a few NMDA receptor antagonists available for clinical use, including ketamine and most importantly, memantine, both of which act as channel blockers. Memantine is used for Alzheimer's disease. The suppression of neuronal cell death has been confirmed by blocking the NMDA receptor channel. Over 1000 different ingredients such as sugars, amino acids, amines and organic acids are included in sake, traditional Japanese alcoholic beverage made from rice.

For looking for new compounds that act as an agonist and an antagonist on NMDA receptor, we investigated the effects of components contained in sake on NMDA receptor. Various amines (agmatine, spermine, putrescine, tyramine, isoamylamine) contained in sake. Spermine is known to inhibit or activate the channel activity of NMDA receptor subunits dependent manner. Only NMDA receptors containing the GluN2B subunits display spermine potentiation. Agmatine contained a lot in sake is considered as a novel neurotransmitter or neuromodulator in the central nervous system. So, we examined the effect of thirteen components (agmatine, spermine, putrescine, tyramine, isoamylamine, 2-phenylethylamine, trimethylamine *N*-oxide, ornithine, betaine, choline, 5-oxoprorine, cysteic acid, and *N*-acetylglutamic acid (NAG)) of sake on GluN1/GluN2A and GluN1/GluN2B subtypes of NMDA receptors, which were expressed in *Xenopus* oocytes. Trimethylamine N-oxide and betaine promoted the activity of both receptors. Agmatine, putrescine, tyramine, ornithine, choline, 5-oxoproline, cysteic acid, NAG, and 2-phenylethylamine inhibited the activity of both receptors. Isoamylamine indicated the opposite effect of spermine. It was indicated that isoamylamine act as an antagonist against the GluN1/GluN2B receptor, but an activator for GluN1/GluN2A receptor. And, it was indicated that these components might be used as medicines for schizophrenia or potential therapeutic agents such as anxiety and dementia.

MTU07-38

Functional reconstitution of glycinergic synapses incorporating defined glycine receptor subunit combinations

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Glycine receptor (GlyR) chloride channels mediate fast inhibitory neurotransmission in the spinal cord and brainstem. Four GlyR subunits (α1-3, β) have been identified in humans, and their differential anatomical distributions lead to a diversity of synaptic isoforms with unique physiological and pharmacological properties. To improve our understanding of these properties, we induced the formation of recombinant synapses between cultured spinal neurons and HEK293 cells expressing GlyR subunits of interest plus the synapse-promoting molecule, neuroligin-2A. In the heterosynapses thus formed, recombinant α1β and α3β GlyRs mediated fast decaying inhibitory postsynaptic currents (IPSCs) whereas α2β GlyRs mediated slow decaying IPSCs. These results are consistent with the fragmentary information available from native synapses and single channel kinetic studies. As β subunit incorporation is considered essential for localizing GlyRs at the synapse, we were surprised that α1-3 homomers efficiently supported IPSCs with β subunit incorporation accelerating IPSC rise and decay times in α2β and α3β heteromers only. Finally, heterosynapses incorporating α1D80Aβ and α1A52Sβ GlyRs exhibited accelerated IPSC decay rates closely resembling those recorded in native synapses from mutant mice homozygous for these mutations, providing an additional validation of our technique. These heterosynapses should prove useful for evaluating the effects of drugs, hereditary disease mutations or other interventions on defined GlyR subunit combinations under realistic synaptic activation conditions.

MTU08 Signal Transduction

MTU08-01

IP3 AND/OR inositol levels changes are separate effects of lithium that may mediate its induced behavioral and cellular changes

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Background: The inositol-depletion-hypothesis for lithium's molecular mechanism of mood-stabilization has not decisively determined whether inositol-depletion *per-se* or phosphoinositols accumulation induces the drug's beneficial effects. Knockout in mice of either of two inositol-metabolism-related genes – *IMPA1* or *SMIT1* (encoding for inositol-monophosphatase-1 and the sodium/*myo*-inositol-transporter, respectively) mimics several lithium's behavioral/biochemical effects.

Methods: Brain phosphoinositols and phosphoinositides labeling following intracerebroventricular administration of ^{3}H-inositol in wildtype, Li-treated, *IMPA1*- and *SMIT1*-knockout mice was assessed. Anti-deppressant- and anti-manic-like behavior and autophagy markers were assessed following central administration of inositoltrisphosphate or inositol-monophosphate.

Results: Lithium-treatment caused increased phosphoinositols labeling in the frontal-cortex and hippocampus but decreased phosphoinositides labeling in the frontal-cortex. *IMPA1*-knockout, like lithium-treatment, caused increased phospho-inositols labeling but no change in phosphoinositides. *SMIT1*-knockout did not affect phosphoinositols, but, like Li-treatment, reduced phosphoinositides in the frontal-cortex. Intracerebroventricular administration of inositoltrisphosphate but not inositol-monophosphate trapped in liposomes caused lithium-like changes in the forced-swim test, prevented by an inositoltrisphosphate-receptor-antagonist, xestospongin-C. Intracerebroventricular administration of inositoltrisphosphate also induced a lithium-like effect in the amphetamine-induced hyperlocomotion test. Finally, similarly to lithium, inositoltrisphosphate affected autophagy markers indicative of an enhanced process.

Conclusions: These results suggest that lithium's inhibition of inositol-monophosphatase-1 affects the phosphatidylinositol signaling-system in two ways: a) depleting inositol followed by decreasing phosphoinositides levels; b) elevating inositol-monophosphate levels followed by accumulation of phosphoinositols. Each or both may mediate lithium-induced behavior.

Based on our previous findings showing attenuated depressive-like behavior in response to autophagy enhancers, we propose the following cascade of events: lithium increases inositoltrisphosphate accumulation which enhances autophagy by desensitization of the inositoltrisphosphate receptors and induces anti-bipolar-like behavior.

MTU08-02

TRPV1 expression in corneal afferent neurons

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Corneal afferent neurons are divided into three main functional types: polymodal nociceptors, cold sensing neurons and pure-mechano nociceptors. We have previously shown that TRPM8 and Piezo2 expression can be used as markers for corneal cold sensing neurons and pure mechano-nociceptors, respectively (Bron, Wood et al. 2014). Whilst TRPV1 expression is often used as a marker for polymodal nociceptors in other tissue systems, there is little evidence for this in the cornea. The aims of the current study were to show that TRPV1 expressing corneal afferent neurons are distinct from cold-sensing (TRPM8) neurons and pure mechano-nociceptors (Piezo2), and to define the molecular phenotype of TRPV1-expressing corneal afferent neurons. We used retrograde tracing to identify corneal afferent neurons in the guinea pig trigeminal ganglion combined with double label *in situ* hybridization and/or immunohistochemistry to determine their molecular phenotype. Approximately 43% of corneal afferent neurons expressed TRPV1, 28% expressed Piezo2 and 8% expressed TRPM8 (n = 3 animals). There was no co-expression of TRPV1 and Piezo2 in corneal afferent neurons, and only 2% co-expressed TRPV1 and TRPM8. We were also able to define at least three separate subpopulations of TRPV1-expressing corneal afferent neurons on the basis of CGRP and/or GFRα3 co-expression (n = 4 animals). Our study shows that TRPV1 expressing corneal afferent neurons are distinct from corneal cold sensing (TRPM8) neurons and pure mechano-nociceptors (Piezo2), and are therefore most likely polymodal nociceptors. Furthermore, the TRPV1-expressing corneal afferent neurons can be subdivided into specific subpopulations based on their molecular phenotype.

Bron, R., R. J. Wood, et al. (2014). "Piezo2 expression in corneal afferent neurons." J Comp Neurol 522(13): 2967–2979.

MTU08-03

Calbindin D28K and S100B have a similar interaction site with the lithium-inhibitable enzyme impase-1: a new drug target site

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Lithium-salts (Li) are the prototype drug for the treatment of bipolar-disorder. Therapeutically-relevant Li concentrations inhibit inositol-monophosphatase (IMPase)-1, a key enzyme in the phosphatidylinositol signaling system. Therefore, IMPase-1 is a hypothesized target of Li's beneficial effects. Calbindin D28k (calbindin) and S100B are both calcium-binding proteins, both shown to enhance IMPase-1 activity. All three proteins, calbindin, S100B and IMPase-1, are abundant in the central nervous system. We have recently reported *in-silico* model results of the IMPase-1/calbindin

complex indicating that the 55–66 amino-acid segment of IMPase-1 anchors calbindin *via* Lys59 and Lys61 with a glutamate in between (the Lys–Glu–Lys motif). We have also demonstrated that six amino acid peptides including the Lys-Glu-Lys motif or part of it inhibit the *ex-vivo* calbindin-enhanced IMPase activity.

We now compared our *in-silico* model of the IMPase-1/calbindin complex with the crystal structure of S100B. Although calbindin and S100B have a low sequence homology (38% using BLAST), they seem to activate IMPase-1 in a similar mode. This raises the possibility that the calbindin and S100B similar domains interact and activate IMPase-1 by binding the same Lys–Glu–Lys motif of IMPase-1. It is reasonable to expect that molecules interfering with the interaction of IMPase-1 with either of its activators will have Li-like beneficial effects.

MTU08-04

New pharmacological tools to investigate oxytocin receptors in neurodevelopmental brain disorders

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Oxytocin (OXT) administration has been shown to regulate socio-emotional behaviors in a number of animal species including humans. OXT treatment has been proposed to improve social cognition in patients with Autism Spectrum Disorders as well as in other conditions characterized by altered social behavior. To validate and optimize OXT treatment in neurodevelopmental brain disorders, the identification of new selective and potent pharmacological tools targeting neuronal OXT receptors (OXTR) is a high priority.

In this study we evaluated new peptidic molecules for their selectivity, capability to activate different intracellular signalling pathways and to specifically label neuronal cells.

We tested peptidic analogs by radioligands binding assays to determine their affinity and receptor selectivity on OXTR/vasopressin receptors, by BRET-based assay to test their coupling specificity and finally by *in vivo* confocal analysis to test receptor trafficking and recycling.

We characterized two functional analogs active on specific Gi isoforms to dissect Gi signalling pathways in neuronal cells, a high affinity agonist and three antagonists to probe mice OXTR in translational approaches and fluorescently labelled analogs to follow receptor internalization and recycling.

Selective and highly potent analogues targeting brain OXTR are instrumental to understand the involvement of the OXT system in neurodevelopmental disorders characterized by social dysfunctions. These compounds will help the design of new drugs to treat or ameliorate specific symptoms or groups of symptoms in these complex diseases.

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MTU08-05

Redox-sensitive activation of PI3K/AKT pathway in RVLM on endoplasmic reticulum stress-associated neurogenic hypertension

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We reported previously that endoplasmic reticulum (ER) stress in the rostral ventrolateral medulla (RVLM), where the sympathetic premotor neurons for maintenance of basal vasomotor tone reside, is engaged in neural mechanism of hypertension. We investigated in the present study the role of the PI3K/Akt signaling in RVLM in the redox-sensitive and ER stress-associated neurogenic hypertension. In comparison to normotensive Wistar-Kyoto rats, expressions of the p85 and p110 subunits of PI3K and phosphorylated form of Akt were significantly greater in RVLM of the spontaneously hypertensive rats (SHR), accompanied with increased expressions of ER stress markers, GRP78 and phosphorylated elF2α. Inhibition of PI3K expression and dephosphorylation of Akt in the RVLM of SHR resulted in a significant decrease in systemic arterial pressure (SAP), alongside suppressions of the augmented GRP78 expression and eIF2α phosphorylation. Protect the RVLM from the ER stress with salubrinal or 3,4,5-trimethoxybenzoic acid, on the other hand, had no effect on the augmented PI3K expression or AKT activity, although the SAP was decreased. Treatment of the superoxide dismutase mimetic, tempol, not only abrogated oxidative stress in RVLM, but also blunted the increased expression of PI3K/Akt signaling and ER stress markers, resulting in an decrease in SAP of the SHR. Collectively these results suggest that the redox-sensitive activation of PI3K/Akt pathway in the RVLM may account for the ER stress-associated neurogenic hypertension in the SHR.

MTU08-06

M1 muscarinic receptors newly incorporated into plasma membrane of CHO cells demonstrate alterations in agonist binding properties

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Four-day treatment of Chinese hamster ovary (CHO) cells expressing muscarinic M_1 receptors (CHO-M_1) with 100 nM amyloid $\beta_{1\text{-}42}$ ($A\beta_{1\text{-}42}$) results in a rather selective decrease in agonist affinity and an increase in the proportion of high-affinity binding sites (Janickova et al., Neuropharmacology 67, 2013). We have now probed whether such treatment influences the incorporation of new receptors to plasma membrane. To this end we inactivated plasma membrane receptors using propylbenzylcholine mustard (PRBCM) in control or CHO-M_1 cells exposed to 100 nM $A\beta_{1\text{-}42}$ for 4 days and monitored the time course of the incorporation of new receptors into plasma membrane using specific binding of membrane impermeable non-subtype selective antagonist ^{3}H-N-methylscopolamine (^{3}H-NMS). We found significantly higher reappearance of the M_1 receptors in $A\beta_{1\text{-}42}$ treated than in control cells 24 h after exposition to PRBCM. In next experiments we explored binding characteristics of newly recruited M_1 receptors in

plasma membrane of control CHO-M_1 cells 6 or 24 h after PRBCM treatment. Newly incorporated receptors exhibited the same affinity for ^{3}H-NMS in membranes prepared from both control and PRBCM treated cells. However, binding of the agonist carbachol demonstrated significantly lower affinity of the high-affinity binding at both time intervals after PRBCM treatment, and higher proportion of the high-affinity binding site 24 h after PRBCM treatment. These changes in agonist binding that resemble changes after $A\beta_{1\text{-}42}$ treatment may indicate that the observed effects of $A\beta_{1\text{-}42}$ may primarily be due to faster turnover of the M_1 receptors.

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MTU08-07

Neuronal depolarization induces tyrosine phosphorylation of TLS/FUS and its translocation to the nucleus

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Translocated in liposarcoma (TLS)/FUS is a RNA/DNA-binding multifunctional protein involved in splicing of pre-mRNAs and transport of fully-processed mRNAs/RNAs between the nucleus and the cytoplasmic organelles. TLS mutations are known to be cause type6 familial amyotrophic lateral sclerosis (ALS6). ALS6 patients, who carry point mutations in the C-terminus of TLS, display a cytoplasmic aggregates containing mutant TLS proteins in the motor neurons. Majority of TLS is localized in the nucleus of the neurons, however, point mutations that disrupt a PY(proline-tyrosine)-NLS at the C-terminus tail significantly impair necessary and sufficient nuclear import of TLS via transportin. To understand how the NLS in TLS/FUS is activated and interacts with tranportin in the nucleus, we investigated tyrosine phosphorylation at the NLS upon neuronal depolarization. Mouse neuronal spinal cord cell line NSC-34 were treated by 75 mM KCl. At 5, 15, and 30 min after the depolarization, the cells were homogenized in either hypotonic buffer or RIPA buffer in the presence of phosphatase inhibitor cocktail and applied to subsequent centrifugations to separate into nuclear and cytoplasmic fractions. Proteins in each cellular fractions and whole cell extracts were quantitatively analyzed by immunoblotting with TLS-specific monoclonal antibody or phosphotyrosine-specific monoclonal IgG. Over 30 min after the depolarization, TLS protein level was not changed while tyrosine-phosphorylated TLS protein (pY-TLS) is increased by 2-fold at 5 min and peaked at 15 min. In the nuclear fraction, the amount of pY-TLS was increased by approximately 2.8-fold at 30 min after the depolarization. This observation was reciprocal to pY-TLS content in the cytoplasmic fraction. Although a tyrosine kinase responsible for the tyrosine phosphorylation of TLS upon hyperactivation of motor neurons has not been identified, our results indicate that PY-NLS plays an important role for necessary nuclear translocation of TLS when motor neurons are jeopardized by non-cell autonomous excitotoxicity.

MTU08-08

CDK5 regulates RAB8-dependent axonal outgrowth via phosphorylation of RAB8 guanine-exchange factor GRAB

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Cyclin-dependent kinase 5 (Cdk5) is a neuron specific Ser/Thr protein kinase that is activated by the binding of either p35 or p39 regulatory subunit. It plays an important role in a variety of neuronal functions including neuronal migration during brain development, synaptic signaling, survival, and neuron death. Cdk5-p35 is also involved in neurite elongation through the supply of membrane components to neurite tip, but it is not fully understood how Cdk5-p35 regulates the membrane transport in growing axons. In general, membrane trafficking is regulated by Rab GTPase family proteins. Rab GTPase is activated by guanine nucleotide exchange factors (GEFs), which stimulate the exchange of guanine nucleotide bound to Rab from GDP to GTP. Among many Rab GEFs, we were interested in GRAB, a GEF for Rab8A, which is also known as a binding protein for Rab11A/B, because GRAB has (S/T)PX(R/K) consensus phosphorylation sequences for Cdk5. When we examined phosphorylation of GRAB by Cdk5-p35 in cultured cells, GRAB was indeed phosphorylated at Ser169 and Ser180 in a Cdk5-dependent manner. Further, their phosphorylation inhibited the interaction of GRAB with dominant negative Rab8A-T22N, suggested that phosphorylation of GRAB by Cdk5-p35 suppresses activity of Rab8. We asked for Rab8A function in axonal elongation. When Rab8A was overexpressed in mouse primary neurons, axonal outgrowth was stimulated. GRAB also regulated axonal outgrowth in a phosphorylation-dependent manner. The nonphosphorylation mutant of GRAB (GRAB-S169/180A) promoted axonal outgrowth, more than its phosphomimic mutant (GRAB-S169/180D) when they were coexpressed with Rab8A-wt. However, there was no difference in axon length between GRAB-S169/180A and GRAB-S169/180D when they were coexpressed with dominant negative Rab8A-T22N or constitutive active Rab8A-Q67L. These results suggest that Cdk5-p35 regulates that Rab8-mediated axonal outgrowth by phosphorylation of GRAB at Ser169 and Ser180.

MTU08-09

Optopharmacological control of endogenous g protein-coupled receptors

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Optopharmacology is a very promising technology to tackle pathophysiological processes. Contrary to optogenetics which requires the use of transgenes, optopharmacology allows for the targeting of endogenous receptors in their native tissues through the use of photoswitchable ligands that can be switched ON and OFF with light. This method provides fine spatial and temporal control of drug activity and offers unique opportunities for *in vivo* investigation. Herein we report the development and characterization of what is, to our knowledge, the first photoswitchable allosteric modulator of a G protein–coupled receptor. Alloswitch-1 is a selective negative allosteric modulator for the metabotropic glutamate receptor mGlu5 which enables the optical control of endogenous mGlu5 receptors, both *in vitro* and *in vivo*.

MTU08-10

Sequestration of GDNF family ligands in inflammatory bone pain

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Recent studies have implicated Glial cell-line Derived Neurotrophic Factor (GDNF) Family Ligands (GFLs) in the pathogenesis of pain. The GFLs (GDNF, neurturin, artemin and persephin) act through the receptor tyrosine kinase RET and one of four accessory subunits (GFRa1-4) which confer ligand specificity (GDNF/GFRa1, neurturin/GFRa2, artemin/GFRa3, persephin/GFRa4). We are currently investigating the role of GFL signalling through these complexes in inflammatory bone pain. The present experiments explored the role of endogenous GFL signalling in inflammatory bone pain by sequestering artemin and GDNF with function blocking antibodies. Sprague-Dawley rats were anaesthetized with isoflurane and inflammation was induced by injection of Complete Freund's Adjuvant (CFA; 50 μl 1:1, oil:saline; $n = 14$) into the tibial medullary cavity. The percentage of weight bearing on the injected hindlimb relative to total weight bearing was determined using an incapacitance meter and a decrease in percentage was used as an index of pain. CFA injection resulted in decreased ipsilateral weight bearing for up to 10 days post-injection, and a return to normal over the next week. At the peak of CFA-induced pain (day 4), the percentage of ipsilateral hindlimb weight bearing was significantly lower than pre-injection values for the same animals and the values for saline injected controls (50 μL; $n = 11$) at this time-point ($P < 0.05$). Artemin and GDNF were sequestered by co-injection of polyclonal anti-artemin (20 μg/10 μl; AF1085; R&D Systems; $n = 7$) or anti-GDNF (20 μg/10 μL; AB212-NA; R&D Systems; $n = 4$) antibodies with CFA into the medullary cavity. Artemin (but not GDNF) sequestration completely abrogated the CFA-induced reduction in ipsilateral weight bearing. Co-injection of control IgG (20 μg/10 μL; R&D Systems; $n = 6$) with CFA had no effect on the CFA-induced reduction in ipsilateral weight bearing. These findings indicate that the artemin/GFRa3 signalling pathway is involved in the pathogenesis of inflammatory bone pain and may be a target for pharmacological manipulations to treat it.

MTU08-11

Manipulation of spatial working memory persistence in mice

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There are considerably ongoing debates regarding specific stimuli such as stress or administration of drugs in enhancing the consolidation of memory. Fluoxetine, a selective serotonin re-uptake inhibitor (SSRI) type of antidepressant, was already investigated for its memory enhancing effect in the fear-motivating passive avoidance task. However, in a stress-free condition, the consolidation-enhancing effect of fluoxetine has not yet been examined. In this study, we investigated the time-dependent administration of fluoxetine and its effect to the extension of memory consolidation in mice under the Barnes maze task, a non-stress-motivating behavioral task. After confirming the complete memory of the behavioral paradigm, fluoxetine (20 mg/kg, i.p.) was administered at the following 4 schedules: 1–7 days, 8–14 days, 15–21 days and 1–21 days after the probe tests. We found that fluoxetine administration in all 4 schedules markedly enhanced memory retrieval compared to the control group. On the other hand, the co-administration of Way 100635, a 5-HT receptor antagonist, significantly reduced the spatial memory enhancing property of fluoxetine, suggesting that the memory (re)consolidation process is mediated via the *serotonergic* system. This memory enhancement was related to the increased PKA-CamKII/CREB pathway, which was shown in the hippocampal tissue by Western blot. Overall, memory consolidation would be enhanced and persisted for a long time partly by manipulating the serotonergic neurotransmitter system. In conclusion, our present study implies that modulating serotonergic system could regulate PKA-CREB signaling and would be expedient way to improve memory persistence.

Keywords: Serotonin; spatial memory; Barnes maze; memory consolidation; PKA:

MTU08-12

Suppression of NMDA-induced alteration of mitochondrial membrane potential by treatment of $GABA_B$ receptor agonist

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Calcium influx through NMDA receptors is critical in synaptic plasticity, whilst massive calcium influx induces neuronal cell death that includes mitochondrial depolarization. Several signal transductions modulate NMDA receptors gating. $GABA_B$ receptors mediate inhibitory transmission via suppressing adenylate cyclase. We have evaluated correlations between those two receptors. Primary cortical neurons were prepared from mouse embryo (E15) and neuronal cell death was estimated by MTT assay. Calcium influx and mitochondrial membrane potential were detected by fluorescence indicators and the fluorescence intensity of each cell was assayed by ImageJ. After exposure to 100 mM, but not 1.0 mM, NMDA, neuronal cell death was observed, whilst calcium influx was similarly occurred. Mitochondrial depolarization was transiently observed by the exposure to NMDA in the concentration dependent manner and that was suppressed by potent $GABA_B$ receptor agonist R(+)-baclofen. The transient increase in mitochondrial membrane potential was not altered by pretreatment with either forskolin or SQ22536. These results suggest that $GABA_B$ receptor activation modulates intracellular circumstances mediating to mitochondrial membrane potential without adenylate cyclase but with other signal pathways including activation of GPCR mediated potassium channels.

MTU08-13

Role of RAS in endothelin-dependent ERK activation in cortical astrocytes

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Endothelin-1 (ET-1) is a mitogen for astrocytes and glioma cells and it is implicated in many CNS pathologies that involve reactive gliosis, through the activation of a complex network of interconnected signaling cascades, that possibly include transactivation of receptor tyrosine kinases (RTKs). A major effector of the two ET-1G-protein-coupled receptors is ERK1/2, an important regulator for both proliferation and differentiation. The present study was designed to explore Ras-dependent signaling mechanisms that lead to activation of ERK1/2 by ET-1 in rat cortical astrocytes in culture. To begin to address this mechanism, we first examined the temporal profile of ERK1/2 activation and found that it comprises of a sharp increase by 5 min and a second amplification that peaked at 30 min, lasting up to 3 h. We then examined Ras activation by ET-1, using affinity precipitation assays with the GTP-Ras-binding domain of Raf-1, and found that Ras activation was evident by 5 min, peaked by 10 min, and negligent by 20 min. The magnitude of Ras activation is regulated by GAPs, like the tumor suppressor neurofibromin, which functions as such through a central domain termed GRD; this domain exists as two variants, I or II, the latter being the prominent transcript in astrocytes. Overexpression of the GRDII domain significantly inhibited the second amplification of the ET-1-dependent ERK activation but not the first peak. We recorded similar effects with siRNA-mediated depletion of neurofibromin. PKC-epsilon, a PKC that we have previously shown to have a key role in coupling GPCRs to activation of RTKs through direct interactions with the cytosolic kinases Src and Fyn, was activated with a temporal profile that preceded activation of ERK, while Fyn activation followed the same pattern. Collectively these results suggest that Ras regulates the second amplification of ERK activation by ET-1, possibly through transactivation of an RTK, while they may offer an explanation for the growth advantage of astrocytes in individuals with mutations in the neurofibromin gene NF1.

MTU08-14

Glutamate uptake characterization in HEPG2 cells

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Glutamate (Glu) the main excitatory neurotransmitter of the Central Nervous System maintain their concentration well regulated through the action of Na^+-dependent Glu transporters known as excitatory amino acid transporters (EAATs). Five subtypes of EAATs have been described named EAAT1-5. Besides EAATs, Na^+-independent, Cl^-, dependent Glu uptake has been described known as system X_c^- this acts as an obligate Cysteine/Glu antiporter. Both systems are well described in Central Nervous System.

A precise and defined relationship between brain and liver has been known for many years, patients with chronic liver disease frequently experience neurological problems. In this scenario, Glu transport becomes important since Glu is the most abundant intracellular hepatic amino acid in the liver and is involved in a wide variety of liver metabolic pathways such as ureagenesis, gluconeogenesis and glutathione synthesis. However Glu uptake in liver has not been thoroughly characterized. With this in mind we measured [^{3}H]-L-Glu uptake in presence and absence of Na^+, and the kinetics constants of both Glu uptake systems were determined. We could elucidate the contribution of the different Glu transporter systems using specific inhibitors in these cells and correlate it to the plasma membrane protein levels of EAATs and system X_c^-.

MTU08-15

Visualization of neurotransmitter uptake in serotonergic neurons

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Serotonin (5HT) re-uptake is the termination of serotonergic neurotransmission. The biggest fraction of serotonin is cleared from the synaptic cleft via high-affinity transporters like the serotonin transporter (SERT). High-affinity transporters are the principal targets for commonly prescribed antidepressant drugs. However, these compounds do not provide satisfactory treatment outcomes for all patients. To study serotonergic neurotransmission at cellular level neurotransmitter release and re-uptake in serotonergic neurons need

to be investigated. We have combined differentiation protocols for the generation of stem cell-derived serotonergic neurons together with confocal microscopy to study the uptake and release of fluorescent substrates known to be selectively taken up by monoaminergic neurons. These substances include: (i) 4–(4-(dimethylamino)styryl)-N-methylpyridinium (ASP+), an analog of the neurotoxin MPP^+; (ii) the fluorescent false neurotransmitter (FFN511); and (iii) serotonin (5-hydroxytryptamine; 5-HT) itself, which is known to emit fluorescence upon excitation at 320–460 nm.ASP+ is taken up into living serotonergic neurons through the serotonin transporter, but not accumulated into synaptic vesicles; FFN511 diffuses in a SERT-independent way into serotonergic neurons and accumulates in synaptic vesicles. Application of ASP+ so far has been used to investigate substrate/transporter interactions. We have recently reported upon studies on FFN511 uptake and release in stem cell derived serotonergic neurons and upon quantitative studies on uptake and release of 5-HT in living serotonergic neurons. We have used this system also to study the effect of selective serotonin re-uptake inhibitors (SSRIs) on substrate uptake in stem cell-derived serotonergic neurons especially for the enantiomers of Citalopram: Es- and R-citalopram.

MTU08-16

The effect of western diet consumption on neuronal activation in the hippocampus and associated brain regions

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Recent studies have shown that the consumption of a "western" diet (WD), a diet high in fat and carbohydrates, can affect cognition with impairments in visuospatial ability, rule learning and working memory detected in animals. The hippocampus is a site for structural abnormalities in early cognitive dementias and is the focus of our laboratory in association with weight gain. In a series of experiments we explored the ability of WD consumption to influence changes in neuronal activation. In the present study two groups of male hooded rats were fed either normal chow or WD (21% fat, 0.15% cholesterol) for 3 months. Basal c-Fos immunohistochemistry was performed in sections of the hippocampus (CA1, CA2&3 and dentate gyrus) with results demonstrating decreased basal neuronal activation in the CA2&3 regions ($p = 0.04$) of the hippocampus. In associated regions, there was no difference in the retrosplenial cortex however a decrease in the prefrontal cortex ($p < 0.01$) was observed in basal neuronal c-Fos expression. In a following experiment two groups of male hooded rats were fed either normal chow or WD for 3 months and activated c-Fos immunohistochemistry was performed. Animals were placed in a novel environment for 10 min and culled 1.5 h later to allow for maximal induction of c-Fos following a novel stimulus. No changes were observed in the hippocampus and the prefrontal cortex in the number of Fos-positive cells, however there was a subsequent increase of neuronal activation in the striatum ($p = 0.02$) and substantia nigra ($p = 0.03$) after exposure to a novel environment in the WD animals. These results demonstrate that our dietary manipulation model has divergent effects on different hippocampal efferent systems.

MTU08-17

The potential role of SNX12 in endosomal vesicle trafficking in the central nervous system

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SNX12, a member of the SNX (sorting nexin) family, has a PX (phox homology) domain that binds phosphatidylinositol 3-phosphate, thereby allowing selective membrane association primarily to the endosome. SNX12 shares the highest homology with SNX3 among the SNX family, and neither of them has a BAR (Bin-Amphiphysin-Rvs) domain. SNX3 has been recently reported to play an important role in retrograde trafficking of Wnt-binding protein, Wntless. Retrograde transport from the endosome to Golgi is mediated by the retromer complex, which is highly expressed in the brain. Evidence indicates that retromer-dependent trafficking is essential for the delivery of selected signaling receptors in neurons. Therefore, we hypothesized that SNX12 may play a role in endosomal protein trafficking in the brain. We found that SNX12 was highly and ubiquitously expressed in the central nervous system, especially in the neurons, and that its expression increased as neurons became more mature. In cultured primary hippocampal neurons and HeLa cells, endogenous SNX12 and overexpressed GFP-SNX12 were co-localized with VPS35, a component of heteropentameric retromer complex in early endosomes. Interestingly, SNX12 knockodown by shRNA in neurons led to an increased level of endogenous SNX3, which suggests the potential for a compensatory function between SNX12 and SNX3. Our data provide preliminary evidence that SNX12 plays a role in endosomal protein trafficking in neurons. Further studies are needed to examine how SNX12 regulates endosomal vesicle trafficking and which cargo molecules are transported by SNX12 in the central nervous system.

MTU08-18

The effects of insulin induced hypoglycaemia on tyrosine hydroxylase phosphorylation in the rat brain and adrenal gland

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Recurrent hypoglycaemia is an unavoidable side effect of intensive insulin therapy leading to diminished plasma adrenaline defence response to subsequent hypoglycaemic episodes. Our study investigated the effects of single and recurrent insulin induced hypoglycaemia on tyrosine hydroxylase (TH) phosphorylation and protein (as a measure of TH activation and catecholamine biosynthesis) in the adrenal gland and certain catecholaminergic brain regions. Male SD rats either received daily saline (control group) or insulin i.p. injections (10 U/kg) (recurrent hypoglycaemia group) on 3 consecutive days; or saline i.p. injections on days 1 and 2 and insulin i.p. injection (10 U/kg) on day 3 (single hypoglycaemia group) ($n = 5$–8 per group). Rats were euthanised at 60 min after the last injection on day 3. Plasma adrenaline (measured by ELISA) was significantly increased in response to single hypoglycaemia; this response was diminished by 41% ($p < 0.01$) in response to recurrent hypoglycaemia. TH phosphorylation at Ser40, Ser31, Ser19 and TH protein were analysed by western blotting. pSer31TH was signif-

icantly increased in the adrenal gland (4–5 fold; $p < 0.0001$), C1 neurons (1.5–2 fold; $p < 0.01$), substantia nigra (2 fold; $p < 0.01$), ventral tegmental area (VTA, 2 fold; $p < 0.01$) and nucleus accumbens (NAc, 1.3 fold; $p < 0.05$) in response to single hypoglycaemia relative to controls; these responses were diminished in response to recurrent hypoglycaemia only in VTA and NAc. Total TH was significantly increased in response to recurrent hypoglycaemia compared to controls in the adrenal gland only (1.7 fold; $p < 0.01$). pSer40TH was not significantly altered in response to either single or recurrent hypoglycaemia in any tissue. The increased pSer31TH in the adrenal gland with no changes in pSer40TH suggests that pSer31TH alone can increase TH activity. The increased pSer31TH in the brain suggests that catecholamines might have been released in response to insulin induced hypoglycaemia. The main effect of recurrent hypoglycaemia to reduce adrenaline secretion might occur at the level of adrenal gland affecting its ability to release catecholamines.

MTU08-19

Phosphorylation of serotonin 1A receptor (5HT1AR) by CDK5 activity

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Mental disorders including depression are one of urgent issues to be addressed. To prevent the onset and develop the treatment, it is important to understand a mechanism of diseases at molecular level. It is generally considered that dysregulation of neuronal activities is an underlying mechanism. Cyclin-dependent kinase 5 (Cdk5) is a neuron-specific Ser/Thr kinase, which is activated by regulatory subunit p35 or p39. Recent reports suggest its function in synaptic activity and association with anxiety and depression. I investigated here a role of Cdk5-p35 in mental disorders by focusing on serotonin 1A receptor (5HTlAR). 5HTlAR is expressed highly in central nervous system and is thought to be involved in psychiatric activity. 5HT1AR is a seven transmembrane G-protein-coupled receptor, which binds to Gi or Go of trimeric G proteins to inhibit adenylyl cyclase or open K^+ channels in neurons. Dysfunction of the serotonin signal is considered as the cause of many mental diseases. So, 5HT1AR has been a target of drug development for anxiety and depression. It is not fully known, however, how 5HTlAR is regulated. There are three possible Cdk5 phosphorylation sites in 5HT1AR. We examined phosphorylation of 5HT1AR by Cdk5-p35. Expression level of 5HT1AR was decreased by co-transfection with Cdk5-p35, but not with kinase negative Cdk5-p35, in COS-7 cells. 5HT1AR was indeed phosphorylated by Cdk5-p35. We constructed non-phosphorylatable Ala mutants, T149A, S245A, and T314A, and examined their phosphorylation. Thr314 was identified as a phosphorylation site in 5HT1AR. These results suggest that Cdk5 controls the serotonin signal through phosphorylation-dependent down regulation of 5HT1AR.

MTU08-20

CDK5 activator, P35 is degraded by proteasome via two pathways

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Cdk5-p35 is a neuron specific Ser/Thr protein kinase. Cdk5-p35 play a role in various neuronal activities such as neuronal migration, membrane transport, and neurite outgrowth. By contrast, abnormal activation of Cdk5 induces neuronal cell death, resulting in neurodegenerative disorders including Alzheimer disease. The activity of Cdk5 must be regulated precisely. p35 is an activation subunit of Cdk5, whose expression levels determine the kinase activity. p35 is a short half-life protein which is degraded by the ubiquitin-proteasome system. However, the degradation mechanism, including E3 ligase for p35, has not been elucidated yet. Identification of E3 ligase for p35 is very important for understanding the regulation mechanism of Cdk5-p35 activity. One of approaches to identify the E3 ligase is to determine ubiquitination sites. Since poly-ubiquitination occurs on Lys residues in proteins, we took a strategy to mutate Lys residues to Arg. Unexpectedly, even though all 23 Lys residues in p35 were replaced by Arg (p35-23R), p35-23R was still degraded by proteasome. p35-23R could bind and activate Cdk5, indicating that the degradation was not due to improper folding of p35-23R. These results suggest that p35 degradation is mediated by both of p35's ubiquitination and non-ubiquitination systems. However an inhibitor of E1 ubiquitin activation enzyme inhibited the degradation of p35-23R. The results suggested that the degradation of p35 requires ubiquitination of other proteins. Next, we studied how p35 is degraded by proteasome without ubiqitination. Tumor supressor Nkx3.1 has ubiquitin-independent degradation degron. We thought p35 also contains proteasomal degradation signal without ubiquitination. We noticed that p35 has an amino acids sequence at 244–260 with a homology to the degron of Nkx3.1. When a critical amino acid Pro in that degron sequence was mutated to Ala, the degradation was reduced. These results indicated the Cdk5 activity is regulated by the degradation of p35 via both pathway. It is a future question when and where these two degradation pathways operate in neurons.

MTU08-21

KCNQ2/3 localization and function at the axon initial segment is disrupted in variants causing epileptic encephalopathy

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KCNQ2/3 are voltage-gated potassium channels underlying the M-current (I_M) regulating neuronal excitability. Genetic mutations in KCNQ2/3 may lead to mild disorders such as Benign Familial Neonatal Seizures (BFNS) or severe disorders such as epileptic encephalopathy. Localization and concentration of KCNQ2/3 channels at the axon initial segment (AIS) tightly regulates normal firing patterns. However, changes in distribution of KCNQ2/3 channels throughout development may play a role in severity of mutations of the KCNQ2 gene. In addition, mutations leading to epileptic

encephalopathy may have different mechanisms that fail, which include gating, trafficking, or rapid degradation, based upon their location in critical regions of the channel subunit. In order to test for this, I performed immunofluorescence labeling in rodent tissue, cultured rat hippocampal neurons, and perform biotinylation assay in CHO cells transfected with mutant KCNQ2 in order to determine whether or not trafficking of the KCNQ2/3 channels are altered or abnormally degraded. In mouse sections, KCNQ2 and KCNQ3 are detectable at AISs of pyramidal neurons (PyNs) in the neocortex and the hippocampal CA3 region at P7, and appear to increase in concentration thereafter. Additionally, immunofluorescence microscopy performed on tissue sections from transgenic mice overexpressing the dominant negative mutant G279S revealed an aberrant labeling pattern: KCNQ2 was completely absent at the AIS and was retained at intracellular puncta in the soma and dendrites. KCNQ3 was partially redistributed to these puncta. However, surface biotinylation assays in CHO cells show no change with pore mutations. KCNQ2 and KCNQ3 proteins are detected at AISs in rodent at time points roughly equivalent to human birth and labeling intensity increased with age. Some mutations may act by preventing surface trafficking and AIS concentration. Since such effects that may not be easily revealed through heterologous expression, further development of *in vivo* models is warranted.

MTU08-22

Inhibiting internalized receptors at the source of pain transmission

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The Neurokinin 1 Receptor (NK_1R) is a mediator of inflammation and central pain transmission that can be stimulated by the neuropeptide Substance P. The NK_1R regulatory cycle requires cell surface activation to mediate G protein-dependent signalling; endocytosis for disassembly of signalling complexes; and receptor recycling or targeting to lysosomes for degradation. Recent evidence also indicates that endosomal NK_1R can form signalosome complexes, initiating signaling events that are spatially and temporally distinct from those initiated at the cell surface. Furthermore, we have demonstrated that signaling events mediated by endosomal NK_1R are consistent with pain transmission. Selectively targeting drugs to endosomes to inhibit intracellular NK_1R signaling therefore offers a novel approach for pain regulation. This study characterizes intracellular NK_1R signaling and evaluates two approaches for endosomal drug delivery: a) conjugating drugs to a membrane-anchoring sterol, cholestanol; and b) packaging drugs into pH-tunable nanoparticles that release cargo in the reduced pH of endosomes. Each system was labeled with fluorescent dye and their intracellular distribution was assessed by live-cell microscopy. Cell signalling and trafficking assays, electrophysiology and pain studies were performed to determine if NK_1R antagonists conjugated to cholestanol or packaged into the hydrophobic core of pH-sensitive nanoparticles could deliver antagonists to endosomes and subsequently improve their inhibitory properties. Our data demonstrate that internalized NK_1R is essential for the full repertoire of receptor-mediated signalling. Furthermore, cholestanol and nanoparticles deliver drugs to endosomes and selectively target NK_1R signalling events of pathophysiological importance. In particular, nanoparticles facilitated rapid endosomal drug delivery whereas cholestanol-conjugation offers prolonged drug residence in endosomes and provided improved pain inhibition in mice, compared to soluble antagonists. These data demonstrate the importance of internalized receptors in pain and presents new tools for achieving analgesia by directing antagonists to relevant intracellular locations.

MTU08-23

Allosteric interactions at the human translocator protein

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Introduction: The 18 kDa translocator protein (TSPO) has become a target for development of drug treatments for anxiety, neurodegenerative diseases and cancer. Limited knowledge of TSPO binding sites has hindered development of these drugs, however recent work suggests pyrazolo [1,5-*a*] pyrimidine acetamides may be a promising class of TSPO ligands (Scarf *et al.*, 2009).

Objectives: We aim to examine the effect of altering the number and position of nitrogen atoms around the pyrazolo [1,5-*a*] pyrimidine acetamide heterocyclic core on affinity and efficacy at the human TSPO.

Methods: Affinity was examined by competitive radioligand binding on human T98G glioblastoma cells using TSPO ligand [^{3}H]-PK 11195, while a bromodeoxyuridine ELISA was used to examine anti-proliferative action.

Results: When nitrogen atoms were reduced to either 1 in the case of indoles or 2 in the case of benzimidazole, complex binding was displayed ($n_H = -2.11 \pm 0.05$ & -0.49 ± 0.15 respectively, $p < 0.05$ for $n_H \neq 1$). Compounds displaying complex binding also positively modulated the anti-proliferative ability of an IC_{10} concentration of PK 11195 without themselves affecting proliferation. This behavior was absent in the pyrazolopyrimidine, imidazopyridine and purine heterocylic cores.

Discussion: We found evidence that changing the number and position of nitrogens in the heterocyclic core of pyrazolo [1,5-*a*] pyrimidine acetamide influences the ability of these derivative compounds to interact allosterically at the TSPO. This is the first evidence of allosteric behavior at the human TSPO and opens an avenue for the production of novel allosteric anxiolytics, anti-cancer and neuroprotective drugs.

Reference:

Scarf A *et al.* (2009) J Med Chem 52: 581–592.

MTU08-24

Suppressor of cytokine signalling 2 (SOCS2) interacts with TRKB receptor and regulates neuronal outgrowth

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Tropomyosin related kinase-B (TrkB) is a member of the receptor tyrosine kinase family which, in response to its ligand Brain Derived Neurotrophic Factor (BDNF), can initiate signalling pathways leading to neuronal survival and neurite outgrowth. Our lab has shown that SOCS2, known as a negative regulator of the JAK/STAT signalling pathway, affects neurite outgrowth by regulating EGF and NGF receptor signalling in PC12 cells and primary cortical neurons. In this study we hypothesised that SOCS2 may also have a regulatory role in neuronal function by regulating TrkB. Full length and mutated constructs of both TrkB and SOCS2 were transfected into HEK 293T cells to determine the regions of each protein required for interaction. Immunoprecipitation experiments were performed and showed that SOCS2 interacted strongly with TrkB, but weakly with TrkB mutants lacking the juxtamembrane or kinase domain. No interaction was observed when both the juxtamembrane and kinase domains were deleted. Immunoprecipitation using SOCS2 mutants and TrkB mutants indicate involvement of the SOCS2 SH2 domain and the Kinase domain of TrkB in forming their interaction. Both SOCS2 and TrkB are abundant in the hippocampal region of the brain. To examine the interaction of SOCS2 and TrkB in these cells, hippocampal neurons from P1 rats were cultured for 14 days, and immunoprecipitation was conducted using anti-TrkB antibodies and Western blot was probed for SOCS2. The results confirm the interaction between SOCS2 and TrkB in this *ex vivo* condition. Finally, neurite outgrowth was used as an indicator to examine the functional role of SOCS2 and TrkB interaction. Hippocampal neurons were transfected with SOCS2 and an SH2 mutant in the presence and absence of BDNF. Results to date show that the deletion of the SH2 domain in SOCS2 reduces neurite outgrowth in the presence of BDNF. Collectively, these data suggest that SOCS2 interacts with TrkB and this interaction affects the neurite outgrowth of hippocampal neurons.

MTU08-25

Er calcium modulates spontaneous firing of cerebellar purkinje neuron

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In neuronal cells, intracellular calcium has crucial role in signal transduction including neurotransmitter release, permeability of ion channels, and various enzyme activity. The source of these intracellular calcium is mainly two; Extracellular calcium, Endoplasmic reticulum(ER) store calcium. The importance of extracellular calcium contribution in neuronal signaling have been well discussed and determined, however, the functional role of ER calcium has not been studied well, relatively. Cerebellar Purkinje neuron is one of the pacemaker cell, which has spontaneous activity. Regulatory up- and down- state of this cell are features for pacemaking property and it is well known that one of the key contributors for this property is the dynamic intracellular calcium concentration. Here, we focused on the ER calcium store, which is activated by various ligands and releases Ca^{2+} to intracellular space through IP3 receptor(IP3R) or ryanodine receptor(RyR). So we investigated the process how ER calcium could contribute to modulate spontaneous firing. First, we applied CPA, a SERCA inhibitor, to block Ca^{2+} source to ER and observed spontaneous firing rate was significantly changed. Then, we tested IP3R and RyR inhibitor to determine from which calcium would mainly contribute to spontaneous firing. Furthermore, we also used many drugs inhibiting store-operating calcium entry (SOCE) and found how the activity was changed.

MTU09 Neurogenesis and Cell Differentiation (Part 1)

MTU09-01

Exogenous carbon monoxide improves neuronal differentiation: a near-death experience

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Cerebral ischemic injuries and neurodegenerative disorders lead to death or impairment of neurons in the central nervous system. Application of stem cell based therapies are promising strategies and are currently under investigation.

Carbon monoxide (CO) is an endogenous product of heme degradation by heme oxygenase. Administration of CO at low concentrations produces several beneficial effects in distinct tissues. Although there is no published data reporting CO as a factor involved in stem cell differentiation several evidences support this hypothesis. This gasotransmitter induces mitochondrial biogenesis which is also broadly described to be involved in metabolic shifts during neuronal differentiation process. Likewise, CO-induced pathways can occur via generation of small amounts of ROS which are also important signaling molecules in neuronal differentiation. CO-releasing molecules (CORMs), which are organic and organometallic compounds with the capacity of delivering CO in a time and tissue-specific manner, have been developed as therapeutic tools for the last decade. Herein CORM-A1 is used for studying neuronal differentiation.

The CO effect on modulation of neuronal differentiation is assessed in three different models with increasing complexity: human neuroblastome SH-S5Y5 cell line, human teratocarcinome NT2 cell line and hippocampal organotypic slice cultures (HOSC). Cell lines were differentiated in post-mitotic neurons by treatment with retinoic acid supplemented with CORM-A1. Cell proliferation and final neuronal population were evaluated by microscopic nuclei counting. Gene expression of neural cell population was analyzed by immunocytochemistry and reverse transcriptase quantitative PCR assays. Mitochondrial population was quantified by PCR. HOSC consists in an *in vivo*-like model and allows the immunohistochemical assessment of cell proliferation, differentiation, migration and death following treatment with CORM-A1.

CO does increase the final yield of post-mitotic neurons, promotes an increase on precursor cell proliferation and inhibits cell death. Furthermore cell mitochondrial population is increased by CORM-A1 supplementation. Further work is needed for assessing the mechanisms underlying CO effects in neuronal differentiation namely by targeting modulation of metabolic pathways, redox alterations and autophagy related pathways.

MTU09-02

The role of CHL1 in ventral midbrain dopamine development

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The ventral midbrain (VM) dopamine (DA) pathways are important for motor and cognitive function. Consequently dysfunction within these networks has been associated with a number of neurological and psychiatric disorders. Abnormal development may underlie these disorders and thereby highlights the significance of understanding the intricate and precise sequence of events that results in their birth and connectivity. While a number of regulators of VM neurogenesis and DA axon guidance are known, the function of adhesion molecules in these contexts remains to be elucidated. A microarray gene list generated in our laboratory from developing ventral midbrain tissue has identified key candidates that may be important during dopamine development. Cell adhesion molecule with homology to L1CAM (CHL1), a member of L1 family, has been shown to influence neuronal survival, proliferation and axon guidance elsewhere in the developing CNS. Here we show CHL1's function in VM DA development. We demonstrate the temporal and spatial expression of CHL1 in the VM during periods of dopaminergic neurogenesis and axon morphogenesis. Given the ability of CHL1 to function in a soluble and bound forms, we examined its function in both contexts on VM primary cultures. CHL1 had no effect on the differentiation of TH^+ dopamine neurons, however selectively promoted dopaminergic (TH^+) neurite elongation and branching, an effect no observed in other (TUJ+) neurons in culture. The bound form of CHL1 promoted TH^+ axon elongation to a greater extent than the soluble form, while the soluble form had greater effect on branching, suggest that soluble and membrane-bound forms of CHL1 could play differing roles in dopamine development. Then, we examined the chemotaxis of CHL1 on VM DA explants. CHL1 had a repulsive effect selectively on (TH^+) axons, but not on the other (TUJ1 +) axons. By studying the CHL1 mutant embryos, we show the fundamental roles of CHL1 in VM DA development and axon guidance. Combined this body of work will increase our understanding of VM DA development and axons morphogenesis.

MTU09-03

Neonatal lethality of neural crest cell-specific rest knockout mice by the reduction of acetylcolinesterase activity in myenteric

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Background: RE1-silencing transcription factor (REST), also known as NRSF (neuron-restrictive silencer factor), is a well-known transcriptional repressor of neural genes. *Rest* null mice have embryonic lethality which prevents further investigations of the functions of the *Rest* gene *in vivo*. We previously generated *Rest* conditional knockout (CKO) mice and reported the genetic ablation of *Rest* leads to *in vitro*-specific derepression of neuronal genes during neurogenesis.

Objective: To further investigate the Rest function *in vivo*, we focused on the development of the neural crest cells (NCCs) induced by the ablation of REST expression because of the multipotential developmental cell fate of NCCs which might increase the chance to detect REST-related phenotypes.

Methods: To examine the effects of *Rest* deletion *in vivo*, we generated mice containing the floxed *Rest* alleles, in which exon 4 encoding the CoRest binding site can be removed by the expression of Cre recombinase. We used a *Wnt1-Cre* allele to excise the floxed *Rest* gene in the early progenitor cells of the developing mouse NC lineage cells *in vivo*. The morphology, histology and gene expression of enteric nerve system were analyzed.

Results: NCC-specific *Rest* CKO mice showed neonatal lethality that were characterized by gastrointestinal tract dilation, while no histological abnormalities except the thinning of the digestive tract as a consequence of the gas accumulation. The gas collected from the swollen digestive tracts of the *Rest* CKO mice contained high concentration of CO_2. They do not have proper gastric retention after oral dye administration and the reduction of acetylcolinesterase (AChE) activity in NCC-derived myenteric plexus in the stomach was detected.

Conclusion: The *Rest* CKO mice indicates a failure of gut function by underdeveloped cholinergic transmission in enteric nerve system. The observed gastrointestinal distension phenotype provides a model for understanding the genetic and molecular basis of NCC defects in humans.

MTU09-04

Quantitating phosphatidylcholine species changes during differentiation of induced pluripotent stem cells into neurons

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Phosphatidylcholines are the most abundant phospholipids of mammalian membranes, representing 58% of the membrane. During neuronal differentiation, the extension of the plasma membrane means that an increase in phosphatidylcholine biosynthesis is essential. Neurite outgrowth is also an important component of neuronal plasticity, as well as for neuronal regeneration after injuries or in neuropathological conditions. Reductions in phosphatidylcholine species have been identified in the brains, cerebrospinal fluid and peripheral tissues of Alzheimer's disease patients but the reason behind this remains unknown. Using electrospray ionisation mass spectrometry we have quantified changes in phosphatidylcholine species of three different cell lines during three stages of neuron differentiation: induced pluripotent stem cells, neural progenitors and neurons. We found unsaturated lyso species PC O-36:2, PC O-34:1 and unsaturated species PC 38:2 significantly decreased over the course of differentiation in all three cell lines. Significant variations were also observed between the cell lines at each stage of differentiation, including differences in the concentration of shorter species between PC 32:2 – PC 34:0 in induced pluripotent stem cells, larger poly-unsaturated species between PC 38:0 – PC O-40:4 in neural progenitors and unsaturated species in neurons. Current studies are focused on modulating the levels of these species to determine their effects on neuronal differentiation, function and survival. Understanding these differences may lead to new ways to restore neuronal plasticity in neuropathological conditions.

MTU09-05

Small molecule approach to direct differentiation of human induced pluripotent stem cells to sensory neurons

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Background: Strategies that exploit induced pluripotent stem cells (iPSCs) to derive neurons have relied on cocktails of cytokines and/or growth factors to bias cell signaling events in the course of fate choice. These are often costly and inefficient, involving multiple steps.

Objective: In this study, we took an alternative approach and selected five small molecule compounds (SMCs) of key signaling pathways to test out a new protocol for the derivation of sensory neurons from human iPSCs.

Results: Within 8 days of the differentiation protocol, iPSC-derived sensory neurons were achieved at > 80% efficiency. The derived cells were positive for cytoskeletal markers common to neurons, Tuj1 and neurofilament, and specific markers for sensory neurons, Islet, peripherin and Brn3a. Whole-cell patch-clamp recordings of the neurons showed firing in response to membrane depolarization, capable of generating action potentials sensitive to tetrodotoxin (TTX). In co-culture with rat Schwann cells in myelinating medium, axon bundles of the derived sensory neurons underwent myelination, showing internodal segments that were positive for myelin associated proteins. The phenotype of the iPSC-derived sensory neurons was sustainable in Neurobasal medium supplemented with maintenance growth factors but without SMCs.

Conclusion: Our rapid and efficient induction protocol promises controlled production of sensory neurons from human iPSCs as a pool for developmental studies and disease modeling.

MTU09-06

Early life diet influences hippocampal microglial maturation and cognitive function throughout life

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The early life nutritional environment can program microglial proliferation within the hypothalamus, with early overfeeding predisposing the individual to a lasting central pro-inflammatory profile that contributes to overactive immune responses long-term. Here we hypothesized that early life overfeeding can induce long-term microgliosis that extends beyond the hypothalamus into regions controlling cognitive function, such as hippocampus, and that microgliosis in this region can disrupt cognition. We tested this idea by manipulating the litter sizes in which Wistar rats were raised so that the pups were suckled in litters of 4 (neonatally overfed) or 12 (control). This manipulation induces long-term obesity and hypothalamic microgliosis in the neonatally overfed. We then examined microglial and neuronal profiles during development and in adulthood, performance in tests of cognitive function, and hippocampal responses to a cognitive task. Neonatally overfed rats have microgliosis in the CA1 region of the hippocampus as early as

14 days after commencing overfeeding. These neonatally overfed rats also have increased microglial density in the dentate gyrus persisting into adulthood. These changes are coupled with differences in markers of neuronal proliferation and survival and with deficits in cognitive function in the radial arm maze. Thus, early life diet can contribute to long-term changes in the hippocampal inflammatory profile, potentially dictating how the animal responds to a cognitive challenge.

MTU09-07

Persistent radial glial cells in the adult human SVZ differentially express fatty acid binding proteins (FABPS)

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During neuronal development, neuroblasts in the subventricular zone (SVZ) use radial glial cells (RGCs) to direct their migration. It is generally accepted that RGCs gradually disappear and transform into astrocytes. We have identified RGCs that persist late into brain development and even into adulthood. Normal human brains (adult and fetal) were compared to Huntington diseased (HD) brains. HD SVZ has an increase in cell proliferation and neurogenesis and as a consequence should have more radial glia.

Aim: Determine the presence of persistent RGCs in adult human SVZ (normal and HD brains) and find the differential role that FABPs (3-5-7-12) play in neurogenesis and neuroblast migration.

Methods: Immunohistochemistry, with antibodies raised against RGC markers including a range of FABPs, was performed on human brains (41–67 years of age and 21–23 weeks of gestation).

Results: The human SVZ expresses an abundance of $FABP5^+$ cells, which colabel with various cell-type specific markers (40E-C, PCNA, GFAP and PSA-NCAM). The majority of $FABP7^+$ cells in the underlying caudate colabel with NeuN. A subset of the $FABP5^+$ cells colabel with radial glial markers. These RGCs were found throughout the adult SVZ with long radial fibers protruding into the caudate nucleus and showed similar expression profiles to the radial glia in fetal human brains. In HD SVZ the RGCs cells were no longer present.

Discussion: The differential expression of FABP throughout neurogenesis indicates the requirement of specific lipids in the different phases of neurogenic development in the SVZ. The persistence of RGCs in the normal adult brain and absence in HD could indicate ongoing use of RG scaffolds in adult neurogenesis. The lack of scaffolding in HD could explain the accumulation of proliferative cells and increased thickness of the HD SVZ.

MTU09-08

Melatonin attenuates methamphetamine-induced decrease in adult hippocampal progenitor cell proliferation via cell cycle inhibition

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Methamphetamine (METH), an extremely addictive stimulant drug, has been found to impair learning ability and spatial memory function as well as hippocampal progenitor cell proliferation while the mechanism underlying of these effects are still unclear. Our recent study showed that melatonin, a hormone mainly synthesized by the pineal gland, regulated the expression of cell cycle regulators and enhanced the proliferation of the adult neural progenitor cells. In this study, the adult hippocampal progenitor cells cultured was used to determine the effect and mechanism of METH induced this effect. Moreover, we also investigated whether the alteration in progenitor cells proliferation and p53 and p21 protein, a cyclin-dependent kinase inhibitor, expression caused by METH treatment could be attenuated by melatonin pretreatment. $p21^{Cip1}$ plays a central role in the proliferation by inhibits cell cycle progression at G1 phase and could be up-regulated by the p53 tumor suppressor. The present study showed that METH treatment caused a decrease in the number of neurospheres and increased the expression of p53 and p21 protein and enhanced the accumulation of p21 in nucleus while melatonin pretreatment ameliorated all of these effects. According to these results, we concluded that melatonin attenuated METH-induced the reduction in adult hippocampal progenitor cells proliferation by decrease p53 and p21 protein expression and by debilitating p21 nuclear accumulation.

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MTU09-09

Catecholamine innervation of the hippocampus and the relevance to adult neurogenesis and dementia in Parkinson's disease

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A key pathological feature of Parkinson's disease (PD) is the progressive degeneration of midbrain dopaminergic neurons, which causes motor dysfunction. However there are a range of 'non-movement' related features (including cognitive dysfunction, dementia and sleep disorder), which are not alleviated by dopamine replacement therapy.

We are currently investigating the hypothesis that reduced hippocampal neurogenesis contributes to cognitive dysfunction in PD. Previous studies have suggested that reduced dopaminergic innervation of the subventricular zone and hippocampus leads to reduced levels of cell proliferation and neurogenesis in these areas.

The neurogenic subgranular layer (SGL) of the hippocampus receives a rich noradrenergic innervation, however, innervation by midbrain dopamine neurons is less well characterised.

Here we present results from on-going studies aimed at characterising the impact of selective lesioning of (i) the midbrain dopamine system or (ii) noradrenergic neurons of the locus coeruleus, on hippocampal neurogenesis.

We also describe results from biochemical, immunohistochemical and retrograde tracing studies in order to better characterise the source of dopaminergic and noradrenergic input to the hippocampus. The results show that while the hippocampus receives bilateral innervation from the locus coeruleus, innervation from the ventral midbrain is largely non-dopaminergic.

MTU09-10

Evidence of neurogenesis from nestin-expressing cells in the adult mouse midbrain

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Whether or not neurogenesis occurs in the adult midbrain is a controversial but important question for developing better treatments for Parkinson's disease (PD). BrdU+ cells here are capable of generating glia, but not neurones. In contrast, we have shown that the electrophysiology and gene expression of previously Nestin-expressing cells in midbrain is remarkably similar to surrounding control neurones, but also distinguished by increased excitability and expression of the pro-neuronal genes Pax6, Msx1, and Ngn2. The aims of the present study were to: (1) confirm these findings at the protein level using immunohistochemistry; and (2) examine the proliferative capacity of Nestin-expressing midbrain cells. Tamoxifen (10 mg/mouse/day in corn oil, p.o.) was administered to adult (> 8-weeks old) Nestin-CreER^{T2} x GtROSA mice to permanently label Nestin-expressing cells with β-galactosidase (β-gal). In mice administered Tamoxifen twice/week from 8 to 16 weeks, 23% of β-gal+ cells were NeuN+, whereas there were no β-gal+/GFAP+ cells (apart from in the ependymal lining of the midbrain aqueduct). 6 weeks following 4 consecutive days of Tamoxifen, 150 Pax6 + cells were identified in two animals, however only 2 were β-gal+. In mice administered Tamoxifen and BrdU (50 mg/kg i.p. twice/day) for 4 consecutive days then left for 2 weeks, there was a higher density of β-gal+ cells in the midbrain (89 ± 4 cells/0.12 mm^2, $n = 5$) than the olfactory bulb (58 ± 8), whereas the density of BrdU+ cells was higher in the olfactory bulb (74 ± 9) than the midbrain (14 ± 4). Notably, there was very little co-localization of β-gal and BrdU in either region. Furthermore, the number of midbrain β-gal+ cells following Tamoxifen increased to 155 ± 25% of control ($n = 4$, $p = 0.012$, z-test) after 6 weeks, and decreased to 50 ± 4.63% of control ($n = 2$, $p = 0.032$, t-test) after 2 weeks when the anti-mitotic agent Ara-C was administered. These data indicate that Nestin-expressing cells in the adult mouse midbrain have a propensity to be or become neurones rather than astrocytes, and that they do proliferate, albeit at much slower rates than BrdU+ cells.

MTU09-11

The organisation, development and connectivity of two callosal projections arising from the mouse somatosensory cortex

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The corpus callosum is the largest fibre tract in the brain and facilitates a wide variety of sensory, motor and cognitive functions. However, despite the importance of this structure in mediating communication between the two cortical hemispheres, many aspects of its organisation, development and connectivity remain unclear. Recently we demonstrated that two major contralateral callosal projections that arise from L2/3 primary somatosensory cortical neurons are differentially affected by manipulations of neuronal activity (Neuron, 2014, 82(6): 1289–1298). However, the factors underlying the distinct behaviours of these callosal projections from a single cortical area remain unclear. Here, we address this question by comparing the spatial extent, developmental sequence, and cortical circuitry of these projections. Using *i*n utero electroporation to selectively label L2/3 neurons and their callosal axons, we demonstrate, for the first time, the full range of projections that result from this labelling method, and find that the two major contralateral callosal projections have differing anterior-posterior extents of innervation. We next investigated their sequence of development and found that they selectively innervate the cortex and branch at similar stages. Finally, using a combination of retrograde labelling, variegated anterograde labelling and optogenetic manipulation, we investigated the location of the cell-bodies that give rise to the projections, as well as the degree of branching these projections undergo and the ultimate functional connections their axons make. We find that these two major contralateral projections predominantly belong to separate cortical circuits, which may explain their differential dependence on developmental cues. This work provides significant insight into the final stages and variations of interhemispheric cortical wiring, and provides a unique model system to study the mechanisms regulating independent callosal projections arising from a discrete population of neurons.

MTU09-12

Differentiation potential of NG2 glia after ischemia is controlled by sonic hedgehog

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NG2 glia constitute a fourth glial cell type in the adult mammalian central nervous system that is distinct from other neural cell types. Several studies have shown that these cells display wide differentiation potential under pathological conditions *in vivo*, where they give rise predominantly to reactive astrocytes. The aim of this study was to identify the growth factors that influence the NG2 glia differentiation after cerebral ischemia. We used transgenic Cspg4-cre/Esr1/ROSA26Sortm14(CAG-tdTomato) mice, in which tamoxifen administration triggers the expression of red fluorescent protein (tomato) specifically in NG2 glia. Differentiation potential (*in vitro* and *in vivo*) of tomato-positive (tomato$^+$) NG2 cells from control or post-ischemic brains was determined using the patch-clamp method and immunohistochemistry in the presence of drugs, which activate or inhibit Sonic hedgehog (Shh) signaling pathway. The ischemic injury was induced by middle cerebral artery occlusion, a model of focal cerebral ischemia (FCI). Tomato$^+$ cells isolated from control brains and cultivated *in vitro* without Shh signaling manipulation displayed membrane and immunohistochemical properties corresponding to several cell phenotypes. They predominantly comprised NG2 glia (45.0 ± 3.9%), astrocytes (22.0 ± 4.1%), oligodendrocytes (18.7 ± 5.0%) and pericytes (13.3 ± 3.8%). On the other hand, NG2 glia isolated from the post-ischemic cortex differentiated *in vitro* preferentially into astrocytes (74.1 ± 5.4%) at the expense of NG2 glia. The activation of Shh signaling in NG2 glia isolated from control brain significantly increased differentiation of NG2 glia into astrocytes, while the inhibition of Shh signaling such differentiation attenuated/diminished. We also manipulated Shh signaling

in vivo after FCI. After Shh signaling activation the number of astrocytes derived from NG2 glia increased in the gliotic scar, while Shh signaling inhibition their number decreased. Taken together, our data indicate that Shh is an important factor that controls the composition of gliotic scar formed around ischemic injury. Support: GACR P303/12/0855, P304/12/G069.

MTU09-13

Optogenetically activated camp signaling induces axonal elongation and branching

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Tight regulation of axonal elongation and target selection is required for the normal development of neuronal circuits. To examine the action of intracellular cAMP on axonal elongation and branching, we used photoactivated adenylyl cyclase (PAC), a microbial enzyme that produces cAMP in presence of blue light, in primary neuronal cultures of rat dentate granule cells. Photoactivation of PAC transfected in cultured neurons for 30 min significantly increased intracellular cAMP levels and thereby promoted the elongation of primary axons and branching of axonal collaterals. We pharmacologically and genetically examined the role of two main downstream targets of cAMP, i.e., protein kinase A (PKA) and exchange protein directly activated by cAMP (EPAC). siRNA-mediated knockdown of PKA inhibited branching of axonal collaterals but not elongation of primary axons, whereas siRNA-mediated knockdown of EPAC inhibited elongation of primary axons but not branching of axonal collaterals. Consistent with these results, immunocytochemical analyses revealed the localized expression of PKA in axonal shafts and EPAC in axonal growth cones. Thus, the cAMP signaling pathway is likely to regulate axonal elongation and branching via EPAC and PKA, respectively. *Supported by the Grant KAKENHI (26460094, 26117504, 2625000).*

MTU09-14

Analysis on mechanisms underlying promotion of neuronal differentiation by ergothioneine in neural stem cells

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Ergothioneine (ERGO) is a food-derived hydrophilic antioxidant, distributed to the brain, and taken up into neural stem cells (NSCs) via carnitine/organic cation transporter OCTN1/SLC22A4. OCTN1-mediated ERGO uptake in mouse NSCs promoted neuronal differentiation by induction of expression of Math1, one of the basic helix-loop-helix (bHLH) transcription factor, via unidentified mechanisms different from antioxidant action of ERGO (Ishimoto *et al.*, *PLOS ONE* 9, e89434, 2014). Elucidation of detailed mechanisms underlying promotion of neuronal differentiation by ERGO may lead to find a novel target for promotion of neurogenesis with an aim to treat neurodegenerative disorders. In the present study, we focused on induction of neurotrophic factors, which promote neuronal differentiation by regulating expression of bHLH transcription factors, as the candidate of the mechanism. Long term exposure of NSCs to ERGO at 500 μM for 9 days significantly increased mRNA expression of Math1 and neurotrophin 5 (NT-5), and tended to increase expression of brain-derived neurotrophic factor (BDNF) and neurotrophin 3 (NT-3). Short term exposure of NSCs to ERGO also increased mRNA expression of NT-5, BDNF and NT-3 depending on the exposure time of ERGO until 12 h, followed by increase in expression of Math1 at 24 h. NT-5, BDNF and NT-3 activate neurotrophic tyrosine kinase receptor type2 (TrkB). To clarify the intracellular signaling pathway related with induction of Math1 by ERGO exposure, NSCs were incubated with ERGO in either the presence or absence of the inhibitor of TrkB or its three downstream signaling pathways, PI3K/Akt signaling, PLCγ-mediated signaling and MAPK/ERK signaling, and expression of Math1 was examined. As a result, the inhibitor of TrkB or ERK tended to suppress induction of Math1 by ERGO. These results suggest that ERGO may promote neuronal differentiation at least partially via activation of TrkB/MAPK/ERK signaling by induction of NT-5. Further studies are required in order to clarify upstream mechanisms underlying induction of NT-5 by ERGO.

MTU09-15

Drebrin knockout results in the impairment of olfaction and adult neurogenesis

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Drebrin is an F-actin-binding protein which plays an important role in regulation of spine morphology. Drebrin consists of two major isoforms, drebrin A and E. We have generated drebrin null-knockout mice (DXKO) and drebrin A specific knockout mice (DAKO). We have previously reported that DAKO shows behavioral abnormality in context dependent fear conditioning test, whereas other behavioral tests are normal as well as spine genesis (Kojima *et al.*, 2010). In this study, we analyzed DXKO using various behavioral tests. DXKO showed abnormal behavior in buried food test and three-chamber social interaction test which were olfactory bulb (OB) related behavior, whereas other fundamental behavioral tests, such as anxiety-related test, motor function test, and visual acuity test, were normal. In Golgi staining, we observed normal dendritic spines in the OB of DXKO, suggesting that the olfaction disorder was not caused by spine abnormality. To examine what kind of abnormality in OB other than spine abnormality attributed to the olfaction disorder, we immunohistochemically analyzed the number of dying cells and mature neurons in the OB. In the OB in DXKO the number of cell death decreased compared to that of wild-type mice (WT), whereas the number of mature neurons did not change. Since in OB newly generated neurons are migrated from subventricular zone (SVZ), we next examined whether arriving of newly generated neurons in the OB

increased or not. One day after injection of BrdU, the number of newly generated neurons in DXKO was smaller compared to that of WT. But, at 4 weeks the cell number was larger in DXKO than WT. Also, the number of Ki-67 positive cell proliferation decreased in SVZ. The above results suggest that the adult neurogenesis in DXKO decreases, whereas the neuronal survival in OB increases. This is consistent with the idea that the decrease of cell production results in the elongation of cell survival in OB (Sui *et al*., 2012). These abnormalities might cause olfaction disorder in DXKO.

MTU09-16

Differentiation potential of neonatal neural stem/progenitor cells is affected by WNT signaling

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The canonical Wnt signaling pathway has an important role in embryonic development and the establishment of neurogenic niches. Here we aimed to elucidate the effect of Wnt signaling on the differentiation potential of neonatal neural stem/progenitor cells (nNS/PCs), isolated from the frontal lobe of the mouse brain, *in vitro*. To manipulate Wnt signaling at different cellular levels, three transgenic mouse strains, enabling tamoxifen triggered activation/inhibition of Wnt signaling, were used. To follow the fate of nNS/PCs, immunocytochemical staining, the patch-clamp technique and calcium imaging were employed. We identified three distinct current profiles among differentiated nNS/PCs. Flat-shaped $GFAP^+$ cells displayed passive time- and voltage-independent K^+ currents, while round $DCX^+/MAP2^+$ cells expressed fast activating outwardly rectifying K^+ currents (K_A) and delayed outwardly rectifying K^+ currents (K_{DR}). Star-like $NG2^+/PDGFR\alpha^+$ cells with a complex current pattern were characterized by inwardly rectifying K^+ currents (K_{IR}), in addition to K_{DR} and K_A. Wnt signaling pathway inhibition caused a marked decrease in the incidence of the cells displaying an outwardly rectifying current pattern (neuron-like cells), while the number of the cells with a passive and complex current pattern increased. In neuron-like cells, we also detected increased K_{DR} and K_{IR} current densities, decreased incidence of cells expressing voltage-dependent Na^+ channels and lowered Ca^{2+} responses to glutamate application. On the other hand, activation of Wnt signaling led to opposite effects in the incidence of the three distinct cell types, current density of K_A in the cells showing a complex current pattern, and in the current density of voltage-dependent Na^+ channels. Taken together, our data indicate that the canonical Wnt signaling pathway promotes nNS/PCs differentiation into cells with neuronal characteristics. Support: GACR P303/12/0855, P304/12/G069 and GAUK 26214.

MTU09-17

Inducible knockout of MEF2A, C, and D from nestin-lineage stem cells impairs hippocampal neurogenesis *in vivo*

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Myocyte enhancer factor-2 (MEF2) transcription factors are implicated in activity-dependent neuronal survival, differentiation, and synapse formation in postmitotic cells during development, but the role of MEF2 in neural stem/progenitor cells (NSPCs) in the adult brain *in vivo* is unknown. We utilized a transgenic mouse model (nestin-$CreER^{T2}$/R26R-YFP mice) in which Mef2A, C, and D were inducibly deleted in nestin-expressing NSPCs and their progeny. Recombined cells in the hippocampal granule cell layer (GCL) were visualized and quantified by YFP expression. In the GCL of wildtype mice (WT), postmitotic neurons expressed Mef2A, C, and D, while Type-1 stem cells and proliferating progenitors did not. Based on this expression, we hypothesized Mef2A, C, and D deletion in nestin-expressing NSPCs and their progeny following Tamoxifen (TAM) administration would result in fewer mature neurons. In WT mice, stereological and morphological analyses revealed an increase in YFP+ neuron number and dendritic formation over time. Contrary to our hypothesis, inducible (i) Mef2 KO mice also displayed an increase in YFP+ neurons over time – but with significantly stunted dendrites – suggesting an uncoupling of neuron survival and dendritogenesis. We also found additional non-cell autonomous effects. For example, WT mice had no difference in proliferating progenitors, adult-born neuron number or dendritic complexity over time, but iKO mice had an early increase in proliferating progenitors, and a later decrease in adult-born neurons with increased dendritic complexity. WT mice also demonstrated a trend for fewer apoptotic cells while iKO mice had a trend for more apoptotic cells. Neither WT nor iKO mice had a significant change in GCL volume over time. These *in vivo* findings indicate a surprising role for Mef2A, C, and D in cell- and non-cell autonomous control of adult hippocampal neurogenesis that is distinct from its role during development.

MTU09-18

Histo-cytometric analysis of prenatal mesencephalic dopaminergic terminal specification based on NURR1 and tyrosine hydroxylase

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Disturbances in Mesencephalic dopamine (MsDA) development and function have been shown in developmental animal models of schizophrenia and have been proposed as antecedents to disease. Using spinning disk confocal microscope and 3D-reconstruction software IMARIS, we quantitatively assessed tyrosine hydroxylase (TH) and its transcriptional regulator Nurr1 in rat mesencephalon from embryonic (E) day 12 to E17. Based on Nurr1/TH fluorescent

intensity per cell, three MsDA subpopulations were identified in the Ms floor plate: a [Nurr1^{high}, TH^{neg}] subgroup in the midline of intermediate zone; a ventral-midline [Nurr1^{medium}, TH^{medium}] subgroup and a bilateral ventral-lateral [Nurr1^{medium}, TH^{high}] subgroup in the marginal zone. They represent MsDA progenitors, developing DA neurons in ventral tegmental area (A10) and developing DA neurons in substantia nigra compact pars (A9) respectively. This is the first time that significant variations of Nurr1/TH expression have been reported in MsDA cells. Likewise, flow cytometric (FCM) analysis of prenatal Ms revealed the same cellular profile: [Nurr1^{high}, TH^{neg}] represent a 2.5% population, 0.4% [Nurr1^{medium}, TH^{medium}] and 0.3% [Nurr1^{medium}, TH^{high}]. This allows the separation of pure MsDA cell subpopulations using fluorescence activated cell sorting (FACS). We are able to isolate total RNA from FACS harvested cells ranging 7.8–8.0 by RNA integrity number (RIN). RNA sequencing for these three MsDA subpopulations is ongoing. This novel histo-cytometric approach allows the transcriptomic profiling MsDA neural lineage in early MsDA development of normal animals and subsequent comparison with schizophrenic animal models, for example developmental vitamin D deficiency and maternal immune activation. Such knowledge regarding embryonic DA neuron subtypes may also be of benefit to future cell therapy for Parkinson's disease.

MTU09-19

Delineating the function of amyloid precursor protein dimerisation in neuritogenesis in SH-SY5Y cells

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The Amyloid precursor protein (APP) is involved in a wide range of functions some of which include neurite outgrowth, neuroprotection, modulation of copper and iron metabolism, modulation of cell to cell contact and myelination. Although APP has been studied extensively its mechanisms of action has not yet been fully elucidated. The dimerisation of APP is known to affect its processing and function. Studies have shown that dimerisation alleviates the neuroprotective effects of secreted APP and can influence the generation of Aβ42. Data from our lab suggests that dimerisation of APP, caused by an APP-L17C mutation, decreases neuritogenesis in SH-SY5Y cells. This phenotype can be rescued after treating the L17C with condition media from WT-APP mutants, which suggest a secretory factor that modulates neurite outgrowth is missing in the APP-L17C mutant. To understand the mechanism involved we investigated whether dimerisation of APP modulates the secretion of factors involved in neurite outgrowth and/or if the dimerisation affects second messenger pathways. We found APP dimerisation regulates neurite outgrowth by changing the expression of miRNAs. We detected differential expression of microRNAs involved in neurite outgrowth in the APP-L17C mutants as compared to wildtype APP cells, 2 weeks of differentiation. The L17C phenotype could be reversed using a RHO pathway inhibitor (Y27632) which implicates APP dimerisation in RHO activation. We have found that APP dimerisation affects several pathways regulating neurite outgrowth and current studies are investigating the exact mechanisms linking APP dimerisation to these changes.

MTU09-20

Adult born neurons: do cells "retire" and does developmental age matter?

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Neurogenesis occurs throughout adulthood in mammals. However, there is controversy about whether or not old neurons "retire" and newly born neurons take their place in neural circuitry. In order to address this controversy, we "birth-dated" newly born neurons in the dentate gyrus of Sprague Dawley rats using two thymidine analogues, (chloro-deoxyuridine (CldU) and iodo-deoxyuridine (IdU)), given at 35, 12, 6 or 4 weeks prior to study at 10 months of age. We then used immunofluorescence to identify active neurons as indicated by co-localization of the thymidine analogue with the protein expressed by the immediate early gene Zif268 and a neuronal marker, either calbindin or NeuN. We found that the cells born when the animals were 2 months old (35 week old cells) expressed Zif268 just as much as those born only 4 weeks prior to study. However, those born at 12 and 6 weeks before study were significantly less active ($p = 0.002$ and $p = 0.01$, respectively). We then asked the question: Does the developmental age of the animal influence the relative activity of newly born cells? Using cells birth-dated at 12 weeks and 4 weeks prior to study in animals aged only 5 months at perfusion, we found no difference in activity levels between the two groups. Surprisingly, this level of activity was also not significantly different from the activity in the 35 or 4 week old cells in the 10 month old animals. These results indicate that neurons born when a rat is young (2 months of age) continue to be highly active in neuronal circuitry. If "retirement" does occur, it appears to be specific to cells born during middle-age.

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MTU09-21

Vitamin d regulates tyrosine hydroxylase expression: n-cadherin may be a possible mediator

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Vitamin D is a neuroactive steroid. Its genomic actions are mediated via the active form of vitamin D, $1,25(OH)_2D_3$, binding to the vitamin D receptor (VDR). The VDR emerges in rat mesencephalon at embryonic day 12, representing the peak period of dopaminergic cell birth. Our prior studies reveal that developmental vitamin D (DVD)-deficiency alters the ontogeny of dopaminergic neurons in the developing mesencephalon. In developmental and toxicological studies it has been proposed that $1,25(OH)_2D_3$ may modulate the differentiation and maturation of dopaminergic neurons; however, to date there is lack of direct evidence. The aim of the current study is to investigate this both *in vitro* using the SH-SY5Y cell line transfected with rodent VDR and *in vivo* using a

DVD-deficient model. Here we show that in VDR-expressing SH-SY5Y cells, $1,25(OH)_2D_3$ significantly increased in a dose- and time-dependent manner the production of tyrosine hydroxylase (TH), the rate-limiting enzyme in dopamine synthesis. In accordance with $1,25(OH)_2D_3$'s anti-proliferative actions in the brain, $1,25(OH)_2D_3$ reduced the percentage of dividing cells from approximately 15% to 10%. Given the reported role of N-cadherin in the direct differentiation of dopaminergic neurons, we examined here whether it may be elevated by $1,25(OH)_2D_3$. We confirmed this *in vitro* and more importantly, we showed DVD-deficiency *decreases* N-cadherin expression in the embryonic mesencephalon. In summary, in our *in vitro* model we have shown $1,25(OH)_2D_3$ increases TH expression, decreases proliferation and elevates N-cadherin, a potential factor that mediates these processes. Accordingly all of these findings are reversed in the developing brain in our DVD-deficiency model. Remarkably our findings in the DVD-deficiency model phenocopy those found in recent model where N-cadherin was regionally ablated from the mesencephalon. This study has shown that vitamin D directly modulates TH expression and strongly suggests N-cadherin may be a plausible mediator of this process both *in vitro* and *in vivo*.

MTU09-22

NFIX regulates the mode of radial glial cell division during hippocampal morphogenesis

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The principal class of stem cell in the developing rodent forebrain are radial glial cells (RGCs). Past research has demonstrated that mice lacking either *nuclear factor one a (Nfia), Nfib* or *Nfix* display an increased number of cortical progenitor cells expressing RGC markers at mid-gestation, implying a key, although undefined, role for this gene family in regulating the mode and rate of RGC division. Here we analyzed the regulatory role of NFIX in RGC biology by examining the generation of hippocampal neurons from the ammonic neuroepithelium of $Nfix^{-/-}$ and control mice. We found that during the early neurogenic period the RGC population was expanded within $Nfix^{-/-}$ mice, and that RGCs exhibited reduced cell-cycle exit and delayed generation of intermediate neuronal progenitor cells. Underlying this phenotype was an increased proportion of RGCs that underwent symmetric self-expanding divisions. The expansion of the RGC pool in $Nfix^{-/-}$ mice prolonged the neurogenic window and led to an increased number of neurons in the postnatal hippocampus. These findings establish that NFIX promotes neuron production by regulating the mode of RGC division.

MTU09-23

Modafinil promotes adult neuronal cell proliferation and modulate multiple signaling pathways during 48 h sleep deprivation

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Neurogenesis in the adult hippocampus is strongly implicated in hippocampal dependent learning and memory formation. It is well established that sleep deprivation impair adult neurogenesis and different cognitive performances. Modafinil is a psychostimulant known to increase vigilance and cognitive capability during sleep deprivation. In this study we investigated the modulatory role of modafinil on adult neuronal cell proliferation during 48 h of total sleep deprivation.

In five groups viz. CC (cage control + vehicle treated), AC (apparatus control + vehicle treated), AC+MDF (apparatus control + modafinil treated), SD (sleep deprived for 48 h + vehicle treated) and SD+MDF (sleep deprived for 48 h + modafinil treated). The rats were sleep deprived using a cage shaking stimulus based on animal activity. Experiments were conducted for reference memory, immunohistochemistry and RT-PCR studies.

Modafinil significantly improved reference memory performance in the Morris water maze during 48 h SD. Numbers of BrdU positive cells decreased significantly after 48 h SD compared to CC and AC. Modafinil significantly increased BrdU positive cells compared to SD group. Numbers of doublecortin (DCX) positive cells were significantly decreased in SD group compared to CC and AC. Modafinil attenuated numbers of DCX positive cells in intermediate and post mitotic stages but not proliferative stage. Colocalization studies revealed no changes of gliogenesis among groups.

RT-PCR analysis of Notch and WNT signaling molecules revealed an overall down-regulation after sleep deprivation whereas modafinil up-regulated expression of Notch 1 (3.8 fold), Notch 2 (5.1 fold), Nrg1 (16.8 fold), Ascl1 (5.6 fold), Dll1 (49.7 fold), Hey1 (1.6 fold), Hey2 (7.6 fold) and Heyl (7.0 fold) compared to SD group.

In conclusion modafinil promote reference memory and adult neuronal cell proliferation during 48 h sleep deprivation. Modulation of the multiple signaling pathways may be played important role in this context.

MTU09-24

Effect of lemur kinase 1A (LMTK1A) on trafficking of endosomal vesicles

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Lemur kinase 1A (LMTK1A), a substrate of CDK5/p35, is a Ser/Thr kinase highly expressed in mammalian brain. LMTK1A consists of an N-terminal kinase domain and a C- terminal long tail. We have previously shown that LMTK1A inhibits neurite outgrowth via modulating the function of Rab11A, a small GTPase regulating recycling endosome traffic. However, it is unknown yet how the kinase activity is involved in neurite outgrowth and what is the function of the C-terminal tail region. In this study, we examined the role of kinase activity of LMTK1A in trafficking of the endosomal vesicles at neurite tip using PC12 and Neuro2A cells. We found that, while the wild type (wt) LMTK1A was predominantly localized in perinuclear region, kinase negative (kn) mutant of LMTK1A is distributed evenly throughout the whole cell cytoplasm. Further, in the neurite tips wt LMTK1A was not transported into the actin-rich cortex and stayed at the ends of microtubules (MTs), whereas a kinase negative mutant distributed both in the actin-rich cortical and

MT-rich precortical areas. In addition, LMTK1A-containing vesicles travelled along the MT to the neurite tip, and the expression of LMTK1A affected the microtubule organization in the tips of neurites. Thus, LMT1A regulates the transport of membrane vesicles from MTs to cortical actin filaments, which may be a critical step of transporting membranes to the tip of neurite, ultimately controlling the neurite outgrowth.

MTU09-25

Developmental scenarios for the evolutionary origin of the corpus callosum

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The most remarkable evolutionary innovation in the brain of placental mammals was the rewiring of interhemispheric connections through the corpus callosum. This provided a shorter route for integration of bilateral circuits, significantly increasing the speed of information processing. We previously found that marsupials share an ancestral pattern of commissural wiring with monotremes, despite being more closely related to placentals. Therefore, here we studied the development of these connections in a marsupial animal model to unravel the main events that could have triggered its evolution. By EdU birth-dating and *in vitro* retrograde labeling, we find that piriform and olfactory structures pioneer formation of the anterior commissure. Then, deep-layer commissural neurons of the neocortex send axons that are followed by upper-layer axons, as revealed by sequential electroporation of reporter genes in pouch-developing young. An interesting finding is the transient co-existence of laterally- and medially-oriented projections, suggesting that a proto-callosal phenotype is embryonically present but largely pruned after weaning. We discuss these findings in terms of general developmental processes directing commissure formation in mammals and possible developmental scenarios explaining evolution of the corpus callosum.

MTU09-26

Metabolism of tamoxifen in mouse brain: implications for fate-mapping studies using tamoxifen-inducible CRE/LOX system

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Tamoxifen-inducible Cre/Lox system is widely used in fate-mapping studies for visualization or functional modification of specific cell-type population at desired time-points. In experiments, in which the cell fate is influenced by any treatment, such as surgery or drug administration, the time window between tamoxifen injection and the treatment should be large enough to allow complete degradation of tamoxifen and its metabolites. Otherwise, they might promote recombination in cells that start express the Cre recombinase as a result of the treatment, which can lead to data misinterpretation. We aimed to define the optimal time window allowing the complete degradation of tamoxifen and its metabolites, such as 4-hydroxytamoxifen, N-desmethyltamoxifen, endoxifen and norendoxifen, in mouse brain after injection of 400 mg/kg tamoxifen i.p. Therefore,we determined EC_{50} of all substances and employed liquid chromatography coupled with mass spectroscopy to determine their degradation kinetics. Our results revealed that tamoxifen and its metabolites were completely degraded within 10 days in young adult C57BL/6 mice (male), while the age-matched FVB male mice displayed faster degradation kinetics and metabolized all substances within 6 days. Moreover, 18 months old C57BL/6 mice were unable to metabolize all substances within 14 days. Interestingly, disruption of blood-brain barrier by middle cerebral artery occlusion 14 days after tamoxifen injection caused a sudden increase in 4-hydroxytamoxifen concentration in injured hemisphere exceeding its EC_{50} value. Taken together, we show that tamoxifen metabolism in mouse brain is age- and strain-dependent, and is affected by the disruption of blood-brain barrier following ischemic injury. These findings indicate the possible weaknesses of fate-mapping of neural cells using tamoxifen-inducible Cre/Lox systems.

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MTU09-27

Bio-engineered scaffolds promote the functional integration of transplanted human ESC-derived cortical progenitors in stroke rats

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The majority of strokes result from loss of blood flow to parts of the brain – the consequence of which is the death of brain cells in these areas. Additionally, the surrounding brain tissue can also become vunerable and die. However, current therapies for stroke are limited – providing only modest efforts to protect against the ongoing cell death, whilst failing to address the initial cell loss.

The bio-engineered scaffolds have been developed as a new therapy for brain repair, which offeres both physical and biological supports to re-build the lost tissue architecture. These scaffolds are gels made from proteins normally present in the brain's extracellular matrix, and are thereby able to fill tissue voids and provide tissue support. Added to this, the scaffolds can incorporate cell transplantation into these gels to replace those lost neurons during the injury. These scaffolds were implanted with cell transplantation in an animal model of stroke for 9 months to assess the repair ability.

These scaffolds have been shown to significantly descrease the atrophy caused by stroke, which show the protection to host neurons against ongoing degeneration in the injured brain. Additionally, the scaffolds can promote both survival and graft volume of hESC-

derived cortical progenitors compared to cell transplantation alone. The study also shows the functional recovery in the injured animals under the scaffolds treatment after 9 months.

Overall, these scaffolds were shown to restore the lost tissue mass, as well as providing structural support and protein delivery to promote the survival and integration of neural transplants in stroke rats. The study will also yield important knowledge for the ability to exploit such conduits for other neural injuries.

MTU09-28

PGC-1alpha directed neuronal differentiation as a therapeutic intervention in alzheimer's disease model

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In the adult brain, new neurons are continuously generated from neural stem cells (NSCs) to maintain critical functions and repair processes. However, the molecular mechanisms that regulate adult NSCs function *in vivo* remain largely unknown. PGC-1α, a master integrator of energy metabolism is known to regulate diverse functions. But so far its role in the regulation of NSCs function remains largely uncharacterized. Therefore, in the present study we evaluated whether PGC-1α integrates with the genes and transcription factors that control proliferation and maturation of NSCs in the hippocampus of adult mice. To discern the role of PGC-1α in NSCs proliferation and differentiation, we used RNA interference and AAV mediated overexpression of PGC-1α in the hippocampus. Furthermore, shRNA mediated knockdown of PGC-1α in hippocampus resulted in decreased pool of NSCs and bromodeoxyuridine (BrdU) positive cell proliferation consequently leading to dysregulation of neurogenesis. Conversely, PGC-1α overexpression *via* viral mediated gene transfer strongly up regulated the mRNA expression of neurogenic genes/transcription factors such as neuregulin, neurogenin, neuroD1 and suppressed the expression of gliogenic gene STAT3. Furthermore, the co-localization of BrdU with doublecortin (DCX), neuronal nuclei (NeuN) were enhanced indicating that PGC-1α is necessary for the production of functional neurons. In continuation with this we also examined the involvement of PGC-1α in the regulation of hippocampal dependent learning and memory processes by generating toxin induced Alzheimer's disease model. AAV mediated over-expression of PGC-1α in AD models resulted in an increase in cognitive function. Taken together, these results demonstrate an essential and highly specific role of PGC-1α in regulating hippocampal neurogenesis and in ameliorating the associated symptoms of AD.

MTU10 Animal Model of Neuropsychiatric Disorders (Part 1)

MTU10-01

SYNGAP1 haploinsufficiency affects pyramidal neuron morphological maturation in developmental brain

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Cortical remodeling is a critical aspect in shaping optimal cerebral connectivity. Neuropsychiatric and neurodevelopmental disorders are associated with genetic mutations that negatively affect neuronal plasticity including structural changes in dendritic spines and in dendritic branching architecture. Sporadic (*de novo*) mutations that induce genetic haploinsufficiency of *SYNGAP1/Syngap1* cause non-syndromic intellectual disability (NSID) with comorbid autism spectrum disorder (ASD) epilepsy. *SYNGAP1* is an essential neurodevelopmental gene that encodes for a protein that potently regulates dendritic spine structure and function.

We hypothesize that genetic haploinsufficiency of *SYNGAP1* disrupts dendritic spine dynamics and neuronal morphology of cortical cells altering the developmental pace of brain maturation.

We show that *SYNGAP1/Syngap1* mutation promotes accelerated rate of spine formation as well as a precocious, adult-like neuronal morphology of layer 5 pyramidal neurons in young mice. Interestingly, the processing of spine pruning also occurs earlier compared to control groups, indicating that fundamental features of neuronal development are accelerated in this model of ID.

MTU10-02

Fluphenazine (a typical antipsychotic) has purgative effect in turkey

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Reported recently is the purgative side effect of fluphenazine in turkey. In view of this, intramuscular fluphenazine was investigated for purgative activity in healthy turkeys. Seven turkeys weighing 2.1 ± 0.5 kg were intramuscularly administered 5, 10, 15, 25, 50, 125 and 200 mg/kg body weight of fluphenazine and observed for a period of 30 min and thereafter for 24 h. The number of fecal droppings from the turkeys in 10 min were 4, 7, 2, 3, 1, 2 and 2 respectively. The purgative effect stopped at the end of 10 min of observation. Pearson correlation of −0.4 showed that the number of fecal droppings were not linear with dose progressions (i.e. the number of droppings were loosely scattered away from the line). But there was a strong correlation of 16% ($r^2 = 0.16$) between dose of fluphenazine and frequency of fecal droppings. However, the probability of one fecal dropping by healthy turkey in 10 min is 1/7. In conclusion, 10 mg/kg of fluphenazine caused 7 fecal droppings in 7 min but at 125 and 200 mg/kg the frequency of fecal droppings was 2. However, at 50 mg/kg there was one frequency of fecal dropping in 7 min. But the first dropping at 125 and 200 mg/kg was soft but the second dropping was watery diarrhea. The volume of excreta increased with the dose, but the frequency of dropping decreased with the increased doses. Other signs observed are standing still, opistothonus, calmness, torticollis and hyperventilation. Therefore, turkey and fluphenazine could be used as models for purgation. The purgative action may be by gastric sedation or anticholinergic.

MTU10-03

Perinatal phencyclidine reduces NWASP and WAVE1 protein expression and reduces levels of myelination markers in the PFC of rats

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Background: *N*-methyl-D-aspartate (NMDA) receptor antagonism by perinatal phencyclidine (PCP) treatment leads to neuronal damage and causes long-term behavioural alterations in rodents. It is routinely used to model pathological processes in the brain that may be present in schizophrenia, such as alterations to dendritic development, and disruptions to myelin processes. Changes to the polymerization and reorganization of the actin cytoskeleton can have significant effects on the morphology and dynamics of the dendrites within the brain. Actin regulation is primarily regulated by neural Wiskott-Aldrich syndrome protein (NWASP), and WASP-family verprolin homology protein-1 (WAVE1).

Methods: We have examined the role of actin related, cytoskeletal proteins NWASP and WAVE1 in a neurodevelopmental model of schizophrenia using PCP to determine if these signaling pathways are altered in the prefrontal cortex and hippocampus throughout different stages of neurodevelopment. Male Sprague Dawley rats were injected subcutaneously with PCP (10 mg/kg) or saline at postnatal days (PN)7, 9 and 11. Rats (n = 6/group) were sacrificed at PN12, 5 weeks or 14 weeks. Relative expression levels of protein expression were examined in the prefrontal cortex and hippocampus of the treated rats.

Results: NWASP, WAVE1 and MBP were decreased ($0.001 \leq p \leq 0.032$) in the prefrontal cortex of PCP treated rats at PN12. At 5 weeks of age, NWASP was reduced in the prefrontal cortex ($p = 0.037$) and WAVE1 was reduced in the hippocampus ($p = 0.006$). At 14 weeks, there were no significant changes in any of the tested proteins ($p > 0.05$).

Conclusions: This is the first report of an alteration in NWASP and WAVE1 proteins in the rat brain, directly following NMDA receptor antagonism by PCP treatment in early development. These findings suggest that alterations in these important scaffolding related proteins may contribute to the development of deficits in myelination and cognitive performance in the brain.

MTU10-04

Selective estrogen receptor modulators, raloxifene and tamoxifen, modulate dopaminergic activity: implications for schizophrenia

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Evidence suggests that estrogen plays a protective role against the development and severity of schizophrenia. Although estrogen may be beneficial in treating schizophrenia, its chronic use is associated with side-effects. Selective estrogen receptor modulators (SERMs) may provide a better alternative to estrogen and be a safer treatment option for both men and women. Our previous research in rats, suggests that estradiol may protect against schizophrenia symptoms by acting on the dopaminergic system. Therefore, we propose that SERMs can also modulate the dopaminergic system, and that this modulation is the basis for their therapeutic effects in schizophrenia. We investigated the effect of raloxifene and tamoxifen on dopaminergic-induced disruptions of prepulse inhibition (PPI). PPI is an operational measure of sensorimotor gating; this gating is a normal protective mechanism in the brain functioning to filter irrelevant information, allowing for coherent thought. Adult female Sprague-Dawley rats were either intact, ovariectomised (OVX), OVX and estradiol-treated, OVX and raloxifene-treated, OVX and tamoxifen-treated. The dopamine D1/D2 receptor agonist, apomorphine (0, 0.1, 0.3 and 1 mg/kg), caused the expected dose-dependent disruption in PPI in intact and OVX rats. However, this PPI disruption was prevented in OVX rats treated with estradiol, raloxifene or tamoxifen. In untreated OVX rats, average PPI was 55% after saline and 36% after 1 mg/kg apomorphine treatment, a reduction of 19%. However, estradiol-treated and raloxifene-treated rats showed only a 7% reduction in PPI, and tamoxifen-treated rats had a 3% reduction in PPI caused by apomorphine treatment. Dopamine D1 receptors, D2 receptors and dopamine transporter binding density was assessed using autoradiography. Estradiol/SERMs differentially altered binding density in the nucleus accumbens. In conclusion, the SERMs, raloxifene and tamoxifen, prevented dopamine-induced disruptions in sensorimotor gating, similar to estradiol. This data lends support to the hypothesis that estrogen/SERMs play a protective role in schizophrenia via modulation of the dopaminergic system.

MTU10-05

RELAXIN-3/RXFP3 and the septohippocampal pathway: effects on learning and memory of viral-based RXFP3 changes in transgenic mice

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The 'septohippocampal system' (SHS) is regulated by GABA-projection neurons of the 'nucleus incertus' (NI), including a population that expresses relaxin-3 peptide, which interacts with specific receptors (RXFP3) on neurons in medial septum (MS), hippocampus and other SHS nodes. Local RXFP3 modulation in MS alters hippocampal ('theta rhythm') activity and spatial memory in rats; via putative actions on GABA/ACh septohippocampal-projection neurons; but similar studies in mice have not been conducted and the nature of RXFP3 effects within the MS/hippocampus is unknown. Thus, we are studying relaxin-3/RXFP3 systems in the SHS of relevant transgenic mice. Using Cre/LoxP recombination methods, we assessed affective behaviour and working memory in mice with targeted deletion of RXFP3 from the dentate gyrus. In an initial cohort, 'floxed-RXFP3' mice injected bilaterally in the dorsal hippocampal hilar layer with AAV1/2-Cre/eGFP displayed similar locomotor activity, anxiety-like behaviour (light/dark box and elevated plus maze tests), and short-term working memory (Y-maze) to AAV1/2-eGFP-injected control mice (n = 8/5, $p > 0.05$). In contrast, this treatment enhanced long-term spatial memory in the Morris water maze, reflected by increased time in the target quadrant during the probe trial; n = 8,5 $p < 0.05$, unpaired t-test), with no differences in spatial learning detected during acquisition trials (n = 8/5, $p > 0.05$), possibly reflecting lower behavioural flexibility whereby mice persist with an 'incorrect solution'. Using *in situ* hybridization histochemistry, we are assessing the overlap of RXFP3 mRNA expression and YFP staining, and changes in RXFP3 mRNA levels in the hippocampi of AAV1/2-Cre/eGFP injected and control mice, as an index of receptor deletion. Further studies are underway to extend these findings, but current data are consistent with the possible regulation of learning and memory retrieval by hilar GABA interneurons and possible RXFP3 modulation of these networks.

MTU10-06

the oligodendroglial abnormalities in mood disorders: a study of human and monkey post mortem brain

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Recent postmortem brain studies of psychiatric disorders revealed neuronal and oligodendroglial abnormalities. These studies usually used stereological methods to count the number of cells in discrete brain regions. However, stereological methods are laborious and intrinsically low throughput, taking typically long periods to complete a large study. We therefore developed a novel quantitative cell-counting method for unfixed, frozen postmortem brains using a flow cytometer. The anisotropic brain tissue was homogenated and collected cells as an isotropic suspention of nuclei. The nuclear suspention was immunostained with nuclear (7-AAD), neuronal (NeuN), and oligodendroglial (olig2) markers. This method was able to count stained nuclei and measure quantitatively their sizes and fluorescence intensities. We applied this method to frozen unfixed postmortem human brains. The gray matter of the frontopolar and inferior temporal cortex from patients with major depressive disorder, bipolar disorder and normal controls were analyzed. Most of the available confounding factors were matched between the groups. We found significant reductions of the number of oligodendrocyte lineage cells in the frontopolar cortex of major depressive disorders and bipolar disorders, whereas no significant

differences were found in the inferior temporal cortex. The result of postmortem brain study from patients with mood disorders suggests that the pathogenesis of the disorders may involve some abnormalities in oligodendrocytes in the frontopolar cortex. To explore the dynamics of cortical oligodendrocytes, we examined brains from crab-eating monkeys (*macaca fascicularis*). First, we estimated the number of oligodendrogenesis in the frontal, temporal, and occipital cortex. We found that the number of oligodendorogenesis was larger in frontal cortex than other regions. Second, we try to establish a nonhuman primate model of cytokine-induced depression by administrating interferon-alpha, which often causes depression in human. In the preliminary study, we observed abnormalities of behavioral and neuropathological changes in the depression model of monkey.

MTU10-07

Comparing repetitive transcranial magnetic stimulation and fluoxetine treatment on the olfactory bulbectomy model of depression

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Depression is one of the leading causes of disability worldwide. With 30% of sufferers not responding to conventional antidepressant treatments there is a growing need for the development of novel therapies to alleviate symptoms in these patients. One of the most promising of these is repetitive transcranial magnetic stimulation (rTMS), however, the mechanisms of action and thus, the most effective treatment parameters remain unknown. This study aimed to replicate clinically used methods of rTMS in an animal model of depression and assess behavioural and neurobiological changes. C57Bl%5C6J mice underwent either surgery to aspirate the olfactory bulbs (olfactory bulbectomy) or a sham surgery. Mice with olfactory bulbectomy received either rTMS (n = 11), sham rTMS (n = 9), fluoxetine (n = 9) or a vehicle treatment (n = 9) administered for 4 weeks starting 2 weeks after surgery. The forced swim test was carried out at three time points: before surgery (baseline), 2 weeks after surgery to confirm the bulbectomy model, and at the end of the treatment period to assess efficacy. After the final behavioural test, mice were euthanased and serotonin levels assessed in the prefrontal cortex using an ELISA. With respect to baseline values; olfactory bulbectomy mice showed significantly higher activity levels in the forced swim test and this was rescued only by rTMS, fluoxetine showed no significant effect. Serotonin levels were significantly increased only in mice treated with fluoxetine. These results indicate a replication of the therapeutic effect of rTMS treatment in a specific animal model of depression, providing a foundation for exploring the mechanisms of rTMS antidepressant effects in human patients. Our findings showing improved behavioural outcomes in the forced swim test without a significant increase in serotonin levels suggest that rTMS may have a mechanism of action that is distinct from serotonin-related antidepressant treatments, consistent with its beneficial effects in drug resistant patients.

MTU10-08

Understanding the DISC1 locus impairment mouse using RNA-SEQ

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Disruption of the 1q42.1 locus that includes the *DISC1* gene has been associated with mental illness, including depression and schizophrenia. To understand how this locus affects brain function, we generated a novel mouse model, designated *Disc1* locus impairment (*Disc1* LI), which was designed to perturb as many *Disc1* isoforms as possible according to current information. This mutant has exons 1, 1b, 2, and 3 deleted, and also carries a 25-base pair deletion in exon 6. In contrast to previous mouse models that have selectively targeted full-length *Disc1* and emphasized hippocampal and cortical deficits, the *Disc1* LI model displays striatal deficits such as volume reduction, and a decrease in Bdnf levels (Jaaro-Peled, submitted). Behaviour was compared between *Disc1* LI and the two related models, 25-base pair deletion in exon 6 only (*Disc1* Δ25 bp) and deletion of exons 1–3 only (*Disc1* Δexons 1–3). The results suggest that there is a synergistic deficit in the *Disc1* LI mouse. Here we describe an RNA-seq study to examine differential gene expression in the frontal cortex of *Disc1* LI mutant and littermate controls. Four different analysis pipelines were applied to the data and results were compared at a false discovery rate of 0.05. Genes that are validated by qRT-PCR are also examined in the frontal cortex of *Disc1* Δ25 bp and *Disc1* Δexons 1–3 mice. Gene expression changes in the striatum will also be compared between the three mutant models using qRT-PCR. The overall aim is to uncover transcriptional differences that may explain the behavioural differences between the models.

MTU10-09

a proteomic study of the nucleus accumbens following sensitisation to MK-801 in the rat

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Schizophrenia is a chronic and debilitating psychotic illness that affects approximately 1% of the population. Abnormalities in glutamatergic function have been consistently linked with schizophrenia aetiology. The most direct evidence for this comes from studies where N-methyl-D-aspartate (NMDA) receptor antagonists, such as phencyclidine and MK-801, were given in low doses to patients. Such treatments exacerbate pre-existing symptoms in patients and induce schizophrenia-like symptoms in healthy subjects. One of the most frequently used pharmacological models for schizophrenia research involves chronic treatment of rodents with these NMDA receptor antagonists. The aim of the present study was to investigate the signalling pathways altered in a rodent model of MK-801 sensitisation. Sprague-Dawley rats treated once daily for 7 consecutive days with MK-801 (0.25 mg/kg, i.p.) displayed a sensitised locomotor response when re-exposed to MK-801 after a 5-day period of abstinence. We used SWATH proteomics (LC-MS/

MS) to positively identify over 1000 proteins in the nucleus accumbens brain region of MK-801 sensitised ($n = 3$) and naïve rats ($n = 4$). Our data show significant alterations in several pathways, including trafficking and activation of glutamate AMPA receptors, trafficking of GluR2-containing AMPA receptors (involved in calcium permeability), cytosolic calcium, synaptic plasticity and disinhibition of SNARE formation in MK-801 sensitised rats compared to naïve controls. These results suggest that sensitisation to MK-801 was associated with changes in the glutamatergic synapse with downstream effects on calcium signalling. This study has provided pharmacological targets in the nucleus accumbens that are currently being investigated in the MK-801 sensitisation rodent model.

MTU10-10

Risperidone-induced weight gain in juvenile female rats through modulating hypothalamic histaminergic/NPY pathways and activity

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Second Generation Antipsychotic drugs (SGAs) such as risperidone are increasingly prescribed (mostly off-label use) to children and adolescents for treating various mental disorders. SGAs cause serious weight gain/obesity and other metabolic side-effects. This study aimed to establish an animal model of risperidone-induced weight gain in female juvenile rats, and to investigate the effects of risperidone on the expression of hypothalamic histaminergic H_1 receptor (H_1R) and neuropeptides, and their associations with weight gain.

Methods: Female Sprague Dawley rats treated orally with risperidone (0.3 mg/kg, 3 times/day) or vehicle (control) starting from postnatal day (PD) 23 ($\pm$1 day) for 3 weeks (a period corresponding to the childhood-adolescent period in humans).

Results: In female juvenile rats, risperidone treatment increased food intake and body weight gain, which started to appear after 12 days treatment. Risperidone also significantly decreased locomotor activity of female rats. Consistently, the risperidone significantly elevated the mRNA expressions of hypothalamic H_1R, neuropeptide Y (NPY), and agouti-related peptide (AgRP) compared to controls, in which H_1R and NPY levels were correlated with weight gain and food intake. However, risperidone did not affect the hypothalamic proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) mRNA expressions.

Conclusion: These results suggested that risperidone elevated body weight gain via regulation of the hypothalamic H_1R, NPY and AgRP pathways, as well as reducing activity in juveniles.

MTU10-11

Differential short- and long-term behavioural effects of chronic antipsychotic treatment in adolescent and adult rats

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The prescription of antipsychotic drugs (APDs) to adolescents has increased dramatically over recent decades, even though the long-term consequences of such an early exposure are not fully understood. This is of particular importance because the brain continues to mature during adolescence, and this period may represent a window of sensitivity to exogenous insults like APDs. The aim of this preclinical study was to examine the hypothesis that the adolescent brain is more vulnerable to APDs than the adult brain. Male rats were treated chronically (21 days) with vehicle or one of three APDs (15 mg/kg clozapine, 1.3 mg/kg risperidone or 0.05 mg/kg haloperidol) as adolescents (postnatal day (PND) 36–56), young adults (PND58–78) or adults (PND80–100). Conditioned avoidance response (CAR) was examined on day 17 of chronic treatment and after a drug-free period (PND118 for all rats) with a challenge dose. Longitudinal MRI scans were performed on the same rats at PND35, PND57, PND79 and PND120 with the brain tissues collected on PND127. On day 17 of chronic treatment, chronic haloperidol induced significantly less escape failures in adolescents than in adults whereas tolerance to avoidance-suppressing effects of clozapine was common to all ages. After the drug-free interval, a sensitization-like CAR suppression was observed selectively in the rats with adolescent risperidone exposure. We did not find any gross brain structural or neurochemical correlates for these behavioural changes. In conclusion, differential behavioural effects of haloperidol and risperidone were observed in adolescents and adults. Long-term increase in adult behavioural sensitivity following adolescent risperidone treatment suggests that adolescence represents a window of vulnerability to this drug. Given risperidone is the most commonly prescribed APD to adolescents, this finding has important clinical implications.

MTU10-12

Maternal immune activation alters molecular indices of the NMDA receptor in the striatum

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Schizophrenia is a debilitating disease that has many risk factors, one of which includes prenatal infection that leads to Maternal Immune Activation (MIA). Offspring of pregnant rats subjected to MIA show deficits paralleling schizophrenic individuals, particularly cognitive and inhibitory neuron dysfunction. The neural areas involved in these behavioural and functional changes may involve the basal ganglia, as these regions are critically affected in schizophrenia. We determined if changes in the NMDA glutamate receptor (NMDAR) may contribute to the neurobiological changes found in MIA offspring within the basal ganglia. Brains of adult offspring from dams treated with the viral infection mimic polyriboinosinic:polyribocytidilic acid (poly I:C) or saline at gestational day 10 (GD10) and 19 (GD19) were processed for binding to NMDAR channel ([^{3}H]MK801 NR2A ([^{3}H]CGP39653) and NR2B ([^{3}H] Ifenprodil) NMDAR subunits, and *in-situ* hybridisation of NR1 and NR2A mRNA, within the dorsal striatum and nucleus accumbens (shell and core separately). Preliminary analysis revealed an overall effect of MIA increasing NMDAR channel and NR2A binding ($F(1, 48) = 12.379$, $p < 0.01$; $F(1, 48) = 4.615$, $p < 0.05$) and decreasing NR1 mRNA expression ($F(1,48) = 6.213$, $p < 0.05$) in offspring. Pairwise comparisons revealed increases in specific binding to NMDAR channel within all basal ganglia areas (males at both time points, all areas $p < 0.05$), and NR2A (GD10 males only, all areas $p < 0.05$). Further investigation will determine if basal ganglia inhibitory markers are affected by MIA. These preliminary findings suggest MIA affects NMDAR levels in both the dorsal and ventral striatum, and may therefore substantiate that subcortical changes may contribute to behavioural changes found in the MIA rats.

MTU10-13

NMDAR antagonism in major depressive disorder and anxiety – a study of efficacy and side-effects of NMDAR antagonists

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Glutamate is the major excitatory neurotransmitter in the central nervous system and its fast actions are mediated by ionotropic receptors such as the NMDA receptors (NMDARs). Currently, the most widely used antidepressants block serotonin and noradrenaline reuptake. This treatment has a delayed onset of 3–4 weeks and only ~60% of patients respond adequately to treatment. Evidence suggests that NMDARs could be a new therapeutic option for treating major depression and possibly also anxiety. It has been suggested that selectivity towards NR2B subunit-containing NMDARs improves the efficacy/side-effect profile. The objective of the current study was to test NMDAR antagonists with different selectivity in mouse tests of antidepressant and anxiolytic efficacy as well as for adverse side effects, using the selective serotonin reuptake inhibitor citalopram as positive control.

MK-801, ketamine, S-ketamine, RO 25-6981, and citalopram were tested in the forced swim test (FST) for antidepressant-like effects. High doses of citalopram (30 mg/kg), RO 25-6981 (10 mg/kg), ketamine (10 mg/kg) and S-ketamine (10 mg/kg) showed an antidepressant-like effect. Preliminary data from the elevated zero maze (EZM) of anxiolytic action indicate that citalopram and RO 25-6981 also possess anxiolytic-like effects, whereas high doses of ketamine shows tendencies towards anxiogenic-like effects. Further studies are needed to confirm this. Locomotor activity was investigated as a potential confounder of FST/EZM using the open field locomotor activity setup (OF). Furthermore, this measure is relevant in the characterization of side effects. Preliminary results from the OF indicate that high doses of citalopram, RO 25-6981 and MK-801, but not ketamine and S-ketamine, result in an increase of locomotor activity.

The NMDAR compounds seem to possess different efficacies on depression- and anxiety-like behavior. Moreover, the side-effect profile appears to differ between the compounds. In nearest future novelty-induced hypophagia and Y-maze testing will be conducted to investigate potential anxiety-like effects and cognitive disturbances of these compounds.

MTU10-14

Resveratrol abrogates insulin resistance-induced pain-depression dyad in rats

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Background: Emerging evidence reveals a strong link between insulin resistance and neurological dysfunction. Insulin resistance leads to dysregulation of neuroendocrine system and thus predisposes to pain-depression dyad.

Objective: This study was designed to gain a deeper insight in the pathophysiology of pain-depression, a co-morbidity associated with chronic fructose-induced insulin resistance syndrome.

Methology: Fructose (15% solution) as a substitute for drinking water for 24 weeks induced insulin resistance in male Wistar rats. Insulin resistance was evident at 6th week and persisted till end of study as assessed by significant increase in body weight, blood pressure, plasma insulin, glucose levels, glycosylated heamoglobin levels, HOMA-IR index and altered lipid profile. Both pain and depression were evaluated after 20th and 24th weeks by von-frey hair test, Randall Sellito, tail immersion test and forced swim test respectively. Treatment with resveratrol (5, 10 and 20 mg/kg) was initiated after 6th week of fructose administration and continued till the end of study.

Results: Fructose-induced hyperinsulinemia was coupled with decreased pain threshold, significant increase in immobility period

along with marked increase in lipid peroxidation and nitrite levels as well as decrease in antioxidant enzyme activities in the brain. This increase in the oxido-nitrosative stress is integrated with the increase in serum TNF-α and IL-1-β levels. Fructose administered rats also showed markedly increased serum substance-P and cortisol levels stating dysregulation of neuroendocrine system. Resveratrol dose-dependently ameliorated insulin resistance-induced pain-depression along with mitigation of oxido-nitrosative stress mediated alterations in cytokine and cortisol levels.

Significance of study: Pearson's correlation revealed a positive correlation between insulin resistance and decrease in pain threshold and increased immobility period. These results provide a novel insight into the possible protective role of resveratrol in insulin resistance-induced pain-depression dyad.

MTU10-15

Does developmental vitamin d deficiency exacerbate the effects of second hit exposures?

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Vitamin D insufficiency affects 1 billion people around the world with pregnant women at high risk of hypovitaminosis D. We have been exploring the effects of developmental vitamin D deficiency (DVD) and exposure to second environmental hits, such as prenatal alcohol exposure, which alone has deleterious effects on development and behaviour. The high prevalence of hypovitaminosis D and alcohol consumption in pregnant women suggest that both insults may take place simultaneously, elevating the offspring's vulnerability to neuropsychological disorders later in life. This study aimed to examine whether the absence of vitamin D would leave the brain vulnerable to the effects of a secondary insult as prenatal ethanol exposure. Four-week old female C57Bl/6J and Balb/c mice were placed on a vitamin D deficient or standard diet for 6 weeks and mated at 10-weeks-old. Females were then exposed to either 10%(v/v) ethanol or water for GD0-8 and offered water for the rest of gestation. Early developmental milestones were assessed at P21 and locomotion was tested at P21 and 70, followed by hippocampal expression of candidate genes *Vglut1-3* in adults. At P21 animals that underwent a single exposure (DVD deficiency or prenatal ethanol) showed hyperlocomotion compared to controls, while animals exposed to both insults showed further hyperlocomotion compared to those under a singular exposure. There was also an overall effect of diet and exposure in adult locomotion, however, in the opposite direction to that found at P21. The expression of *Vglut1* and *3* was not altered due to treatment, however, *Vglut2* showed a significant three way interaction of Strain x Diet x Exposure ($F_{1,89} = 4.364$, $p < 0.05$). We are currently following up these findings by examining P0 brain gene expression microarray to visualize altered genes. We propose that DVD deficiency leads to increased vulnerability to second hit exposures and further studies are warranted to study the effects of these modifiable environmental risk factors on brain development and morphological abnormalities.

MTU10-16

Estradiol regulates gamma-band oscillations in the hippocampus and related cognitive functions

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Cognitive deficits in schizophrenia are functionally disabling and there is currently no treatment to address this core symptom. Schizophrenia patients exhibit reduced gamma-band oscillations (30–80 Hz) during the execution of cognitive tasks. Gamma-band oscillations are generated by parvalbumin positive (PV+), fast-spiking interneurons, which are reduced in schizophrenia brains. Estrogens have beneficial effects on cognitive function and we previously showed that estradiol regulates the expression of PV+ interneurons in the hippocampus and influences related cognitive function. The current study demonstrates for the first time that estradiol regulates gamma frequency oscillations in the dorsal hippocampus and hippocampal dependent memory in female mice.

We recorded brain activity from the dorsal hippocampus of female sham ovariectomized (OVX) mice, OVX mice and OVX mice with estradiol replacement, while they were performing Y-maze, a short-term hippocampal-dependent memory task. OVX mice showed a significant reduction in gamma-band activity, specifically during decision making, which was accompanied by a significant deficit in short-term memory. Estradiol replacement rescued these deficits. Together with our previous findings, we suggest that estradiol mediates hippocampal gamma oscillations via the expression of PV+ interneurons.

This study unraveled a mechanism underlying beneficial effects of estrogen on cognitive function in schizophrenia.

MTU10-17

Enhanced adenosine A1 receptor expression promotes antidepressant effects mediated by HOMER1A upregulation

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Major depressive disorder is among the most commonly diagnosed disabling mental diseases. Several non-pharmacological treatments of depression, such as sleep deprivation (SD), electroconvulsive therapy and deep brain stimulation, evoke a pronounced increase in adenosine concentration and/or upregulation of adenosine A_1 receptors (A_1R) in the brain. To test directly whether increased A_1R signalling mediates antidepressant effects, we generated a transgenic mouse with enhanced doxycycline-regulated A_1R expression, specifically in forebrain neurons. Up-regulating A_1R led to pronounced acute and chronic resilience towards depressive-like behaviour in various tests. Conversely, A_1R knock-out mice displayed an increased depressive-like behaviour and were

resistant to the antidepressant effects of SD. We show further that A_1R upregulation, SD or imipramine and ketamine treatment increases homer1a expression in medial prefrontal cortex (mPFC). Specific siRNA knockdown of homer1a in mPFC enhanced depressive-like behaviour and prevented the antidepressant effects of A_1R up-regulation, SD and acute imipramine and ketamine treatment. In contrast, viral overexpression of homer1a in the mPFC had strong antidepressant effects. Thus increased expression of homer1a is a final common pathway mediating the antidepressant effects of SD, enhanced A_1R expression and antidepressant medications such as imipramine and ketamine.

MTU10-18

RELAXIN-3/RXFP3 signalling promotes motivational drive and stress resilience in mice

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The neuropeptide relaxin-3 is expressed by broadly projecting neurons within the pontine *nucleus incertus*, and signals through its widely expressed G-protein coupled receptor, RXFP3. Relaxin-3/RXFP3 signalling can modulate a range of limbic, septohippocampal, and hypothalamic circuits to influence motivation, stress responses, and other modalities related to behavioural arousal. Using transgenic and wild-type (WT) mice in combination with newly developed pharmacological tools, we first assessed the involvement of this system in motivational drive. Central injections of RXFP3 antagonist reduced motivated food seeking ($p < 0.05$) and consumption ($p < 0.001$) in WT mice, while in salt (sodium) depleted WT mice, RXFP3 antagonist treatment reduced the motivation to consume a 0.3 M NaCl solution ($p < 0.001$) – an effect also observed in sheep ($p < 0.001$). Furthermore, relaxin-3 and RXFP3 knockout (KO) mice displayed reduced motivation to run on voluntary home-cage running wheels ($p < 0.001$), and to press a lever to obtain sucrose reward in an operant chamber ($p < 0.05$). Secondly, stress resilience was assessed, revealing that relaxin-3 and RXFP3 KO mice were hypersensitive to stress-induced insomnia ($p < 0.001$) and stress-induced changes in alcohol consumption ($p < 0.05$), respectively. Furthermore, central infusion of an RXFP3 agonist reduced elevated levels of anxiety-like behaviour induced in WT mice by the benzodiazepine receptor inverse agonist, FG-7142. To better determine the potential mechanisms which underlie these actions, studies are ongoing to identify the neurochemical phenotype of RXFP3 neurons using mice which express yellow fluorescent protein within RXFP3-positive cells. Taken together, these studies provide further evidence that relaxin-3/RXFP3 signalling influences key neuronal circuits to promote motivational drive and stress resilience, and highlight the potential of the relaxin-3/RXFP3 system as a therapeutic target.

MTU10-19

The drive to drink - MMP-9 as a positive controller in alcohol reward-driven behaviors

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The main criterion of alcohol dependence is loss of control over drinking. Addiction occurs when alcohol-related cues are processed abnormally. Long-term drinking implies dysfunction in brain circuitry including the central nucleus of the amygdala (CeA). Human studies implicate extracellular matrix protease, MMP-9, in alcohol addiction, with alcoholism linked to higher MMP-9 levels. Moreover, MMP-9 has been implicated in CeA-related reward-driven behaviors. As "hijacked" reward pathways may develop in addiction, it is pivotal to understand these mechanisms. To test if addiction involves MMP-9, we used ethanol self-administration animal model that mimics human alcohol addiction (high motivation, craving, relapse, loss of control over drinking). We carried out continuous behavioral monitoring of C57BL/6 MMP-9 knockout mice (ko) along with their wildtype (wt) siblings living in social groups in IntelliCage System. Animals were tested for behaviors in a new and familiar environment. They were trained to consume ethanol and tested for motivation for reward. Motivational tests were followed by free access to alcohol, withdrawal and relapse periods. To verify if modulation of MMP-9 level is important in aberrant structural plasticity in amygdala, we analyzed dendritic spine morphology in this region. To better define the role of MMP-9 in motivation for rewards, lentiviral vector overexpressing autoactivating MMP-9 was microinjected in CeA of wt and ko animals that next underwent training. Our results reveal that both genotypes develop alcohol-drinking behavior, yet they differ in particular aspects of it. MMP-9 ko mice have decreased progressive ratio breakpoint of effort exerted to obtain alcohol reward indicating lower motivation. Additionally they performed less attempts to obtain alcohol in signaled non-reward period (withdrawal). Chronic alcohol drinking also produced alterations in dendritic spine shape within the central amygdala of both wt and ko. Interestingly more pronounced changes were observed in wt animals affecting mostly mushroom spines which had enlarged spine head in high alcohol consumers. These results support the involvement of MMP-9 in mediating motivation-related alcohol addiction and highlight the role of central amygdala in these phenomena.

MTU10-20

RELAXIN-3/RXFP3 systems and central stress- and anxiety-related circuits in mice

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Relaxin-3 is a highly-conserved neuropeptide expressed in the nucleus incertus (NI) of rat and mouse. Studies in rats demonstrated that central administration (icv) of RXFP3-A2, an RXFP3 agonist, reduced general anxiety; and icv or local injections of relaxin-3 into

hypothalamus (PVN) increased indices of HPA axis activity. However, the relaxin-family receptors involved and the direct/indirect nature of these effects are unclear. We addressed this issue in mice to determine whether these effects are conserved and to take advantage of transgenic mouse lines to explore mechanisms of action. Male C57BL/6J mice implanted with icv guide-cannulae were tested in elevated plus-maze (EPM), large open-field (LOF) and light/dark box (L/D box) after treatment with RXFP3-A2, with time spent in the aversive zone used as an anxiety index. In 'naïve' mice, RXFP3-A2 (1 nmol), had no significant effect on performance in EPM, LOF or L/D box. Mice pre-treated with the anxiogenic drug, FG-7142 (30 mg/kg, ip,) displayed increased anxiety in the L/D box compared to control, which was reduced by RXFP3-A2 (1 nmol; $p < 0.01$). Blockade of endogenous relaxin-3/RXFP3 signalling with an RXFP3 antagonist (R3(B1-22)R; 4 nmol) increased anxiety-like behaviour in the LOF ($p < 0.01$), but not EPM or L/D box. In initial studies of RXFP3 signalling and the HPA axis, icv and local administration of RXFP3-A2 (1 nmol, icv; 0.1 nmol, iPVN) did not alter serum corticosterone levels. Furthermore, RXFP3 knockout mice displayed a similar corticosterone profile to wildtype littermates after a 15-min restraint stress. These data are consistent with preliminary observations in an RXFP3-Cre/YFP reporter mouse line that CRF-immunoreactive neurons in PVN do not express RXFP3-associated YFP. Our findings suggest differences in aspects of relaxin-3/RXFP3 systems between mice and rats and differential effects of RXFP3 on hypothalamic/non-hypothalamic stress- and anxiety- circuits.

MTU11 Neurodegenerative Disease (Part 1)

MTU11-01

The control of firing pattern of the midbrain periaqueductal gray neurons

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The PAG is a critical relay of the limbic brain and determines motor expression of emotions [such as vocalization, laughing, crying, fear and anxiety]. This includes facial modulation, laryngeal, pharyngeal, respiratory and cardiovascular control, control of autonomic systems required for expression of emotions. For this, the PAG maintains strong connections to the pre-motor inter-neuronal control circuits located in the caudal brainstem and spinal cord. Previous research examined the motor expression of emotions and associated autonomic changes produced from various parts of the PAG. However it is not known what type of activity patterns the PAG neurons express *in vivo*. In this study the PAG was mapped for extracellular neuronal activity within its various sub-regions in the rat and cat during triggered emotional and autonomic expression. Electrophysiological characteristics such as burst frequency & adaptation, power & amplitude density, threshold activation constants, inter-spike intervals and the topography of neuronal circuit function were investigated. The PAG was found to be predominantly quiescent in the resting state. Silent PAG cells could be activated by iontophoresis of DL-Homocysteic acid (DLH) an excitatory amino acid glutamate agonist. Cells made to fire in this manner, ceased activity when either DLH ejection was terminated or by co-iontophoresis of muscimol (GABA agonist). Spontaneously active cells were few, restricted to the dorsal PAG and on recording extracellularly, typically fired in a slow and irregular pattern. Activation of either behavioral (e.g. vocalization, fear) and/or autonomic interventions caused immediate activation of PAG neurons mainly in the lateral and ventrolateral PAG. In such instances, lateral and ventrolateral PAG cells showed two distinct types of activity patterns; 1) single spike firing and 2) burst firing. The cells fired both tonically and phasically when correlated with specific autonomic output such as the diaphragm EMG. Predominantly the non-bursting PAG neurons had a near normal distribution around 200 to 250 msec, while burst-firing cells typically showing a bimodal distribution. The functional implications of PAG circuit physiology are discussed in terms of descending motor and autonomic control of emotional expression.

MTU11-02

The role of FTLD-associated proteins in neurite and synapse health and function

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In the last 7 years a number of proteins have been identified that are pathologically or genetically associated with frontotemporal lobar degeneration (FTLD), including TDP-43 and C9ORF72. The normal function of these proteins and their involvement in the degeneration of neurons in disease is not yet well understood, however there is accumulating evidence that they are involved in neurite outgrowth and maintenance. The aim of this study was to provide insights into whether TDP-43 and C9ORF72 have neurite-related roles. Immunohistochemical analysis and Western blotting, combined with cellular fractionation techniques were utilized to study the expression and localisation of FTLD proteins *in vivo* and *in vitro*. We demonstrated that C9ORF72 was present within neurites of cortical neurons where it had a punctate expression throughout the microtubule and actin cytoskeleton and also within brain tissue. To determine if C9ORF72 was present in synapses we performed preparation of synaptosomes. C9ORF72 was rich in synaptosome fractions along with synaptophysin, PSD95 and GAD67 and was absent from membrane fractions. C9ORF72 was also present within the nucleus. Although predominantly nuclear, TDP-43 was also present with a punctate pattern in neurites and Western blotting demonstrated that expression was high during neurite outgrowth and synapse formation. Our current studies are investigating the role of TDP-43 in neurite outgrowth and branching. Our study suggests that C9ORF72 and TDP-43 are present within neurites where they may play roles in synapse formation and neurite outgrowth, respectively. Determining a role for proteins such as TDP-43 and C9ORF72 within neurites will better help us to understand disease processes.

MTU11-03

Cognitive performance testing of P38 MAP kinase knockout mice using the touchscreen operant chamber

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Mitogen-activated protein (MAP) Kinases regulate a wide range of biological functions, including in the central nervous system. Here we addressed the role of a distinct MAP kinase, p38 MAP kinase, in regulating cognitive functions in adult mice by testing female p38 MAP kinase knockout mice using a touchscreen-based operant chamber system. All mice were tested subsequently for hippocampus-dependent memory, recognition memory and attention performance in the object-location paired associates learning (dPAL), the pairwise discrimination (PD) and the 5-choice serial reaction time task (5-CSRTT), respectively. Both p38 knockout mice and wild-type littermates performed the three tests with an equal number of trials and in an equal amount of time. p38 knockout mice achieved significantly lower correct trials in the dPAL task. However, p38 knockout mice showed similar performance in the PD task as compared with wild-type mice. Furthermore, p38 knockout mice showed low performance in the 5-CSRTT, in particular at short reaction time. Therefore, our study suggests that p38 MAP kinase, while being dispensable for recognition memory, is critical in mice for hippocampus-dependent memory tasks and attention-dependent.

MTU11-04

TDP-43 mediated synaptic alterations in the pathogenesis TDP-43 proteinopathies

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TPD-43 mediated neurodegeneration results from the pathological miss-processing and/or functional change of this RNA-binding-protein. Recent research has indicated that TDP-43 alterations may have an early, underappreciated, pathological role at the synapse. To investigate the role of mutant TDP-43 at the synapse we characterised the pre- and post-synaptic pathology occurring in the cortex of the TDP-43^{A315T} mouse model of TDP-43 proteinopathy with regional immunohistochemistry and spine density analysis. Spine analysis was investigated in TDP-43^{A315T} YFP-H fluorescent mice. Pre-synaptic pathology was investigated using immunohistochemistry (Synaptophysin, GluR1, GluR4, VGluT and VGAT1). Dendrite spines in the TDP-43^{A315T}YFP-H mice were investigated on the Zeiss LSM-510-Meta confocal microscope using Neurolucida™ software. Whilst there was no significant difference in Synaptophysin, GluR1 and Glu4 labelled puncta, Glutamatergic and GABAergic pre-synaptic vesicle transporters were significantly reduced at day 90 (symptom onset) in the TDP-43^{A315T} mice compared to wild-type controls. These changes were specific to motor cortex and not present in somatosensory cortex. However, there was significant total reduction in spine densities in somatodendritic and apical TDP-43^{A315T} x YFP-H dendrites at this symptom onset, day 90, time-point. There was no significant difference in spine densities during the development of the TDP-43^{A315T} x YFP-H mouse, investigated at day 30. Our investigations highlight potential pathogenic roles for mutated TDP-43 at the synapse. Understanding the role that TDP-43 plays in synaptic dysfunction may reveal new therapeutic windows for intervention in TDP-43 proteinopathies.

MTU11-05

Functional analysis of amyotrophic lateral sclerosis-associated mutations of profilin 1 in primary mouse neurons

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Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease and familial ALS accounts for 10% of cases. The identification of new familial ALS mutations in the actin-binding protein profilin 1 directly implicates actin dynamics and regulation in the pathogenesis of ALS. However, how these mutations cause ALS is unknown. To help elucidate the pathomechanisms, we are studying the functional role of ALS-associated profilin1 mutations in both *in vitro* and *in vivo* models. Currently no mouse models are available for the study of the ALS-associated profilin 1 mutations. We are currently developing four new transgenic mice that overexpress profilin 1 or mutant profilin 1 with either a motor neuron specific promoter or a pan-neuronal promoter. Expression constructs for *in vitro* overexpression for functional analysis in primary mouse neurons have also been developed. Our data from studies using primary hippocampal mouse neurons, show that profilin 1 C71G expression results in increased dendritic tree length and arborisation in developing central nervous system neurons. This increase in branching is compartment specific as there was no significant change in axonal branching. Preliminary functional analysis on mature cultured neurons grown for 19 days *in vitro*, showed an increase in dendritic spine density in hippocampal neurons expressing profilin 1 C71G, compared to control vector transfected cells. Additionally, aggregations of profilin 1 have been observed in cultured hippocampal neurons transfected with profilin 1 C71G. Our study is the first to show dendrite-specific morphological changes in neurons expressing profilin 1 with an ALS-associated mutation. Studying the functional consequences of these ALS-associated profilin 1 mutations will help us to unravel some of the mysteries underlying the progression of this complex neurodegenerative disease.

MTU11-06

Kinase activity modulation to restore cognitive impairment and prevent neurodegeneration in a mouse model of down′s syndrome

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Down syndrome (DS) is caused by trisomy of chromosome 21 (HSA21) leading to genome-wide imbalanced gene expression. In DS, cognitive disability is present since early development and progressively increases with ageing. Moreover, individuals with DS show degenerative changes and high prevalence of Alzheimer-type dementia. Currently there is no effective therapy to improve learning and memory or to prevent neurodegeneration later in life.

Several studies in a trisomic mouse model of DS (Ts65Dn) indicate that environmental enrichment (EE), a housing condition that enhances social, sensorimotor and cognitive abilities, induces neuroplasticity and synaptotrophic effects that can partially rescue cognitive impairments. However, as also observed in DS individuals, the effects of EE on young Ts65Dn mice are limited, probably due to inadequate transition of initial plasticchanges into stable synaptic contacts.

The dual specificity tyrosine-phosphorylation-regulated kinase 1A (Dyrk1A) has been postulated as a candidate gene implicated in multiple DS phenotypes. It encodes a serine-threonine kinase that phosphorylates critical substrates in neuroplasticity, cognitive function and neurodegeneration. Our laboratory has previously shown that Dyrk1A kinase activity is modulated by EE.

Here we propose that the normalization of Dyrk1A activity using (−)-Epigallocatechin-3-Gallate (EGCG), the most abundant catechin found in green tea, will potentiate the effects of EE inTs65Dn mice. To test this hypothesis we analyzed the effect of combined EGCG-EE on the cognitive performance of Ts65Dn mice.

Our results indicate that the combined use of EGCG-EE significantly improves visuo-spatial and cholinergic-dependent cognitive function in young and middle-age trisomic mice. We are now assessing the neurochemical, structural and functional correlates of the treatment effects. The data indicates that combined use of EGCG-EE in trisomic mice potentiates the effects of EE. In young adult Ts65Dn mice, it restores the neuroplasticity potential and in middle-age mice it prevents the onset of cholinergic neurodegeneration. We conclude that Dyrk1A is a key factor modulating activity-dependent neuroplasticity and represents a promising therapeutic target for intellectual disability and neurodegeneration in DS.

MTU11-07

Neuroprotective effects of α-mangostin against scopolamine-induced cognitive deficits

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Cholinergic neurons, particularly in the basal forebrain, are markedly depleted in Alzheimer's disease (AD) which is associated with cognitive deficits. Muscarinic antagonist scopolamine (SCO)-induced cognitive deficits is commonly used as a model for AD. The extract from the fruit rind of mangosteen (*Garcinia mangostana* L.) was recently reported to improve spatial memory in SCO-induced amnesic rats. α-Mangostin (α-MG) is an aprenylated xanthone derivative from the fruit rind of mangosteen. The effects of α-MG on learning and memory performance were thus investigated in SCO-induced amnesic rats. Eight groups (n = 8 each) of 8-weeks-old male Wistar rats were i.p. injected with normal saline solution (1 mL/kg), donepezil (2 mg/mL/kg; positive control), α-MG (50 mg/mL/kg), or α-MG (100 mg/mL/kg) followed by i.p. injected with SCO (2 mg/ml/kg) or normal saline solution (1 mL/kg) an hour later. Thirty minutes later, the learning and memory performance were assessed using Morris water maze test. All rats received four trials per day. These procedures were repeated for 7 days. On day 7, all rats were tested in the probe trial. We found that donepezil and α-MG (50 and 100 mg/ml/kg) given to rats before SCO administration could ameliorate adverse effects of SCO by decreasing time to find platform on training session and increasing both time spent and number of entries into the target quadrant in probe trial session when compared to their control group. These findings indicated that donepezil and α-MG could improve spatial memory impairment. Pretreatment with donepezil and α-MG provided neuroprotective effects against SCO-induced memory deficits and neuronal impairment in rat brain. Future work will be required to determine whether the effects of α-MG on cholinergic system in hippocampus, cerebral cortex, and basal forebrain are related with an amelioration of the SCO-induced memory deficits.

MTU11-08

NMDA preconditioning alters the functionality of the adenosine A2A receptor in mice

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N-methyl D-aspartate (NMDA), administered at subtoxic dose, plays a protective role against neuronal excitotoxicity, a mechanism described as preconditioning. Since adenosinergic receptors activation influence the achievement of NMDA preconditioning in the hippocampus, we aimed to evaluate the potential functional interplay between adenosine A_{2A} receptors ($A_{2A}R$) activity and NMDA preconditioning. Binding properties and expression of $A_{2A}R$, and glutamate uptake was assessed in the hippocampus of mice subjected to NMDA preconditioning. Male adult Swiss mice received saline (NaCl 0.9%, i.p.) or a nonconvulsant dose of NMDA (75 mg/kg, i.p.). After 24 h the binding of [^{3}H]CGS21680 was performed in hippocampal membrane extracts preparations. Adenosine receptors expression was analyzed by western blot. In addition, 24 h after NMDA preconditioning, animals were treated with the $A_{2A}R$ agonist, CGS21680 (0.05 mg/Kg, i.p.) and subjected to contextual fear conditioning test. Glutamate uptake in hippocampal slices obtained from preconditioned mice was performed after treatment with CGS21680. Statistical analyses were performed with one-way ANOVA followed by unpaired t-test or two-way ANOVA followed by Newman-Keuls test. The immunodetection of $A_{2A}R$ in total hippocampal homogenates showed no significant differences evoked by NMDA preconditioning and did not alter $A_{2A}R$ maximum binding for the selective ligand [^{3}H]CGS21680 (Saline: Bmax = 2.4 ± 0.3 fmol/mg protein, NMDA: Bmax = 3.6 ± 0.7 fmol/mg protein). However, the treatment of mice with CGS21680 increased the time of freezing during the exposure of animals to the new environment ($p < 0.05$). NMDA preconditioning did not affect the freezing time of mice *per se*, but it prevented the response observed after the activation of $A_{2A}R$. Furthermore, post-activation of $A_{2A}R$ by CGS21680 blocked the increase of glutamate uptake induced by NMDA preconditioning ($p < 0.05$). These results suggest a change of functionality of $A_{2A}R$ and highlighting the role of cross-talk between glutamatergic and purinergic transmissions.

MTU11-09

Toll-like receptor 4 activation exacerbates injury following repetitive mild traumatic brain injury

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Repeated mild traumatic brain injury (rmTBI) or concussion, is associated with the development of the neurodegenerative disorder, chronic traumatic encephalopathy (CTE). CTE is characterised by the accumulation of hyperphosphorylated tau within the brain, associated with cognitive and behavioural deficits. The exact mechanisms behind how concussion may promote neurodegeneration in some individuals remains poorly understood, and it is likely that there may be external factors which increase the risk of developing the disease. One such factor maybe activation of the innate immune receptor, toll-like receptor 4 (TLR4), by augmenting the inflammatory response and promoting neuronal injury. To investigate, animals were subject to three rmTBIs spaced 5 days apart, induced using the diffuse impact acceleration model, with a 450 g weight released from 1 m. Sham animals underwent surgery, but received no injury. At 24 hrs or 5 days following the last injury rats were administered either the classical TLR4 agonist, lipopolysaccharide (LPS, 0.1 mg/kg), or an equal volume of saline. One day after receiving LPS, rats were assessed for anxiety on the open field before being perfuse-fixed and the brains examined for phosphorylated tau, axonal injury and microglial activation. Inducing TL4 activation via LPS injection, at either 1 or 5 days post-injury led to a significant increase in anxiety in rmTBI animals ($p < 0.05$), an effect that was not seen in shams. This was accompanied by a significant increase in phosphorylation of tau in the cortex under the

impact site ($p < 0.05$) in LPS treated rmTBI animals, with a small non-significant increase also seen in LPS treated shams. Interestingly only the animals treated with LPS at 1 day after injury showed increased microglial activation and axonal injury, suggesting that timing of TL4 activation is important. We conclude that TLR4 activation following rmTBI affects the acute neurological response to the injury, which is dependent on the timing of activation and could potentially play a role in the development of CTE.

MTU11-10

Beyond the REDOX imbalance: contributes to an oxidative stress modulation GLUT3 impaired in huntington's disease

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A failure in energy metabolism and oxidative damage are associated with Huntington's disease (HD). Ascorbic acid released during synaptic activity inhibits use of neuronal glucose, favouring lactate uptake to sustain brain activity. Here, we observed a decreased expression of GLUT3 in STHdhQ111 cells (HD cells) and R6/2 mice (HD mice). Localisation of GLUT3 was decreased at the plasma membrane in HD cells affecting the modulation of glucose uptake by ascorbic acid. An ascorbic acid analogue without antioxidant activity was able to inhibit glucose uptake in HD cells. The impaired modulation of glucose uptake by ascorbic acid was directly related to ROS levels indicating that oxidative stress sequesters the ability of ascorbic acid to modulate glucose utilisation. Therefore, in HD, a decrease in GLUT3 localisation at the plasma membrane would contributes to an altered neuronal glucose uptake during resting periods while redox imbalance should contributes to metabolic failure during synaptic activity.

MTU11-11

Glycogen accumulation induces neurodegeneration. lafora disease

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Glycogen is a branched polymer of glucose that constitutes the sole carbohydrate reserve in mammals. It is synthesized by glycogen synthase (GS), the only mammalian enzyme able to polymerize glucose. Within the brain, glycogen is found mainly in astrocytes, while most neurons do not show detectable levels under physiological conditions. However, in some diseases, glycogen is abnormally accumulated in neurons.

Lafora disease (LD) is a fatal neurodegenerative condition characterized by the accumulation of aberrant glycogen in several cell types, including neurons. LD is caused by mutations affecting two enzymes: malin and laforin. Both enzymes interact functionally to promote the degradation of GS and its activator Protein Targeting to Glycogen (PTG). The causal role of glycogen accumulation in neurodegeneration in LD remains controversial, since the malin-laforin complex may have additional functions to that of the regulation of glycogen synthesis, such as the control of autophagy; in fact, KOs of malin and laforin present autophagy impairment.

To study whether the accumulation of glycogen is primarily responsible for the neurodegeneration in LD we have generated several mouse models with altered capacity to accumulate glycogen in neurons. Our findings reveal that glycogen accumulation is indeed responsible for the neurodegeneration of the malin KO model, as well as for the impaired autophagy. These results identify the regulation of glycogen synthesis as a key target for the treatment of LD and other glycogen-associated neurodegenerative diseases.

MTU11-12

moderation of enhanced MGLUR1 mediated synaptic signalling restores motor learning in a mouse modeof human spino-cerebellar ataxia

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Cerebellar ataxias are a rare and incurable group of neurodegenerative disorders. Several ataxias, including SCA1, are inherited polyQ disorders caused by expansion of unstable CAG repeats. Using a transgenic Purkinje neuron (PN)-specific mouse model of human ataxia, SCA1 (82Q expansion in the gene for ataxin-1) that is also doxycycline repressible (82Q OFF-ON) we aim to identify specific early driver(s) of ataxia progression.

Motor performance and gait analysis revealed mild ataxia in 6 and 12 week old 82Q ON mice ($p < 0.01$, one and two-way ANOVAS) whereas 12 week old mice where 82Q expression is repressed during weeks 0–6 (82Q OFF-ON) behaved normally.

In contrast, PNs from pre-symptomatic 82Q OFF-ON mice and all ataxic 82Q ON mice exhibited abnormally long-lasting mGluR1-mediated synaptic currents ($p < 0.0001$, two-way ANOVAS), suggesting that enhanced mGluR1 signalling occurs before the onset of ataxia.

To determine the functional significance of enhanced mGluR1 function we administered a very low dose of a potent, negative allosteric modulator of mGluR1, JNJ 16259685 (0.03 mg/kg, sub cutaneous) to 6 and 12 week 82Q ON mice prior to an acute motor learning test. JNJ (but not vehicle) treatment significantly improved performance in 82Q ON mice ($p < 0.0001$, two way ANOVAS) whilst the performance of JNJ and vehicle-treated wild type mice was unaffected.

We conclude that mGluR1 is an early cellular mechanism that may mark the beginning of SCA1 neurodegeneration and may be a useful therapeutic target for treatment during the early stages of ataxia.

MTU11-13

Assessment of retinal degeneration and lipofuscin accumulation in the CLN6 mouse model of neuronal ceroid lipofuscinosis

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Neuronal accumulation of autofluorescent intracellular debris, called lipofuscin, in all Neuronal Ceroid Lipofuscinosis (NCL) variants leads to visual impairment, as well as mental and motor deficits. The aim of this study was to assess the development of ocular pathology in a mouse model of late infantile NCL, variant CLN6^{nclf}. CLN6^{nclf} and C57BL6/J mice were investigated at 2, 4 and 8 months of age. Retinal function was studied through electroretinography. Ocular morphology was assessed by retinal fundus pictures and histology. Interactions of microglia and the spatial location of lipofuscin was investigated by immunohistochemistry and the detection of autofluorescence respectively. Gliosis was determined by labelling of Müller cells for glial fibrillary acidic protein (GFAP). Retinal fundus photography showed the presence of abnormal ocular lesions in CLN6^{nclf} mice from 2 months of age. Retinal function in CLN6^{nclf} mice decreased in a time dependant manner with both photoreceptor and inner retinal neuronal deficits apparent from 2 months. Histological evaluation of the retina showed autofluorescent lipofuscin accumulation within the retinal pigment epithelium (RPE) and retinal neurons. By 8 months there was a significant reduction of the photoreceptor layers while inner retinal structure remained intact. At this time, Müller glial cells showed increased gliosis and microglia were activated, especially in the outer retinal layers. Early change in photoreceptor function prior to loss of photoreceptor neurons, in conjunction with accumulation of lipofuscin within the RPE in CLN6^{nclf} mice suggests impairment of the RPE may lead to subsequent visual dysfunction in this mouse model. Showing various age related macular degeneration (AMD) like features, the CLN6^{nclf} mouse model, holds promise as a fast progressing model of this ocular disease.

MTU11-14

Cumulative functional copper deficiency in spinal cords of amyotrophic lateral sclerosis (ALS) model mice and sporadic ALS cases

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Objectives: We recently reported that increasing copper bioavailability both therapeutically and genetically acted to protect spinal cord motor neurons, whilst concomitantly improving locomotor function and survival of ALS model mice. These outcomes indicate that copper deficiency contributes to the pathogenesis of ALS. For the present study we directly assessed the extent of copper deficiency in ALS.

Methods: We assessed levels and copper-dependent activity of the anti-oxidant SOD1, the ferroxidase ceruloplasmin and mitochondrial cytochrome c oxidase in the spinal cords of SOD1G37R ALS model mice from pre-symptom through to late symptom stages of disease progression. We also assessed post-mortem spinal cord samples from human cases of sporadic ALS. For mouse tissue analyses, non-transgenic littermate and wild-type SOD1 overexpressing mouse spinal cords, and non-disease affected livers were used as controls. Spinal cords from healthy controls were included in the human tissue analyses.

Results: All analyses revealed a strong disparity between protein levels measured and their copper-dependent activity, consistent with a broad functional copper deficiency in ALS. This disparity was evident at an early age in the ALS model mice and was cumulative as disease progressed.

Conclusions: These results indicate the cause of ALS phenotype in mutant SOD1 mice is not restricted to mutant SOD1 toxicity and may be driven, at least in part, by broader consequences of functional copper deficiency. The fact that the human ALS tissue we examined included only sporadic cases of the disease indicates the role of functional copper deficiency in ALS extends beyond mutant ALS cases.

MTU11-15

A pure compound from moringa oleifera: a possible antidote to vanadium neurotoxicity

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Moringa oleifera is widely distributed throughout Africa and Asia, forms part of the diet and has high level of antioxidative property. Vanadium is a neurotoxic metal of the transition series, and has been shown to cause astrogliosis, lipid peroxidation and demyelination in various regions of the brain. The nervous system has a high level of lipid content, low intrinsic anti-oxidative abilities which coupled with the high rate of metabolic activity makes the brain susceptible to oxidative stress. Purification of the methanolic extract of *Moringa oleifera* leaf was done by liquid-liquid fractionation, gravity column and High Performance Liquid Chromatography. Nuclear Magnetic Resonance was used to elucidate structure and antioxidant potential was assessed using Ferric reducing Antioxidant Potential. The effect of the pure compound (MIMO2) was evaluated in cell culture using HT22 cells. The effect of MIMO2 (0.5 and 0.25 μM) and vanadium 100 μM was assessed in the cells using Dihydroethidium (DHE) staining (oxidative stress assay), and COMET assay (to assess DNA damage). The effect of the crude extract was also assessed. Vanadium administration caused an increase in oxidative stress which was ameliorated by MIMO2 by up to 40% when assessed with DHE. Assessment of DNA damage using the COMET assay resulted in about 33% amelioration with the administration of MIMO2, while the crude extract 0.2 mg/ml gave a protection of about 25%. DHE staining revealed a pronounced loss of dendritic processes of cells with the administration of vanadium. This was considerably lessened with the administration of MIMO2, but less with the administration of the crude extract. MIMO 2 appeared to give a better protection against vanadium toxicity relative to the crude extract.

MTU11-16

Identification of proteins interacting with amyloid precursor protein for neurotoxicity and neuroprotection

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The amyloid precursor protein (APP) is a type I transmembrane molecule that is proteolytic processed into a range of fragments including the neurotoxic amyloid beta (Aβ) peptide associated with Alzheimer's disease and the neuroprotective soluble APPα (sAPPα) species. We hypothesise that APP and its processed fragments would interact with specific ligands to carry out its functional activities. Identifying these APP ligands is important for determining the molecular basis for Aβ toxicity and sAPP neuroprotection. To achieve this aim we have employed affinity capture using the biotin:streptavidin system. Aβ ligands: we wanted to test the binding profiles of different **Aβ oligomeric** species since they have different neurotoxic activities. The trimer and tetramer species are significantly more toxic than the monomer and dimer forms (Jana, Cappai, Ciccotosto unpublished). We used purified biotinlyalted Aβ40 monomer, dimer, trimer and tetramer species bound to streptavidin agarose resin and this was incubated with mouse brain lysates. Bound proteins were identified by mass spectrometry:database interrogation. **sAPP ligands:** we used a biotinylated peptide encoding APP residues 96–110. This peptide confers neuroprotection *in vivo* against traumatic brain injury (Corrigan *et al*. J Neurochem 2014, 128: 196–204). We wanted to compare the binding profile APP96-110 against APP96-110mutant which is an inactive mutant. The biotinylated peptides bound to streptavidin agarose resin were incubated with mouse brain lysates and bound proteins identified by mass spectrometry:database interrogation. Results: A number of specific proteins have been identified from both the Aβ and sAPP binding experiments. Their status as bona fide Aβ or APP96-110 binding ligands is being confirmed by immunoprecipitation. Confirmed binders will be tested in cell based experiments by knocking down (siRNA) or overexpression to confirm their respective role in either Aβ toxicity or sAPP mediated neuroprotection.

MTU11-17

Single chain recombinant antibodies to target TDP-43 interactions

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Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disorder characterized by the progressive degeneration of motor neurons. A landmark was the discovery in 2006 of Transactivation response DNA-binding protein of 43 kDa (TDP-43) as a major component of ubiquitinated inclusions found in most ALS cases. TDP-43 is predominantly a nuclear protein that can bind DNA and RNA, and it has been implicated in regulating mRNA splicing, stability as well as gene transcription. The pathogenic pathways of TDP-43 are not well understood. We discovered that TDP-43 is a co-activator the p65 subunit of nuclear factor kappa B (NF-kB), a key regulator of genes involved in innate immunity, cell survival and inflammation. We obtained evidence that TDP-43 interacts with p65 through its RNA recognition motif 1 (RRM1) domain.

We propose to develop a therapy based on delivery of single chain (scFv) antibodies that can target specifically the TDP-43 RRM1 domain with the dual action to block the TDP-43 interaction with p65 NF-κB and to attenuate formation of TDP-43 aggregates. So, we have derived monoclonal antibodies binding specifically the RRM1 domain of TDP-43. From the mRNA of hybridoma, we have derived vectors expressing recombinant scFv antibodies that include variable IgG heavy and light chains joined by a linker, a human myc epitope and a nuclear localization signal. The scFv antibodies are able to block the interaction between TDP-43 and NF-kB p65 as determined by ELISA and immunoprecipitation assays. When expressed in cultured cells, the scFv antibodies were detected in the nucleus and cytoplasmic fractions. The scFv antibodies were able to reduce the levels of TDP-43 without causing cellular toxicity. Preliminary experiments based on transfection into BV2 microglial cells provided evidence that vectors encoding scFv antibodies against TDP-43 RRM1 can attenuate the activity of NF-kB-luciferase reporter. These results support the feasibility to use these scFv antibodies for blocking the TDP-43 interaction with p65 NF-kB.

MTU11-18

Degradation of inhibitory chondroitin sulphate proteoglycans improves graft integration in Parkinsonian mice

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The lack of guidance cues together with the presence of inhibitory molecules within the adult central nervous system creates an environment that is restrictive to axonal growth following injury. Consequently, whilst clinical trials in Parkinson's disease (PD) patients have demonstrated the capacity of fetally derived dopamine neurons to survive, integrate and alleviate symptoms, the full potential of these grafts have been hindered by the non-permissive host environment resulting in incomplete reinnervation of the striatum and failure to restore basal ganglia circuitry. One such inhibitory molecule is the chondroitin sulphate proteoglycan (CSPG), a protein that has been shown to impede axonal growth both during development and after injury. Digestion of CSPG's, by delivery of the bacterial enzyme chondroitinase ABC (ChABC), can improve axonal regrowth following a number of neural injuries. Here we examined whether ChABC could similarly improve axonal growth of transplanted dopamine neurons in an animal model of PD. Acute delivery of ChABC, into the medial forebrain bundle, degraded CSPG's along the nigrostriatal pathway. Dissociated ventral mesencephalon from embryonic mice was transplanted into the substantia nigra with animals also receiving a single dose of either ChABC or Penicillinase (control) at the time of transplantation. ChABC treatment had no effect on graft survival but resulted in enhanced axonal growth along the nigrostriatal pathway and reinnervation of the target striatum, compared to control mice. This study demonstrates that removal of axonal growth inhibitory molecules could significantly enhance a grafts ability to form

long-distance connections and induce improved functional outcomes.

MTU11-19

New insights in quantitative analysis of phosphorylation of tau in ad model mouse and tauopathy brains by PHOS-tag SDS-page

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Aggregates of hyperphosphorylated Tau are commonly found in brains of tauopathies such as Alzheimer disease (AD), frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17), corticobasal degeneration (CBD), progressive supranuclear palsy (PSP) and Parkinson's disease. Therefore, understanding of the pathological environment that induces hyperphosphorylation of Tau is important. Most previous studies have employed phospho-specific antibodies to detect Tau phosphorylation, which, while useful, have weakness in quantitative and combinatorial phosphorylation analysis of multiple sites. Here, we applied Phos-tag SDS-PAGE to characterization of Tau phosphorylation in AD or FTDP-17 model mice. P301L mutant Tau in JNPL3 mice was hyperphosphorylated only in Sarkosyl-insoluble aggregates. Phosphorylation levels of Tau in 5xFAD mice were similar to those of wild type mice. We also analysed phosphorylation of Tau in AD and CBD patients. AD at Braak stage V had a slightly higher phosphorylated Tau, whereas AD at Braak stage VI showed hyperphosphorylation states of Tau. Unexpectedly, there were relatively large amounts of unphosphorylated Tau in normal human brains. Further, Sarkosyl-soluble Tau was not hyperphosphorylated in AD and CBD patient's brains, whereas Sarkosyl-insoluble Tau was highly phosphorylated. The phosphorylation profiles of AD and CBD patients was different a little. These results suggest that the phosphorylation states of Tau in brains are not so high and may be hyperphophorylated after when it is incorporated into aggregates.

MTU11-20

Peptidome and proteome analysis of cerebrospinal fluid and serum in guillain-barre syndrome

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Guillain-Barre syndrome is an acute inflammatory polyneuropathy PNS disorder, believed to be autoimmune-associated, though very unusual for that being monophasic, self-restraining and practically never relapsing. The axonal GBS *form is associated with anti-glycoside autoantibodies production* triggered by *Campylobacter jejuni infection through molecular mimicry mechanism.* The pathogenesis of the major GBS form - an acute inflammatory demyelinating polyneuropathy (AIDP) associated with completely reversible peripheral nerve demyelination is different and completely unapprehended. Our earlier experiments showed that the biochemical diagnostic symptom of AIDS – the increase in protein concentration in cerebrospinal fluid – was associated among others with increase in antibodies and their fragments and slight upregulation of other immune-associated GeneOnthology groups indicating immune-response activation. However, overall no protein cluster showed dramatic increase leading to assumption of semi-specific blood protein leakage into CSF due to the damage of haemato-encephalitic barrier. This, however, was contradicted by CSF and blood peptidome comparison. We found just several hundreds of unique endogenous blood peptides in GBS serum, which is believed to result from high blood exopeptidase activity multiplied by kidney and liver filtration. In SGB CSF we observed more than 2500 unique peptides from about 700 proteins, both of them very different from the ones in blood. Intriguingly, in SGB CSF we observed increase in endogenous peptides of CAM proteins, which bind together myelin cover and axon membrane. Anti-CAM auto-antibodies were earlier shown in AIDS, supposedly as a result of molecular mimicry with unknown pathology similar to axonal GBS principle. However, finding these peptides in CSF could only be attributed to their generation locally, while no CNS damage was reported for GBS. Thus, peptidome data clearly indicate cell-destructive processes in CNS, which might also account for protein concentration increase. To further investigate the subject we are now analyzing CSF and blood for antibodies against reported as possibly associated with AIDP diseases as well as CAM proteins in order to find cross-correlation between proteomic, peptidomic and immunological data.

MTU11-21

Restoration of biochemical, behavioral and ultrastructural changes in aging rat brain: neuroprotective role of 17β -estradiol

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Objective: Aging in females and males is considered as the end of natural protection against age related diseases like osteoporosis, coronary heart disease, diabetes, Alzheimer's disease and Parkinson's disease. These changes increase during menopausal condition in females when the level of estradiol is decreased. The objective of this study was to investigate neuroprotective potential of 17β estradiol (E2) treatment on the activities of acetylcholinesterase and monoamine oxidase, membrane fluidity, neurolipofuscin, glucose transporter-3 (GLUT3) expression and testing learning memory, occurring in brains of female rats of 3 months (young), 12 months (adult) and 24 months (old) age groups, and to see whether these changes are restored to normal levels after exogenous administration of 17β estradiol (E2).

Methods: The aged rats (12 and 24 months old) (n = 8 for each group) were given subcutaneous injection of 17β estradiol (0.1 μg/g body weight) daily for 1 month. Learning was tested in a Morris water maze with expression of synaptic molecules synaptophysin and synapsin I and ultrastructural studies of brain region by MRI.

Results: The results obtained in the present work revealed that normal aging was associated with significant increases in the activity of monoamine oxidase and neurolipofuscin accumulation in aging rats, and a decrease in acetylcholinesterase activity, membrane

polarization and GLUT3 expression. E2 treatments improved attention and memory functions of the aging rats with enhanced the levels of synaptic molecules synaptophysin and synapsin I. Ultrastructural studies revealed that the changes were more pronounced in the aged treated rats in terms of presence of lipofuscin and lysosomal degradation. Our data showed that exogenous administration of E2 brought these changes to near normalcy in aging female rats.

Conclusions: It can therefore be concluded that E2's beneficial effects seemed to arise from its, antioxidant and antilipidperoxidative effects, implying a therapeutic potential drug for age related changes.

MTU11-22

Human prion diseases in brazil

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Global surveillance of CJD and its subtypes was recommended by WHO for a better understanding of the iatrogenic CJD causes as well as the distribution of hereditary CJD forms. Prion diseases have been under compulsory notification in Brazil since 2005. So far, we have received 434 blood samples from notified cases of suspected CJD. They were analyzed by direct genomic sequencing to identify mutations and polymorphisms in the *PRNP* gene. The average age of our patients was 60.3 years (10–94 year), males representing 52% of the cases. PRNP polymorphisms analysis at codon 129 showed that 51% of cases were homozygous for methionine, 30% were heterozygous and 19% were homozygous for valine. Regarding genetic diseases, we found fifteen patients with CJD, in which the mutation E200K (nine cases), D178N (two cases), T183A (one case), V180I (one case) and octarepeat insertion (two cases) were detected. We also diagnosed two patients with GSS syndrome (P102L) and three patients with fatal familial insomnia (129M+178N). After clinical evaluation, imaging exams, the presence of 14.3.3 protein, genetic and immunohistochemistry analysis, the notified cases were classified according to the WHO criteria. Among of them, 8.3% were classified as sporadic CJD, 39% as probable CJD, 18% as possible CJD, 4.6% as genetic prion disease, 23.6% as suspected CJD and 6.5% were non-CJD. This study provides the first epidemiologic data about human prion diseases in Brazil. Similar to any other country the availability of brain tissue from these patients is a limiting factor to confirm the diagnosis of prion diseases. In this way, the present work represents an important tool for prion-prevention policies and shows great importance for future implementation of clinical trials.

MTU11-23

Modulatory effects of tumor necrosis factor α on neurotrophins in myelinating spinal cord and dorsal root ganglion cultures

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Neuropathic pain, caused by a lesion of the nervous system, is often reported as having a continuous burning character along with the appearance of abnormal sensory signs, such as allodynia or hyperalgesia. It is induced by various exogenous and endogenous molecules e.g. pro-inflammatory cytokine [Tumor Necrosis Factor-alpha, (TNF-α)] and Neurotrophins [Nerve Growth Factor, (NGF) - Brain Derived Neurotrophic Factor, (BDNF)]. NGF acts as a pathogenic pain mediator, its levels are elevated in several painful conditions. BDNF shows similar hyperalgesic effects to NGF and is involved in the central sensitization and synaptic plasticity in the spinal cord. TNF-α alters many genes during inflammation and other disease conditions. However, molecular and cellular mechanisms involving cytokines and neurotrophin cross-talk underlying neuropathic pain (NPP) is still unclear. We hypothesize that in case of neuropathic pain condition, TNF-α initiates the pain and modulates neurotrophins (NGF and BDNF) level which regulates the pain condition. We monitored the transcript as well as protein levels of neurotrophins and its receptors in myelinating spinal cord culture and dorsal root ganglion neuron culture after exogenous TNF-α exposure using RT-PCR, Western blot and Immunocytochemistry. Our results demonstrate involvement of TNF-α in the regulation of neurotrophic factors (BDNF and NGF) in these culture models. Outcome of this study will help in further understanding the cross talk mechanism of cytokine and neurotrophins in neuropathic pain development which might be useful in addressing the therapeutic window for NPP treatment.

MTU11-24

Nogo receptor 1 (NGR1) deletion in axons halts axonopathy and demyelination during experimental autoimmune encephalomyelitis (EAE)

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Multiple Sclerosis (MS) is a neurological disorder that is associated with inflammatory demyelination and axonal damage in the central nervous system. Recently, we identified NgR1-dependent phosphorylation of the collapsin response mediator protein 2 (pCRMP-2) as a molecular mechanism during axonal degeneration during neuroinflammation. However, while NgR1-dependent signalling appears to be prominent in facilitating axonal damage, it is yet to be determined whether this pathogenic event occurs as a consequence of primary degeneration or, subsequent to demyelination. Here, we show that transduction of retinal ganglion cells (RGCs) with an AAV2 encoding improved Cre (AAV2-iCre-eGFP)

limits optic nerve axonal degeneration in $ngr1^{flx/flx}$ mice during the peak stage of EAE, when compared to transduction with control virus (AAV2-eGFP). Conversely, transduction of RGCs with an AAV2 encoding full-length mouse NgR1 (AAV2-mNgR1-eGFP) potentiates optic nerve axonal degeneration in $ngr1^{-/-}$ mice when compared to AAV2-eGFP injected nerves. In these optic nerves, pCRMP-2 is localised exclusively in degenerative axons, a finding also evident within the plaque core of human progressive MS white matter lesions. As a corollary, we found that there was consequential preservation of myelin integrity in the optic nerves of AAV2-iCre-eGFP injected $ngr1^{flx/flx}$, whereas significant demyelination occured in the optic nerves of AAV2-mNgR1-eGFP injected $ngr1^{-/-}$ mice. Our data further suggest that MS pathophysiology may indeed be propagated through NgR1-dependent pCRMP-2, thereby impeding CRMP-2 physiological function, through kinesin-1 facilitation of anterograde axonal transport. CRMP-2 bound kinesin-1 motor protein levels are decreased in MS brain tissue, which may be a predictor of subsequent axonal degeneration. As such, we conclude that pCRMP-2-dependent axonal degeneration may be a direct result of NgR1 signalling during EAE progression leading to disrupted axonal transport of physiological cargo from the cell body.

MTU11-25

Estimating spatial sensitivity profiles for electrical stimulation of retinal ganglion cells

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The bionic eye aims to restore lost vision resulting from photoreceptor loss by stimulating surviving retinal ganglion cells (RGCs) via an array of microelectrodes implanted within the eye. The desire to provide patterned vision and the increasing number of electrodes in new devices necessitates stimulation using multiple electrodes simultaneously. However, simultaneous stimulation poses a number of problems due to cross-talk between electrodes and uncertainty regarding the resulting activation pattern. Here we present a model and methods for estimating the responses of RGCs to patterned electrical stimulation. Whole cell *in vitro* patch clamp recordings were obtained from 20 RGCs with various morphological types in rat retina. The retina was placed onto an array of 20 stimulating electrodes. Biphasic current pulses with 500 us phase duration and 50 us interphase gap were applied to each electrode at a frequency of 10 Hz, with the amplitude of current on each electrode sampled from a Gaussian distribution. Spike triggered covariance (STC) analyses on 80% of the response data were used to identify the parameters of a linear-nonlinear model. The remaining data were used to validate the model. STC analysis revealed the spatial sensitivity profile for electrical stimulation for each cell. The average model prediction root-mean-square error was 7% when compared to validation data. The linear spatial filter of our model reveals the combination of electrodes that resulted in the highest activation sensitivity for each recorded RGC. The accuracy of the model indicates that the linear-nonlinear model is appropriate to describe the responses of RGCs to electrical stimulation.

MTU11-26

Cypermethrin induces early-onset neurodegeneration and cognitive impairment in rats: upregulated levels of oxidative stress, GSK3

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Introduction: Cypermethrin, a widely used synthetic pyrethroid, induces neurotoxicity during development. However, whether cypermethrin causes neurodegeneration at an early age remains uninvestigated. We hypothesized the role of cypermethrin to promote neurodegeneration which ultimately leads to cognitive impairements by inducing oxidative stress and Glycogen synthase kinase-3 beta (GSK-3β).

Methods: We treated 24-day old rats with cypermethrin (25 mg/Kg) for 3 weeks, and examined oxidative stress parameters using enzymatic-assay and modulation in different protein levels by western blotting and immunohistochemistry in frontal cortex and hippocampus.

Results: We found that the treatment of cypetrmethrin enhanced the oxidative stress levels in the rat brain marked by increased lipid peroxidation and nitrite levels which induced the astroglial activation as evident from enhanced GFAP levels. This further leads to the up-regulation of the kinase protein GSK-3β. We further probed for any involvement of this kinase in regulating cypermethrin mediated neurodegeneration. Investigating the mechanism of action revealed a GSK-3β mediated enhanced phosphorylation of tau protein to form neurofibrillary tangles. Tangle formation served as the causative factor of neuronal death which leads to learning–memory deficits.

Discussion: Therefore, Cypermethrin evokes early-onset taupathy culminating into cognitive impairements, where upregulated oxidative stress, astroglial activation and gsk3 beta play critical roles.

MTU11-27

Effect of retinoic acid on telomerase activity in SH-SY5Y cell

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Although telomerase is best known for its function of maintaining telomeres in dividing cells, non-telomeric functions of the protein part TERT emerged recently. Recent studies have also recently shown that TERT protein is expressed in neurons. We therefore aim to investigate telomerase activity in differentiate SH-SY5Y cells seeded on laminin (10 μg/ml) and collagen (10 μg/ml) plated 12well plate using 10 μM Retinoic acid (RA) and 10 nM Tetradecanoylphorrbol-13-acetate (TPA). To determine the telomerase activity, the cells were harvested and cell counts were done

using hemocytometer. 100 µl pre-cooled lysis reagent was added and incubated on ice for 30 min and centrifuged at 15,000 × g for 20 min at 4°C. Supernatant was collected, protein concentrations determined, and various concentrations of cell extract was used for the telomerase assay using the Telomerase PCR ELISA procedure. The absorbance of the samples was measured at 450 nm using an ELISA microtiter plate reader. Our results showed that SH-SY5Y cells seeded on collagen coated plated were better differentiated than those on laminin coated plate. RA was far more potent in differentiating SH-SY5Y cells into mature neurons than TPA or a combination of RA+TPA. Ki67 staining showed no sign of proliferation in RA treated group after 10 days unlike the other groups where there was 11.8%, 8.2%, and 15.1% proliferation for TPA, RA+TPA, and serum free medium respectively. β-III tubulin staining also supported this observation as all undifferentiated cells were all differentiated into mature neurons with the presence of β-III tubulin in the RA treated group unlike the other group unlike the other groups treated with TPA, RA+TPA, or just medium. Telomerase activity was also reduced in RA differentiated SH-SY5Y cells when compared to the other groups treated with TPA, RA+TPA, or medium. Our observations therefore show that SH-SY5Y cells are a perfect model for normal human nerve cells that express telomerase activity.

MTU11-28

Kolaviron was protective against NAN3 induced oxidative stress in the prefrontal cortex

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In this study we have evaluated the neuroprotective and regenerative properties of kolaviron (phytochemical isolated from *Garcina kola*) in neurons of the prefrontal (Pfc) before or after exposure to sodium azide (NaN_3) induced oxidative stress. Separate groups of animals were treated as follows; kolaviron (200 mg/Kg) for 21 days; kolaviron (200 mg/Kg for 21 days) followed by NaN_3 treatment (20 mg/Kg for 5 days); NaN_3 treatment (20 mg/Kg for 5 days) followed by kolaviron (200 mg/Kg for 21 days); 1 ml of corn-oil (21 days-kv vehicle); NaN_3 treatment (20 mg/Kg for 5 days). Exploratory activity associated with Pfc function was assessed in the open field test (OFT) following which the microscopic anatomy of the prefrontal cortex was examined in histology (Haematoxylin and Eosin) and antigen retrieval Immunohistochemistry to show astroglia activation (GFAP), neuronal metabolism (NSE), cytoskeleton (NF) and cell cycle dysregulation (p53). Subsequently, we quantified the level of Glucose-6-phosphate dehydrogenase (G6PDH) and lactate dehydrogenase (LDH) in the brain tissue homogenate as a measure of stress-related glucose metabolism. Kolaviron (Kv) and Kolaviron/NaN_3 treatment caused no prominent change in astroglia density and size while NaN_3 and NaN_3/Kv induced astroglia activation and scar formation (astrogliosis) in the Pfc when compared with the control. Similarly, Kolaviron and Kv/NaN_3 did not alter NSE expression (glucose metabolism) while NaN_3 and NaN_3/Kv treatment increased cortical NSE expression; thus indicating stress related metabolism. Further studies on enzymes of glucose metabolism (G6PDH and LDH) show that NaN_3 increased LDH while kolaviron reduced LDH in the brain tissue homogenate. In addition kolaviron treatment before *($P < 0.001$)* or after ($p < 0.05$) NaN_3 treatment also reduced LDH expression; thus supporting its role in suppression of oxidative stress. Interestingly, NF deposition increased in the Pfc after kolaviron treatment while Kv/NaN_3 showed no significant change in NF when compared with the control. In furtherance, NaN_3 and NaN_3/Kv caused a decrease in NF deposition (degeneration). Ultimately, the protective effect of KV administered prior to NaN_3 treatment was confirmed through p53 expression; which was similar to the control. However, NaN_3 and NaN_3/Kv caused an increase in p53 expression in the Pfc neurons (cell cycle dysregulation). We conclude that kolaviron is not neurotoxic at 200 mg/Kg BW. Furthermore, 200 mg/Kg of kolaviron administered prior to NaN_3 treatment (Kv/NaN_3) was neuroprotective when compared with Kolaviron administered after NaN_3 treatment (NaN_3/Kv).

MTU11-29

Consecutive mapping of glucose and copper metabolism in mouse brain with F-18 FDG and 64CUCL2-PET/CT

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Copper is a nutritional trace element required for development and function of mammalian brain. Positron emission tomography (PET) is a versatile tool for mapping of cerebral copper metabolism in living animals, using radioactive copper as a tracer.

Objective: of this study was to compare cerebral glucose and copper metabolism in mouse brain with consecutive PET/CT using F-18 FDG or copper-64 chloride ($^{64}CuCl_2$) as a tracer.

Methods: Balb/C mice (Female, 12 weeks old, $N = 5$) were subjected to PET/CT after injection of F-18 FDG, using a small animal PET/CT scanner. After 24 h post injection of F-18 FDG, the mice were injected with $^{64}CuCl_2$ and subjected to second PET/CT. PET/CT images of the mice were reconstructed, and F-18 FDG and ^{64}Cu radioactivity in various regions of mouse brain were quantified with PET. Cerebral ^{64}Cu radioactivity of the Balb/C mice was also compared with the cerebral ^{64}Cu radioactivity of the *Atp7b*$^{-/-}$ knockout mice (7–8 weeks old, $N = 4$), a mouse model of Wilson's disease.

Results: Distinct biodistribution of F-18 FDG and $^{64}CuCl_2$ was visualized in mouse brains, with abundant F-18 FDG in the cortex and low ^{64}Cu uptake in the cortex of mouse brains. F-18 FDG activity in the white matter of mouse brain is lower than cortical F-18 FDG activity, but ^{64}Cu radioactivity in the white matter of the mouse brain was actually not lower than, or higher than cortical ^{64}Cu radioactivity. Furthermore, ^{64}Cu radioactivity in the brains of Balb/c mice was higher than ^{64}Cu radioactivity in the brains of *Atp7b*$^{-/-}$ knockout mice, likely secondary to hepatic sequestration of ^{64}Cu caused by Atp7b gene mutation.

Conclusion: PET is a useful tool for mapping of cerebral copper metabolism in mice, and potentially in humans. This new $^{64}CuCl_2$-PET/CT technology may be useful for delineation of the role of copper ions in physiology of human brain and pathophysiology of neurodegenerative disorders, a frontier in metalloneurochemistry.

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MTU11-30

Survival motor neuron protein upregulation slows disease progression in a mouse model of amyotrophic lateral sclerosis

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Loss of survival motor neuron (SMN) protein causes motor neuron degeneration in childhood disorder spinal muscular atrophy. We recently demonstrated that SMN loss occurs in motor neurons of amyotrophic lateral sclerosis (ALS) patients and superoxide dismutase 1 (SOD1) ALS mouse model, suggesting a role for SMN depletion in ALS pathogenesis. Furthermore, SMN overexpression improved locomotor function and motor neuron survival in SOD1 mice[1]. We therefore examined the time course of SMN expression and effect of SMN overexpression in a second mutant TDP-43^{A315T} mouse model of ALS. SMN expression was analysed in pre-symptomatic and symptomatic TDP-43^{A315T} mice and wild-types using Western blotting and immunohistochemistry. TDP-43^{A315T} mice were crossed with transgenic PrP-SMN mice overexpressing SMN. Double transgenic TDP-43^{A315T};PrP-SMN mice and control genotypes TDP-43^{A315T} and PrP-SMN were examined for weight loss, motor function and survival. Spinal cords were analysed for motor neuron counts and glial cell activation. SMN protein levels were increased by 1.5-fold and 2-fold, respectively, in spinal cords of pre-symptomatic and symptomatic TDP-43^{A315T} mice. There was a corresponding accumulation of nuclear and cytoplasmic SMN complexes in spinal motor neurons of TDP-43^{A315T} mice. SMN overexpression significantly increased survival of female TDP-43^{A315T} mice by 2 months, compared to control TDP-43^{A315T} mice. This was associated with significant rescue of motor neurons and attenuation of astrocyte and microglia activation in spinal cords. We conclude that SMN upregulation slows neurodegeneration, neuroinflammation and disease progression in the TDP-43^{A315T} mouse model of ALS. This, together with results from SOD1 model, paves the way for future therapeutic testing of SMN enhancing agents in ALS.

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MTU11-31

MMP-9 action and dendritic spines remodeling during post-traumatic epileptogenesis

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Epilepsies are a group of disorders characterized by the appearance of spontaneous recurring seizures. In 20% of patients, epilepsy develops as an effect of traumatic brain injury (TBI) which triggers long-lasting cascade of events called epileptogenesis. Development changes in organization of neural circuits, may lead to imbalance between excitatory and inhibitory neurotransmission and increased risk for seizures. Recent evidences indicate important role of extracellular matrix metalloproteinase-9 (MMP-9) in neuronal circuitry remodeling and synaptic plasticity. Moreover post-traumatic aberrant plasticity includes changes in spine morphology and density. Our previous data indicate progressive cortex (Cx) degeneration, structural changes in the hippocampus (Hp) and elevated level of MMP-9 activity in ipsilateral Cx and Hp after TBI (14 days post-injury). The aim of the present study was to evaluate the spine density and dendritic spines morphology alterations after TBI. For this we used controlled cortical impact (CCI) as an animal model of TBI. 7 and 14 days post-CCI, mouse brain sections were fixed and stained using lipophilic dye – DiI. High resolution pictures were analyzed with SpineMagick! Software. Spine density was significantly decreased both 7 and 14 days post-injury in ipsilateral Cx and CA1 field of Hp. Interestingly, in ipsilateral dentate gyrus (DG) of CCI animals the number of spines peaked. As massive neurodegeneration is also observed in lesion area, such spine loss may further exacerbate cortical network stability. Dendritic spine shape remains in a strong relation with its function. Previous reports indicate MMP-9 involvement in remodeling of dendritic spine shape *in vitro*/*vivo*. As levels of MMP-9 are altered in brain after trauma, we aimed also at characterizing variations in dendritic spines shape. Length to width ratio of spines in ipsilateral Cx, CA1 and DG was decreased indicating spine shortening. Moreover we observed increase of spine head width in ipsilateral CA1 and DG. Our study indicates that MMP-9 action might be important for major dendritic spines reshaping observed after brain injury. In consequence these aberrations may lead to altered sensibility of neuronal circuitries and in consequence triggering of seizures.

MTU11-32

A LA-ICPMS time course analysis of changes in cerebral metals following a controlled cortical impact

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Traumatic brain injury (TBI) is complicated by the prominent involvement of the biological transition metals Iron (Fe), Copper (Cu) and Zinc (Zn). Non-heme bound Fe has been demonstrated to be abnormally elevated immediately after TBI, and the toxic liberation of zinc after brain injury has also been confirmed in many studies. Similarly, Cu has been shown to be deficient following brain trauma. Taken together, these alterations in brain metal levels are likely to contribute to the hallmark pathologies that occur within the brain post-injury (such as cell death and diffuse axonal injury) and also to both the acute and chronic neuropsychological sequelae (such as depression, anxiety and cognitive loss) that are prominent in brain injured patients. To further investigate the spatial and temporal metal dyshomeostasis that occurs following brain trauma, we have surveyed Fe, Zn and Cu levels using laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) in mice following a controlled cortical impact TBI. The ipsilateral hemisphere was compared to contralateral hemisphere and regional areas radiating towards the center of the brain from lesion site were analyzed. Significant regional and time point specific elevations of Fe, Zn and Cu were detected immediately and up to 28 days after TBI. These data point to the need for a multi-modal approach to maintaining metal ion homeostasis across the time course of injury in order to ameliorate the associated behavioural deficits that are likely mediated by these alterations in metal levels.

MTU11-33

effect of melatonin on the alteration of clock gene and melatonin receptor expression in hippocampus of the aging rats

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Melatonin (MEL), a neuroendocrine signal of darkness and an antioxidant, acts as an endogenous circadian time cue. In addition, there are claims that MEL has anti-aging properties. Aging has potent effects on many circadian rhythms accompanied by impairment in memory processing. Interestingly, the hippocampus, a brain area that plays an important role in learning and memory, can express circadian rhythm of clock gene and melatonin receptor. In this study, we investigated the effect of aging on clock genes and melatonin receptor in the rat hippocampus. In addition, the effect of MEL on the expressions has been also investigated. The expression of clock genes; *Per1*, *Bmal1* and *Rev-erbα* as well as melatonin receptor; *Mt1* and *Mt2* were studied by using real-time PCR in 3 age groups (2, 12 and 24 months) at 12 h apart (Zeitgeber time (ZT) 08 and 20). MEL was treated in drinking water during the nighttime for 2 months on age-induced desynchronization in expression of these genes. Expression of *Per1* and *Rev-erbα* mRNA showed significant higher values at ZT08 whereas *Bmal1* and both melatonin receptor showed at ZT20. In middle group, *Rev-erbα* and *Bmal1* did not change but *Per1*, *Mt1* and *Mt2* showed loss of rhythmicity. However, in old group, these entire genes showed significant different from other groups with lower expression and abolition of rhythmic expression. Furthermore, MEL administration was able to restore these effects. The present study suggests that melatonin may improve age-induced changes in the expression of clock genes and melatonin receptor in the hippocampus.

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MTU11-34

Neurodegenerative consequences of episodic metabolic stress in oligodendrocytes

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Multiple Sclerosis (MS) is a demyelinating disease of the CNS, typically with clinical manifestation in early adulthood. Historically, MS is characterized as an autoimmune disease, but evidence from new animal models and clinical trials has indicated other potential etiologies. To test a potential oligodendrocyte contribution to disease, our lab has developed the *OBiden* (*OBi*) mouse model. In this model, mice develop normally until 2 months of age, and then we induce metabolic stress in mature oligodendrocytes once per week throughout their adult life. To test the secondary neurodegenerative affects of our primary oligodendrocyte stress we have used *in vivo* MRI to identify significant 3rd ventricle enlargement in the *OBi* mice, as well as possible gray matter lesion development. We have also performed longitudinal behavioral tests and find cognitive decline that presumably arises from the combination of oligodendrocyte stress and secondary neurodegeneration. Thus, the mice exhibit significant increases in a depression-like endophenotype, which is a common symptom in MS patients. In addition, we find deficits in short-term recognition memory of our mice using a novel object test, which is another pathology consistent with cognitive decline in MS patients. We are also analyzing the molecular characteristics of gray and white matter in the *OBi* mice to define consistently affected pathways or common gene expression signatures in the degenerating CNS. Using western blot and immunocytochemistry, we find increased neurofilaments in degenerating gray matter decreases in dendritic stabilizing proteins including MAP2. Finally, we observe focal activation of microglia and astrocytes in gray and white matter, which is reminiscent of glial scarring shown in several neurodegenerative diseases. Currently, we are sequencing the transcriptome of degenerating and normal gray matter in *OBi* and *MS* patients to identify gene expression and signaling pathways that are perturbed in neurons as a result of oligodendrocyte stress.

MTU11-35

Fucoidan protects hippocampal neurons and its synaptic profile in rats subjected to transient global cerebral ischemia

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Fucoidan is a fucose rich sulphated polysaccharide widely available in sea weeds. Fucoidan was well known for its anti-inflammatory, antioxidant, anticoagulant, antithrombotic, antiviral and antitumor activity through research studies. Present study is designed to investigate the role of fucoidan as a neuroprotectant against transient global cerebral ischemia induced pyramidal cell damage in hippocampal CA1 region and learning and memory function in rats. The rats were subjected to 4 vessel occlusion (4VO – Bilateral common carotid artery and bilateral vertebral artery occlusion) for 15 min, sham rats were exposed to the same procedure except common carotid artery occlusion. Fucoidan was administered to the treatment group, at the dosage of 50 mg/kg body weight intraperitoneally 1 h after 4VO. On post operative day 7, rats were assessed for spatial and non-spatial learning and memory function through radial arm maze, novel object recognition task and their brain tissues were processed for histopathological and biochemical assessment. Fucoidan treated rats showed significant improvement in both spatial and non- spatial learning and memory task than the lesioned rats. The severe oxidative stress that the rats underwent resulted in elevated lipid and protein oxidation is indicated through high malonaldehyde and protein carbonyl levels in the lesion group than the fucoidan treated group. Significant increase in CA1 pyramidal neuron density and its synaptic density and spine count was observed in fucoidan treated rats, this may be due to fucoidan's ROS quenching, membrane protective properties. In conclusion this study suggests that fucoidan could be the safe and

economical drug of choice in addressing the issues associated with cerebral ischemia induced cognitive decline.

MTU11-36

Activity of NS-309 in pilocarpine treated chronically epileptic rat slices *in vitro*

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Introduction: Pilocarpine model of epilepsy is widely studied animal model generally used to investigate effect of any agent for its effectiveness as an anticonvulsant in context to temporal lobe epilepsy or complex partial seizure. NS-309 is a SK-channel opener and never been studied for anticonvulsant activity in chronic epilepsy paradigm. We studied NS-309 in brain slices (entorhinal cortex) from pilo-treated chronic epileptic and also slice from control animals.

Methods: Male Wistar rats (4 weeks older) were injected pilocarpine 340–350 mg/kg (i.p.) 30 min after methylsolpoamin (1 mg/kg sc.) injection. Post status epilepticus state, diazepam 10 mg/kg (i.p.) was injected to terminate the status epilepticus. Control group received saline instead of pilocarpine treatment. After 2 months of pilocarpine injection animals were video recorded to confirm occurrence of spontaneous recurrent seizure in treated animals. Only those rats were used in this study that shown spontaneous seizures. Animal were decapitated and brain slices of 400 micron thickness were cut and maintained in an interphase setup at 35 ▫ C supplied with carbogen and aCSF for at least 2 hrs before recordings. 4-AP (100 μM) was used for consistent seizure-like events (SLEs) induction. Local field potential measurements were recorded by placing glass microelectrode in medical entorhinal cortex.

Results: In slices from pilocarpine treated rats, NS-309 at 100 (n = 9) and 50 μM (n = 6) blocked 4-AP induced SLEs in all the slices. In control slices from saline treated rats NS-309 at 100 μM showed similar activity by suppressing SLEs in six out seven all the studied slices (n = 7), however at 50 μM dose it blocked SLEs in only 50% of slices (n = 6) $p < 0.05$.

Conclusion: These results from pilocarpine and control groups suggested that NS-309 possesses anticonvulsant activity not only in slices from control rats but also in chronic epileptic rats. It indicates that NS-309 might have potential action on chronic seizures. Although, owing further studies to find out side effects on other system.

MTU11-37

Stereotactic delivery of M1000 prions to the dorsal CA1 in mice induces greater fear extinction and spatial memory disturbance

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Prion diseases are a group of fatal neurodegenerative diseases with the unique features of being transmissible. Significant improvements towards understanding the spreading and propagation have been done but little still known about the neurotoxic mechanisms that lead to neuronal death and ultimately to the cognitive symptoms, such as memory loss. Neurotoxic effects have been reported *in vivo* and *in vitro* with synthetic recombinant prions but direct neurotoxicity upon early stages of infection with brain-derived prions has never been reported, giving credit to the hypothesis that neurotoxicity is depended only upon *de novo* propagation. To verify this hypothesis C57/BL6 mice were injected with M1000 prions in dorsal CA1 and assessed for memory and behavioural changes within an acute time window after the inoculation, excluding any possible substantial effect derived from endogenous prion propagation, such as sequestration of local PrP protein as substrate. For this reason the animals were all tested within 21 days after the inoculation, considered as the beginning of significant *de novo* prion propagation. Classic fear conditioning paradigm shows an acute inocula effect in the hippocampus with greater extinction rate of the tone memory, but not context, when compared to normal brain homogenate-injected controls. Spatial learning, assessed with Barnes maze test, showed a milder prion effect in the learning phase although a significant decrease in accuracy is observable during the test phase. Other less cognitively demanding hippocampal-dependent tests such as Y maze, burrowing and nesting failed in detecting further effect of prion inoculation. Together these results report for the first time that brain-derived prions have an acute effect on the hippocampus transiently disturbing the memory functions. The mechanisms of prion toxicity are still unknown but immunohistochemical analysis of the hippocampi are still in progress and it will provide insights about neurotoxic mechanisms involved in acute exposure to prion and provide new therapeutic target for more conventional prion diseases.

MTU11-38

Expansion of suppressor tregs by IL-2/IL-2 MAB complexes slows disease progression in the mutant SOD1 mouse model of ALS

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Mutations in superoxide dismutase 1 (SOD1) cause amyotrophic lateral sclerosis (ALS), a progressive disease characterised by motor neuron degeneration, muscle weakness and paralysis. While ALS targets motor neurons, other cell types including peripheral immune cells, are involved. Activation of T-regulatory cells (Tregs) may be a disease modifier of ALS and transplantation of Tregs is neuroprotective in mutant SOD1 mice. Here, we investigate the therapeutic potential of Treg expansion in mutant SOD1 mice using an interleukin-2 (IL-2)/IL-2 antibody (IL-2 mAb) complex with rapamycin (rapa).

Methods: $SOD1^{G93A}$ mice (n = 20/group) were administered IL-2/IL-2 mAb complex (1 μg/5 μg) + rapa (1 mg/kg), rapa alone or vehicle 2–3 times weekly from 60 days of age. Peripheral blood was analysed for T-cell populations using FACS. Motor function, weight loss and survival were compared between groups. Motor neuron counts and glial activation were determined using immunohistochemistry.

Results: Treatment with IL-2/IL-2 mAb + rapa significantly prolonged survival of male $SOD1^{G93A}$ mice ($p < 0.01$), compared to rapa and vehicle groups. IL-2/IL-2 mAb significantly increased CD4 + FoxP3 + Treg numbers by 6-fold in blood and 2-fold in spleens of SOD1 mice, but not CD8 + T-cells, confirming selective Treg expansion. Furthermore, these Tregs showed increased CTLA4 and GITR expression, confirming a suppressor phenotype. Lastly, there was reduced microglial activation in spinal cords of $SOD1^{G93A}$ mice treated with IL-2/IL-2 mAb + rapa.

Conclusion: We demonstrate IL-2/IL-2 mAb complexes stimulate selective expansion of suppressor Tregs in mutant SOD1 mice, leading to attenuated microgliosis and increased survival. These data suggest that expansion of endogenous neuroprotective Tregs using this approach may be an effective therapeutic strategy for ALS.

MTU11-39

Does reduced synaptic spine density in pyramidal neurons underlie symptoms of a neurodegenerative lysosomal storage disorder?

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Sanfilippo syndrome is a paediatric neurodegenerative lysosomal storage disorder which results from a deficiency in one of four lysosomal enzymes responsible for the degradation of heparan sulphate (Neufeld and Muenzer, 1989). Characterised by neurocognitive regression, with prominent behavioural disturbance, effective therapeutic intervention is lacking and death results by early adulthood. (Valstar *et al*., 2008). While its biochemical aetiology is well defined, the mechanisms subserving progressive neurological dysfunction remain poorly understood. We have used mouse models of Sanfilippo type A and type B to examine the hypothesis that neurological decline results from impaired neuronal communication, due to discrete changes in cell structure i.e. aberrant dendrite and dendritic spine morphology, rather than a gross loss of neurons, based on evidence by Wilkinson *et al*., (2012). In Golgi-Cox-stained brain sections, taken from ~20-week old mice, we observed a significant reduction in the density of dendritic spines on primary, secondary and tertiary dendrites of murine Sanfilippo type A and B cortical pyramidal neurons, with a reduction in tertiary dendrite length also present in the latter. Whilst these changes temporally correlate with onset of neurological deficit in Sanfilippo type A (Crawley *et al*., 2006), further studies are needed to determine their functional impact and whether they precede clinical disease. Furthermore, the end-mechanisms underlying these observations require interrogation and are the subject of ongoing investigations within our group.

MTU11-40

Discovery and characterization of potent biphasic α5$GABA_A$ receptor modulators

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Introduction: Therapeutic drugs targeting GABA type-A chloride channel receptors ($GABA_A$Rs) are used as anxiolytics, sedatives, and antiepileptics. These drugs are allosteric positive modulators of $GABA_A$Rs that enhance GABA activity. As these drugs non-selectively target many $GABA_A$R subtypes causing unwanted side effects, subunit-specific drugs are now desirable. Positive modulators selective for α5-containing $GABA_A$Rs (a5$GABA_A$Rs) have shown promising results in treating cognitive symptoms of schizophrenia and age-related dementia, whereas α5$GABA_A$R negative modulators have proven worth as cognition enhancers, in post-stroke recovery and prevention of general anesthetic-induced amnesia, without causing sedation and anxiety.

Aim: The aim of this project is to discover and characterize novel α5$GABA_A$R-selective compounds.

Methods: This was accomplished by screening a library of synthetic compounds on HEK293 cells, transfected with α5β3γ2L $GABA_A$Rs and an anion-quenchable yellow fluorescent protein (YFP), using a high-throughput fluorescence-based anion influx assay. Modulation on other $GABA_A$R subtypes (α1β2γ2L, α5β2γ2L) and drug binding sites were explored via functional studies using two-electrode voltage clamp electrophysiology (TEVC). To prepare for TEVC, *Xenopus borealis* frogs were anesthetized with MS-222 and surgically incised to obtain the oocytes, which were digested and injected with $GABA_A$R mRNAs.

Results: TEVC recordings revealed that isomeric compounds RM 68, RM 69 and RM 70 are potent $GABA_A$R modulators. These isomers, except RM 69, demonstrated biphasic modulation, selectively potentiating α5$GABA_A$Rs in the nanomolar (sometimes picomolar) range, but inhibiting $GABA_A$Rs non-selectively at higher micromolar concentrations. Removing mercaptoacetaldehyde from RM 68 to give RM 96 eliminated its α5$GABA_A$R selectivity. Flumazenil managed to neutralize the potentiation of GABA responses by the 4 compounds, suggesting that the potentiating effect of these compounds was modulated via the benzodiazepine site. RM compounds can be potential leads to design novel subtype specific drugs for α5-related disorders and to study the pharmacology of α5$GABA_A$Rs.

MTU11-41

The role of PFN1 in the pathogenesis of ALS

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Amyotrophic lateral sclerosis (ALS) motor neuron disease is uniformly fatal, usually within 5 years. Most ALS cases are

sporadic (SALS) whereas ~10% are familial (FALS). Mutations in *PFN1* (profilin 1) have recently been identified in some ALS patients however only few genetic studies confirmed that *PFN1* mutations could cause ALS. PFN1 is a nucleotide exchange factor regulating the polymerization of actin network which is involved in multiple cellular pathways. In order to support the role of PFN1 in the pathogenesis of ALS we sequenced *PFN1* in 720 French ALS patients: 120 FALS and 600 SALS. Two mutations were identified: the M114T mutation that segregated with the disease in two affected siblings in one FALS (0.8%) and the E117G variant in two SALS (0.3%) and 1/500 control (0.2%). Interestingly, neuropathological analyses performed on a patient carrying the E117G variant showed the presence of neuronal intranuclear protein inclusions, immunopositive for alpha-actin, which could result from a disruption of PFN1-mediated nuclear actin export. To study the functional impact of these mutations *in vitro* and *in vivo* we produced 3 lentiviral vectors expressing wild-type or mutant PFN1. These constructs were used to overexpress PFN1 in HEK293T cells: only PFN1 mutants induced the formation of cytoplasmic TDP43 positive inclusions. These vectors have also been used to establish new mouse models using lentiviral transgenesis technology with high efficiency: 80% of the progeny carried the transgenes. Phenotypes in these transgenic mice are currently followed using body weight, motor behavior and electromyography recordings. Quantitative analysis of motor neuron survival, motor axon diameter and neuromuscular junctions will be performed as well as aggregate formation and gliosis. Results will be correlated for each animal to the level of transgene expression, especially in motor neurons.

MTU11-42

Neurotrophin regulation of Alzheimer's disease pathology

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Alzheimer's disease (AD) is a devastating neurodegenerative disease characterized pathologically by the accumulation of amyloid-β (Aβ) protein and hyperphosphorylation of tau. These two key proteins are major contributors to neuronal toxicity and disease progression; however, the causal factors initiating this toxic cascade in sporadic disease are unknown. We hypothesize that the degeneration of basal forebrain cholinergic neurons (BFCN) and a decrease in neurotrophin availability, which occur coincidentally with the disease, are key regulators of aberrant Aβ accumulation and tau hyperphosphorylation that constitute the molecular basis of AD pathology. Here we show that loss of BFCN innervation to the hippocampus results in a decrease in brain-derived neurotrophic factor (BDNF) protein and an increase in soluble Aβ levels in the hippocampi of presymtomaptic APP/PS1 transgenic AD mice. Similarly, in presymptomatic tau transgenic pR5 mice, BFCN loss results in decreased BDNF levels and an increase in hyperphosphorylated tau epitopes. Both transgenic lines also show learning and memory deficits in a Morris water maze task only after BFCN lesioning. The present work is helping to gain a better understanding of the molecular mechanisms underpinning AD progression by resolving the relationship between BFCN denervation, decreases in neurotrophin availability and the initiation of AD pathology.

MTU11-43

Isolation of brain derived extracellular vesicles for studying pathobiology & identifying biomarkers of neurodegenerative disease

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Extracellular vesicles (EVs) are released by all cells in the nervous system in both normal and pathogenic settings. Growing evidence indicates that EVs are prominent mediators of neurodegenerative diseases such as ALS, prion, Alzheimer's and Parkinson disease. EVs have been shown to traffic neurodegenerative disease associated proteins such as the prion protein, beta-amyloid and alpha-synuclein from their cells of origin to the extracellular environment where they can be up taken by recipient cells to mediate cell-to-cell communication. In addition to contributing to disease development EVs also have potential use as a diagnostic. In order to understand the role EVs play in the neurodegenerative brain we devised a protocol (modified from Perez-Gonzalez JBC 2012) for isolating EVs secreted into the extracellular space of murine and human control and diseased brain tissue. These brain-purified vesicles were characterised as exosome-like vesicles by standard techniques to rule out cross contamination with microparticles or microsomes. Their proteomic and genomic profile was determined, in addition to functional impact on recipient cells. We have demonstrated that frozen human and mouse brain tissues yield EVs with similar characteristics to exosomes isolated from either conditioned media or bodily fluids. The proteomic and genomic profile of brain derived EVs provided insights into their function and suggests that EVs have a key role in transferring disease relevant molecules. In the last decade there has been an increasing trend of research into EVs which has expanded into neurodegenerative diseases. EVs play an important part in communication in the brain however this has been difficult to show experimentally. Here we begin to examine the contribution of EVs to neurodegenerative disease propagation and pathology by isolation direct from brain tissue and reveal the importance of EVs in the neurodegenerative brain.

MTU11-44

Functional recovery in new mouse models of ALS/FTLD after clearance of TDP-43 pathology

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Cytoplasmic TDP-43 pathology in brain and spinal cord is the pathological hallmark of both amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD-TDP). However, currently available mouse models of TDP-43 proteinopathies largely fail to recapitulate key pathological features of human disease including cytoplasmic phosphorylated TDP-43, insoluble TDP-43 accumulation, and down-regulation of endogenous nuclear TDP-43, combined with the progressive neuron loss and motor dysfunction

leading to death seen in ALS. Furthermore, it remains unknown whether or not clearance of TDP-43 pathology would be beneficial following disease development, which is relevant due to the relatively late stage of disease at which ALS and FTLD-TDP are usually diagnosed in patients. We therefore developed new mouse lines with the human *NEFH* promoter driving doxycycline (Dox)-suppressible brain and spinal cord expression of cytoplasmically-localized human TDP-43 (hTDP-43ΔNLS). These mice show phosphorylated TDP-43 pathology in motor cortex, spinal cord and hippocampus as early as 1 week after induction of hTDP-43ΔNLS expression, and develop limb tremor accompanied by hindlimb clasping at 2–3 weeks, along with a progressive decline in motor task performance. Brain and muscle atrophy begins at 4 weeks, and spinal cord motor neuron loss is detected at 6 weeks, with gradual weight loss leading to disease endstage at a median of 10.3 weeks. In order to delineate the contribution of TDP-43 pathology to disease progression, we used Dox to suppress hTDP-43ΔNLS expression after 6 weeks, when significant pathology is seen and neurodegeneration has occurred. Remarkably, TDP-43 pathology was rapidly cleared, nuclear TDP-43 returned and neuron loss was halted, allowing muscle reinnervation, motor task improvement and dramatic lifespan extension. Clearance of TDP-43 pathology is therefore beneficial even at late disease stages, indicating that targeting cytoplasmic TDP-43 is a worthy avenue for therapeutic development in ALS and FTLD-TDP.

MTU11-45

Characterizing how post translational modifications can alter the serine protease inhibition of the KPI domain in app

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The amyloid-β precursor protein (APP) is a ubiquitous type-I transmembrane protein that is the progenitor of amyloid-β peptides; a primary component of amyloid plaques found in the brains of Alzheimer's disease (AD) patients. From the 3 predominant splice isoforms of APP expressed in the brain, the larger isoforms contain a Kunitz protease inhibitor (KPI) domain (e.g. APP_{751} and APP_{770}). These larger isoforms of APP are up regulated in AD and potently inhibit serine proteases. We discovered that metal content and glycosylation status can post-translationally alter the protease inhibitory effects of APP_{751}. The KPI domain has been identified to inhibit a variety of serine proteases including trypsin, chymotrypsin, kallikrein, plasmin and human factor XIa. Using an *in vitro* fluorescence-based assay, we now also identify another serine protease matriptase-2 that is also inhibited by APP_{751}. Of all bioavailable metals tested (e.g. iron, zinc and calcium), zinc enhanced the inhibitory effects of APP on only kallikrein and human factor XIa. In contrast, deglycosylation of APP_{751} was able to decrease the effects of APP to inhibit all proteases. The novel inhibitory role of KPI-containing APP on matriptase-2 activity has implications in the regulation of cellular iron. Matriptase-2 is a type-II transmembrane protease that controls hepcidin through cleaving hemojuvelin from the cell surface. Previous evidence through genetic inactivation of matriptase-2 reveal a reduced iron loading in various mouse models. Inhibition of matriptase-2 by APP_{751} may have a similar outcome and suggests an alternative pathway in which iron homeostasis is maintained as well as how alterations in iron occur with AD.

MTU11-46

Therapeutic potential of n-acetylcysteine in huntington's disease: effects on glutamate uptake and mitochondrial function in mice

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Huntington's disease (HD) is a neurodegenerative disorder caused by a CAG-repeat expansion encoding an extended polyglutamine tract in the huntingtin protein. Oxidative stress and excitotoxicity have previously been implicated in the pathogenesis of HD. We hypothesized that *N*-acetylcysteine (NAC) may reduce both excitotoxicity and oxidative stress through its actions on glutamate uptake and antioxidant capacity. The R6/1 transgenic mouse model of HD was used to investigate the effects of NAC on HD pathology. Acute NAC administration was found to have antidepressant-like effects, whilst chronic administration delayed the onset and progression of motor deficits, as shown using three independent measures. *In vivo* microdialysis in combination with pharmacological manipulation were used to assess the effect of acute NAC on glutamate uptake. The expression of glutamate transporter proteins, GLT-1 and system xc-, were not changed by chronic NAC treatment, implying that the therapeutic effect of chronic NAC was not due to the reduction of excitotoxicity through the regulation of glutamate uptake. However, assessment of mitochondrial function in the striatum and cortex revealed that R6/1 mice show reduced mitochondrial respiratory capacity specific to the striatum. This deficit was rescued by chronic treatment with NAC. There was a selective increase in markers of oxidative damage specific to striatal mitochondria, which was also rescued by NAC. In conclusion, acute NAC administration displayed antidepressant-like effects, which are likely due to the effects on glutamate uptake, whilst chronic NAC treatment is able to delay the onset of motor deficits in the R6/1 model of Huntington's disease by ameliorating mitochondrial dysfunction. Thus, NAC shows promise as a potential therapeutic agent in HD.

MTU11-47

Tetramethylpyrazine nitrone protects retinal ganglion cells against N-methyl-D-aspartate induced injury

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Purpose: 2-[[(1,1-dimethylethyl)oxidoimino]-methyl]-3,5,6-trimethylpyrazine (TBN) is a derivative of tetramethylpyrazine armed with a nitrone moiety, with dual functions of blocking Ca^{2+} overload and scavenging free radicals. Here we investigate whether TBN

protects retinal ganglion cells (RGCs) against N-methyl-D-aspartic (NMDA) induced injury in rat and explore the underlying mechanism.

Methods: Injury of RGCs was induced by intravitreal injection of NMDA. TBN, or its solvant, or memantine (positive control) was administrated, i.p. daily for 5 days after the NMDA injection. At various time points, TBN concentration in rat aqueous humor was detected by HPLC. The survival rate of RGCs was evaluated by counting Brn3a-positive cells. Retinal functions were tested by electroretinogram (ERG) and visual behavior evaluated by black-white box. Expression level of apoptosis related proteins Bax, Bcl-2, cleaved caspase3 and caspase3 was checked by western blotting. Reactive oxygen species (ROS) levels were accessed by CellROX expression on RGCs. Changes of NMDA-induced Ca^{2+} influx was detected by calcium imaging on RGCs on whole-mount retina.

Results: TBN quickly entered the aqueous humor and reached a peak concentration around 45 min after drug administration. TBN significantly increased the survival rate of RGCs. Furthermore, TBN increased the amplitude of photopic negative response to flash, and extended the duration of animals staying in black box after NMDA injury. The elevated ratio of cleaved caspase-3/caspase-3 and bax/bcl-2 induced by NMDA was restored by TBN. The effects of TBN were very similar to those of memantine. Our preliminary data showed that TBN decreased the ROS in NMDA-injured retina, as well as the NMDA-induced calcium overload.

Conclusion: TBN protected RGCs against NMDA injury in rat in both their morphologies and functions. TBN may protect RGCs by inhibiting the mitochondrial related apoptotic process, possibly by scavenging free radicals and inhibiting Ca^{2+} overload. Thus TBN may serve as a promising candidate to treat glaucoma in clinic.

MTU11-48

P53/HMGB1 complexes regulates mitochondria dysfunction-triggered striatal neurodegeneration via autophagy and apoptosis activation

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Background: Administration of the mitochondrial inhibitor 3-nitropropionic acid (3-NP) induces striatal pathology mimicking Huntington disease (HD) *in vivo*. Previous studies showed that 3-NP triggered p53 and Bcl-2-depedent autophagy activation and cell death. However, the mechanisms of cell autophagy and apoptosis induced by metabolic impairment are not fully understood. High-mobility group box 1 (HMGB1) protein, a chromatin-binding nuclear protein and damage-associated molecular pattern molecule, is important in oxidative stress and downstream autophagy, apoptosis and cell death signaling. Accumulation of HMGB1 at sites of oxidative DNA damage is associated with DNA repairing. The present study investigated the p53 and HMGB1 form a complex which regulates autophagy activation and cell death induced by 3-NP.

Methods: We intoxicated rat striatum with 3-NP by stereotaxic injection and measured its effects on p53/HMGB1 and pro-apoptotic proteins caspase-3 and LC3. Also, to elucidate the role of the p53/HMGB1 in 3-NP-induced striatal degeneration, we then treated primary striatal neurons in culture, in which neuronal death was replicated by application of 3-NP. To determine the interaction between HMGB1 and p53 in response to cell stress, we immunoprecipitated whole cell lysates with HMGB1or p53 antibody and probed for p53 or HMGB1 by western blot. Finally, 3-NP-induced cell viability was detected after p53 or HMGB shRNA treatment.

Results: The results showed that p53, HMGB1, caspase-3 and LC3 increased after 3-NP treatment. In addition, 3-NP-induced elevations in LC3 and caspase-3 were significantly reduced by the p53 inhibitor PFT or HMGB1 inhibitor Glycyrrhizin, and PFT or Glycyrrhizin also significantly inhibited 3-NP-induced striatal damage. Similarly, 3-NP also induced the expression of p53/HMGB1, caspase-3, LC3 in striatal neurons. Furthermore, we found that increased complex formation between HMGB1 and p53 following 3-NP-induced mitochondria dysfunction by immunoprecipitation assay, and knockdown of p53 in striatal cells increases expression of cytosolic HMGB1 and induces autophagy. Conversely, knockdown of HMGB1 increases p53 cytosolic localization and decreases autophagy. Finally, we found 3-NP-induced elevations in cell death were significantly reduced by the p53 or HMGB1 shRNA. At the same time, exogenous expression of p53 or HMGB1 can reverse this trend.

Conclusions: Taken together, our data support that p53/HMGB1 played roles in signaling both autophagy and apoptosis in neurodegeneration induced by mitochondria dysfunction.

MTU12 Psychiatric Disorders and Drug Abuse (Part 1)

MTU12-01

Repeated exposure to a serotonin 1B/1A agonist facilitates acquisition of MDMA self-administration

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There is tremendous variability between subjects in the latency to acquisition of MDMA self-administration. We have previously shown that disruption of central serotonin systems, either through lesions or through genetic knockout of the serotonin transporter, facilitate the acquisition of MDMA self-administration, suggesting that MDMA-produced serotonin release is inhibitory to the reinforcing effects of MDMA. One mechanism by which MDMA-produced serotonin release might produce this effect is through adaptations in specific serotonin receptor subtypes. The 5-HT_{1A} and 5-HT_{1B} receptors are both well localised to regulate dopamine release, and have been implicated in modulating the reinforcing effects of many drugs of abuse. In this study we tested the effect of repeated exposure to the 5-$HT_{1B/1A}$ agonist, RU 29469, on acquisition of MDMA self-administration, as well as on behavioural responses to 5-HT_{1A} and 5-HT_{1B} activation in rats. Repeated administration of RU 24969 significantly decreased the latency to acquisition of MDMA self-administration, and increased the proportion of animals that acquired the behaviour. This pretreatment failed to alter RU 24969-produced adipsia, a measure of 5-HT_{1B} response. In contrast, repeated exposure to RU 24969 shifted the dose-effect curve for hyperactivity produced by the 5-HT_{1A} agonist, 8-OH-DPAT, rightward, suggesting a desensitisation of 5-HT_{1A} receptor mechanisms. These data suggest that the reinforcing effects of MDMA are modulated by 5-HT_{1A} receptor mechanisms.

MTU12-02

The first alcohol drink triggers MTORC1-dependent synaptic plasticity in nucleus accumbens dopamine D1 receptor neurons

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Alcohol is an intrinsically reinforcing substance that can shape behavior towards pathological drinking. While it is known that increased mesolimbic dopaminergic (DA) activity reflects alcohol's reinforcing property, the enduring cellular alterations following the initial alcohol experience have not been elucidated. We previously showed that mTORC1, a kinase that controls dendritic translation and is implicated in synaptic plasticity, is activated in the nucleus accumbens (NAc) following an acute alcohol challenge, and mTORC1 inhibition reduces binge alcohol drinking (Neasta *et al*., 2010, PNAS). Here, we examined the specificity and enduring physiological consequences of alcohol-induced mTORC1 activation. NAc neurons are differentiated by their expression of either DA D1 receptors (D1R neurons) or D2 receptors (D2R neurons), so we first determined how selective DA receptor activation affects mTORC1 signaling in the NAc. Systemic administration of a D1R but not D2R agonist activates the mTORC1 pathway in the NAc and results in the translation of genes involved in excitatory neurotransmission, including *GluA1*, *Homer2,* and *PSD-95*. Furthermore, D1R activation increases levels of GluA1 and Homer in an mTORC1-dependent manner. Similarly, voluntary alcohol drinking activates the mTORC1 pathway selectively in D1R neurons, and increases synaptic expression of GluA1 and Homer. Because of mTORC1's involvement in synaptic plasticity, we tested whether a single alcohol challenge alters synaptic transmission in NAc D1R or D2R neurons. The initial voluntary alcohol drinking session or non-contingent alcohol administration leads to an enduring enhancement of the AMPA/NMDA ratio selectively in NAc D1R neurons, and this effect is blocked by inhibiting either D1Rs or mTORC1. Finally, we provide evidence that alcohol triggers plasticity via increased postsynaptic AMPAR activity. Collectively, our data imply that alcohol enhances synaptic strength on NAc D1R neurons via D1R-mediated activation of mTORC1, which results in local protein translation and postsynaptic remodeling. We propose that this plasticity in the NAc after a single alcohol experience reflects a neural imprint of alcohol's reinforcing property.

MTU12-03

Cocaine self-administration enhances D1 medium spiny neuron activity and drives drug seeking via microtubule signaling cascades

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Reward learning is robust, and cue/context presentation following abstinence elicits seeking for the previously paired reward. Reward learning has been attributed to the formation of new synaptic connections on D1 and D2 containing medium spiny neurons (MSN); however, the mechanism by which this process occurs, and how it is dysregulated in addiction, is not understood. Recently it was determined that microtubules are involved in dendritic spine structure/stability suggesting that they may have an important role in this process. Utilizing cocaine self-administration paired with calcium imagining and viral approaches, we dissected the cell-type specific mechanisms driving microtubule signaling and determined how these pathways converge to alter MSN function and drive addictive behaviors. Following self-administration and 24 hr withdrawal, Src phosphorylation (Y416) was increased, while other microtubule associated proteins (MAPs) were unchanged. Overexpressing Srcin1 (which reduces Src activity) reduced the motivation to administer cocaine, suggesting a direct link between Src levels and reinforcement. Following longer withdrawal, levels of beta-tubulin and EB3, a MAP that regulates microtubule polymerization, were elevated, and positively correlated with NR2B levels, a marker of spine stability. Further, microtubules modulate the ability to associate a drug with contextual cues, as evidence by the ability of a

microtubule inhibitor in the NAc to reduce conditioned place preference. This is likely due to a D1 specific effect as calcium imaging indicated that contextual information about drug administration is mediated by D1 activity. We hypothesize that cocaine self-administration leads to the formation of dendritic spines on D1 MSNs, and that during abstinence/withdrawal microtubule entry into spines enhances EB3-dependent signaling cascades leading to long-term stabilization of spines and long-lasting cue-reward associations. Additionally, here we show that inhibiting microtubule polymerization reduces cocaine reward, suggesting that microtubule inhibitors may serve as efficacious pharmacotherapies to prevent relapse.

MTU12-04

Evaluation of the brain damage and behavioral changes after ethanol withdrawal and allopregnanolone infusion in rats

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Background: Alcohol dependence can cause several damages, including neurodegeneration. After alcohol withdrawal, symptoms such as anxiety and depression may appear, and the GABAergic neurosteroid allopregnanolone (ALLO) is cited as one drug that can regulate behavior and the GABAergic system. The aim of this study was to verify if alcohol withdrawal could cause DNA brain damage and oxidative stress and to evaluate ALLO effects on behavioral changes after acute ethanol withdrawal.

Methods: Male Wistar rats were subjected to an oral ethanol (8%; v/v) or sucrose self-administration during 21 days followed by 5 repeated 24-h cycles (ethanol withdrawal and ethanol reinstatement). Behavioral changes were analyzed after ALLO injections (0; 0.375; 1.0; 2.5 mg/Kg i.p) in the open field (OFT) and forced swim test (FST) after 6 h of alcohol deprivation. After euthanize, brain areas were dissected to perform comet assay and reactive oxygen species (ROS) test.

Results: DNA damage was significantly higher in all structures after ethanol consumption compared to controls (pre-frontal cortex: 314.3 ± 26.6, $p = 0.021$; cerebellum: 329.0 ± 31.1, $p = 0.006$; hypothalamus: 371.0 ± 24.2, $p = 0.002$; striatum: 393.2 ± 3.1, $p < 0.001$; hippocampus: 308.1 ± 31.7, $p = 0.029$). The increase in ROS production was also observed in all brain areas ($p < 0.05$). After ALLO infusion there was no alteration in the immobility time during the FST ($p = 0.491$), neither in the locomotion during the OFT ($p = 0.824$).

Conclusions: The intermittent low doses of ethanol exposure model induced DNA damage and increase ROS production observed in several brain areas connected with the limbic system. Those areas have an important control in anxiety and depressive-like manifestations. Nevertheless the ALLO doses used were not able to recover the behavioral alterations caused by ethanol.

MTU12-05

Long-term effects of childhood/adolescent antipsychotic drug treatment on dopamine binding in adult rats

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Background: Use of antipsychotic drugs (APDs) in childhood/adolescence is currently exponentially increasing worldwide, despite serious limitations in scientific evidence on the safety/efficacy during this critical neurodevelopmental period. APDs have a wide receptor binding profile, including a high affinity for the dopaminergic (DAergic) neurotransmitter (NT) system in order to regulate neurotransmission. Current short-term investigations into APD use in juvenile rats have found alterations to signalling pathways including DA, essential to APD therapeutic efficacy and also heavily involved in multiple neurodevelopmental processes. This study therefore investigated the long-term effects of childhood/adolescent APD on DA receptor binding and re-uptake.

Methods: An established animal model for APD treatment in young rats was used to model clinical treatment in humans. Rats ($n = 6$/group) were treated with the APDs Aripiprazole, Olanzapine and Risperidone from postnatal day (PD) 22–50 equating to the human childhood-adolescent time period. Animals were then sacrificed on PD106. Quantitative autoradiographic methods were used to detect the density of [^{3}H]SCH23390 and [^{3}H]WIN35428 binding to D1R and DAT respectively.

Results: Compared to the controls, Risperidone significantly decreased DAT binding in the prefrontal cortex, caudate putamen and nucleus accumbens of male (all $p < 0.05$), but not female rats (all $p > 0.05$).

Conclusions: Childhood/adolescent APD treatment with Risperidone had a long-term effect of decreasing DAT binding in the prefrontal cortex, caudate putamen and nucleus accumbens of adult male rats. This clearly indicates potential effects of APD use in the critical childhood/adolescent neurodevelopmental window on long-term DA NT signalling. Further investigations into the neural mechanisms involved, along with potential reasons for observed differences across drug treatment groups and genders in DAT binding may shed further light on potential chronic effects of APD use in the young population. (M. De Santis was supported from a scholarship from Australian Rotary Health).

MTU12-06

Blocked fear renewal in adult rats by disconnection of the infralimbic cortex-amygdala-hippocampus circuitry during extinction

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Extinction of conditioned fear refers to the decrease in conditioned responding to a fear-eliciting stimulus due to the repeated presentation of that stimulus without any adverse outcome. This process underlies exposure therapies that treat anxiety disorders. It is

widely accepted that extinction involves the formation of a new memory of "safety" that competes with the original fear memory ("danger") in adult rats, and does not erase the original fear learning. This is supported by the phenomenon of renewal, where rats exhibit the return of fear when tested in a context different to that in which they received extinction. It is thought that the new learning during extinction involves amygdala communicating to the ventral hippocampus (vHPC) and infralimbic cortex (IL), which leaves the original fear memory intact. We temporarily disconnected this circuitry during extinction by simultaneously inactivating the IL and vHPC by infusing muscimol, a $GABA_A$ agonist, or saline prior to extinction. Cannulas were implanted in the IL and the vHPC in one of three configurations: ipsilateral, contralateral and bilateral. We observed that rats treated with saline displayed renewal of extinguished fear, as did rats infused with muscimol in the ipsilateral condition. Rats that received contralateral or bilateral infusions of muscimol prior to extinction failed to display renewal of fear. These results suggest that extinction may reverse or "erase" the original fear memory when the IL-amygdala-vHPC circuitry is disrupted. This finding is consistent with the idea that extinction erases the original fear memory in juvenile rats, where the connections between those brain regions are immature.

MTU12-07

Differential effects of modafinil and methamphetamine on epigenetic and glutamatergic markers expression in the prefrontal cortex

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Chronic use of methamphetamine (METH) leads to long-lasting cognitive dysfunction in humans and animal models. Modafinil is a wake-promoting compound approved for the treatment of narcolepsy and being prescribed *off label* for the treatment of METH dependence. There is increasing evidence that epigenetic defects play a major role in the pathogenesis of psychiatric and substance abuse disorders. Interestingly, several psychotropic drugs in clinical use exhibit epigenetic effects in addition to their commonly understood mechanisms of action. We previously demonstrated that modafinil can rescue METH-induced deficits on visual memory retention through a mechanism that involves restoration of ERK signaling in the medial prefrontal cortex (mPFC). In the present study, we evaluated mPFC epigenetic regulators of gene expression that modify the local state of chromatin, including histone deacetylases (*Hdac*), DNA methyltransferases (*Dnmt*), and methylcytosine dioxygenases (*Tet*). We also quantified histone 3 acethylation, glutamate receptor AMPA (*Gria*) and NMDA (*Grin*) subunits and *c-Fos* expression. METH (1 mg/kg, sc) was administered as a single dose or repeatedly (once daily for 7 days and 4 days of withdrawal). Additionally, we evaluated the effects of a single dose of modafinil (90 mg/kg, ip) given alone or after METH withdrawal. Our results show that METH and modafinil exert differential effects on epigenetic marker expression in the mPFC, with METH altering a larger set of epigenetic regulators than modafinil. These differences could be related to the METH-induced cognitive impairments and mPFC abnormalities. Finally, our results suggest that modafinil, given in the presence or absence of METH, appears to initiate differential transcriptional and epigenetic programs that might contribute to its beneficial cognitive effects.

MTU12-08

4I (N-(3-chloro-2-methylphenyl) quinoxalin-2-carboxamide), a novel 5HT3 antagonist, reverses diabetes-induced depressive phenotype

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Depression associated with diabetes is becoming a severe risk factor for all-cause mortality yet, it remains poorly treated. It may partly be attributed to the inefficient efficacy of the current pharmacotherapy. Evidences that brain serotonergic deficits are associated with altered psychological behavior in diabetes and that $5HT_3$ receptor (5HT3R) antagonists have serotonergic facilitatory effects, this study examined, in mice, the effects of 4i (N-(3-Chloro-2-methylphenyl) quinoxalin-2-carboxamide), a novel 5HT3R-antagonist in ameliorating diabetes-induced depressive phenotype. Experimentally, (1) to evaluate the antidepressant effects of 4i, mice with 8-weeks of diabetes (induced by a single injection of streptozotocin, 200 mg/kg, i.p.) were treated with vehicle or 4i (0.5–1 mg/kg/day, i.p.) or fluoxetine (a positive control, 10 mg/kg/day, i.p.) for 4-weeks and subjected to neurobehavioral assays followed by biochemical estimation of serotonin levels in midbrain, prefrontal-cortex and cerebellum. (2) To evaluate the role of 5HT3R, diabetic mice (received 4i, 1 mg/kg/day, i.p.) were pretreated with a selective 5HT3R-agonist, 1-(m-Chlorophenyl)-biguanide (mCPBG, 10 mg/kg/day, i.p.) and subjected to the same neurobehavioral and biochemical assays. Results showed that diabetic mice exhibited a significant behavioral deficit, including depression-like behavior in forced-swim test, anxiety-like in open-field test and sociability deficits in social-interaction test. Diabetic mice also exhibited a significant decrease in serotonin contents in these brain regions. 4i (1 mg/kg), similar to fluoxetine, prevented these behavioral anomaly and normalized brain serotonin levels, but 4i (0.5 mg/kg) ameliorated only diabetes-induced depressive-like behavior and serotonin deficits, and not anxiety-like behavior and social deficits. mCPBG impaired 4i (1 mg/kg) mediated response against diabetes-induced behavioral deficits and increase in brain serotonin levels. 4i demonstrated a dose-dependent activity and this effect was associated with 5HT3R-antagonism-mediated enhanced serotonin levels in discrete brain regions. These findings suggest the robust and potential activity of 4i in ameliorating diabetes-induced depression, which may involve normalization of serotonin deficits in discrete brain regions. Moreover, this study gives a conception of "5HT3Rs as novel target" in the pathophysiology of diabetes-induced depressive disorders, the antagonism of which may provide new insights into the therapy.

MTU12-09

Septal glucagon-like peptide 1 receptor expression determines suppression of cocaine-induced behaviour

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Glucagon-like peptide 1 (GLP-1) and its receptor GLP-1R are a key component of the satiety signalling system and long-acting GLP-1 analogues have been approved for the treatment of type-2 diabetes mellitus. Previous reports demonstrate that GLP-1 regulates glucose homeostasis alongside the rewarding effects of food. Both palatable food and illicit drugs activate brain reward circuitries, and pharmacological studies suggest that central nervous system GLP-1 signalling holds potential for the treatment of addiction. However, the role of endogenous GLP-1 in the attenuation of reward-oriented behaviour, and the essential domains of the mesolimbic system mediating these beneficial effects, are largely unknown. We hypothesized that the central regions of highest *Glp-1r* gene activity are essential in mediating responses to drugs of abuse. Here, we show that *Glp-1r*-deficient *(Glp-1r*$^{-/-}$*)* mice have greatly augmented cocaine-induced locomotor responses and enhanced conditional place preference compared with wild-type (*Glp-1r*$^{+/+}$) controls. Employing mRNA *in situ* hybridization we located peak *Glp-1r* mRNA expression in GABAergic neurons of the dorsal lateral septum, an anatomical site with a crucial function in reward perception. Whole-cell patch-clamp recordings of dorsal lateral septum neurons revealed that genetic *Glp-1r* ablation leads to increased excitability of these cells. Viral vector-mediated *Glp-1r* gene delivery to the dorsal lateral septum of *Glp-1r*$^{-/-}$ animals reduced cocaine-induced locomotion and conditional place preference to wildtype levels. This site-specific genetic complementation did not affect the anxiogenic phenotype observed in *Glp-1r*$^{-/-}$ controls. These data reveal a novel role of GLP-1R in dorsal lateral septum function driving behavioural responses to cocaine.

MTU12-10

Effects of adjunctive raloxifene treatment on brain activity during facial emotion processing in schizophrenia

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Estrogen has been implicated in the development and course of schizophrenia with most evidence suggesting a neuroprotective effect. The selective estrogen receptor modulator raloxifene has been shown to reduce symptom severity in schizophrenia and preserve neural activity in healthy older adults. The present study was designed to determine the extent to which adjunctive raloxifene treatment would alter abnormal neural activity during facial emotion processing in schizophrenia. Twenty people with schizophrenia (14 men, 6 women) received fMRI during facial emotion identification in conjunction with a thirteen-week, randomized, double-blind, placebo-controlled, crossover trial of adjunctive raloxifene treatment (120 mg/day orally). Region of interest analyses, focused on brain areas associated with the processing of angry versus neutral faces, were performed to assess differential activity between the raloxifene and placebo conditions. Adjunctive raloxifene significantly increased activation in the hippocampus and inferior frontal gyrus compared to the placebo condition (FWE $p < 0.05$). There was no significant difference in performance accuracy or reaction time between active and placebo conditions. This study provides the first evidence that adjunctive raloxifene treatment can influence neural activity in brain regions associated with emotional processing deficits in schizophrenia. These findings support the hypothesis suggesting a role of estrogen in schizophrenia and provides the first evidence for the potential role of adjunctive raloxifene in reversing emotion processing abnormalities and impaired social functioning in men and women with schizophrenia.

MTU12-11

OREXIN2 (OX2) receptors in nucleus incertus mediate stress-induced reinstatement of alcohol-seeking in alcohol-preferring (IP) rat

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Alcoholism is a chronic relapsing disorder and a major global health and societal problem. Stress is a key precipitant of relapse in human alcoholics and in animal models of alcohol-seeking. The brainstem *nucleus incertus* (NI) contains a population of relaxin-3 neurons that are highly responsive to psychological stressors. Indeed, the ascending NI relaxin-3/RXFP3 signalling system is implicated in stress-induced reinstatement of alcohol-seeking in rats, a model of relapse (Ryan PR *et al*., PNAS (USA) 110, 20789–94, 2013). The NI receives an orexinergic innervation from the lateral hypothalamus and expresses orexin$_1$ (OX_1) and orexin$_2$ (OX_2) receptor mRNA. Moreover, the orexin system is implicated in stress-induced relapse to drug and alcohol-seeking (Baimel C *et al*., Br J Pharmacol 172, 334–348, 2015). Therefore, in alcohol-preferring (iP) rats, we examined the effect of bilateral injections into the NI of the OX_2 receptor antagonist, TCS-OX2-29 or the OX_1 receptor antagonist, SB-334867 on stress-induced reinstatement of alcohol-seeking. We also determined the effects of orexin-A on NI neuron activity, using whole cell patch-clamp recordings in rat brain slices. Bilateral NI injections of TCS-OX2-29 attenuated stress-induced reinstatement of alcohol-seeking (RM one-way ANOVA, effect of treatment on lever pressing, $F_{(2,26)}$ 18.67, $p < 0.0001$). In contrast, intra-NI injection of SB-334867 had no significant effect. In line with these data, orexin-A depolarized a majority of NI

neurons recorded (relaxin-3 positive and negative) in rat brain slices, an effect maintained in the presence of tetrodotoxin and synaptic-blockers, indicating a direct postsynaptic action. These effects were blocked by bath application of TCS-OX2-29, but not SB-334867. These results suggest that stress activates an orexin input to the NI that contributes towards stress-induced reinstatement of alcohol-seeking, mediated predominantly via OX_2 receptor signalling.

MTU12-12

effect of the dual orexin receptor antagonist TCS1102 on intravenous nicotine self-administration and reinstatement in the rat

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Background: Smoking is the leading preventable cause of morbidity and mortality worldwide. Animal models have shown orexin antagonists reduce alcohol, cocaine and opioid seeking behaviour. Recent evidence also shows that nicotine promotes orexin release. We therefore examined impact of a dual orexin antagonist on nicotine self-administration and reinstatement.

Method: Male Sprague-Dawley rats (n = 16) were implanted with chronically indwelling jugular vein catheters and intracranial guide cannulae targeting the lateral ventricle. After recovery, they received 10 days of self-administration training where each active nosepoke (FR1) was reinforced with a 30 μg/kg/100 μL infusion of nicotine. On separate test days, rats received i.c.v. infusion of 1, 3 or 10 μg TCS1102 or vehicle 10 min prior to self-administration. After self-administration testing and a total of 29 days of nicotine self-administration, they underwent extinction (at least 6 days until 2 consecutive days of ≤30% active nosepoke responding), during which time active nosepokes had no consequences. Relapse-like behaviour was precipitated by presentation of drug-associated cues, a priming injection of nicotine (0.3 mg/kg, s.c.) or both the cue and prime, with extinction sessions in between. Rats received i.c.v. infusion of 10 μg TCS1102 or its vehicle prior to each reinstatement session.

Results: Central administration of 10 μg TCS1102 prior to self-administration had no significant effect on responding for nicotine. Reinstatement was precipitated by drug-associated cues, a priming injection of nicotine or a cue+prime compound. Compound reinstatement was significantly lower in TCS1102 treated animals relative to vehicle controls but there was no significant difference between the vehicle and TCS1102 treatment groups for cue-induced or nicotine-primed reinstatement sessions. Importantly, there was no effect on locomotor activity.

Conclusion: The dual orexin antagonist, TCS1102, selectively reduced compound reinstatement over other nicotine-seeking and self-administration behaviours. This suggests that dual orexin antagonists, like the recently approved suvorexant, may have limited clinical utility for nicotine addiction.

MTU12-13

localised injection of MK-801 into limbic brain regions increase high frequency neural oscillations in distant brain regions

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Objectives: NMDA receptor hypofunction is a potential molecular mechanism involved in the pathophysiology of schizophrenia. Administration of NMDA receptor antagonists to rats induce well-validated endophenotypes of schizophrenia, such as hyperlocomotor activity and cognitive dysfunction, promoting this as an appropriate animal model. NMDA receptor antagonists also increase high frequency brain oscillations, particularly in the gamma frequency range (30–80 Hz). The relationships between aberrant gamma oscillations and behaviour caused by NMDA receptor antagonists is not well-understood, but elucidating this may be relevant for schizophrenia, since this disorder is also associated with disturbances in gamma oscillations. Here, we investigated how NMDA receptor hypofunction in different brain regions influence gamma oscillations and behaviour.

Methods: Wistar rats (n = 30) were surgically implanted with depth recording electrodes into the prefrontal cortex, dorsal hippocampus, and nucleus accumbens, as well as injection cannulae into one of these regions. After recovery, the effects of intra-brain infusion of the selective NMDA receptor antagonist MK-801 (0–50 micrograms) on gamma oscillations within and between regions, and on locomotor behaviour were assessed.

Results: Injection of MK801 dose-dependently increased gamma oscillations in all three brain regions regardless of where the drug was infused, compared to a control injection of aCSF [$p < 0.001$ for all regions]. However, phase coherence between regions was not significantly altered ($p > 0.05$ for all comparisons). MK801 also dose-dependently increased locomotor activity when injected into the three regions ($p < 0.001$).

Conclusion: Local NMDA receptor hypofunction in limbic brain regions is sufficient to increase gamma oscillations in distant regions, causing both local and large scale circuit disruption possibly involving thalamic relay neurons. We also determined that locomotor activity can be equally influenced by blocking NMDA receptors in several brain regions. Our findings suggest that localised NMDA receptor hypofunction, such as that observed in schizophrenia, could be responsible for disruptions in global brain activity and connectivity.

MTU12-14

MGLUR5 dysregulation in schizophrenia and cognition: from gene to protein

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Due to the hypothesized role of metabotropic glutamate receptor 5 (mGluR5) in the cognitive pathology of schizophrenia, we explored the possibility of mGluR5 dysregulation in regions associated with cognitive function, and the effects of common variants within the regulatory 3' untranslated region (3'UTR) of the *GRM5* gene on cognitive function in schizophrenia. We examined total protein levels of mGluR5 and mGluR5 regulatory proteins (Norbin, Tamalin and Preso1) in the dorsolateral prefrontal cortex (DLPFC) and CA1 region of postmortem schizophrenia and control brain tissues (n = 37/group NSW Tissue Resource Centre). We also examined the association of novel SNPs located within the regulatory 3'UTR of *GRM5* with schizophrenia and nine measurements of cognitive function (n = 268/group; Australian Schizophrenia Research Bank). In both the DLPFC and CA1, mGluR5 protein was increased (22–42%; $p < 0.001$); conversely, mGluR5 regulators were reduced in the DLPFC (29–30%) and increased in CA1 (34–83%; $p < 0.009$). In addition, *GRM5* SNP rs60954128 was associated with schizophrenia in males ($p < 0.011$), and the minor allele of rs3824927 was associated with increased cognitive impairment in male schizophrenia patients ($p < 0.040$). Both SNPs also exerted differential effects on several cognitive domains in men and women with schizophrenia ($p < 0.042$). We thus report altered levels of mGluR5 and mGluR5 regulatory proteins in the DLPFC and CA1 regions in schizophrenia. Together with sex-specific effects of *GRM5* variants (located in the regulatory region of the gene) on cognitive function in schizophrenia patients, these findings provide strong evidence of mGluR5 dysregulation in schizophrenia, and for the first time, that *GRM5* has a modulatory effect on cognitive function in humans.

* equal contribution.

MTU12-15

sex-specific cognitive and motivational profiles following sucrose consumption during adolescence in rats

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Excessive consumption of sugar sweetened drinks is a major contributor to the development of obesity and is proposed to evoke enduring changes in the brain's reward system, which in turn can cause perturbations in cognition. Adolescents are the highest consumers of sugar sweetened drinks. Adolescence is a critical period for postnatal brain maturation, thus sucrose consumption may have persistent cognitive effects. In this study we sought to determine the impact of daily sucrose consumption in young male and female rats (N = 12 per group) on spatial and non-spatial memory and on the motivation to perform instrumental responses to gain food rewards as adults. Rats were exposed to a sucrose solution for 2 h a day for 28 days across adolescence [postnatal days (P) 28–56] and underwent behavioural testing as adults (P70 onward). Exposure to sucrose during adolescence significantly impaired place recognition memory, which was used as an assay of spatial memory, in both male and female rats ($p < 0.001$) indicating that sucrose consumption disrupted hippocampal function. This impairment was greater in male than female rats ($p < 0.05$), suggesting an interaction between gender and sensitivity of the hippocampus to disruption. Exposure to sucrose had no detectable effect in either male or female rats on a perirhinal-dependent, object recognition memory task. Rats were then trained to lever press for palatable rewards and tested on a progressive ratio schedule to assess motivation to respond for reinforcement. Female rats exposed to sucrose during adolescence had higher breakpoints on the progressive ratio schedule than controls ($p < 0.05$), indicating that they were more motivated to respond for food rewards as adults, suggestive of craving. However, the opposite was observed in male rats exposed to sucrose, their breakpoints were significantly lower than controls ($p < 0.05$), thus were less motivated to perform instrumental responses, indicating anhedonia. These experiments indicate that sucrose exposure during adolescence induced different behavioural profiles in adult male and female rats.

MTU12-16

Vulnerability to the reinforcing effects of MDMA: results of self-administration studies in rats

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There is tremendous variability in the latency to acquisition of self-administration of most drugs of abuse by laboratory animals. This is particularly true of MDMA self-administration. Some rats seem to be more susceptible to the reinforcing effects and acquire self-administration relatively quickly whereas others are relatively resistant and acquire self-administration slowly, or not at all. We have attributed this variability to between-subject differences in the effect of MDMA on serotonin (5-HT) and dopamine (DA). It is generally accepted that self-administration proceeds as a function of the magnitude of the DA response but MDMA-stimulated DA is relatively small compared to other self-administered drugs. Thus, differences in the DA response might explain the variability in MDMA self-administration. Alternatively, differences in the propensity to self-administer MDMA might reflect differences in the magnitude of the 5-HT response. MDMA preferentially stimulates release of 5-HT, but 5HT is generally inhibitory to self-administration. We have investigated both of these possibilities using behavioural and neurochemical assays and our results suggest that differences in both MDMA-simulated 5-HT and DA release contribute to the variability in sensitivity to the reinforcing effects and abuse liability of MDMA.

MTU12-17

agmatine rescued autistic behavior in valproic acid induced animal model of autism:new implication for the ASD

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Autism spectrum disorder (ASD) is becoming the most challenging developmental disorder, characterized by two core symptoms: social communication deficits and restricted repetitive behavior. Due to the numerous risk factors and complex symptoms that make up the mechanism of ASD, identifying the etiological process and the appropriate therapeutic targets remain a formidable challenge. One of the most popular hypotheses which attempts to explain the mechanism of ASD is the concept of excitatory-inhibitory neuronal imbalance.

Excitatory-inhibitory neuronal imbalance (E/I imbalance) was not only observed in humans, but also present in most animal models of ASD. Here, we tested the hypothesis that modulation of E/I imbalance using agmatine can be a potential therapeutic target for ASD. This hypothesis is based on two grounds. Firstly, valproic acid induced animal models (VPA animal model) showed an increased number of gluatamatergic receptors. Secondly, agmatine, a metabolite of L-arginine, functions as an NMDA blocker. As for the VPA model, valproic acid subcutaneously injected to pregnant SD rats at gestational day 12. Afterwards, we performed behavioral studies following a single intraperitoneal injection treatment of agmatine on pups at their third week of life.

As a result, we found that a single treatment of agmatine mitigated the appearance of the aberrant phenotypes common in ASD, including impaired social behavior, and repetitive behavior and hyperactive. Moreover, we also found that single treatment of agmatine ameliorate overly active downstream signaling of NMDA receptor including ERK1/2 and αCaMKII. These results suggest that agmatine, modulating overly active excitatory neural transmission, could be a potential target for therapeutics aiming to regulate the autistic behavior.

MTU12-18

More than replacement therapy: amphetamine treatment rescues the behavioral and neurochemical consequences of cocaine

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Cocaine abuse results in the disruption of dopamine system function, and it has been postulated that this hypodopaminergic state underlies maladaptive behaviors in addiction. Amphetamine (AMPH) has been examined as a potential treatment for cocaine addiction, with promising behavioral results, in rodents, monkeys and humans. It has been proposed that AMPH acts as an agonist replacement therapy; however, the mechanisms underlying AMPH's effects are unknown. Thus, the goal of the current study was to elucidate the neurochemical mechanisms underlying AMPH's ability to reduce cocaine related behaviors. Animals self-administered cocaine for 14 days and were treated with mini-pump delivered AMPH (5 mg/kg/day, s.c.) or saline throughout the 14 days of self-administration or throughout 7 days of abstinence following self-administration. Cocaine self-administration resulted in escalation of cocaine intake, which is thought to model the transition from recreational use to addiction; however, treatment with AMPH completely prevented escalation. Using fast-scan cyclic voltammetry in the nucleus accumbens core we found that cocaine self-administration reduced phasic dopamine release and attenuated cocaine's ability to inhibit the dopamine transporter and increase synaptic dopamine levels. AMPH treatment blocked the development of these alterations when administered during cocaine self-administration. Additionally, when administered during the withdrawal period (after cocaine self-administration), AMPH completely reversed changes in cocaine potency and phasic dopamine signaling induced by cocaine self-administration. Finally, using a behavioral economics assessment of drug taking and seeking, we found that AMPH treatment reduced the reinforcing efficacy of cocaine for at least 7 days following cessation of treatment, demonstrating that AMPH-induced reversal of the neurochemical consequences of cocaine self-administration has long-lasting behavioral benefits. Our data show that AMPH reduces cocaine self-administration, at least in part, by reversing the cocaine-induced alterations to the dopamine transporter and phasic dopamine signaling. These data may be able to define a mechanism for some of AMPH's therapeutic effects, which could drive the design of more targeted pharmacotherapies with less abuse liability.

MTU12-19

Delineation of rostromedial tegmental nucleus (RMTG) in rats and mice via nociceptin/OFQ expression and anatomical connectivity

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The rostromedial tegmental nucleus (RMTg), or tail of the ventral tegmental area (tVTA), has a strong inhibitory influence on midbrain dopamine neurons. Accordingly, RMTg has been implicated in drug reward and withdrawal, as well as aversive behaviors. Previously, RMTg has been identified anatomically using retrograde tracing from VTA, GABA expression, and psychostimulant-induced Fos activation in rats. However, it is unclear whether RMTg neurons are neurochemically distinct from GABA neurons in the midbrain, or whether RMTg is a caudal extension of GABAergic neurons in VTA. Further, the anatomical location of RMTg has not been well-characterized in the mouse. Here, we show that RMTg neurons in rats and mice express mRNA for prepronociceptin (PNOC), the precursor for nociceptin/orphanin-FQ. *In situ* hybridization labeling of PNOC was observed in the vast majority of RMTg neurons projecting to VTA (those double-labeled with the retrograde tracer CTB) but only in a minority of neurons outside RMTg or within VTA. In mouse, we show that RMTg is defined via PNOC expression, GABA expression (via reporter gene), retrograde tracing from VTA, and location of lateral habenula afferent fibers (via AAV-mediated mCherry expression). Further, optogenetic stimulation of RMTg in mice drives a conditioned place aversion. Altogether, these data delineate RMTg in mice and rats, and show that RMTg is neurochemically distinct from neighboring GABAergic neurons, indicating it may play a distinct functional role as well.

MTU12-20

Neurochemical phenotyping of rxfp3 + cells in the mouse bed nucleus of the stria terminalis

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The bed nucleus of the stria terminalis (BNST) is a key stress-sensitive region within the neurocircuitry of reward-seeking with strong projections to the ventral tegmental area (VTA). The BNST is a highly heterogeneous structure with discrete subpopulations of neurons that express different neurotransmitters and peptides. The BNST contains cells that express relaxin family peptide 3 receptor (RXFP3), the cognate receptor for the neuropeptide relaxin-3. However, the neurochemical phenotype of RXFP3 + cells in the BNST, and whether they are local or VTA-projecting neurons, has yet to be examined in detail. To ascertain the neurochemical content of BNST RXFP3 + cells, we have employed an RXFP3-Cre/YFP transgenic reporter mouse in which the expression of yellow fluorescent protein (YFP) is mediated by Cre recombinase driven by the RXFP3 promoter. YFP expression was first mapped throughout the extent of the BNST, revealing dense populations of RXFP3 + cells in the anteromedial and dorsal division of the posteromedial BNST. Immunohistochemistry for the glial marker Glial Fibrillary Acidic Protein (GFAP) divulged no double-labelled cells, signifying that RXFP3 + cells are neuronal. Immunostaining for VGLUT2 revealed that all examined YFP+ cells were also VGLUT2 + , a strong suggestion that a population of BNST RXFP3 + neurons is glutamatergic. We also observed a subpopulation of YFP+ cells that expressed the main inhibitory neurotransmitter GABA, indicating heterogeneity in RXFP3 + neurons. YFP+ cells also colocalised with Striatal Enriched Tyrosine Phosphatase (STEP), a marker for CRF+ neurons in the oval BNST [1]. Orexin fibres terminated in close proximity to, but seemingly not directly onto, RXFP3 + cells. Current studies are underway to determine if RXFP3 + cells are intrinsic to the BNST or whether they project to the VTA. These neuroanatomical studies will paint a clearer picture of the phenotype and connectivity of BNST RXFP3 + cells in the mouse brain.

1. Dabrowska, J *et al*., (2013) Biol Psychiatry 74, 817–826.

MTU12-21

A silent synapse-based mechanism of alcohol addiction

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Addiction, defined as a state of compulsive drug use, is a pathological form of memory. It shares many commonalities with natural appetitive learning, however, drugs of abuse, unlike natural rewards, create extremely durable memories, making drug use the sole goal of existence. One potential mechanism underlying addiction is drug-induced adaptations at glutamatergic synapses within the brain reward circuitry. We have shown that exposure to addictive substances (cocaine) generates nascent excitatory synapses in the nucleus accumbens (NAc) – a brain structure responsible for motivated behaviors. These AMPA receptor (R)-silent synapses might represent enhanced capacity for learning and could be key substrates for the development of addiction. Given the role of hippocampus in declarative memory formation processes, we investigated the formation and fate of silent synapses in the establishment of short- and long-term alcohol-associated memories. We used a short-term (4 weeks) and chronic (12 weeks) self-administration model of alcohol addiction, where mice, housed larger groups in the IntelliCage system, were constantly monitored for their alcohol consumption as well as their motivation to reward. We have discovered that ethanol consumption elevates the number of silent synapses in the hippocampus of short-term and chronically treated mice. Moreover, the number of silent synapses further increased in mice exposed to chronic ethanol treatment that displayed extremely high motivation for reward. This data suggest that silent synapses might represent an ongoing circuitry reorganization process that primes neurons for enhanced memory formation that in the future might lead to the out-of-control ethanol use.

MTU12-22

Ethanol administration modulates expression of the α4 subunit of neuronal nicotinic acetylcholine receptors in the amygdala

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Alcohol addiction is a complex and debilitating disorder that affects over a million Australians over the age of 15. The processes that underpin alcohol use disorders (AUDs) primarily lead to a distinct physical desire to consume alcohol to points beyond capacity, irrespective of social, emotional or physical damages. Progressive research into the mediation of alcohol addiction via the neuronal nicotinic acetylcholine receptors (nAChRs) is accelerating our understanding of alcohol dependence, to elucidate and optimise potential therapeutic targets. This study utilises acute administration of ethanol and the well-established and described two bottle choice, drinking in the dark, self-administration paradigm. Through application of these paradigms in mice with functional yellow fluorescent labelled α4 nAChR subunits, we have been able to show evidence that the expression of the α4 subunit protein of nAChRs in the amygdala is increased just hours after acute exposure to ethanol. Repeated challenges that occur with chronic alcohol consumption lead to attempts by the brain to maintain stability within the neural network, but at a cost. By using a 12 week self-administration paradigm we have also shown that unlike an acute binge episode, chronic consumption of ethanol causes a decrease in the expression of the α4 subunit protein in the amygdala during withdrawal. As the amygdala is intrinsically linked with components of the mesolimbic dopaminergic pathway and its reaffirmation of alcohol dependence, any dysregulation seen in these cholinergic receptors after both acute and chronic ethanol consumption that persists into protracted abstinence may set the tone for vulnerability to craving by activation of stress-induced reinstatement circuits now driven by a hypo/hyperfunctioning system.

MTU12-23

Behavioral and $GABA_A$ receptors subunits mrna effects of cocaine in 6-hidroxidopamine lesioned male and female rats

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Introduction: Cocaine abuse and dependence are more precocious in Attention Deficit Hyperactivity Disorder (ADHD) patients and females and males show behavioral differences in both disorders. These disorders are related to altered functions in the dopaminergic and GABAergic brain pathways. Our objective was to evaluate effects of cocaine self-administration in male and female rats in a model to ADHD on the behavior and mRNA expression of $GABA_A$ receptor subunits in the prefrontal cortex.

Methods: Male(M) and female(F) Wistar rats were randomized to intrathecal 6-OHDA (100 μg free base) or SHAM 10 μL (0.1% ascorbic acid) and after the rats were placed daily in operant conditioning sessions for oral cocaine-sucrose self-administration during 27 days (0, 0.2, 0.3, 0.4 mg/ml-cocaine – 15 mg/mL-sucrose). After euthanize, pre frontal cortex was dissected to perform Real-time quantitative PCR (qPCR) to quantify mRNA expression of GABA A subunits (α1,α2,γ2).

Results: Females showed more daily active lever presses than males ($p < 0001$) and 6-OHDA-lesioned animals consumed less sucrose self-administration ($p < 0.05$) than SHAM animals. When cocaine was added to sucrose solution, the 6-OHDA- lesioned animals presented less lever presses than the non-lesioned animals considering cumulative responses in the active lever (F/SHAM -1415.23 ± 32.86; M/SHAM -1180.84 ± 32.86; F/6OHDA-917.33 ± 28.17; M/6OHDA – 538.12 ± 28.12; $p < 0.001$). Females rats, irrespective lesion, showed higher mRNA expression of γ2-$GABA_A$ than males ($p = 0.003$).

Conclusions: 6-OHDA lesion decrease sucrose self-administration, and that cocaine behavioral effects on sucrose reinforcement is sensitized in 6-OHDA lesioned males. Cocaine effects on sucrose self-administration are less intense in females and expression of γ2-$GABA_A$ is higher in females, irrespective of previous dopaminergic lesion or not. Further studies should elucidate the participation of the pre-synaptic dopaminergic neurons in sucrose-cocaine consumption in female and males rats and changes subunits $GABA_A$.

MTU12-24

Involvement of mitochondrial dysfunctions in ASD and ADHD

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Mitochondrial involvement in the development of ASD and ADHD phenotypes is suggested, based on sparingly available data for the former, and circumstantial evidences for the latter disease group. However, this hypothesis is never tested. We prepared mitochondrial transgenic cell lines, cybrids by fusing platelets from healthy persons and patients with ρ0 neuronal cell line, depleted of their mitochondrial genes. Mitochondrial gene inheritance in cybrids was examined employing long-template PCR, differentially using external primers and internal primers, which respectively transcribed mitochondrial or nuclear DNA sequence. PCR products of both the primer sets indicated successful transfer of mitochondria, whereas absence of PCR product of external primers denoted ρ0 cells. PicoGreen® staining produced punctate fluorescence restricted to cytosol, and strong green fluorescence in nuclei indicated inheritance of mDNA by ρ0 cells. This is further supported by Mitotracker Green® staining of networked mitochondria in SH-SY5Y and control cybrids, unrestricted fluorescence in ρ0 cells and fragmented mitochondrial staining in ASD/ADHD cybrids. Rate of oxygen consumption by cells or by mitochondria was significantly reduced in ASD/ADHD cybrids, compared to controls. A significantly reduced ATP6 and ATP8 levels and loss of mitochondrial membrane potential, and an increased superoxide generation supported the organelle's dysfunction in ASD/ADHD cybrids. In differentiated cybrids serotonin level was found to be high in ASD and ADHD cybrids but the expression of monoamine oxidase A and B was unaffected. This is first study to demonstrate mitochondrial dysfunctions in ASD/ADHD. Loss of mitochondrial integrity and functions could be a major underlying mechanism for the disease phenotype development.

MTU12-25

Selective estrogen receptor modulation increases hippocampal activity during probabilistic association learning in schizophrenia

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People with schizophrenia show probabilistic association learning impairment in conjunction with abnormal neural activity. The selective estrogen receptor modulator (SERM) raloxifene preserves neural activity during memory in healthy older men and improves memory schizophrenia. Here, we tested the extent to which raloxifene modifies neural activity during learning in schizophrenia. Nineteen people with schizophrenia participated in a twelve-week randomized, double-blind, placebo controlled, cross-over adjunctive treatment trial of the SERM raloxifene administered orally at 120 mg daily to assess brain activity during probabilistic association learning using functional magnetic resonance imaging (fMRI). Raloxifene improved probabilistic association learning and significantly increased fMRI BOLD activity in the hippocampus and parahippocampal gyrus relative to placebo. A separate region of interest confirmatory analysis in 21 patients versus 36 healthy controls showed a positive association between parahippocampal neural activity and performance in patients, but no such relationship in the parahippocampal gyrus of healthy controls. Thus, selective

estrogen receptor modulation by raloxifene concurrently increases activity in the parahippocampal gyrus and improves probabilistic association learning in schizophrenia. These results support a role for estrogen receptor modulation of mesial temporal lobe neural activity in the remediation of learning disabilities in both men and women with schizophrenia.

MTU12-26

Methamphetamine, MDMA and bath salts induce cytotoxic effects in bovine brain microvessel endothelial cells

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Designer drugs such as synthetic psychostimulants are a worldwide problem. These drugs include methamphetamine (METH), 3,4-methylenedioxymethamphetamine (MDMA) and commercial preparations of synthetic cathinones including mephedrone, methylone and 3,4-methylenedioxypyrovalerone (MDPV), among others, typically referred to as "bath salts." Studies suggest that psychostimulants may exert neurotoxic effects by altering monoamine systems in the brain. In addition, it has been shown that METH and MDMA adversely affect the integrity of the blood-brain barrier (BBB), but there are currently no reports about the effects of synthetic cathinones on theBBB. The aim of this study was to compare the effects of METH, MDMA andMDPV on bovine brain microvessel endothelial cells (bBMVEC) as an *in vitro* model of the BBB. Confluent bBMVEC monolayers (cultured 10–14 days) were treated with increasing concentrations of METH, MDMA and MDPV (500 μM–2.5 mM) for 24 h. METH and MDMA increased LDH release only at the highest concentration (2.5 mM), whereas MDPV was cytotoxic at all concentrations. MDMA decreased cellular proliferation only at 2.5 mM, while similar effects of METH and MDPV observed at 1 mM. MDPV and MDMA produced cytotoxicity as measured by increases in nitric oxide (NO) and reactive oxygen species (ROS). Morphological analysis revealed different patterns of cell damage. For instance, METH induced vacuole formation at 1 mM in conjunction with disruption of the monolayer at 2.5 mM. MDMA induced disruption of the endothelial monolayer from 1 mM without vacuolization. On the other hand MDPV induced disruption of this monolayer at doses > 500 μM without vacuole formation; at 2.5 mM, the few remaining cells lacked endothelial morphology. These data suggest that even though these synthetic psychostimulants have similar effects on monoaminergic systems, they each induce BBB toxicity by different mechanisms, with MDPV affecting BBB permeability by inducing cytotoxicity in brain endothelial cells. With the emergence of "bath salts" on the drug scene, it is mportant to understand the physiological implications of its abuse to aid clinical treatment.

MTU12-27

MGLUR2 possitive allosterism possibly complements the hallucinogenic mechanism of action of ayahuasca

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Ayahuasca is a hallucinogenic beverage employed by many tribes throughout the amazonian basin in their rituals. This admixture consists of at least two different plants: *B. caapi* and *P. viridis*. The mechanism of action of the alkaloids present in Ayahuasca is well established: *B. caapi* provides a series of beta-carbolines –mostly harmine and tetrahydroharmine (THH)–, that inhibit monoaminoxidase A (MAO-A) rendering the dimethyltryptamine (DMT) from *P. viridis* orally active. DMT hallucinogenic effect is mediated mainly by serotonin receptor subtype 2 (5-HT2AR; Carbonaro et al. Psichopharmacol, 2015). Experiments conducted in our group in collaboration with the PDSP showed that analogues built on the beta-carboline scaffold, in addition to MAO-A inhibition, produce positive allosteric modulation (PAM) on metabotropic glutamate receptor subtype 2 (mGlu2R). And so does THH, but not harmine, both contained on Ayahuasca. Recent studies have established that 5-HT2AR-mediated hallucinogenesis require the formation of a 5-HT2AR-mGlu2R heterodimer. This complex could also be involved in the psychosis associated with schizophrenia (González-Maeso et al. Nature, 2008). Co-administration of 5-HT2AR hallucinogenic ligands and mGlu2R antagonists increase hallucinogenic-like behavior in mice, but to the best of our knowledge, no experiments have been conducted with mGluR2 PAMs (Moreno et al. Neurosci Lett, 2011). THH potentiates the effect of glutamate in mGlu2R without any significant intrinsic activity. This effect occurrs at concentrations of tetrahydroharmine achieved in plasma after Ayahuasca ingestion. Additionally, tetrahydroharmine showed positive brain permeability in our experimental models. At the present time, we are aiming to determine whether the action of THH as mGlu2R PAM contribute to the overall effect of Ayahuasca, perhaps expanding the current paradigm in the mechanism of action of beta-carbolines present in the beverage to a two-levels coadjutant role, MAO-A inhibition and mGlu2R PAM. Additionally, we have found that pinoline behaves both as a 5-HT2AR agonist and mGlu2R PAM. Endogenous occurrence of this beta-carboline is controversial, but its structure could represent an interesting pharmacological tool for studying the 5-HT2AR-mGlu2R heterocomplex and its implications on psychosis.

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MTU13 Mechanism of Neuroprotection (Part 1)

MTU13-01

Prevention of retinal ganglion cell degeneration by a selective calpain-2 inhibitor in a mouse model of acute glaucoma

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Primary angle-closure glaucoma (PACG) is a major cause of blindness in the world and is expected to become more prevalent as longevity increases. Angle-closure glaucoma produces a rapid rise in intraocular pressure (IOP), which results in retinal ischemia and retinal ganglion cell (RGC) death. However, the mechanisms by which increased IOP leads to RGC death are not currently understood. Previous studies have also shown that NMDA receptor blockade as well as several calpain inhibitors reduced RGC loss after retinal ischemia. However, none of the studies have specifically addressed the roles of the two major calpain isoforms in the retina, calpain-1 (or μ-calpain) and calpain-2 (or m-calpain). In this study, we first determined the time-courses of calpain-1 and calpain-2 activation following increased IOP by analyzing changes in immunoreactivity for the selective breakdown product of spectrin (SBDP) generated by either calpain-1 or calpain-2 and for PTEN, a calpain-2 selective substrate in the inner plexiform layer (IPL) containing dendrites and synapses of RGC. We compared these time-courses in wild-type (wt) andcalpain-1 knock-out (ko) mice. Interestingly, calpain-1 was rapidly activated in IPL at 2 h following increased IOP, while calpain-2 activation was delayed and was only observed in the retina at 4 h after increased IOP. Increased IOP for 1 h resulted in about 42% decrease in the number of RGC in wt mice after 3 days. Calpain-1 ko mice exhibited more degenerating RGCs (about 63% decrease) than wt mice, further supporting the notion that calpain-1 activation is neuroprotective. Systemic injection of a selective calpain-2 inhibitor (C2I), 30 min before and 2 h after increased IOP prevented calpain-2 but not calpain-1 activation, and promoted RGC survival (about 14% decrease in RGC number). Our data indicate that calpain-2 activation plays a critical role in acute glaucoma-induced RGN degeneration and support the idea that a selective calpain-2 inhibitor has the potential to prevent acute glaucoma-induced blindness.

MTU13-02

Hydrogen sulphide: protective or deleterious?

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H_2S has been reported to be protective against cell injuries/death induced by oxidative stress, toxins such as rotenone and 6-hydroxydopamine (6-OHDA), inflammation and amyloid-β. On the contrary, we have reported that H_2S enhanced cell death in cells under oxygen and glucose deprivation (OGD). In addition, H_2S production increased in ischemic tissues and the reduction in H_2S resulted in significant protection against infarction. Recent study showed that H_2S can serve as an electron donor that can enhance mitochondria electron transport chain (ETC.) and promote cellular bioenergetics. This leads to our hypothesis that the protective effect of H_2S is oxygen-dependent. In our present study, we overexpressed H_2S synthesizing enzyme, cystathionine beta synthase (CBS) in SH-SY5Y cells to produce H_2S endogenously when its substrates homocysteine and cysteine are exogenously supplied. Our results showed that endogenous H_2S enhanced cell death induced by OGD, but reversed cell death induced by rotenone or 6-OHDA treatment. This protection was accompanied by diminished reactive oxygen species (ROS) levels. Antimycin at both 0.5 and 1 μM reversed rotenone or 6-OHDA induced cell death. Furthermore, when cells subjected to OGD were reoxygenated, cell death was reversed. These results support the idea that H_2S, by acting as an electron donor, may be protective by enhancing mitochondrial function. However, under ischemic condition, the same action of H_2S may enhance ROS accumulation and thus become deleterious.

MTU13-03

TIMP-1 loaded nanoparticles: a therapeutic strategy for neuroprotection

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Introduction: There is a marked, deleterious increase in expression of Matrix Metalloproteinase-9 (MMP-9) during numerous pathologic conditions such as ischemic stroke, epilepsy and various excitotoxic/neuroinflammatory processes. Therefore, inhibition of MMP-9 is considered as a potential therapeutic target for neuroprotection. Currently available chemical inhibitors of MMP-9 are poorly specific and have many off-targets leading to unanticipated side effects. As development of specific inhibitors is always a challenging task therefore, we planned to evaluate neuroprotective effects of an endogenous inhibitor of MMP-9, Tissue Inhibitor of Matrix Metalloproteinase-1 (TIMP-1), which is a 28 KDa protein. However, the major obstacles of using TIMP-1 as a neuroprotective agent are its *in vivo* short half-life and low brain permeability. Hence, we planned to explore a nanotechnological approach for delivery of TIMP-1, by using poly lactic-co-glycolic acid (PLGA) based Nanoparticles (NPs), so in the future it can be developed as a neuroprotective agent.

Results: Here, we have developed TIMP-1 loaded PLGA NPs which can deliver TIMP-1 in a sustained release manner and can cross the blood brain barrier (BBB). These NPs were coated with polysorbate 80 (Ps80) to improve their BBB penetration. We evaluated these NPs for their *in vitro* and *in vivo* BBB penetration by using primary rat brain endothelial cell model and by tail vein injection in mice respectively. The *in vitro* and *in vivo* results have

shown that NPs are non-toxic to endothelial cells and they have BBB penetration. Finally, we evaluated their neuroprotective effects on organotypic hippocampal slice culture using propidium iodide staining and LDH assay which have shown that TIMP-1 and TIMP-1 loaded have neuroprotective effects against Kainic Acid (KA) induced excitotoxicity. Moreover, we have shown through gelatinase assay that these effects are mediated through MMP-9 inhibition.

MTU13-04

Melatonin ameliorates branched chain fatty acids-induced neurotoxicity in rat brain synaptosomes

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Phytanic acid (PA) and Valproic acid (VPA) are branched chain fatty acids (BCFAs). PA plays an important role in disease with peroxisomal impairment and VPA is used as an antiepileptic drug and a mood stabilizer. Although, VPA is a relatively safe drug, but its use in higher concentration is associated with idiosyncratic neurotoxicity. We have used synaptosomal preparation of rat brain as an *in vitro* model to evaluate the effect of BCFAs induced neurotoxicity. Synaptosomes are commonly used to study of synaptic functions. Melatonin is also known to be an endogenous free-radical scavenger and a broad-spectrum antioxidant being especially effective in restraining free-radical damage in the brain. The action of melatonin as an antioxidant make it attractive for studying a potential protective role against PA and VPA mediated neurotoxicity by using preparation of synaptosomes isolated from brain of rats as an *in vitro* model. In our study, we investigated the protective effect of melatonin could ameliorate PA and VPA induced alterations in oxidative stress biomarkers in synaptosomes. In this study, co-treatment exposure with melatonin significantly restored the lipid peroxidation (LPO) levels and protein carbonyl (PC) contents. It also replenished the PA and VPA induced neurotoxic effects on altered non-enzymatic antioxidant defence reduced glutathione (GSH) and neurotoxicity markers. Moreover, the inhibitory effect elicited by PA and VPA on cell viability (MTT) and membrane potential was totally prevented by co-incubation with melatonin. We observed that PA and VPA induced oxidative stress in synaptosomes as indicated by an increase in the production of cellular reactive oxygen species (ROS) and melatonin was able to inhibit ROS generation. The results of our investigation emphasize the potential use of melatonin as a nutraceutical and mitigatory agent against BCFAs induced oxidative stress.

MTU13-05

Walnuts reduce the levels of soluble amyloid beta oligomers and increase its clearance in mouse model of Alzheimer's disease

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Soluble oligomers of amyloid beta (Aβ) are neurotoxic in the brain of patients with Alzheimer's disease (AD). We have previously reported that a walnut-enriched diet improves learning skills, memory, and motor coordination, and it reduces anxiety in the Tg2576 transgenic mouse model of AD (AD-tg). In this study, we analyzed the levels of total Aβ and soluble Aβ oligomers in the brain, and of Aβ 40 and Aβ 42 in the blood from AD-tg mice fed a diet with or without walnuts and from wild-type mice. From the age of 4 months, the experimental AD-tg mice were fed diets containing 6% (T6) or 9% walnuts (T9) equivalent to 1 or 1.5 oz, respectively, of walnuts per day in humans. The control groups, i.e., AD-tg and wild-type mice, were fed a diet without walnuts (T0, Wt). Aβ levels were assessed at the ages of 4 (before starting diet with walnuts), 14 and 18 months. At 4 months, Aβ levels (brain and blood) were similar in T0 and Wt groups. At the age of 14 and 18 months, T0 had significantly higher Aβ levels than Wt mice. In the brain, AD-tg mice on diet with walnuts had significantly lower levels of soluble Aβ oligomers compared to T0 mice. The effect of 9% walnuts was significantly higher than that of 6% walnuts. In the blood samples, the levels of Aβ 40/42 were increased in T6 and T9 mice compared to T0 mice, suggesting that walnuts in the diet can increase the clearance of Aβ from brain to the blood. These findings suggest that dietary supplementation with walnuts may have a beneficial effect in reducing the risk, delaying the onset, or slowing the progression of AD by reducing the levels of soluble Aβ oligomers in the brain and increasing Aβ clearance.

MTU13-07

A role for BDNF in mediating adolescent gabaergic interneuron expression

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Cognitive deficits are a common, crippling and prodromal symptom of schizophrenia. Despite this, there is currently no pharmacological treatment available. Gamma-aminobutyric acid (GABA)ergic interneurons in the forebrain play a major role in maintaining cognitive functioning and their expression and function have been found to be altered in schizophrenia patients. Brain-derived neurotrophic factor (BDNF) is a vital neurotrophin that is essential for the development and function of GABAergic interneurons, including guiding their development throughout puberty, a critical period of interneuron maturation. This time period, extending into early adulthood, is also the period with highest incidences of schizophrenia onset. Sex hormone surges during adolescence and exerts organisational effects on the developing brain. Females are noted to have fewer cases, later onset and milder symptoms compared to males and the protective effect potentially arises from the female sex hormone estrogen, which has been shown to induce BDNF expression. However, little is known about the developmental changes of GABAergic interneurons during the dynamic adolescent period in either male or female frontal cortex. Contrasting the trajectories between the sexes may encapsulate the difference that underlies male vulnerability. Here, we examined the interplay between gender and BDNF on GABAergic interneuron development by looking at the expression of GABAergic interneuron markers parvalbumin, somatostatin, calretinin and the GABA synthesising enzyme GAD67 in the medial prefrontal cortex of male and female mice, both wild-type and BDNF heterozygotes, from juvenile age through to adulthood. Compared to wild-types, parvalbumin expression was reduced in male BDNF heterozygous mice but was not altered in female BDNF heterozygotes. Somato-

statin upregulation was delayed in female but not male BDNF heterozygotes. Neither calretinin nor GAD67 expression differed from WT in either sex. This study show case the dynamic and sex-dimorphic influence BDNF has on the maturation processes of GABAergic interneurons across adolescence and may contribute to explaining the palpable sex difference in onset and symptom severity.

MTU13-08

Neuroprotection against neurotoxicity: inconsistent morphological parameters

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For several decades now, the subject of neuroprotection against neurotoxicity has received a great deal of attention in view of potential neuroprotective therapies. However, most of these potential therapies have failed subsequent clinical trials, proofing frustrating and ineffective. Several studies have demonstrated numerous drugs, chemical agents and natural plant extracts to be effective for treating acute stroke in animal models by targeting the inflammatory processes and mediators that contribute to the production of brain injury following ischemic stroke; ion channel modulators such as calcium channel blockers; glutamate receptors such as anti-excitotoxic agents or apoptosis (programmed cell death) inhibitors; neurotrophic factors or production of highly reactive oxygen free radicals as their mechanisms of neuroprotection. Although evidence from preclinical studies has been exciting and many drugs have progressed to multi-center clinical trials, none of the neuroprotective agents has been proven to be clinically beneficial. One important discrepancy that stands out in many of these studies is an inconsistency in a demonstrable morphology following evidence of neuroprotection. Noting that cells may rapidly become nonfunctional after the onset of injury (irrespective of the etiology), although they are still viable, with potentially reversible damage; a longer duration of injury may eventually lead to irreversible injury and cell death, this cell death typically precedes ultrastructural, light microscopic, and grossly visible morphologic changes. Other factors affecting findings in neuroprotection/neurotoxicity studies include study design – in-vivo versus in-vitro; mode of administration of toxic agent; bioavailability of agent; neuronal integrity and role of supporting glia. By identifying a demonstrable morphological parameter from neuroprotection following neurotoxicity, we may be able to set guidelines for future effective Neuroprotective/Neurotoxicity studies that will diminish the discrepancies between preclinical studies and clinical trials.

MTU13-09

Tau depletion prevents brain damage in a mouse model of stroke with reperfusion

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Stroke is a major cause of death. The majority are ischemic strokes resulting from arterial occlusion or hemorrhages. Even if the therapeutic restoration of the blood flow is successful, reperfusion of the brain areas surrounding the ischemic core is associated with progressive damage of neurons. Excitotoxicity contributes to brain damage after stroke. However, the molecular mechanisms involved are incompletely understood. We have previously shown that amyloid-β (Aβ)-induced excitotoxic neuronal damage in Alzheimer's disease (AD) is mediated by tau. Here, we show that tau also mediates neuronal damage following reperfusion after stroke in mice. We used tau-deficient ($tau^{-/-}$) mice together with two experimental models of stroke, permanent middle-cerebral artery occlusion (MCAO) and transient MCAO for 1.5 h with subsequent reperfusion. While experimental stroke with reperfusion resulted in progressively worsening neurological deficits and significant brain damage in wild-type, $tau^{-/-}$ mice showed protection from progression of mild neurological deficits and markedly reduced infarct sizes 24 h after MCAO. In contrast, permanent MCAO caused severe neurological deficits and large infarct sizes in both wild-type and $tau^{-/-}$ mice. This suggests a threshold for the protection from excitotxiciy by tau depletion. Accordingly, $tau^{-/-}$ mice are protected from severe excitotoxic seizures induced by low but not high doses of pentylenetetrazol. Taken together, lack of tau prevents brain damage in a mouse model of stroke with reperfusion, implicating tau in acute brain damage beyond its role in AD.

MTU13-10

Adult vitamin D deficiency is associated with impaired cognition, oxidative stress and vulnerability to second hit exposures

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Glutathione, the body's main endogenous antioxidant, neutralises reactive oxygen species, free radicals, and other toxins. While oxidative stress has been linked to a number of neuropsychiatric disorders, vitamin D supplementation upregulates antioxidant defense mechanisms, including increasing glutathione levels which may contribute to neuroprotective properties. The aims of this study were to determine if adult vitamin D (AVD) deficiency impaired glutathione synthesis and cognitive processes, and leads to increased vulnerability to second hit exposures, such as social stress. Adult male BALB/c mice were fed either a vitamin D deficient diet or a diet containing vitamin D (1000 IU/kg) for 10 weeks. Glutathione was measured via HPLC and protein expression changes were analysed using ITRAQ proteomics. Mice were either food restricted and trained on the 5 choice serial reaction task to measure attention or underwent a social defeat paradigm for 10 days followed on the eleventh day by a social interaction test. AVD-deficient mice had a reduction in total glutathione levels compared to controls, and protein expression changes in the gamma-glutamyl cycle, which is responsible for the production of glutathione. The proteomics data also revealed expression changes in proteins important for glutamate and GABA metabolism. In the attention task, AVD-deficient mice made significantly fewer correct responses, compared to control mice, when stimulus duration was reduced. They also made more omissions and took longer to make correct responses. Finally, AVD-deficiency exacerbated the effects of social stress in a social interaction test. We have now shown that AVD deficiency leaves the brain vulnerable to oxidative stress and provided further evidence of changes in glutamate and GABA metabolism following AVD deficiency. We have also shown that these changes may be important in cognitive processes, with AVD deficient mice exhib-

iting mild cognitive impairment. Furthermore, we have shown that AVD deficient mice are more vulnerable to social stress compared to controls. Therefore, oxidative stress may be a key underlying mechanism behind vitamin D deficiency's effects on the brain.

MTU13-11

Oligodendroglial exosomes protect neurons and influence axonal transport

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In the CNS, myelination acquires an intimate communication between oligodendrocytes and neurons not only for a fast saltatory conduction but also to maintain axonal integrity, which is impaired in mice lacking the myelin proteins PLP and CNP. We examined the role of oligodendroglial exosomes in axon-glia communication and their specific impact on neuronal physiology. Exosomes are nano-sized, endosomal-derived vesicles carrying a specific set of lipids, proteins, and RNAs to target cells. Recently, we have provided evidence that neuronal electrical activity controls glial exosome release resulting in subsequent neuronal uptake and functional retrieval of the exosomal content. In addition, functional assays revealed a role in neuroprotection by desensitizing neurons towards cellular stress. Here, we analyze the underlying mechanisms of exosome-mediated neuroprotection using different experimental approaches. We demonstrate that oligodendroglial exosomes ship protective cargo to neurons increasing their stress tolerance, potentially via the transfer of protective enzymes such as catalase and superoxide dismutase. Moreover, phosphorylation arrays revealed altered signal transduction pathways involved in cell survival upon exosome administration. In order to evaluate the exosomal influence on axonal transport, we analyzed the movement of BDNF-containing vesicles in neurons by time-lapse microscopy. We found a reduced percentage of pauses exhibited during overall movement as well as a significantly lower number of static vesicles upon exosome treatment and oxidative stress exposure compared to control cells. It has been shown that axonal transport is affected in PLP-deficient mice. Intriguingly, exosomes derived from PLP and CNP nulI oligodendrocytes do not benefit neuroprotection. We therefore addressed the question whether these exosomes impact axonal transport.

In summary, our study indicates that oligodendroglial exosomes mediate neuroprotection by the transfer of protective biomolecules to neurons, activation of prosurvival signaling pathways and by promoting axonal transport.

MTU13-12

Neuroprotective propensity of bacopa monniera *in vitro* (PC12 cells) and *in vivo* (wistar rats)

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Scopolamine is a competitive antagonist of muscarinic acetylcholine receptors and thus classified as an anti-muscarinic and anticholinergic drug. PC12 cell lines possess muscarinic receptors and mimic the neuronal cells. These cells were treated with different concentrations of scopolamine for 24 h and were protected from the cellular damage by pre-treatment with BME.

Our results elucidate that pre-treatment of PC12 cells with BME ameliorates the mitochondrial and plasma membrane damage induced by scopolamine as evidenced by MTT and LDH leakage assays respectively. Further, Cognitive-enhancing activity of BME was evaluated against scopolamine-induced amnesic rats *in-vivo* by the novel object recognition test (NOR), elevated plus maze (EPM) and by Morris water maze (MWM) tests. Scopolamine (2 mg/kg body wt, i.p.) was used to induce amnesia in rats. BME at three different dosages (i.e., 10, 20 and 40 mg/kg body wt.) improved the cognition of rats as evaluated by behavior studies. BME administration has normalized the neurotransmitters levels that were altered by scopolamine administration in hippocampus of rat brain. BME supplementation also ameliorated scopolamine effect by down-regulating AChE and up-regulating BDNF and muscarinic M1 receptor expression in brain hippocampus and PC12 cells confirms the potent neuroprotective role. BME administration showed significant protection against scopolamine-induced toxicity by restoring the levels of antioxidant and lipid peroxidation. These results indicate that, cognitive-enhancing and neuromodulatory propensity of BME is through modulating the expression of AChE, BDNF, MUS-1 and also by altering the levels of neurotransmitters levels in hippocampus of rat brain.

MTU13-13

Targeting PPAR-gamma modulates the peripheral neuropathic pain: possible biochemical, mitochondrial evidences

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Neuroinflammation and oxidative stress has been suggested to play key role in the development and maintenance of the neuropathic pain. Mitochondrial dysfunction was regarded to play crucial role in the development of oxidative stress and neuroinflammation. The present study was designed to explore the role of neuroinflammation, oxidative stress in the spinal nerve ligation (SNL) induced neuropathic pain with the help of various behavioral, biochemical, mitochondrial and cellular alterations in rats. In the present study, unilateral lumbar L5 and L6 spinal nerves were ligated to induce neuropathic pain in rats. Behavioral parameters were assessed on the day before ligation and successively on day 7th, 14th, 21st and 28th post ligation. Neuroinflammatory, oxidative stress parameters and mitochondrial enzyme functions were assessed on day 28 after behavioral observations. SNL resulted in significant increase in mechanical allodynia, mechanical hyperalgesia, cold allodynia and heat hyperalgesia as assessed by Vonfrey, Randall Selitto, Acetone drop and Hot plate tests respectively. SNL also resulted in significant increase in oxidative stress parameters (increased lipid peroxidation, nitrite, reduced superoxide dismutase, catalase and glutathione) in lumbar spinal cord. Mitochondrial enzyme complexes activities were significantly inhibited by SNL. Pioglitazone (a PPAR gamma agonist) treatment (10 and 20 mg/kg, i.p.) for 28 days significantly reversed the various behavioral,

biochemical and mitochondrial alterations in SNL treated animals. Results of the present study shown that ameliorative potential of pioglitazone in SNL induced behavioral and mitochondrial alterations which may be further attributed to inhibition of oxidative stress and mitochondrial dysfunction in rats.

MTU13-14

Metformin can alleviate the age-related retinal vulnerability to pressure injury by reducing oxidative stress level

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Metformin is an extensively used anti-diabetic agent; beyond its hypoglycemic effect metformin possesses some neuroprotective properties as well. Metformin mechanisms of action are not fully understood, but it is acknowledged that AMPK activation is required for many of metformin effects. The AMP-activated protein kinase (AMPK) proposed to play a central role in various neuroprotective interventions. The aim of this study is to verify whether metformin could limit retinal ganglion cell (RGC) age-related vulnerability to pressure-induced injury. First set of experiments involved aged mice treated with 300 mg/kg metformin in drinking water for 6 weeks ($n = 8$ and 10 metfromin and control respectively) while second cohort of aged mice received metformin intraperitonealy (IP) in 2 different doses and frequencies (250 mg/kg daily and 200 mg/kg 3 days per week; $n = 7$ and 11 metformin and control respectively) for 3 weeks. Pressure injury involved an acute elevation of intraocular pressure (IOP) through cannulating the anterior chamber of the eye. Retinal function was assessed using electroretinography (ERG) before and after IOP injury. Protein levels of oxidative stress markers and AMPK measured using immunoblotting. While Metformin in drinking water was not able to inhibit retinal dysfunction, IP metformin almost fully reversed age-related inner retinal dysfunction following injury ($p < 0.001$). IP metformin treatment significantly diminished oxidative stress markers expression in injured retinas ($n = 5$/group). Surprisingly, activated form of AMPK (phospho-AMPK) expression in retina was comparable between IP metformin and control ($n = 5$/group). In conclusion: Our studies showed that oral metformin does not provide any protection against IOP injury whereas intraperitoneal metformin significantly improves the old eye's response to IOP injury. Oxidative stress reduction plays a role in this neuroprotective effect of metformin. Although phospho-AMPK expression was similar in IP metformin and control mice tissues it could simply reflect the instability of phospho-AMPK as tissues harvested 24 hour after last IP injection.

MTU13-15

Melatonin enhances autophagic activity via rela P65 deacetylation activity by sirtuin 1 in senescence-like state in SH-SY5Y cells

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Autophagy is a major route for the bulk degradation of abnormal cytosolic macromolecules and organelles. This process plays a pro-survival role by maintaining cellular homeostasis. However, autophagy diminishes in a senescent cell which is considered as an aging characteristic. Melatonin is a hormone mainly secreted by the pineal gland and plays a wide range of physiological functions. This hormone exerts its anti-aging effect, possibly through the regulation of Sirtuin1 (SIRT1) pathway. The deacetylation ability of SIRT1 is important for controlling several transcription factors function including NF-κB. Apart from inflammation, NF-κB can regulate autophagy by inhibiting Beclin1, the initiator of autophagy. Although numerous studies have revealed the role of melatonin in regulation of autophagy, very limited experiments showed that melatonin could increase autophagic activity via SIRT1 in a senescent model. In this study, the pro-autophagic effect of melatonin through deacetylation activity of SIRT1 on RelA p65 was investigated in hydrogen peroxide (H_2O_2)-induced cellular senescent SH-SY5Y neuroblastoma cells. Our results demonstrated that melatonin could increase protein level of SIRT1, Beclin1, and LC3-II, a hallmark protein of autophagy, and reduced the level of acetylated-Lys310 in p65 subunit of NF-κB in SH-SY5Y cells treated with H_2O_2. Furthermore, in the presence of Sirtuin1 inhibitor, melatonin failed to increase the autophagic markers. The present data indicated that melatonin enhanced autophagy via SIRT1 pathway. Taken together, we provided a novel mechanism of melatonin in modulating autophagy which could be a potent target for anti-aging therapy.

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MTU13-16

Pharmacokinetic properties of a dimeric inhibitor of postsynaptic density protein-95 in rats

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Pharmacological inhibition of the interaction between the post-synaptic density protein-95 (PSD-95), neuronal nitric oxide synthase (nNOS) and the *N*-methyl-*D*-aspartate receptor (NMDAR) has recently emerged as a promising therapeutic strategy in the treatment of stroke. In the current study we present pharmacokinetic data for UCCB01-145; a newly developed dimeric inhibitor that perturbs the

PSD-95/nNOS/NMDAR interaction by binding the PDZ1-2 domains of PSD-95. In addition, we outline experiments investigating the effects of PSD-95 inhibition following traumatic brain injury (TBI) in rats. UCCB01-145 is labelled with 5-carboxyfluorescein and uptake into the CNS was determined after IV injection into the tail vein of rats (7.5 mg/kg). Rats were sacrificed at 15, 30, 60 and 120 min and drug concentrations were determined in plasma and brain by HPLC with fluorescence detection. In plasma AUC = 136 μM × min, and $t_{1/2\beta}$ = 37.6 min. In brain AUC = 39.7 nmol/g x min, C_{max} = 0.398 ± 123 (SEM) nmol/kg, and t_{max} = 60 min. Unbound fraction of drug in brain (f_u) = 0.115, and AUC for unbound drug = 4.56 nmol/g x min. The results of the current study indicate that UCCB01-145 permeates the blood-brain barrier and remains in the brain within a therapeutically relevant time window in rats. Pharmacological inhibition of PSD-95 has shown to be neuroprotective in animal models of stroke and in humans with iatrogenic stroke. Since the pathogenesis of stroke partially overlaps with secondary injury mechanisms leading to cell death after TBI, it is possible that the beneficial effects of PSD-95 inhibition will translate from stroke to brain trauma. We are currently investigating this by administering dimeric inhibitors of PSD-95 in the controlled cortical impact model of TBI in rats.

MTU13-17

Pair housing reverses the detrimental effect of social isolation in aged mice after stroke

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Background: Age is an important non-modifiable risk factor for stroke. Stroke rates double every decade after age 55. Social isolation (SI) exacerbates behavioral deficits, slows functional recovery and worsens the histological injury after stroke in young animals, primarily by increasing the inflammatory response. However, the inflammatory response differs in aging, and whether the detrimental effects of SI are also seen in aged animals is unknown. We hypothesize that acute and chronic post stroke SI will worsen stroke pathology and recovery in aged mice and that pair housing (PH) will reverse these effects.

Methods: Eighteen-month-old male C57BL/6 mice were pair housed (PH) for two weeks prior to stroke and randomly assigned to various housing conditions immediately after stroke. Behavioral analysis was done weekly beginning at day 7. Mice were sacrificed either at 72 hours or 4 weeks after a 60-minute middle cerebral artery occlusion (MCAO) or sham surgery (n = 9–10/group).

Results: Mice isolated after stroke (ST-ISO) had significantly greater infarct volumes and neurological deficit scores ($p < 0.05$. n = 13/group) compared to pair-housed (PH) stroke mice, and significantly higher plasma IL-6 levels compared to SH-PH ($p < 0.001$, n = 13/group) or ST-PH mice ($p < 0.05$) 72 h after stroke. Levels of IL-6 and the amount of post-stroke atrophy were similar between groups 4 weeks post-stroke (n = 9–14/group). A significant interaction [F (1, 28) = 259.6, $p < 0.001$] between housing and stroke was found in the Novel Object Recognition Task (NORT) at day 14. Pair housing reinstated the expression of brain-derived neurotrophic factor (BDNF),synapsin-1 and myelin basic protein (MBP) vs. isolated animals.

Conclusions: Social isolation immediately after stroke enhanced early injury and delayed behavioral recovery. Pair housing reversed these effect by restoring BDNF and myelin levels. This suggests that the beneficial effects of pair housing extend to aged animals. Further, these BDNF-mediated effects are facilitated by increased activation of synapsin 1.

MTU13-18

Nerve growth factor (NGF) and artemin regulate neurite outgrowth differently both in normal and injured adult rat sensory neurons

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NGF promotes neural regeneration and functional recovery after nerve injury but the potential therapeutic benefits are limited by hyperalgesia. Many NGF-sensitive sensory neurons also express GFRα3/Ret, which are co-receptors for artemin, a member of the glial cell-line derived neurotrophic factor (GDNF) family of ligands (GFLs). The positive effects of artemin include enhanced regeneration, recovery of sensory functions, and reductions of sensory hypersensitivity in rodent models. However, this broad activity is inconsistent with GFRα3 expression being restricted to a subset of NGF-sensitive or peptidergic C-fibres and so, the underlying biological mechanisms remain unknown. We have studied NGF and artemin using *in vitro* neurite outgrowth assays in adult rat dorsal root ganglion (DRG) neurons, before and after preconditioning by *in vivo* nerve injury. In this peptidergic neuron type, NGF increased neurite initiation, elongation and branching but artemin could only increase neurite initiation and was less potent(n = 3). Inhibitors of PI3-kinase and Src-kinase completely blocked this effect of artemin, but could only reduce the effects of NGF(n = 3). Preconditioning with *in vivo* axotomy is commonly used to induce a pro-regenerative state in DRG neurons. This facilitated spontaneous neurite initiation and outgrowth in somatic sensory afferents, which had a ceiling effect that occluded further facilitation by NGF or artemin. Visceral sensory afferents responded differently to preconditioning as baseline neurite initiation was below the ceiling, which allowed further increases by NGF or artemin(n = 3), but no new effect of artemin on neurite extension was seen(n = 3). These data conclude that artemin facilitates neurite initiation in the peptidergic sensory neurons, but it is less potent than NGF and does not mimic its neurotrophic actions. This selective *in vitro* activity of artemin is inconsistent with the broader pharmacological effects of *in vivo* systemic treatment being mediated by GFRα3/Ret signaling in peptidergic sensory neurons.

MTU13-19

Melatonin modulates methamphetamine-induced NFκB activation and TNFα overexpression through the MT1/MT2 receptor

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Methamphetamine is a well-known psychostimulant drug of abuse that causes a serious public health worldwide. In addition to its addictive effect, methamphetamine exposure has been shown to be associated with neuroinflammation of several brain areas. Several lines of evidence indicate that TNFα plays an impoe role in methamphetamine-induced neuroinflammatory processes, resulting apoptotic cell death. Many investigators have demonstrated the anti-neuroinflammatory effects of melatonin by mechanism which is still unclear. The objective of this present study is to evaluate the effect of melatonin on methamphetamine-induced NFκB activation and TNFα overexpression in SH-SY5Y cell line. We found that pretreatment with 100 nM melatonin could prevent IκB degradation and NFκB nuclear translocation caused by methamphetamine exposure. These protective effects were reversed by pre-incubation with 1 μM luzindole, a melatonin MT1/MT2 receptor antagonist. Furthermore, methamphetamine-mediated TNFα overexpression was also suppressed by pretreatment with melatonin, and pretreatment with luzindole diminished this attenuating effect. From these findings, we proposed that melatonin exerts its protective effects on methamphetamine-induced neuroinflammation through the MT1/MT2 membrane receptor *in vitro*.

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MTU13-20

Protective role of neuregulin 1 in inflammatory cytokine induced endothelial barrier hyperpermeability

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Neuroinflammation contributes to the diverse pathophysiology conditions including stroke and traumatic brain injury, resulting in neurodegeneration and loss of neurological function. The response of the microvascular endothelium often contributes to neuroinflammation. Neuregulin-1 (NRG1) is an endogenous growth factor with multiple functions in the embryonic and postnatal brain. Within the brain, NRG1 functions have been studied most extensively in neurons and glia. Recently, NRG1 signaling has been found to be important in the function of brain microvascular endothelial cells. In current experiments we have investigated the pathways through which NRG1's acts on endothelial barrier. Our data show that NRG1-β enhances barrier function by increasing the barrier tightness which was measured by trans-endothelial electrical resistance (TEER). Furthermore, NRG1-β protects against inflammatory cytokine induced decrease in TEER and augment of barrier leakage. An investigation of key signaling proteins suggests that by binding to its receptor ErbB3 on endothelial cell membrane, NRG1-β effects on endothelial permeability through RhoA activation and myosin light chain kinase phosphorylation and reservation of cell periphery-associated cytoskeleton. The fact that NRG1 interacts with RhoA,- mediated pathways, which affect key cellular processes including permeability regulation, lamellopodia formation, actin cytoskeleton maintainence and remodeling, suggests that NRG1 plays a major role in the function of brain microvascular endothelial cells in the setting of neuroinflammation.

MTU13-21

In vivo activation of adenosine monophosphate kinase might have neuroprotective potential in experimental diabetic neuropathy

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This study evaluated the role of pharmacological efficacy of small molecule activator of adenosine monophosphate kinase (AMPK) (A769662) in Streptozotocin (STZ) induced diabetic neuropathy in rats by assessing the neuro behavioural, functional, biochemical and molecular assessment. Motor and sensory nerve conduction velocities (MNCV & SNCV) were obtained using power lab in sciatic posterior tibial conducting system. Sciatic nerve blood flow was assessed using laser doppler oxy meter. Mechanical hyperalgesia was assessed by examining the paw withdrawal threshold by von frey and randal selitto callipers. Thermal hyperalgesia was examined by evaluating the reactivity of tail to hot and cold water stimuli. Treatment of STZ induced rats with A769662 at two doses (15 & 30 mg/kg, i.p.) during the last two weeks of 8 weeks old diabetic rats significantly enhanced MNCV, SNCV and NBF in the sciatic nerves when compared with age matched diabetic rats. Von frey and randal selitto assessment showed that A769662 has shown considerable increase in the paw withdrawal threshold in STZ induced diabetic rats. Similarly, AMPK activator also enhanced the tail flick latencies towards cold and hot stimuli. Overall, the results obtained in the study have indicated that A769662 could alleviate the mechanical and thermal hyperalgesia and nerve conduction deficits associated with diabetic neuropathic pain. Further biochemical evidences showing the link between AMPK and mitochondrial function and *in vivo* expression of AMPK and its downstream signalling proteins is underway to validate the use of AMPK activators in diabetes associated peripheral neurological complications.

MTU14 Autonomic-Autonomic/Neuroendocrine Systems

MTU14-01

Central neuronal pathways activated by leptin and resistin

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Obesity is a state of the organism characterized by excessive body weight. Obesity is a public health burden at present, with most advanced countries suffering from an unprecedented rise in obesity rates. Two adipokines, hormones secreted by adipose tissue, include leptin, and resistin. These adipokines are known to act in the hypothalamus, a region of the brain important for cardiovascular function, feeding behaviours and energy regulation. Leptin also activates other areas of the brain including the midbrain and medulla oblongata. Whether these areas are also activated by resistin is not clear. We hypothesised, that resistin and the combination of resistin and leptin will activate the same neurons that are activated by leptin in some brain nuclei but may differ in others and this may reflect functional differences. This study investigated the neurons in paraventricular nuclei PVN), arcuate nuclei (ARC) and supraoptic nuclei (SON) in the hypothalamus, periqueductal gray (PAG), nucleus of the solitary tract (NTS), rostral ventrolateral medulla (RVLM) and raphe pallidus nuclei (RPaN), that are activated by centrally administered resistin and leptin alone, and when those adipokines are administered in combination. The adipokines were administered intracerebroventriculaarly into anesthetised rats. Three hours later the rats were killed and the brains perfused. Sections of hypothalamus, PAG and medulla were processed immunohistochemically to detect the protein Fos, a marker of neuronal activation. Leptin and resistin alone dramatically increased neuronal activation in all areas examined. When leptin and resistin were combined, the increase in Fos positive neurons in the ARC, and in the lamina terminalis (OVLT and MnPO), was significantly greater than following each adipokine individually. The results suggest that leptin and resistin may act on different neurons in the ARC and lamina terminalis.

MTU14-02

Sensory signalling of gut contractility

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Sensations from the gut are carried by afferent axons travelling in vagal and spinal nerves. Discomfort and pain from the gastrointestinal tract are readily evoked by large distensions imposed on the gut. However, distensions also activate contractile activity, through activation of enteric neural reflexes. Here we recorded how extrinsic sensory pathways are activated by contractile activity generated by applied distension. Short (2–3 cm) tubular segments of ileum intestine were obtained from humanely killed guinea pigs and set up, *in vitro*. Video recordings recorded movements of the gut wall, shown as spatiotemporal maps; transducers recorded intraluminal pressure at either end; conventional extracellular recordings were made from mesenteric nerves and the segment was arterially perfused at 200ul/min. The gut lumen was distended by 5,10,20 or 40 cm water and effects on afferent firing determined. Small distensions (5,10 cm water) evoked bursts of peristaltic contractions; 20 cm water distensions evoked peristaltic contractions that quickly fatigued and 40 cm water distensions (noxious range) evoked large increases in diameter without measurable contractions. Two patterns of sensory firing were evoked by peristaltic contractions (32 units, n = 9). Twelve units had bursts of firing coinciding with peak diameters which occurred while pressure was still rising. Another 5 units showed peak firing occurring during contractions, when diameter was shortening. No units had firing closely associated with intraluminal pressure. With larger distensions (20 and 40 cm water) firing became continuous (in 24/32 units at 40 cm water) and less related to individual contractions. More units were activated by higher distending pressures than lower pressures. Sensory firing from the small intestine is more clearly associated with diameter, rather than intraluminal pressure changes, during distension-evoked contractions.

MTU14-03

Prolactin-dependent regulation of enkephalin expression in the tuberoinfundibular dopaminergic neurons of the lactating mouse

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During late pregnancy and lactation the tuberoinfundibular dopaminergic (TIDA) neurons, located in the arcuate nucleus of the hypothalamus, exhibit a remarkable plasticity, with the induction of enkephalin expression and corresponding reduction in dopamine synthesis. This study aimed to test the hypothesis that this lactation-associated plasticity is dependent on prolactin. Quantitative RT-PCR on tissue micro-punched from the arcuate nucleus of the mouse showed a significant (approximately 10-fold) increase in pro-enkephalin A mRNA expression in late pregnant (day 18) compared to diestrus mice. This enhanced enkephalin expression was maintained into lactation (day 7) and was mirrored by a decrease in tyrosine hydroxylase mRNA levels. To determine if enkephalin protein levels were also increased a series of 30um hypothalamic sections were prepared from colchicine-treated (30ug icv) diestrus or lactating mice and processed for met-enkephalin immunohistochemistry. Data analysis showed that the number of enkephalin positive cells in the arcuate nucleus markedly increased, from 6 $\pm$ 2 in diestrus, to 67 $\pm$ 7 in lactation (n = 5). Suppression of endogenous prolactin for 24 h by bromocriptine administration (200ug/sc every 8 h) in lactating mice suppressed the rise in the number of enkephalin positive cells to 32 $\pm$ 7 (n = 5–7). Conversely, administration of exogenous prolactin to these animals overcame the inhibitory effect of bromocriptine with the number of enkephalin positive cells rising to 91 $\pm$ 5 (n = 5–7). The suggestion raised by these studies that the lactation-associated rise in enkephalin expression is prolactin-dependent was further supported by data

showing that mice carrying a neuron-specific prolactin-receptor deletion failed to exhibit this response, with the number of enkephalin positive neurons in the arcuate nucleus remaining unchanged from that observed in diestrus, null controls. This study demonstrates that the number of TIDA neurons expressing enkephalin is increased during late pregnancy and lactation through a prolactin receptor dependent mechanism. The suppression of the dopaminergic activity and rise in enkephalin-expression in the TIDA neurons has potentially profound implications for the maintenance of prolactin secretion during lactation.

MTU14-04

Enteric inhibitory motor neurons in the guinea pig are insensitive to the action potential blocker lidocaine

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The enteric nervous system mediates many intestinal motor patterns due to the coordinated activity of excitatory and inhibitory enteric motorneurons. Lidocaine is a local anaesthetic that acts on neurons by blocking the generation of action potentials from the cytoplasmic side acting on voltage dependent Na channels. We investigated the action of lidocaine on intestinal motor patters and on transmission from enteric motor neurons mechanically and electrophysiologically. We used segments of small and large intestine taken from guinea-pigs killed humanely. From video recording of gut segments placed in warmed oxygenated Krebs, spatiotemporal maps of changes in diameters (DMaps) were constructed to visualize motor patterns. The colonic migrating motor complexes were blocked by tetrodotoxin (0.6 μM) but not by lidocaine (1 mM). They were also blocked by L-NOARG (100 μM) and by a P2Y receptor antagonist (MRS2179; 10 μM), indicating that enteric inhibitory neurons were still active after lidocaine. In the small intestine, distension-evoked peristaltic contractions were blocked by both lidocaine and tetrodotoxin, as was cholinergic excitatory transmission to longitudinal muscle strips. Intracellular electrode recording, showed that transmission of the enteric inhibitory neurons, recorded as inhibitory junction potentials, were not affected by 300 μM lidocaine and only minimally reduced by 1 mM lidocaine, but were blocked by tetrodotoxin. Similarly in strips of colon after hyoscine (10 μM), the nerve mediated relaxations evoked by transmural electrical stimulation were not blocked by lidocaine. The results reveal that the action potentials of the enteric inhibitory motor neurons appear to be mediated by lidocaine insensitive, voltage dependent sodium channels. The existence of lidocaine-resistant motor complexes suggest that other enteric neurons, upstream of inhibitory motor neurons, may also utilise a lidocaine-resistant sodium channel. Antibodies raised against the lidocaine insensitive channels may confirm their presence in specific subsets of enteric neurons.

MTU14-05

Projections from the inferior colliculus drive putative sympathetic, respiratory and motor populations in the ventral medulla

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We have recently shown that disinhibition of deep layers of the inferior colliculi unmasks coordinated cardiovascular, somatomotor and respiratory responses to multimodal sensory stimuli in the anesthetized rat. As these effects were maintained following extensive decerebration, we proposed that they were mediated by a previously undescribed projection from the inferior colliculus to pontine, brainstem or spinal cardiorespiratory and motor control nuclei. Here we directly examine that hypothesis. Efferent projections from the inferior colliculus were labelled by an AAV vector that drives fluorescent reporter expression. Three weeks after injection into the inferior colliculi, rats were sacrificed and brains examined for evidence of terminal labelling in regions known to drive sympathetic, respiratory or motor outputs. Vector injections confined to the region that drives physiological responses resulted in extensive pontine and medullary projections, with little evidence of projections innervating targets in the spinal cord or rostral to the hypothalamus. The greatest density of terminal labelling was observed within the rostral ventromedial medulla and Raphe nuclei and included putative synaptic contacts with spinally projecting neurons. In contrast, no labelling was apparent in other respiratory or cardiovascular control nuclei in the ventrolateral medulla. The functional significance of these projections was confirmed in electrophysiological experiments *in vivo*, in which stereotypical sympathetic, respiratory and motor responses evoked by disinhibition of the colliculus, recorded in the splanchnic, phrenic and sciatic nerves respectively, were blocked by microinjection of GABA agonists in the region of densest terminal labelling. We conclude that a previously undescribed projection from the colliculus to the RVMM/Raphe may play a key role in driving co-ordinated cardiorespiratory and motor responses to alerting sensory stimuli.

MTU14-06

Neuromechanical factors involved in the formation and propulsion of fecal pellets in the guinea-pig colon

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Background: The neuromechanical processes involved in the formation and propulsion of fecal pellets remains incompletely understood.

Methods: In isolated segments of the guinea-pig proximal and distal colon, we analysed motor patterns, using video imaging, during oral infusion of liquid, viscous material or solid pellets.

Results: Colonic migrating motor complexes (CMMC) in the proximal colon, separated liquid or semisolid contents into elongated shallow boluses. At the colonic flexure these boluses were segmented into shorter pellet shaped boluses. In the empty

distal colon, the spontaneous distal colonic migrating motor complexes were replaced by pellet shaped boluses with oral infusion of liquid or viscous material. These were propelled at speeds proportional to the surface area of the boluses. Solid pellets were propelled at a speed that increased with size to a maximum (corresponding to the size of natural pellets). Pellet speed could be reduced by increasing the load. Hexamethonium insensitive, but TTX sensitive, circular muscle contractions confirmed fluid propulsion by neurally mediated pathways that do not involve cholinergic interneurons.

Conclusions: CMMCs are responsible for the slow propulsion of the semisolids contents in the proximal colon, while the formation of pellets at the colonic flexure involves a content dependent modulation of content independent distal colonic migrating motor complexes. Bolus size and consistency affects propulsion speed suggesting that propulsion is not a reflex action but rather a process involving an adaptable neuromechanical loop.

MTU14-07

Opioid receptors into medial amygdaloid nucleus modulate neuroendocrine responses evoked by restraint stress in rats

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Introduction: The medial amydaloid nucleus (MeA) modulates several physiological and behavioral processes, including autonomic and endocrine changes during aversive situations. The restraint stress (RS) causes significant increase in neuronal activity of the MeA when compared to other amygdaloid nuclei. In addition, lesions in MeA reduced the RS-activated neurons in PVN. The opioid system participates of the mediating neuroendocrine responses, including those associated with aversive situations. Furthermore, it was showing the presence of peptides and opioid receptors in amygdala, suggesting the existence of a functional opioid neurotransmission on that structure. Based on the facts mentioned above, the hypothesis of this study is that the MeA opioid neurotransmission is involved in the modulation of hormonal responses evoked by RS.

Methods: Male Wistar rats (240–280 g) were used. Guide cannulae were implanted bilaterally in the MeA for drug or vehicle (artificial cerebrospinal fluid, aCSF, 100 nL) microinjection. 10 minutes before microinjection of drugs or vehicle into the MeA, rats were subjected to RS. For collect of samples to corticosterone assays, rats were decapitated at time 0, 20 or 60 min of restraint stress and the blood were collected in EDTA tubes (1 mg/mL of blood). Plasma samples were used to measured the corticosterone level by enzyme immunoassay.

Results: The MeA pretreatment with 0.03 nmol of nor-BNI (κ-opioid antagonist - ($F_{1,29}$=39,86, $p < 0.0001$) or 0.03 nmol of UPF-101 (ORL-1 antagonist - $F_{1,26}$=24,46, $p < 0.0001$) potentiated the increase in corticosterone levels, while the microinjection of 0.03 nmol of cyprodime (μ-opioid antagonist - $F_{1,28}$=0,51, P = 0,48) or 0.03 nmol of naltrindole (δ-opioid antagonist - $F_{1,29}$=0,24, P = 0,62) did not change de neuroendocrine and cardiovascular responses induced by RS when compared with vehicle group.

Conclusion: The current results demonstrate that opioid neurotransmission mediates the MeA inhibitory influence on restraint-evoked neuroendocrine changes.

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MTU14-08

Differential effects of subchronic and chronic corticosterone administration on circadian glucocorticoid rhythms IN C57BL/6 mice

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Introduction: Circulating glucocorticoid levels are regulated by the hypothalamic-pituitary-adrenal (HPA) axis activity and exhibit a circadian rhythm characterised by a peak at the start of the active phase (dark cycle for rodents). The activity of the HPA axis increases under the exposure to a stressor, and multiple exposures to stress alters HPA axis activity. Thus, we wanted to examine whether the duration of sustained HPA axis activation impacts on the normal circadian rhythm of circulating glucocorticoids.

Methods: C57BL/6 male mice were administered 25 mg/l of corticosterone (CORT) via their drinking water for either 7 days or 28 days as a model of acute or chronic HPA axis activation respectively. Control animals received untreated drinking water. Blood was collected from mice at 6am, 10am, 2 pm, 6 pm and 10 pm to quantify serum CORT levels using EIA. qPCR was used to examine gene expression in the hypothalamus and pituitary.

Results: 28 days, but not 7 days, of CORT administration significantly shifted the circadian pattern of corticosterone. Compared to controls, treated mice had significantly higher serum corticosterone levels during the dark phase, and significantly lower levels during the light phase ($p < 0.0001$). 7 days of CORT administration was sufficient to cause a shift in the circadian pattern of dopamine D2 receptor gene expression in the pituitary.

Implications: Our preliminary findings indicate that the physiological consequences of chronic stress modeled by 28 days of CORT-administration are in fact preceded by subtle changes in the pituitary. More specifically, subchronic stress induces a change in dopamine D2 receptor mRNA levels and this could lead to increased pomc1 gene expression and ACTH production. This is the first study linking the stress-induced shift in the circadian rhythm of glucocorticoids with dopamine D2 receptor expression.

MTU14-09

Neuroendocrine tumors and secretion: what potential links?

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Neuroendocrine tumors are associated with secretion dysfunction. Catecholamine hypersecretion of pheochromocytoma is responsible for the elevated severe hypertension and stroke risk. Although this aspect is well known by clinicians, it has never been explored at the cellular and molecular level.

Here, we have analyzed the aberrant secretion of catecholamine at a single cell level by applying the highly sensitive amperometry technique on human pheochromocytoma resection. We have

observed a drastic increase of exocytotic events in tumoral cells comparing to healthy human chromaffin cells. These data demonstratefor the first time that hypersecretion is a direct consequence of a deregulation of the secretagogue-induced secretion and not simply a mass effect due to the proliferation of tumoral cells.

Finally, according to their widely accepted involvement in tumorigenesis and their important function in neuroendocrine secretion, Rho GTPases and their regulator pathways appear as good candidate to be involved in secretion defect and/or development of pheochromocytoma. Our results show a decrease of the GTPases Rac1 and Cdc42 activities in human pheochromocytoma compared to healthy tissu. Moreover, by investigating protein expression changes in tumor through a mass spectrometry approach, we have demonstrated that ARHGEF1 and FARP1, two guanine nucleotide exchange factors that activate Rho GTPases are down-regulated. We then confirmed by *in vitro* experiment in PC12 cells that down-regulation of ARHGEF1 and FARP1 triggers the inactivation of Rac1 and Cdc42, respectively.

Altogether, our results demonstrate the importance of secretion in tumoral development, a deregulation of the secretory activity at a cellular level, and an alteration of the Rho GTPase pathways in pheochromocytoma.

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MTU14-10

Proteomic changes in the adrenal medulla following a single episode of glucoprivation: a time-course study

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Catecholamines are released from the adrenal medulla in response to stress. Glucoprivation selectively activates only the adrenergic chromaffin cells in order to release adrenaline which then mobilises glucose. This response is activated via the sympathetic innervation of the medulla and via the actions of corticosterone. The aim of this study was to determine the proteomic changes that are evoked in the adrenal medulla at five time points after the administration of 2-deoxy-D-glucose (2DG). 18 male Sprague Dawley rats received 2DG (400 mg /kg ip) and were then anaesthetised (sodium pentobarbitone 100 mg/kg) immediately at time 0 min or at 20 min, 50 min, 4 h, 8 h, or 24 h later (n = 3 per group). The adrenal glands were removed, the adrenal medulla extracted and frozen. Extracted proteins were fractionated on SDS-PAGE gels and in-gel digested using trypsin. Extracted peptides were separated on nano-flow liquid tandem mass spectrometry (nano-LC-MS/MS, LTQ-XL ion trap). Spectra were searched against *rattus norvegicus* protein sequence database using GPM software. Normalised spectral abundance factors (NSAFs) from biological triplicate analysis are used to perform quantitative analysis. Identified proteins were analyzed using R and Ingenuity Pathway Analysis to determine differential protein expression and their interactions in various pathways. About 900 proteins were identified reproducibly at each time point. When results at each time point were compared to control 78 (20 min), 138 (50 min), 163 (4 h), 136 (8 h) and 107 (24 h) proteins were differentially expressed. The function of all proteins were identified using a range of databases in order to determine the cellular processes changed. Proteins at 20 min were mostly down regulated and related to mitochondrial function and steroidal signalling. Cytoskeleton changes were strongly evident at 4 h. Up regulation of transcription and translational processes was evident at 24 h. A range of cellular processes are altered by glucoprivation in a time dependent manner with the effects of a single episode evident even 24 hours after the stimulus.

MTU14-11

Regulation of sympathetic nerve activity by adipokines

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Obesity and overweight conditions are becoming major health issues worldwide. Obesity can be linked to many complications such as cardiovascular disease. In an obese and an overweight condition the level of adipose tissue is elevated. Adipose tissue secretes adipokines, such as leptin and resistin, which are important in regulating metabolic and cardiovascular functions. Therefore, as the level of adipose tissue increases, the levels of leptin and resistin in the plasma are elevated. In this study we investigated the effects of acute administration of leptin and resistin, alone and in combination, on renal sympathetic nerve activity (RSNA), mean arterial pressure (MAP) and heart rate (HR) in anaesthetised rats. Under general anaesthesia (isoflurane 2% and urethane 1.4–1.6 g/kg), the femoral vein and artery of male Sprague-Dawley rats were cannulated for MAP and HR recording. The renal nerve was then prepared for recording of RSNA. Leptin alone (7 μg), resistin alone (7 μg) and the combination of both resistin and leptin were administrated intracerebroventricularly (ICV). Leptin alone and resistin alone increased RSNA by a similar degree (40–60%). The administration of leptin and resistin combined; had a significantly greater effect on RSNA (maximum increase approximately 160%), compared to the responses to either drug administered alone. These findings show that the combination of both leptin and resistin leads to significantly greater increases in RSNA, suggesting a synergistic effect between leptin and resistin in regulating RSNA. Since both leptin and resistin are increased in obesity and overweight conditions, together they could be making even greater contributions to the increases in RSNA observed in obese and overweight conditions.

MTU14-12

Quantitative immunohistochemical co-localization of TRPV1 and cgrp in varicose axons of murine oesophagus, stomach and colorectum

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In the gastrointestinal tract of mammals, spinal afferent neurons, with cell bodies in dorsal root ganglia detect sensory stimuli, including those that give rise to pain. Many of these neurons express calcitonin gene-related peptide (CGRP) and transient receptor potential cation channel subfamily V member 1 (TRPV1) in their cell bodies and axons, which has led to the use of CGRP and TRPV1 as peripheral markers of spinal afferent neurons. However there are also populations of enteric neurons that express CGRP. In

this study, we used double-labeling immunohistochemistry to quantify the coexistence of CGRP and TRPV1 in varicose axons in the myenteric plexus of the murine oesophagus, stomach and colorectum. In a separate series of experiments, antisera to CGRP were applied to preparations after a period of 5 days organotypic culture, to allow for degeneration of extrinsic neurons. In fresh-fixed tissue, we observed that the majority of CGRP-immunoreactive (IR) varicosities within myenteric ganglia of the lower oesophagus (97 ± 1%), stomach (95 ± 1%) and colorectum (91 ± 1%) were also TRPV1-IR. Similarly, the majority of TRPV1-IR varicosities within myenteric ganglia of the lower oesophagus (95 ± 1%), stomach (91 ± 1%) and colorectum (96 ± 1%) were also CGRP-IR. In organ cultured preparations, CGRP immunoreactivity was present in colorectum, but not stomach or oesophagus, beside sparse nerve fibre fragments. In colorectum, the density of CGRP immunoreactivity was diminished but numerous branching varicose nerve fibres, as well as and enteric nerve cell bodies were labelled. The primary observation of this study is that in the myenteric plexus layer of murine oesophagus, stomach and colorectum, CGRP and TRPV1 are almost exclusively expressed together in varicose axons. Results from organ-cultured tissue are consistent with an extrinsic origin of CGRP in all regions, with additional CGRP-immunoreactivity arising from enteric neurons in colorectum.

MTU14-13

Somatostatin 2A receptors are not expressed on functionally identified respiratory neurons in the ventral respiratory column

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Exogenous somatostatin (SST) has profound inhibitory effects on the ventrolateral medullary (VLM) networks responsible for respiratory rhythm generation. Somatostatin 2a receptors (sst2a) are expressed in the pre-Bötzinger Complex, and have therefore been proposed as a feasible neurochemical marker of respiratory neurons. However, no data regarding to overall pattern of sst2a expression in the VLM are available. Here we comprehensively describe the distribution of sst2a with respect to classical markers of respiratory (glycine transporter 2 mRNA, neurokinin 1 receptor (NK1R)) or cardiovascular (Phox2b, tyrosine hydroxylase (TH)) function in respiratory subnuclei of the ventrolateral medulla and on functionally identified respiratory neurons. Extracellular recordings were made from respiratory-locked neurons in the Bötzinger Complex, pre-Bötzinger Complex, and rostral ventral respiratory group in urethane-anaesthetised, vagotomised and artificially ventilated rats. After functional classification neurons were juxtacellularly labelled with fluorescent dextran or neurobiotin; recovered neurons were processed for sst2a immunoreactivity. Overall, sst2a immunoreactive neurons were found in a column that started at the ventral aspect of the facial nucleus and extended caudally for several millimetres. sst2a expression strongly co-localized with TH and Phox2b in the rostral VLM C1 region. However, sst2a immunoreactivity was virtually absent in the Bötzinger Complex, with no co-localisation of sst2a on glycinergic neurons. In the pre-Bötzinger Complex we saw NK1R colocalisation in 54 ± 5% of sst2a-positive neurons (n = 4 rats), but many NK1R positive neurons were also sst2a-negative. 52 functionally identified respiratory interneurons were recovered, including 11 pre-inspiratory pre-Bötzinger Complex neurons. Regardless of discharge pattern, none were sst2a-immunoreactive. Our evidence suggests that sst2a is not strongly associated with classical markers of respiratory function in the VLM or in neurons with a phasic discharge pattern. We conclude that sst2a expression is a poor marker of respiratory function in this region.

MTU14-14

Distinct organization of upper airway vagal circuits in the brain: evidence from conditional viral tracing and physiology

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Irritations of the airways evoke brainstem-mediated reflex changes in breathing and complex sensorimotor responses dependent upon ascending inputs to higher brain centers. Airway vagal afferents terminate in the nucleus of the solitary tract (Sol) and the paratrigeminal nucleus (Pa5) however, whether these nuclei are comparable in terms of afferent inputs, central ascending projections or physiological function is unknown. In Sprague-Dawley rats (n = 4) retrograde tracing with fluorescently tagged cholera toxin B revealed neurons in the jugular vagal ganglia project predominately to the Pa5, while nodose vagal ganglia neurons terminate in the Sol. Electrical stimulation of the trachea in urethane anesthetized rats evoked stimulus dependent apneic responses (control n = 4; E_{max} reduction in respiratory rate = 3 ± 2 breaths/min) which were abolished after inhibition of the Sol by microinjection of $GABA_A$ agonist, muscimol (n = 5, E_{max} = 62 ± 5, p = 0.001), but only partially inhibited from the Pa5 (n = 5; E_{max} = 36 ± 6, p = 0.01). To determine the ascending projections of airway specific Sol and Pa5 neurons we constructed a conditional HSV-1 H129 virus that switches fluorescent reporters in the presence of Cre allowing the connectivity of subsets of neurons to be visualized. Following inoculation of the airways with HSV-1 H129, the Sol (n = 11) projected to the zona incerta, paraventricular and lateral hypothalamic nuclei, while Pa5 (n = 12) neurons terminated in the submedius and ventrobasal thalamus, regions assocated with sensiromotor control. Taken together, these data suggest distinct pathways governing airway sensations exist, likely playing differing roles in the processing of respiratory responses.

MTU14-15

Putative sympathetic premotor neurons in the rostral ventrolateral medulla are not somatotopically distributed

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Peripheral sympathetic nerves that innervate distinct peripheral targets (e.g. cardiac, renal, and splanchnic beds) exhibit differential sensitivities to metabolic, baroreceptive, and somatic stimuli, and may be independently activated or inhibited in different physiological conditions. These observations suggest that spinal sympathetic

preganglionic neurons that relate to particular sympathetic outputs are driven by distinct pools of central premotor neurons. Bulbospinal neurons in the RVLM have been proposed as a likely site of such differentiation, as their spontaneous activity underlies sympathetic tone in diverse peripheral sympathetic nerves, yet they exhibit diverse functional properties. Here we compare the evidence for somatotopic (in which bulbospinal neurons in one region of the RVLM tend to control drive to one region of the body) or viscerotopic (in which bulbospinal neurons supply drive to SPN with similar functional properties at multiple levels of the spinal cord) organisational schemes using terminal-specific retrograde viral vectors. We injected recombinant herpes simplex-1 vectors that drive the expression of green or red fluorescent proteins into the interomediolateral column of the T2 (cardiac, head & neck) and T10 (adrenal, kidney, mesentery) levels of the spinal cord and mapped the expression of retrogradely transduced RVLM neurons that project to T2, T10, or both. We observed high levels of viral uptake with a total of 116 ± 16 neurons transduced per animal (n = 5 rats, every fourth section counted). Although the number of T2-projecting neurons was higher than the number of neurons transduced from T10 injections (6.5 ± 0.7 vs 4.4 ± 1.2 neurons per section, $p < 0.05$), we saw no obvious difference in their stereotaxic distribution, suggesting a somatotopic organisational scheme is unlikely. However, in all experiments we observed a consistent double-labelled population (14 ± 2% of transduced neurons), suggesting that innervation of multiple spinal targets by a single RVLM neuron is a common occurrence, supportive of an organotypic distribution.

MTU14-16

Bilateral injection of clonidine into the amygdala substantially impairs alerting-induced tail artery vasoconstrictions in rats

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Few studies have investigated whether release of noradrenaline in the amygdala is necessary for the occurrence of autonomic responses to natural (unconditioned) alerting stimuli. In the present investigation in conscious unrestrained Sprague-Dawley rats we acutely injected clonidine, an alpha2 adrenergic agonist, bilaterally into the amygdaloid region and measured the effect on sudden falls in tail artery blood flow elicited by salient possibly threatening alerting stimuli. Pulsatile tail artery blood flow was measured by a Doppler ultrasonic flow probe chronically implanted around the base of the artery, with the signal accessed via a headpiece and a swivel device. Bilateral injection of clonidine (5, 10 and 20 nmol in 200 nl saline) into the amygdala decreased the amplitude of the sudden falls in tail artery blood flow elicited by standardized alerting stimuli in a dose-dependent manner, from 84 ± 3% of baseline to 13 ± 4% of baseline (log linear regression, $p < 0.0001$). The coefficient of variation of the flow signal was also decreased in a dose-dependent manner from 65 ± 5 to 38 ± 5 (log linear regression, $p < 0.001$). Pretreatment with idazoxan (75 nmol/100 nl bilaterally into the amygdala) substantially prevented the reduction in tail flow after clonidine 20 nmol/100 nL (65 ± 4% vs 12 ± 5%, $p < 0.0001$). Clonidine, via activation of presynaptic alpha2 impairs release of noradrenaline from the axon terminals of the locus coeruleus neurons that innervate the amygdala. Thus our study strongly suggests that noradrenergic neurotransmission in the amygdala is of major importance for the occurrence of sympathetically-mediated cutaneous vasoconstriction induced by salient or threatening alerting stimuli.

MTU14-17

Multifunctional confirguration of the nucleus retroambiguus in the rat *in vivo*

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The nucleus retroambiguus (NRA) is an anatomical structure located caudal to the obex primarily defined in the cat. NRA is suggested to play a critical role as a relay nucleus in the limbic-autonomic control pathway [1]. Electrophysiological research on NRA is scant due to lack of its identification in the rat, the current primary mammalian animal model for neuroscience investigations. The present study aimed to identify the NRA in the rat and examine its role in sensorimotor integration. As the midbrain periaqueductal gray (PAG) projects exclusively to the NRA (not to other cells in the spinomedullary region) [2] in the cat, mapping the PAG projections to the area just caudal to the obex was used as a method to identify the NRA in the rat. Neuroanatomical tract tracing revealed a distinct distribution pattern of labelled fibres and terminals bilaterally in the region extending 0–2 mm caudal to the obex. Stereotaxic motor mapping (via chemical stimulation) of the identified NRA region produced distinct laryngeal, abdominal and pelvic floor activation responses. Six different types of respiratory-related neurons were extracellularly recorded in the NRA. They were classified as I-DEC, I-CON, I-AUG, E-DEC, E-CON and E-AUG, based on their temporal relationship with the diaphragm activity and in conjunction with their frequency discharge. Vagal stimulation and peripheral chemosensory activation differentially modulated the activity of all the neurons, the modulation accompanied with changes to motor patterns of laryngeal, respiratory and pelvic floor systems. Our results show that NRA homologue indeed exists in the rat. The NRA neurons play a multifunctional role in the sensorimotor integration of autonomic responses to behavioural expression.

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MTU14-18

Nucleus incertus networks and their influence on arousal and cognition: dreadd studies

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Anatomical and physiological data suggest the hindbrain *nucleus incertus* (NI) plays a key role in arousal, attention, memory and

behavioural state control, in relation to stress responses (Ma and Gundlach, 2015). These studies aimed to determine how ascending NI circuits influence behaviour and brain activity, particularly in limbic and septohippocampal networks.

We used adeno-associated viral (AAV) vector delivery of 'Designer Receptors Exclusively Activated by Designer Drugs' (DREADDs) to pharmacogenetically stimulate or inhibit NI neuronal networks and examined the neurochemical, physiological and behavioural changes in adult male rats. In rats with strong expression of the excitatory DREADD, hM3Dq-mCherry, in NI neurons (n = 16), injection of the pharmacologically-inert 'designer drug' clozapine-N-oxide (CNO; 3 mg/kg; i.p.) produced long-lasting cortical EEG desynchronization independent of movement, which was associated with increased locomotor activity in a novel environment ($p < 0.05$). CNO-induced NI activation was also associated with increased Fos-immunopositive NI neuronal soma ($p < 0.01$), and *in vitro* whole cell current-clamp recordings revealed consistent depolarisation of hM3Dq-mCherry-transduced NI neurons to CNO, confirming functional activation of NI neurons using excitatory DREADD.

In studies using the inhibitory DREADD, behavioural analyses of hM4Di-mCherry transduced rats (n = 5/group) revealed that NI inhibition by CNO (10 mg/kg; i.p.) impaired retrieval of spatial working memory in the Morris water maze, reflected by time in the target quadrant, compared to the performance of saline-treated hM4Di-mCherry and CNO-treated mCherry control rats ($p < 0.01$). These latter findings are consistent with reports that NI blockade with lidocaine impairs spatial working memory in rats (Nategh et al., 2015). Current studies are assessing effects on spatial memory when hM3Dq- or hM4Di-positive NI projections to the medial septum are activated or inhibited with local CNO administration, to better establish the contribution of NI driven septal pathways in cognition.

MTU14-19

Long-term consequences of neonatal overfeeding on female reproductive maturation and function

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Overfeeding in early life has long-lasting effects on adult health, including the development and functioning of the hypothalamic-pituitary-gonadal (HPG) axis. Leptin, an adipocyte-derived hormone and cytokine, mediates the impact of metabolic status on reproduction. Leptin is essential to the formation of the hypothalamic neural circuitry involved in the control of food intake, along with its crucial role in reproductive physiology at all levels of the HPG axis. Importantly, leptin acts as a permissive factor on the onset of puberty, particularly in females, primarily via its action on kisspeptin-producing neurons within the hypothalamus. Our laboratory has demonstrated that neonatal overfeeding in the rat, induced by reduction of litter size during the suckling period, programs a persistent increase in body weight and circulating leptin, as well as other long-term neuroendocrine and neuroimmune consequences. We have previously shown postnatal overfeeding accelerates the onset of puberty in female rats, but this was not associated with changes in hypothalamic kisspeptin. We have therefore begun to examine the peripheral role of leptin and its receptor in reproductive maturation in rats suckled in small litters of 4 (SL), compared to control litters of 12 (CL). Neonatal overfeeding had a differential effect on the expression of the leptin receptor in the neonatal and adult ovary, suggestive of developmental differences in the role of leptin in ovarian function. Importantly, neonatal overfeeding induced premature follicular maturation and exhaustion, potentially reflective of early reproductive senescence. Therefore, our data suggest early life overfeeding has a long-lasting programming effect on reproductive development and function, potentially leading to altered reproductive efficiency.

MTU14-20

OREXIN-1 receptor distribution in the mouse brain stem: co-localization with tyrosine hydroxylase and NNOS

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We systematically mapped the neuroanatomical distribution of the OX1 receptor in the pons and medulla in a reporter mouse line in which green fluorescent protein (GFP) was inserted into the orexin-1 receptor (OX1) locus. In the medulla, we observed OX1-GFP expression in the cuneate, dorsal motor nucleus of the vagus (10N), gracile nucleus, solitary tract nucleus and medullary raphe areas. In the pons, the greatest expression was found in the locus coeruleus (LC) and dorsal raphe nucleus (DRN). High to moderate expression was found in the A5 noradrenergic cell group, pedunculopontine tegmental nucleus (PPTg), laterodorsal tegmental nucleus, and the periaqueductal gray. Double-labeling with neuronal nitric oxide synthase (NOS1) revealed extensive co-localization in cell bodies and fibers of the 10N, A5 cell group and the PPTg. OX1 and tyrosine hydroxylase co-labelling was found in the LC, DRN and the lateral paragigantocellularis cell group in the ventral medulla. Our findings faithfully recapitulate the findings of OX1 mRNA expression previously reported. This is the first study to systematically map the neuroanatomical distribution of OX1 receptors within the mouse hindbrain.

MTU14-21

Electroacupuncture at ST25 inhibits jejunal motility via sympathetic pathways: role of TRPV1

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Background/Aims: Electroacupuncture (EA) at abdominal acupoint, ST25, is effective to treat intestinal dysmotility in the clinic, the underlying mechanisms of its action remain to be explored. The aim of this study was to investigate whether EA at ST25 affects jejunal motility *in vivo* and its underlying mechanism.

Methods: The effects of EA were measured in male Sprague-Dawley rats (EA intensities: 1 mA, 3 mA, 5 mA, 7 mA, and 9 mA), some of which were treated with propranolol or clenbuterol and in male TRPV1 (capsaicin receptor) knockout mice (EA intensities: 1 mA, 2 mA, and 4 mA).

Results: (1) Anesthetized rats exhibited three types of fasting jejunal motor pattern (type A, type B, and type C), and only type C rats responded to EA stimulation. (2) In type C rats, EA at ST25

significantly suppressed the motor activity of the jejune in an intensity-dependent manner. (3) The inhibitory effect of EA was weakened by propranolol (β adrenoceptor antagonist) and disappeared with clenbuterol (β adrenoceptor agonist) induced inhibition of motility, suggesting that the EA effect on motility is mediated via a sympathetic pathway. (4) Compared with wild-type mice, EA at ST25 was less effective in TRPV1 knockout mice, suggesting that this multi-modal sensor channel participates in the mechanism.

Conclusion: EA at ST25 inhibited jejunal motility in an intensity-dependent manner, via a mechanism in which sympathetic nerves and TRPV1 receptors play an important role.

MTU15 Sensory Systems (Part 1)

MTU15-01

Assessing cochlear amplifier adaptation to noise

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We have previously identified that the temporary threshold shift (TTS) mediated by the $P2X_2$ receptor pathway in the WT mouse is oto-protective and represents purinergic hearing adaptation. This was done by measuring the Auditory Brainstem Response (ABR) hearing threshold, which does not resolve the specific role of the cochlear amplifier (i.e. the outer hair cells). Therefore, the objective of this study was to establish whether cochlear amplifier adaptation contributes to sustained purinergic hearing adaptation. This was undertaken by measuring the rate of development of noise-induced increase in cubic Distortion Product Otoacoustic Emissions ($2f_1$-f_2 DPOAE) thresholds about 16 kHz, in WT mice and mice lacking the purinergic $P2X_2$ receptor ($P2X_2KO$). Results: There was no difference in baseline DPOAE hearing threshold between WT and $P2X_2KO$ mice. However, a significant difference was observed between the genotypes after 18 mins and 38 mins of cumulative noise-exposure to 79 dB SPL (sound pressure level) noise. The ABR threshold developed in a similar manner to what we have previously reported, and followed the same trend as the development of the DPOAE threshold. Conclusion: These data suggest that the cochlear amplifier is part of the underlying mechanism for purinergic hearing adaptation, and that the $P2X_2$ receptor-mediated adaptation to sound is upstream of the inner hair cell – type I spiral ganglion neuron afferent synapses. Protocols approved by the UNSW Australia Animal Care and Ethics Committee; Primary support: NHMRC grant APP1089838.

MTU15-02

50B11 cells as a peripheral sensory neuron model for sphingolipid and neurotrophin signalling

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Stimulation and peripheral sensitization of nociceptive neurons with cell bodies in dorsal root ganglia (DRG) is critical for the development of chronic and neuropathic pain.

Investigations of nociceptive DRG neurons are limited by the absence of a suitable cellular high-throughput model.

Here we characterised an immortalized DRG cell line (50B11), as a model for the investigation of sphingolipid and neurotrophin signalling in nociceptive DRG neurons.

We used q-RT-PCR, Western blotting and multiple-labelling immunohistochemistry to characterise the expression of sensory nociceptive neuron markers at the transcriptional and translational level, and determined the effects of forskolin and nerve growth factor (NGF) on phenotypic changes in 50B11 cells.

Stimulation of 50B11 cells with forskolin (50 μM, $n = 3$) induced a significant but transient increase of differentiated cells (from 20 ± 2 to 55 ± 3.5 %) within 24 h indicated by the increase in neuritogenesis (from 38 ± 5 to 58 ± 5 μm). The increase in neurite length of differentiated cells remained constant whereas the percentage of differentiated cells decreased over 48 h. Co-treatment with NGF (100 ng/ml) had no additional effect on the percentage of differentiated cells or neurite length. Differentiated cells showed co-labelling for the markers of nociceptive neurons, isolectin B4 and calcitonin-gene related peptide.

Quantitative RT-PCR (n = 5) showed significant changes in the relative mRNA expression for brain derived neurotrophic factor (Bdnf) and Sphingosine kinase 2 (Sphk2) in response to forskolin/NGF with an increase of Bdnf-mRNA and a decrease of Sphk2-mRNA but no changes in response to forskolin alone. Expression of the sphingosine kinase 1 isoform was lower compared to Sphk2, and showed no significant changes in expression. However, forskolin but not forskolin/NGF significantly increased mRNA levels for the neurotrophin receptor p75 with a similar trend in the low expressed neurotrophin TrkA receptor.

Taken together our data indicate the presence and NGF-dependent and independent regulation of sphingolipid synthesizing enzymes as well as neurotrophins and their receptors.

MTU15-03

TRPA1-mediated analgesic effect of a novel semicarbazide-sensitive amine oxidase inhibitor in a mouse model of chronic neuropathy

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Transient Receptor Potential Ankyrin 1 (TRPA1) cation channel is a key pain transducer on polymodal nociceptors activated by cold and irritants, such as formaldehyde. It is generated by semicarbazide-sensitive amine oxidase (SSAO) catalysing oxidative deamination of primary amines. Although this suggests a potential link between TRPA1 activation and SSAO products, this has not been investigated and its role in pain has not been proposed. We investigated the role of TRPA1 and the effect of our novel oxime SSAO inhibitor SZV-1287 in neuropathy.

Mechanonociceptive threshold of the paw was measured by dynamic plantar aesthesiometry, cold sensitivity by withdrawal latency from 0^{O}C water in wildtype ($TRPA1^{+/+}$) and TRPA1 gene-deficient ($TRPA1^{-/-}$) mice. Traumatic mononeuropathy was induced by partial ligation of one sciatic nerve under anaesthesia.

SZV-1287 (20 mg/kg i.p.) was administered on the 7th postoperative day.

The basal mechanonociceptive thresholds did not differ in $TRPA1^{+/+}$ and $TRPA1^{-/-}$ mice, but the cold withdrawal latency of the $TRPA1^{-/-}$ was significantly longer. Approximately 40–50% mechanonociceptive threshold decrease (hyperalgesia) developed in both groups. This was significantly reduced by SZV-1287 in wildtype mice, but no effect was observed in the knockouts. Nerve ligation induced 55–60% cold allodynia in $TRPA1^{+/+}$ mice, but significantly smaller, only 25–30% in $TRPA1^{-/-}$ one. However, this was not influenced by SzV-1287 administration.

We provide the first evidence that SSAO is involved in pain. Its products mediate neuropathic mechanical hyperalgesia via central activation/sensitization of the TRPA1 ion channel, since no effect was observed on cold allodynia developing by peripheral sensitization. These results open novel analgesic drug developmental perspectives.

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MTU15-04

Use of a transgenic CGRPα reporter mouse to correlate the function and neurochemical content of colorectal afferent neurons

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Several neurochemical classes of colorectal afferents have been distinguished, including a large population expressing CGRP. Most electrophysiological recordings from colorectal afferents lack identification of their neurochemical phenotype. We used a novel transgenic mouse, in which functional properties of primary afferents could be directly correlated with their neurochemistry. Thus, we generated a knock-in mouse expressing the fluorescent reporter, mCherry, under the CGRPα promoter. Decentralised preparations of L_6 and S_1 dorsal root ganglia attached to the colorectum were set up *in vitro*. The bowel was cannulated to apply graded intraluminal distensions, whilst recording intraluminal pressure. Sharp intracellular electrophysiological recordings were made using 5,6-carboxyfluorescein-filled micropipettes to identify nerve cell bodies. Sixty-five colorectal afferents were identified by antidromic action potentials evoked by electrical stimulation of the mesentery or low-grade distension (conduction velocity 0.5 ± 0.2 m/s, 52 in L_6, n = 29). Most discharged action potentials to intra-somal depolarization (18/23 tested, rheobase 0.31 ± 0.18nA), showed hyperpolarization-evoked inwardly rectifying I_H-like currents (19/23 cells) and had passive membrane properties typical of DRG neurons (E_m -55 ± 4.7 mV, R_{input} 62 ± 28 mΩ). Twenty-two neurons were distension-insensitive (tested to 60cmH_2O). Focal electrical stimuli confirmed they had axons in the gut wall (4/4 cells). Seven of 8 distension-insensitive neurons expressed mCherry (max. cross-sectional area 1149 ± 488 μm^2). Forty-two of 43 distension-sensitive neurons were low threshold (10cmH_2O; L_6:36, S_1:7), wide dynamic range, firing up to 75 Hz at 40cmH_2O (max. instantaneous frequency; amplitude: 60 ± 8 mV, half-peak duration: 1.3 ± 0.4 ms, R_{input}: 64 ± 33 mΩ). Most low-threshold afferents expressed mCherry (36/46 cells, max. cross-sectional area 958 ± 404 μm^2). In conclusion, we have developed a preparation that can be used to rapidly determine the functional, electrophysiological and neurochemical phenotypes of colorectal afferent neurons.

MTU15-05

Red light improves sensory and motor recovery following spinal cord injury in rats

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We aimed to investigate the effect of red light treatment on sensory and locomotor recovery following spinal cord injury. 8-week-old Wistar rats received a mild (10 g, 25 mm weight drop) or moderate (10 g, 50 mm) hemi-contusion injury centred over the dorsal horn at T10 of the spinal cord, then received a daily 30 min sham or 670 nm (red) light treatment. Sensory recovery was assessed at 7 days post injury (dpi) using electrophysiology and mechanical hypersensitivity testing, and locomotor recovery assessed daily using the Basso, Beattie, and Bresnahan (BBB) rating scale. The magnitude of surface potentials recorded at the gracile nuclei evoked by electrical stimulation of the sural nerve was significantly reduced ($p = 0.012$; n = 7), and latency significantly increased ($p < 0.001$; $n = 7$) ipsilateral to the injury at 7 dpi in the sham-treated group compared to normal intact animals. 670 nm treatment restored both magnitudes and latencies of evoked potentials back to levels of normal intact animals. 50–60% of animals developed hypersensitivity at 7 dpi to an innocuous mechanical stimulus on the dorsum following spinal cord injury in both groups. Of the animals that developed hypersensitivity, 670 nm treatment significantly decreased the severity of hypersensitivity at 7 dpi compared to the sham-treated group ($p = 0.034$; n = 12), specifically on dermatomes at the level of injury ($p = 0.021$ contralateral to the injury) and below the level of injury on both ipsilateral ($p = 0.020$) and contralateral sides ($p = 0.035$). All animals showed spontaneous locomotor recovery following the injury but the 670 nm treated group demonstrated significantly better outcomes commencing from 3 dpi (n = 10). 670 nm light treatment improves both tactile and pain pathway functional outcomes following spinal cord injury, as well as enhancing locomotor recovery. It therefore may offer a simple and cost effective treatment that can improve both sensory and motor deficits following spinal cord injury.

MTU15-06

Indexing neurovascular coupling by combining electroencephalogram and functional transcranial doppler

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Neuronal function and cerebrovascular supply are usually assessed in isolation however their relationship to each other is critical for brain function. Methods that do exist are typically expensive and unsuitable for populations where the technique would be most valuable, such as the very young and very old. We investigated neurovascular coupling in young healthy adults

(n = 12) by pairing electroencephalogram (EEG) and transcranial doppler (TCD). EEG measures neural function via the recording of electrical activity at the scalp, while transcranial doppler measures uses ultrasound to record blood flow velocity in major cerebral arteries. Visual evoked potentials (derived from EEG data) were recorded over the occipital lobe and blood flow velocity was recorded from the posterior cerebral arteries using TCD, while participants completed a passive checkerboard task, and two active visual half field tasks (identifying either tools or symmetrical shapes). The early N75-P1 evoked potential components showed the closest relationships with posterior blood flow velocity (change relative to baseline), in both the passive and active tasks. This combined EEG-TCD technique, which is non-invasive, inexpensive and suitable for most populations, stands to make valuable contributions to the understanding of neurovascular coupling in health and disease.

MTU15-07

Imaging serotonergic, CGRP, and NOS positive fiber terminals in the dorsal horn of the mouse spinal cord with clarity

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The present study aimed to use the recently developed CLARITY technique to map the serotonergic, CGRP, and NOS positive fibers in the dorsal horn. It was found that serotonergic fibers mainly extended from the intermediate layers towards the dorsal horn and travelled along the rostrocaudal axis, terminating along their paths. CGRP positive fibers were mainly found in the superficial layer of the dorsal horn, extending towards the other side. In lamina 5, CGRP positive fibers projected towards the superficial layers vertically and laterally. The majority of these fibers in the superficial layer travelled perpendicular to the rostrocaudal axis of the spinal cord and terminated along their paths. NOS positive fibers shared similar characters with serotonergic fibers in the dorsal horn but they were more evenly distributed. A small number of positive neurons were also found in lamina 4. The present study provided 3D images of the serotonergic, CGRP and NOS positive fibers in the clarified spinal cord tissue. This revealed the full path of labeled fibers and their terminals within the image frame, which expands our knowledge in the organization of these fiber systems in the spinal cord.

MTU15-08

Reproducible surface potentials in the dorsal column nuclei evoked from peripheral nerves

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The dorsal column nuclei (DCN) relay and process sensory information before it travels to the thalamus and, subsequently, the cortex for conscious perception. We are interested in how the DCN process sensory information. Our aims were 1) to classify DCN surface potentials evoked from nerves of different anatomical locations carrying cutaneous and mixed afferent populations; 2) to demonstrate reproducibility of signal features when evoked from "like" nerves. We used single electrical pulses (0.01 ms; 0.53–1.1 mA) to recruit all fibres from left and right sural and peroneal nerves of 8 week old, urethane anaesthetised, male Wistar rats whilst recording evoked surface potentials from the DCN. 11 repeated trials were recorded from each nerve and signals were band pass filtered (3333–4750 Hz) and time-normalised offline. Intra-animal comparisons were performed on signals from the 11 repeated trials, while inter-animal comparisons were performed on the mean of these 11 signals as a representative response for each animal. Correlation coefficients of intra-animal comparisons ranged from 0.80 to 0.87, and inter-animal correlation coefficients (mean ± SEM) were right sural = 0.69 ± 0.0057; left sural = 0.67 ± 0.0060; right peroneal 0.66 ± 0.0060; and left peroneal = 0.66 ± 0.0061. While there were more events evoked from the peroneal nerve at the 15–55 ms window, post stimulus, compared to the sural nerve ($p < 0.01$), there were no left/right differences between "like" nerves. K-means clustering of event latencies, from all animals, showed that robust events were similar between sides of the same nerves. Knowledge of how sensory information is processed in the DCN could 1 day inform the development of a neural prosthesis capable of recording sensory information in the periphery and artificially reproducing it in the DCN, as a strategy to restore sensory perception to sufferers of spinal cord injury.

MTU15-09

Synaptic contacts between the diffuse cone bipolar cell type DB3A and ganglion cells in the marmoset retina

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Bipolar cells are retinal interneurons that transmit visual information from the photoreceptors to the ganglion cells. In primate retina, at least 10 different types of cone bipolar and about 20 types of ganglion cells are present. Bipolar cells involved in colour vision are known to make selective connections with ganglion cells. It is not known, however, whether similar selectivity exists for other visual pathways. Here, we measured the connectivity of the diffuse bipolar type DB3a to OFF ganglion cell types in the retina of marmoset monkeys (*Callithrix jacchus*). The ganglion cells were transfected *via* particle-mediated gene transfer of an expression plasmid for the postsynaptic density 95-green fluorescent protein (PSD95-GFP) and kept in culture for 3 days. Subsequently, retinas were fixed and processed for immunohistochemistry to label the DB3a cells. The preparations were imaged with a confocal microscope and retinal ganglion cells were classified using

morphological criteria. The synaptic contacts between bipolar and ganglion cells were analysed by determining the number of PSD95-GFP puncta located in the immediate vicinity of labelled bipolar terminals. A total of 174 DB3a axon terminals was analysed. On average, a DB3a axon terminal made 18 ± 4 (95% confidence interval) contacts with OFF parasol ganglion cells (n = 3), 8 ± 2 contacts with broad thorny ganglion cells (n = 3), 9 ± 5 contacts with recursive bistratified ganglion cells (n = 2) and 6 ± 3 contacts with small bistratified ganglion cells (n = 3). These data suggest the DB3a cell preferentially contacts the OFF parasol cell. The parasol pathway serves contrast and motion perception, and this study is consistent with the idea that the functional output of the parasol pathway is modulated by the input of the DB3a cell.

MTU15-10

Sensory discrimination in dendrites

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Processing of sensory information by the cortex is fundamental to brain function. The laminar organization of projections into the sensory cortex results in pyramidal neurons receiving feedback sensory input onto their distal apical and tuft dendrites. *In vitro* data tells us that the transformation of this distal synaptic input into neuronal output is not trivial and we now know that distal dendrites are able to generate non-linear events called dendritic spikes. However, despite their influence on neuronal excitability, little is known about how non-linear integration influences the global processes relevant to sensory processing *in vivo*. To address this, we are investigating a task which requires an animal to learn the relationship between a set of sensory stimuli and then associate this with a reward. We predict that long range inputs from higher order cortical regions play a role in successfully performing this task. Therefore we investigated dendritic activity underlying tactile frequency discrimination task in the tuft dendrites of layer 2/3 pyramidal neurons in the forepaw somatosensory cortex. Using Ca^{2+} imaging and patch-clamp electrophysiology *in vivo*, we determined whether sensory neurons are frequency tuned to forepaw stimulation by generating frequency response curves. In addition we measured the extent to which dendritic Ca^{2+} activity could be evoked by the varying frequencies, and by basic combinations of tactile stimuli. These results will provide a basis for us to further study the role that non-linear dendritic integration plays in sensory discrimination in the awake state.

MTU15-11

Transcranial magnetic stimulation (tms) generates GABAβ-mediated inhibition in layer 5 dendrites

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Transcranial magnetic stimulation (TMS) is a noninvasive technique used to modify neural processing. Although brain function is drastically altered, little is known about the cellular effects of TMS. To test how TMS affects cortical function, the calcium response to sensory stimulation in a population of layer 5 pyramidal neuron dendrites and layer 1 interneurons were measured during TMS using a fiberoptic system ("periscope") in urethane-anaesthetized rats. Hindpaw stimulation alone led to a biphasic increase in layer 5 dendritic calcium activity (295 ± 16 dF/F*ms; n = 12). However, when coupled with TMS (single 500 μs pulse), the integral of the dendritic calcium response to hindpaw stimulation decreased by 32 ± 11 % ($p < 0.05$, $n = 11$). Cortical application of the $GABA_B$ antagonist CGP52432 prevented the inhibitory effect of TMS, indicating that the decrease in L5 dendritic activity is due to activation of dendritic $GABA_B$ receptors. Interneurons within cortical layer 1 also had an increase in calcium during hindpaw stimulation (786 ± 203 dF/F*ms; n = 12). However, in contrast to layer 5 dendrites, layer 1 interneurons also had an increase in calcium in response to TMS (549 ± 208 dF/F*ms; n = 9) which was suppressed by cortical CNQX application (by 39 ± 14 %; n = 8). Taken together, these results illustrate that layer 1 interneurons are indirectly activated by TMS and therefore are the source of layer 5 dendritic inhibition during TMS.

MTU15-12

An in-vivo whole nerve electrophysiological preparation to explore the function of bone afferent neurons in the rat

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The aim of the present study was to develop an *in vivo*, whole-nerve electrophysiological preparation to study the physiology of bone afferent neurons in the rat. For this purpose, we have identified a fine nerve that enters the medullary cavity of the rat tibia through the nutrient foramen on its posterior aspect. Histological analysis revealed this nerve to the rat tibia is exclusively composed of small diameter (< 6 μm) myelinated and unmyelinated fibres, indicating that *afferent* fibres of this nerve are nociceptive in function. We performed a series of dissections to identify the parent nerve from which the nerve to the rat tibia arises ($n = 7$). In all cases, it arose as a small branch of the posterior inter-osseous nerve, which is classically defined as a muscular branch of the tibial nerve. The posterior inter-osseous nerve was easy to isolate from surrounding tissues between tibialis posterior and flexor hallucis longus, deep in the posterior compartment of the leg. Whole nerve electrophysiological recordings were made from the posterior inter-osseous nerve at this site with a platinum hook recording electrode (n = 5). Increasing intra-osseous pressure by infusion of isotonic saline into the medullary cavity elicited multi-unit activity in the posterior inter-osseous nerve. Normal intra-osseous pressure was approximately 20–25 mmHg, and the threshold for multi-unit activity in the nerve was approximately 20 mmHg above this level. The frequency of multi-unit activity increased in response to increasing intra-osseous pressure, suggesting that single units in the nerve increase their firing frequency to encode pressure and/or that single units have different thresholds to activation with some preferentially activated at higher thresholds. This preparation is currently being used to determine how mechanically-sensitive bone afferent neurons respond to inflammation, and application of both algesic and sensitizing agents.

MTU15-13

Red light pretreatment reduces mechanical hypersensitivity and improves locomotor recovery following sciatic nerve chronic constriction injury

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Chronic neuropathic pain is a prevalent, costly and debilitating condition that currently has no effective pharmaceutical or surgical treatment. The objective of this study was to investigate the effects of pretreatment with red (670 nm) light on pain and locomotor deficits following a chronic constriction injury. 8 week old male Wistar rats received either a daily 30 minute sham, or 670 nm light treatment, for 10 consecutive days. Unilateral chronic constriction of the right sciatic nerve using 4 chromic cat gut ligatures was performed under isoflurane anaesthesia following the third day of treatment. Sensory and motor assessments, using mechanical hypersensitivity testing (withdrawal to graded nylon filaments) and footprint analysis, respectively, were performed ~2 hours post injury, then every second day 2 hr prior to daily treatment. Prior to surgery, there was no significant difference between the sham and 670 nm treated groups in withdrawal thresholds ipsi- or contralateral to the injury. Post injury however, the light treated group displayed a significant increase in withdrawal thresholds ipsilateral to the injury compared to the sham treated group ($p < 0.001$). Footprint functional indices significantly improved from day 7 post injury ($p < 0.05$). 670 nm light may be useful to reduce neuropathic pain symptoms as well as promote motor functional recovery in nerves undergoing a chronic constriction injury.

MTU15-14

Peripheral nerve injury alters the excitability of calretinin positive dorsal horn neurons

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The spinal dorsal horn is a key region in the processing of sensory information. Substantial neuron diversity exists, making identification and subsequent analysis of discrete populations difficult. To address this issue we have used a transgenic mouse line that expresses enhanced green fluorescent protein (eGFP) under the control of the calretinin (CR) promoter to study a specific population of DH neurons. In the current study we use a model of neuropathic pain (spared nerve injury; SNI) to identify electrophysiological changes to CR^+ neurons following induction of neuropathic pain. SNI is a widely used model of neuropathic pain where two branches of the sciatic nerve are cut (tibial and common peroneal). This causes sensitisation of the preserved (sural) nerve branch territory due to central sensitisation. These experiments assess the extent to which CR^+ neurons are involved in the generation of altered pain states (hyperalgesia and allodynia). Briefly, CReGFP animals received sham or SNI surgery and underwent von Frey mechanical threshold testing to monitor the development of neuropathic pain. Animals were sacrificed one week post surgery, acute spinal cord slices were prepared and patch clamp recordings were made from CR^+ neurons. Results; Action potential (AP) discharge phenotype shifted from predominantly delayed firing (78%, 14/18) to include both single spiking and initial bursting phenotypes in the SNI group with only half exhibiting the delayed firing phenotype (50%, 8/16). This is also reflected in the latency between the onset of the stimulus and the first AP, which was significantly decreased in SNI animals (448.3 ms ± 76.8 vs 292.8 ms ± 101.2; $p < 0.05$). Finally, the frequency of spontaneous excitatory postsynaptic currents was increased in SNI animals versus sham recordings (5.92 Hz ± 0.5 vs 6.9 Hz ± 0.5; $p < 0.05$). Together, these preliminary data suggest that peripheral nerve injury increases the excitability of CR^+ DH neurons by altering excitatory drive and AP discharge characteristics. These changes in CR^+IN excitability may contribute to the development of central sensitisation and neuropathic pain nerve injury.

MTU15-15

Electrical maturation of sensorimotor processing in the human foetus

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The spinal cord is critical for modifying and relaying *sensory* information to, and *motor* commands from, higher centres in the central nervous system to initiate and maintain contextually relevant locomotor responses. Our understanding of how spinal sensorimotor circuits are established during *in utero* development is based largely from studies in rodents. In contrast, there is little functional data on the development of sensory and motor systems in humans. Here, we use patch-clamp electrophysiology to examine the development of neuronal excitability in human foetal spinal cords (10–18 weeks gestation; WG). Transverse spinal cord slices (300 μm thick) were prepared and recordings were made from neurons in either the ventral (VH) or dorsal (DH) horn at 32°C. Action potentials (APs) could be elicited in VH neurons throughout the period examined, but only after 16WG in DH neurons. At this age, the majority of DH neurons discharged a single AP (6/9) regardless of the magnitude of the injected current, whereas VH neurons usually exhibited tonic AP discharge (15/23) that increased in frequency with increasing current injection. At 16–18WG, APs from DH neurons had significantly smaller heights (29.7 ± 6.7 vs. 47.3 ± 3.4 mV) and afterhyperpolarization amplitudes (-6.5 ± 1.5 vs. -18.8 ± 2.0 mV) compared to VH neurons. Between 10 and 18 WG the intrinsic properties of tonic firing VH neurons changed markedly, with decreases in input resistance (795 ± 145 vs. 237 ± 83MΩ, 10–12 vs. 16–18WG) and increases in AP amplitude (44.8 ± 3.6 vs. 56.5 ± 1.8 mV) and AHP amplitude (-9.22 ± 2.4 vs. -23.3 ± 1.7 mV). Together, our data suggest the developing foetal DHNs are unlikely to be responsive to stimulation of small diameter afferents by 16 WG, whereas VHNs are capable of supporting sustained discharge. The later finding is consistent with reports of muscle activity in the very young foetus.

MTU15-16

Habitual reading direction influences visual search ability differently in the left and right halves of the visual field

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During reading, one makes fast saccades separated by fixational pauses, during which visual information is acquired from the text. Attentional scanning during the approximately 250 msec of each fixation is known to be mostly to the right of fixation (right visual field; RVF) when reading left-to-right scripts such as English. This may explain the correlation in habitual English readers between visual search speed and the size of the representation of the central 12 degrees of the visual field on the *left* primary visual cortex (Verghese et al. (2012) NeuroImage, 93:47–52). Since visual attention is essential for reading and people generally spend many hours reading each day, we hypothesized that visual attention may be allocated asymmetrically depending on the individual's reading direction. Hence, when tested with an attention task, left-to-right readers and right-to-left readers (eg., Farsi readers) may be attentionally biased towards the RVF and LVF respectively. On the other hand, bidirectional readers fluent with both scripts would have equal performance in the two hemifields.

Methods: Three groups of participants - left-to-right, right-to-left and bidirectional readers - were required to find a target (small vertical bar) amongst heterogeneous distractors for orientation search (large vertical and horizontal bars) and homogenous distractors for feature search (small horizontal bars only).

Results: Left-to-right readers showed better performance for targets presented in the RVF (Two-way ANOVA, $p = 0.0131$) while right-to-left readers performed better in the LVF (Two-way ANOVA, $p = 0.0079$). Bidirectional readers showed no significant performance difference between the two sides (Two-way ANOVA, $p = 0.6250$). For the feature search, no significant difference was observed between the two sides in all groups.

Conclusion: The findings support the idea that the visual field asymmetry in search ability may be caused by the habitual direction of reading. This has important implications for understanding the environmental influences on brain development.

MTU15-17

Cortical gating of sensory responses in the amygdala

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In quiescent states such as anesthesia and slow wave sleep cortical networks show rhythmic synchronized activity. In sensory cortices this rhythmic activity shows a stereotypical pattern that is recapitulated by stimulation of the appropriate sensory modality. The amygdala receives sensory input from a variety of sources, and in anesthetized animals, neurons in the basolateral amygdala (BLA) show slow rhythmic synchronized activity. Extracellular field potential recordings show that these oscillations are synchronized with sensory cortex as well as the thalamus, with both the thalamus and cortex leading the BLA. Using whole cell recordings *in vivo* we show that pyramidal neurons and interneurons in the BLA show spontaneous up-states. Footshock and auditory stimulation in the down-state also evoked up-states, and voltage clamp recordings show that evoked up-states fully recapitulate those occurring spontaneously. These results suggest that neurons in the BLA receive convergent input from networks of cortical neurons with slow oscillatory activity. Both somatosensory and auditory stimulation can engage activity in the same networks that project to the BLA. Thus, rhythmic activity in the BLA can be recapitulated by different sensory modalities, suggesting that distinct sensory modalities may have similar representations in the BLA.

MTU15-18

Canonical toll-like receptor mechanisms regulate vagal sensory neuron excitability and growth: role for TRP channels

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Toll-like receptors (TLRs) and other pattern recognition receptors are widely expressed in many cell types, including neurons. In excitable cells, TLR activation produces membrane depolarization, indicative of TLRs coupling to excitatory ion channels that mediate the inward currents. In the present study we set out to assess whether vagal sensory neurons express functional TLR3 and/ or TLR7, important for the recognition of viral RNA. Furthermore, we tested the hypothesis that TLR mediated responses in vagal sensory neurons involve the common sensory neuron TRP channels, TRPV1 and/ or TRPA1. Mouse vagal sensory neurons were dissociated and cultured for up to 4 days for (a) electrophysiological recordings, (b) Ca^{++} imaging studies and (c) neurite outgrowth assays. Bath application of Poly I:C (TLR3 agonist, 50 μg/mL) or Imiquimod (TLR7 agonist, 30 μg/mL) produced rapid inward currents associated with increases in resting membrane potential in patch recordings, transient elevations in intracellular Ca^{2+}, and significant reductions in neurite outgrowth in a subset of sensory neurons. Using cells harvested from knockout mice we confirmed that canonical signalling mechanisms involving Trif and MyD88 adaptor proteins and interferon regulatory factor (IRF) 3 and 7 transcriptional elements are needed for TLR3 (Trif/ IRF3) and TLR7 (MyD88/ IRF7) electrophysiological and neurite responses. Furthermore, pharmacological inhibition of TRPV1 (1 mM SB-366791) partially prevented Poly I:C evoked electrophysiological responses but had no effect on neurite outgrowth whereas inhibition of TRPA1 (1 mM HC-030031) completely blocked both the electrophysiological responses and neurite collapse evoked by poly I:C. By contrast, neither HC-030031 nor SB-366791 altered Imiquimod-mediated responses in either assay. Taken together, these data support the notion that vagal sensory neurons possess functional TLRs which signal via standard TLR signalling processes to regulate sensory neuron growth and excitability. Furthermore, the data suggest that TLR3-evoked functional responses require coupling with neuronal TRPA1, and possibly TRPV1, whereas neither of these TRP channels are needed for TLR7 evoked responses.

MTU15-19

Sensory mechanisms of obstruction-induced detrusor overactivity

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Several hypotheses have been put forward to explain the mechanisms of detrusor overactivity each of which, directly or indirectly, implicates increased sensory signaling from the bladder. The aim of the study was to determine sensory contributions to bladder overactivity. We used a model of gradually-developing bladder outlet obstruction in male guinea pigs to produce detrusor overactivity. Conscious voiding in metabolic cages and micturition contractions in urethane-anaesthetized control, sham-operated and obstructed guinea pigs were measured during continuous cystometry, followed by c-Fos expression in S1 sacral spinal cord neurons. Single unit extracellular recordings were made, *in vitro*, from pelvic afferent nerves in flat sheet bladder preparations. Obstructed guinea pigs showed a significant increase in conscious voiding frequency (1.83 ± 0.3 per hour, $n = 15$, $p < 0.0001$) and a decrease in average voiding volume (1.16 ± 0.23 mL per void, $n = 15$, $p < 0.0001$) compared to sham-operated animals (0.48 ± 0.07 per hour and 5.08 ± 0.39 mL, $n = 19$, respectively). In anaesthetized animals, the interval between micturition contractions during continuous cystometry was not different from obstructed (428 ± 37s, $n = 8$) or sham-operated groups (401 ± 36 s, $n = 5$). However, the number of non-voiding contractions (with amplitude of 2–6 cmH_2O) was 3.5 times higher in the obstructed group. Expression of c-Fos in S1 sacral spinal cord neurons did not differ between control (26 ± 7 cells, n = 8), sham-operated (22 ± 3.9, $n = 9$) or obstructed (22 ± 5.1cells, $n = 10$) animals. Using the spike-following frequency method, no increase in excitability of low threshold stretch-sensitive afferents was found in obstructed bladders, compared to controls. However, high threshold vascular afferents were sensitised in obstructed bladders, firing 4.7 ± 1.5 Hz at 60 g load ($n = 6$, $p < 0.001$) compared to control (1.7 ± 1.2 Hz, $n = 5$). The data suggest that increased signaling from obstructed bladders is transmitted by low threshold stretch-sensitive afferents responding to increased contractile activity (but not intrinsically sensitised), and by sensitised high threshold vascular afferents.

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Poster Sessions Wednesday/Thursday
WTH01 Glia (Part 2)

WTH01-01

Deficits in the signalling as a possible mechanism underlying impaired myelination in the brain of growth restricted rat pups

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Background: Intrauterine growth restricted (IUGR) infants have an increased risk of developing cerebral palsy (CP). White matter injury is the prominent neuropathology in infants with CP. We, and others have previously shown, in IUGR animal models, that maturation of oligodendrocytes is delayed, resulting in hypomyelination. Molecular mechanisms causing this developmental delay are not known. Thyroid hormone (TH) signalling is dysregulated in IUGR human fetal brains. We hypothesize that decreased TH signalling leads to increased expression of inhibitory *Wnt/Notch* pathways, resulting in delayed oligodendrocyte maturation and myelination in the IUGR rat brain.

Method: At embryonic day 18 (term = 22 days), pregnant Wistar rats underwent bilateral uterine vessel ligation to generate IUGR pups at postnatal (P) days 2 (n = 8), 7 (n = 5) and 35 (n = 5); sham surgeries generated controls. Brains were fixed, sectioned and immunostained to identify pre-myelinating (NG2-positive) and myelinating (myelin basic protein [MBP]-positive) oligodendrocytes (P7 only). Pathways that promote [MCT8, TH receptor a (*TRa*), *Sox10*, myelin regulatory factor (*MyRF*)] or inhibit (*Wnt*, *Notch*) oligodendrocyte development were assessed via qPCR in predominantly white matter regions.

Results: For IUGR vs. control, there was a significant: (i) decrease in NG2- and MBP-positive cell density at P7; (ii) decrease in *MBP* mRNA at P7 and P35; (iii) decrease in *MCT8*, *TRa*, *Sox10*, *MyRF* mRNA and an increase in *Axin2*, *Notch1/2*, *Jagged1* and *Hes5* mRNA at P7. There was no difference in (i) *MCT8* or *TRa* mRNA at P2; (ii) no change in D2 and D3 mRNA at P7 and P35.

Conclusions: In IUGR rat pups, TH signalling and pathways that promote oligodendrocyte maturation and myelination are down-regulated, while those that inhibit oligodendrocyte maturation are up-regulated. Further elucidation of these mechanisms could identify potential therapeutic targets to restore myelination in human infants born IUGR, and susceptibility to CP.

WTH01-02

Ethanol causes translocation of glast from cytoplasm to plasma membrane: the effect is blocked by baclofen

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We have been investigating actions of ethanol on the cells of developing nervous tissue. In particular, we have been examining effects of ethanol on developing rat astrocytes in culture. One of the roles of astrocytes *in vivo* is to remove the excitatory neurotransmitter L-glutamate from extracellular space to prevent "hyperglutamatergic" state that could result in neurotoxicity. Cultured astrocytes express mainly the glutamate transporter GLAST. GLAST in astrocytes is subject to regulation by its substrates present in the surrounding medium. For example, D-aspartate which is readily transported by GLAST, will cause a translocation of GLAST molecules from cytoplasm to the plasma membrane [1] and this is thought to activate glutamate transport. We have been studying these shifts by quantitatively evaluating the distribution of fluorescence-labelled GLAST antibodies between the plasma membranes and cytoplasm using deconvolution microscopy. While testing a hypothesis that ethanol interferes with the translocation/activation of GLAST we found that a 45-min exposure to ethanol alone, at concentrations which may be reached during heavy drinking (50 and 100 mM), triggered a shift of GLAST towards the plasma membrane. The effect was blocked by a $GABA_B$ receptor agonist baclofen (100–300 μM) which has been discussed as a potential therapeutic agent in the treatment of alcoholism [2]. In addition, ethanol (50 and 100 mM) increased the aspect ratio (major axis/minor axis) and decreased circularity of the astrocytes. These changes were also blocked by baclofen. The reversal of ethanol effects by baclofen was, at least in part, inhibited by a $GABA_B$ antagonist CGP35348, implying that it was mediated by $GABA_B$ receptors.

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WTH01-03

PAK3 is implicated in the switch from oligodendrocyte precursor cells to differentiated oligodendrocytes

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Background: P21 activated kinase 3 (PAK3) is a serine/threonine kinase belonging to group I of the PAK family and acts downstream the Rho GTPases: Rac and Cdc42. PAK3 is mainly expressed in the brain and its role has been investigated in neurons but not in glial cells. Many mutations of the *pak3* gene have been described in non-syndromic intellectual disability diseases (ID). The

causes of intellectual deficits in patients with *pak3* mutations are still not fully identified. A transcriptome analysis revealed *pak3* expression in oligodendroglial cells. However, the role of PAK3 protein in these cells and in ID remains unknown.

Methods: We used *pak3* null mice to investigate, *in vivo* and *in vitro*, the function of PAK3 in proliferation, migration and differentiation of oligodendroglial cells.

Results: We found PAK3 expression in both oligodendrocyte precursor cells (OPCs) and mature oligodendrocytes. *In vivo* analysis of pak3 knockout brain revealed a significant reduction in the density of differentiated oligodendrocytes in the white matter with respect to WT at postnatal stages. *In vitro* experiments showed no major defects in proliferation or migration of OPCs derived from *pak3* KO mouse, despite high expression of PAK3 in these cells. However, the ability of *pak3*-null OPCs to differentiate into mature oligodendrocytes was significantly reduced.

Conclusion: PAK3 protein is highly expressed in OPCs and *pak3* loss-of-function impairs the switch of OPCs into mature oligodendrocytes both *in vivo* and *in vitro*. Our results highlight PAK3 as new regulator of OPC differentiation into oligodendrocytes.

WTH01-04

Genetic knockout of astrocyte LKB1: implications for glioma and stem cell research

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Our lab recently identified a single nucleotide polymorphism in the STK11 gene as a novel risk factor for multiple sclerosis in women. STK11 encodes the liver kinase b1 (LKB1) protein, a serine/threonine kinase involved in many vital signaling pathways including those controlling cell metabolism, growth, and polarity. It is also a known tumor suppressor, and mutations in STK11 are found in numerous forms of cancer in peripheral tissues. To study the role of LKB1 in the central nervous system, we acquired a strain of mice with STK11 flanked by loxP sites. Using an adeno-associated virus driving Cre recombinase expression, we knocked out LKB1 from primary cortical astrocytes cultured from postnatal day 1 mouse pups. Subsequently, the LKB1 knockout astrocytes (ALKO cells) began to proliferate rapidly and demonstrate characteristics of transformed cells: loss of contact-inhibition, anchorage-independent proliferation, enhanced migratory capability, and immortalization. These cells also displayed sensitivity to metformin treatment, increased inflammatory responses to cytokines, and altered cellular morphology. Interestingly, the ALKO cells also began to lose expression of glial fibrillary acidic protein (GFAP – a marker of mature astrocytes) and re-express the stem cell markers Sox2 and Oct4, localized to the nucleus. In preliminary experiments we have also successfully driven the expression of oligodendrocyte- and neuron-specific markers. These findings have two major implications. The first is that decline in LKB1 expression or activity may play a role in glioma pathology, and that this cell line may be useful as a glioma model. The second is that inhibition of LKB1 activity may represent a novel way of reprogramming astrocytes back into into multi- or pluripotent cells. Further studies to explore the relevance of LKB1 dysfunction in human gliomas, map the multi- or pluripotent potential of these cells, and validate their use as a model system are underway. Additionally, we are currently attempting to replicate the results using siRNA knockdown of LKB1 expression.

WTH01-05

Morphological analysis of microglial and astrocyte populations in the superficial dorsal horn of spinal cord in aged mice

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One of the most problematic pain states to treat is neuropathic pain caused by lesions in the peripheral or central nervous system. Neuropathic pain becomes more common as we age with up to 50% of patients suffering neuropathic pain in middle age or older. New pain treatments are required to address the growing clinical and social issue of neuropathic pain in the elderly. Animal studies have confirmed that nerve injury-induced neuropathic pain is also more intense and prolonged in aged animals. A shortcoming of this literature is that experiments studying the cellular mechanisms underlying neuropathic pain are limited to young adult animals (1–3 months). From this work it is clear that microglia and astrocytes are critical in linking nerve injury to central sensitization and neuropathic pain. Given what is known about neuropathic pain and aging, we cannot simply extrapolate this "young" literature to old animals. We assessed the morphology and activation of glial populations in the spinal cord of aged mice. Aged (28–30 months) and young (3 months) mice were deeply anesthetized (ketamine, 100 mg/kg) and trascardially perfused (4% PFA). Spinal cords were sectioned and immunolabelled with markers of microglia and astrocytes (IBA1 and GFAP, respectively). Sections were imaged and analysed using in house software to provide a semi-automated quantitative assessment of glial morphology. We found there were more microglia in aged spinal cords (12 ± 1 per ROI vs. 6 ± 2 per ROI, aged vs. young), and these cells had more retracted processes (38 ± 2 mm vs. 47 ± 3 mm, aged vs. young). Astrocyte morphology also differed in aged spinal cords with a more extensive and hypertrophied appearance (876 ± 47 vs. 642 ± 48 mm^2, aged vs. young). This morphological analysis confirms that microglia and astrocytes are in an activated state in the aged spinal cord. This mirrors many of the features observed in a neuropathic spinal cord and suggests that aging pain circuits may be neuropathic at baseline.

WTH01-06

The anti-coagulant warfarin increases glial cell activation

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The commonly used anti-coagulant warfarin reduces clotting by inhibiting vitamin K recycling necessary for activation of gamma-glutamyl carboxylase (GGC), which carboxylates and activates clotting proteins. Since vitamin K and GGC have roles in other physiological processes including within the CNS, we tested the hypothesis that chronic treatment with warfarin would lead to alterations in indices of neuroinflammation.

Methods: Adult healthy mice were treated with warfarin for up to 4 weeks, after which brain sections were examined for signs of inflammation, sulfatide content, and expression of genes relevant to vitamin K regulated processes. As a control, we compared the effects of warfarin to those of the anti-coagulant dabigatran etexeliate (DE), which in contrast to warfarin does not reduce vitamin K levels but directly inhibits thrombin. Statistical comparisons were made by 1-way ANOVA and Student's *t*-tests.

Results: We observed that after 4 weeks, warfarin, but not DE induced significant microglial and astroglial activation in different brain regions (cortex, cerebellum, and hippocampus) while in contrast DE showed trends towards reducing the basal levels of glial inflammation. Ultra HPLC analysis of samples measured after 1 week of treatment showed that warfarin, but not DE, reduced cerebellar levels of the C18:0 sulfatide. Quantitative PCR analysis showed that both warfarin and DE caused changes (both increases and decreases) in the expression of a panel of genes involved in vitamin K dependent processes, in different brain regions and with the largest changes in the cerebellum.

Conclusions: These results suggest that warfarin, but no DE, induces alterations within the CNS including increased neuroinflammatory responses.

WTH01-07

Calcium channel inhibitors limit lipid peroxidation and preserve myelin structure in nerve vulnerable to secondary degeneration

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Following injury to the central nervous system, cells beyond the primary injury site undergo secondary degeneration, with Ca^{2+} overload and oxidative stress contributing to further loss of neurons, compact myelin and function. Given that Ca^{2+} enters cells through multiple ion channels, therapeutic strategies to reduce Ca^{2+} influx are likely to require inhibition of several Ca^{2+} channels. We have previously assessed efficacy of various combinations of three Ca^{2+}channel inhibitors for treatment of secondary degeneration: using lomerizine, YM872 and/or oxATP to block voltage gated Ca^{2+}channels, Ca^{2+}permeable AMPA receptors and purinergic $P2X_7$ receptors respectively. Using partial transection of the optic nerve of adult PVG rat to model secondary degeneration *in vivo*, we demonstrated that the three Ca^{2+}channel inhibitors in combination were required to preserve behavioural function, node of Ranvier length, and limit myelin abnormalities, 3 months following injury. However, it is not clearly understood if and how combinations of ion channel inhibitors impact upon oxidative stress and structural contributors to axon and myelin integrity. Here we demonstrate that treatment with the three Ca^{2+}channel inhibitors in combination minimised oligodendrocyte precursor cell loss, and reduced lipid peroxidation 3 days following injury ($p \leq 0.05$). In comparison, node length was preserved by all combinations of the Ca^{2+} channel inhibitors ($p \leq 0.05$), and paranode length was reduced by most combinations, at 3 days. Importantly, novel redox proteomic analysis demonstrated changes in ratios of oxidised methionines, with significant increases in oxidation observed in white matter proteins important for axonal structure and myelin integrity, 3 days following injury. Taken together our results indicate that early prevention of lipid peroxidation and protection of oligodendrocyte precursor cells is associated with long term preservation of function. Preventing the oxidative changes of secondary degeneration is likely to be an important component of efficacy of combinatorial strategies involving Ca^{2+} channel inhibitors.

WTH01-08

Efficient derivation of myelinating oligodendrocytes from NKX2.1-GFP human embryonic stem cell reporter line

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Transplantation of human embryonic stem cell (hESC)-derived oligodendrocyte precursors (OPCs) has been considered as a potential therapeutic strategy for acquired demyelinating, or inherited hypomyelinating disorders. However, the yield of homogeneous hESC-derived OPCs is a limiting factor of current protocols in attempt, to exclude any unwanted immature or other lineage cells that may form gliomas. To increase the homogeneity of hESC-derived OPCs, we utilised the NKX2.1-GFP hESC reporter line and sorted GFP+ and GFP- cells through fluorescent-activated cell sorting at the neural precursor stage of differentiation, based on their peak GFP expression. Subsequently, these two populations underwent further differentiation towards the oligodendroglial lineage under defined conditions. The differentiation potential towards the oligodendroglial lineage was compared between GFP+ and GFP- cell populations. Their oligodendroglial lineage commitment was determined at specific time points during each stage of differentiation by the presence of various antigens which included Nestin, PDGFRα, NG2, O4, MBP, GFAP, β-III tubulin, and Epcam by flow cytometry and immunocytochemistry (ICC). Furthermore, the expression of transcription factors that drive oligodendrogenesis such as Sox10, Olig2 and Nkx2.2 were demonstrated by ICC and qRT-PCR. Our data show that a high yield of homogeneous OPCs could be derived from the NKX2.1-GFP hESC reporter line subsequent to GFP-based sorting (60% of PDGFRα+/NG2 + OPCs from GFP+ populations compared to 2% of PDGFRα +/NG2 + OPCs from GFP- population). The yield of O^{4+} pre-myelinating oligodendrocytes was significantly higher in GFP+ populations (45%) compared to the GFP- population (< 1%). Furthermore, these O^{4+} pre-myelinating oligodendrocytes from GFP+ populations were able to myelinate 30% of axons from rat retinal ganglion cells. Consequently, myelinating oligodendrocytes were efficiently derived from the NKX2.1-GFP hESC reporter line, suggesting a positive role for NKX2.1 during *in vitro* human oligodendrogenesis. Further studies based on these preliminary data may enhance the understanding of oligodendrocyte development and contribute to therapeutic mechanisms for patients with demyelinated or inherited hypomyelinating disorders.

WTH01-09

Developmental expression of GLT1D: a newly discovered and highly abundant glutamate transporter

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We have identified a new, functional and abundant form of the dominant glutamate transporter GLT1, (called GLT1d), which may represent up to 40% of total GLT1 in the adult brain. This study explores the developmental expression of GLT1d in the rodent brain. The development of glutamate homeostatic mechanisms is

tightly linked to the onset of glutamatergic neurotransmission. We have compared the GLT1d expression profile during development with GLT1a (the canonical form of GLT1) and glutamine synthetase (which catabolises accumulated glutamate in astrocytes). We have performed western blotting on whole brain lysate samples from C57BL/6 mice aged from day of birth (P1) to adult. Western blots were probed using specific antibodies against GLT1d, GLT1a and glutamine synthetase.

Results: We demonstrate that GLT1d is just demonstrable in western blots at P1, but significant levels are evident from ~ P7, with a rapid rise in expression thereafter, peaking at around P28, with a slight decline thereafter to adulthood. Using the same brain lysates, we demonstrate that GLT1a is similarly just detectable at birth and peaks slightly earlier, at around P21 whilst GS is initially detectable from P1 and similarly rises with a peak at P21. GLT1d, GLT1a and GS levels decrease slightly to adulthood after each respective peak.

Conclusions: We conclude that very low levels of GLT1d are present at birth but the rise in postnatal expression of GLT1d is in broad register with the rise in other components of the glutamate homeostatic system. Levels of GLT1d mRNA and cellular localization of the GLT1d protein are both the subject of ongoing investigations. We suggest that GLT1d will play a significant role in normal glutamate homeostasis in the developing and adult brain.

WTH01-10

Hyaluronic acid regulates astrocytes shape via CD44 receptor

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The interaction of astrocytes and its extracellular environment is crucial to fulfill their function in physiological and pathophysiological conditions. The changes in the function of astrocytes are tightly coupled to the changes in their morphology (for instance: the glutamate clearance in synaptic cleft, migration, astrogliosis). The hyaluronic acid (HA) is the main component of the extracellular matrix in the brain. The major receptor for HA is CD44, a type I transmembrane glycoprotein. CD44 is expressed by a subset of astrocytes, but the functions and mechanisms of action of the protein within these cells are poorly understood. Dynamic reorganization of the actin cytoskeleton is proposed to be responsible for morphological changes of astrocytes, and it is controlled by small GTPases of the Rho family, including Rac1. In the present study we investigated the influence of hyaluronidase, knock-down of CD44 by specific shRNA, and CD44 overexpression, on astrocyte morphology. Our results showed that hyaluronidase treatment, as well as the knock down of CD44, in astrocytes result in "stellate"-like morphology, whereas overexpression of CD44 causes increase in cell body size and change cell shape of astrocytes into more regular. We used the FRET-based biosensor and a dominant negative mutant of Rac1 to investigate the involvement of Rac1 activity in these hyaluronidase- and CD44-dependent morphological changes of astrocytes. Both, hyaluronidase treatment and knock-down of CD44 enhances, while the overexpression of CD44 reduces the Rac1 activity in astrocytes. Moreover, the morphological changes evoked by both treatments were blocked by inhibition of Rac1 activity. These findings indicate that regulation of Rac1 activity is responsible for hyaluronidase and CD44-driven morphological changes of astrocytes.

WTH01-11

The alteration in differentiation pattern of oligodendrocytes contributed to disease pathogenesis of ALS

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Oligodendrocytes provide structural support and allow rapid signal transduction in axons in the central nervous system. During development, oligodendrocytes differentiated from oligodendrocytes progenitor cells (OPCs) to mature oligodendrocytes that produce myelin. In adult, oligodendrocytes undergo constant turnover, in which oligodendrocytes are formed from the adult OPCs pool in a similar manner as that during development. This is either to replace the damaged oligodendrocytes or to myelinate the axons that weren't myelinated during development. The disruption of such turnover process is known to be involved in demyelinating disease such as multiple sclerosis, in which the damaged oligodendrocytes could not be replaced. Recent studies found the presence of ALS mutant (both SOD1 and TDP43) in the cytoplasm of oligodendrocytes in spinal cord from ALS patients. An increase proliferation rate of OPCs in mSOD1 mice was observed together with a decrease in myelination. We hypothesis that the oligodendrocytes in mSOD1 mice has an altered differentiation process which contributed to the decrease in myelin production, in which potentially involved in the disease pathogenesis of ALS.

To study the differentiation process, we performed immunohistochemistry in spinal cord collected from 8 and 20 weeks old mSOD1 mice and WT mice using antibodies against markers for the specific differentiation stages. Among the markers studied, we observed an increase number of GPR17 (marker for pre-mature oligodendrocytes) labeled oligodendrocytes in the 20 weeks mSOD1 mice compare to the WT. These differences were not observed in the 8 weeks old mice. A morphology studies demonstrated that the GPR-17 positive cells found in 20 weeks old mSOD1 mice have a more branching morphology compare to the WT, indicating that these cells are at a more advance differentiation stages.

Overall we provided evidences for a potential disruption in the differentiation process of oligodendrocytes in the mSOD1 mice. More studies will be required to understand the mechanism that affects this differentiation and how this links to the pathogenesis of the ALS.

WTH01-12

Pharmacological characterisation of new kainate receptor antagonits

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Kainate receptors (KARs) are one of the excitatory L-glutamate gated ion-channels. KARs are widely distributed in the CNS. KARs

are tetrameric assemblies of five different subunits GluK1-5. Presynaptically KARs regulate the release of glutamate and γ-aminobutyric acid (GABA) and postsynaptically they modulate fast excitatory synaptic transmission. However, the lack of selective pharmacological tools has hindered progress in the understanding of the functions of specific KAR subunits. Here we characterise the pharmacological properties of two newly synthesised willardine derivatives (UBP3000 and UBP3001) as KAR antagonists.Method-Calcium fluorescence assay HEK293 cells stably expressing homomeric GluK1 (Q), GluK2 (Q), and GluK3 receptors were used and compounds were tested using a Ca^{2+} influx assay according to manufacturer's instructions (Molecular Devices, UK). IC50 and Ki values for reference antagonists (UBP310 and ACET) were also monitored. Concentration-response curves were analysed using GraphPad Prism 5.03 software, with slope factor fixed at 1, and top and bottom fixed at 100% and 0% inhibition, respectively. Results We found that UBP3000 and UBP3001 are selective antagonists of GluK1 vs. GluK2 and GluK3 receptors. For GluK1 receptors UBP3000 and UBP3001 had IC50 values of 80 ± 21 nM and 171 ± 44 nM, with calculated Ki values of 34 ± 12 nM and 45 ± 14 nM (n = 4), respectively. Furthermore, negligible antagonistic activity on GluK2 receptors was displayed by UBP3000 and UBP3001 when tested up to a concentration 100 μM and the % of antagonism was 6.8 ± 0.6%, 3.8 ± 0.5% (n = 4), respectively. However, no significant antagonist effects of the willardine derivatives (100 μM) were observed on L-glutamate (400 mM) induced calcium influx in GluK3 cells (n = 4). Conclusion Our results demonstrate that UBP3000 and UBP3001 are novel potent and selective GluK1 antagonists and are potentially useful new tools to study KARs and their involvement in synaptic transmission, neuronal plasticity and development.We anticipate that continued development of compounds in the same series will provide new tools to explore functions of specific subunits of KARs and will contribute to the study of their physiological roles in the CNS.

WTH01-13

Reelin, through its receptor ApoER2, regulates Schwann cell migration by activating Rac1; involvement of Tiam1 and Par3

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ApoER2 and its ligand, Reelin play relevant roles in the central nervous system. Reelin guides' migration of neurons during development stimulates neurogenesis and synaptic plasticity in the adult brain. When Reelin binds to the extracellular domain of ApoER2 it induces the recruitment of the cytosolic protein Dab1, which is phosphorylated by Fyn. Subsequently the pathway includes the activation of PI3K, Akt and cell cytoskeleton related proteins including the small GTPases of the Rho family and LIMK1. In this study we investigated the role of ApoER2/Reelin during cell migration of Schwann cells, process critical for development and regeneration of peripheral nervous system (PNS). We first demonstrated that Schwann cells express different splicing variants of ApoER2 and the ligand reelin. Moreover, reelin triggers a signaling pathway in these cells. In addition, in two assays directed to study cell migration, wound healing and Boyden chamber, we found that Reelin significantly stimulated migration. This migration would be dependent in Rac1, since this GTPase was activated by reelin in a time dependent manner. In addition cells expressing the FRET probe Raichu-Rac showed high Rac activity at the edge of the cells and *lamellae*. We found that ApoER2 associates with the polarity complex protein Par3 in a splice-variant dependent way. As Par3 recruits Tiam1, a specific GEF for Rac1 we knockdown Tiam1 observing an inhibition in the Reelin-induced migration. Similarly the migration induced by Reelin was affected by knocking down Par3 indicating that Par3 and Tiam1 are key regulators of the reelin-induced schwann cell migration. It is known Reelin can signal via ApoER2 or VLDL receptor. By using a specific siRNA for ApoER2 we demonstrated that Reelin requires ApoER2 to induce migration. Our working model considers that upon Reelin binding to ApoER2, Par3 would be recruited to the receptor tail, activating Tiam1 and Rac1 specifically to the leading edge of the Schwann cell plasma membrane. Taken together, this research demonstrates a new function for Reelin at the PNS with probable implications in development and regeneration. (Supported by FONDECYT #1150444, Post-doctoral #3130373 and MINREB P-07-011-F).

WTH01-14

Signalling and crosstalk of AMPA/Kainate, mGlu5 and $GABA_B$ receptors in oligodendrocyte precursor cells

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We have characterised group I metabotropic glutamate (mGlu) and $GABA_B$ receptor ($GABA_BR$) signalling in oligodendrocyte precursor cells (OPCs) and investigated interactions between these and ionotropic glutamate receptor (iGluR)-mediated responses. Activation of mGlu5 in cultured primary OPCs produced a rise in intracellular Ca^{2+} $[Ca^{2+}]_i$ that involves both intracellular Ca^{2+} stores and extracellular Ca^{2+}. Repeated agonist application induced desensitization of this response. $GABA_BR$ activation failed to produce an increase in $[Ca^{2+}]_i$, but decreased the mGlu5-mediated $[Ca^{2+}]_i$ rise. Kainate application elicited an inward current in *in situ* white matter OPCs and an increase in $[Ca^{2+}]_i$ in cultured OPCs. This response was dependent on extracellular Ca^{2+} and was at least partially mediated by AMPA-type iGluRs. Interestingly, group I mGluR activation did not affect kainate receptor (KAR)-mediated $[Ca^{2+}]_i$ responses in white matter OPCs, suggesting that mGluR may differentially regulate iGluR expression in white and grey matter OPCs. In cultured OPCs however, group I mGluR activation did increase kainate-induced $[Ca^{2+}]_i$ rises. This mGluR-mediated effect could be blocked by the activation of $GABA_BR$. These results suggest that mGluR and $GABA_BR$ signalling pathways in OPCs can interact and that this interaction can have effects on the modulation of AMPAR/KAR-mediate iGluR responses by group I mGluRs. With both $GABA_BR$ and AMPAR/KAR being involved in the control of OPC proliferation and maturation, and AMPAR/KAR-mediated OPC death being implicated in white matter damage, the interactions between mGluR, $GABA_BR$ and KAR/AMPAR in OPCs are likely to be important for the regulation of OPC behaviour in normal and pathological conditions.

WTH01-15

Characterising the relationship between TrkB and Fyn kinase: critical mediators of BDNF's promyelinating effect on oligodendrocyte

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Fyn kinase is required for oligodendrocyte differentiation *in vitro* and myelination *in vivo* and has been shown to target neuronal TrkB to lipid rafts following treatment with BDNF. We have found that TrkB regulates Fyn kinase protein levels in HEK293 cells. To further investigate the relationship between TrkB and Fyn kinase, we have generated conditional knock-out mice where TrkB is deleted at different stages of the oligodendroglial lineage and are currently assessing if Fyn protein levels are altered *in vivo*.

Additionally, we have interrogated the signalling pathway that BDNF utilises to enhance oligodendrocyte myelination and have previously identified TrkB receptors and Erk1/2 as signalling mediators. Here, using the *in vitro* myelination assay, we show that Fyn is another mediator of the promyelinating effect of BDNF that acts upstream of Erk1/2. In order to interrogate the effect of Fyn we virally manipulated oligodendrocyte precursor cells to overexpress wild type (WT) or kinase dead (KD) Fyn, and used these cells in the *in vitro* myelination assay. The expression of KD Fyn attenuated the BDNF-induced myelination, whereas the expression of WT Fyn enhanced basal myelination and did not respond to the addition of exogenous BDNF as assessed by an increase in the number of myelinated axonal segments. BDNF's activation of Fyn in myelinating co-cultures is a TrkB dependent effect, as shRNA-mediated knockdown of TrkB in oligodendrocytes prevents Fyn autophosphorylation.

Finally, we are undertaking *in vitro* myelination assays utilising TrkB null oligodendrocytes and have found they exhibit a reduced capacity for myelination under basal conditions. We are currently assessing whether virally engineered over-expression of WT Fyn kinase can rescue their hypo-myelinating phenotype. Collectively our data suggest that Fyn and TrkB are mediators of the pro-myelinating effect of BDNF and have a complex regulatory relationship which may contribute to the compensation mechanisms seen in the important process of CNS myelination.

WTH01-16

Expression of functional ionotropic glutamate and GABA receptors in astrocytes of the ventrobasal thalamus

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Astrocytes may express transmitter receptors, which allow them to sense and to respond to neuronal activity. By now, astrocytic neurotransmitter receptor expression was characterised only in a few brain regions. Here we provide a detailed analysis of AMPA and $GABA_A$ receptors in astrocytes of the mouse ventrobasal thalamus. Receptor currents were investigated using the patch clamp-technique combined with single cell RT-PCR to explore receptor subunit expression.

To improve voltage-clamp control and avoid indirect effects, freshly isolated cells were employed using juvenile hGFAP/EGFP mice. Application of the AMPA receptor agonist, kainate, supplemented with cyclothiazide (CTZ), which modulates AMPA receptors, lead to a 6-fold potentiation of the kainate-induced responses. I/V relations of the kainate/CTZ-induced receptor responses were linear with a reversal potential close to 0 mV and were almost completely blocked by the AMPA receptor blocker GYKI 53655. Two sub-populations of astrocytes were found, expressing or lacking AMPA receptors. AMPA receptor-bearing astrocytes displayed a lower density of inwardly rectifying K^+ (Kir) currents than those cells lacking the receptors. The relative Ca^{2+}-permeability of the receptors, P_{Ca}/P_K, was determined using Na^+-free, high Ca^{2+} (50 mM) bath solutions and amounted to $P_{Ca}/P_K = 0.15$. Thus, AMPA receptors of juvenile thalamic astrocytes show a low Ca^{2+}-permeability. Single-cell transcript analysis of AMPA receptors revealed a prevalent expression of GluA2 and GluA3 subunits.

$GABA_A$ receptors currents were found in each cell. The cells were exposed to muscimol which produced desensitizing inward currents that were sensitive to bicuculline. Most frequently, expression of the subunits α2, α5, ß1, γ1 and γ3 was observed.

Our findings add to the emerging concept of functional heterogeneity between astrocytes within and across brain regions.

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WTH01-17

A novel subtype of astrocytes regulates neuronal excitability via release of gliotransmitters

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Astrocytes play active roles in the regulation of synaptic transmission. Neuronal excitation can evoke Ca^{2+} transients in astrocytes, and these Ca^{2+} transients can modulate neuronal excitability. While only a subset of astrocytes appears to communicate with neurons, the types of astrocytes that can regulate neuronal excitability are poorly characterized. We found that ~30% of astrocytes in the brain express transient receptor potential vanilloid 4 (TRPV4), indicating that astrocytic subtypes can be classified on the basis of their expression patterns. When $TRPV4^+$ astrocytes are activated by ligands such as arachidonic acid, the activation propagates to neighboring astrocytes through gap junctions and by ATP release from the $TRPV4^+$ astrocytes. Following activation, both $TRPV4^+$ and $TRPV4^-$ astrocytes release glutamate, which acts as an excitatory gliotransmitter to increase synaptic transmission through group 1 mGluR. Our results indicate that $TRPV4^+$ astrocytes constitute a novel subtype of the population and are solely responsible for initiating excitatory gliotransmitter release to enhance synaptic transmission.

WTH01-18

GFAP splice variant expression in the developing rodent brain

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Glial fibrillary acidic protein (GFAP) is an intermediate filament expressed in the cytoskeleton of grey and white matter astrocytes throughout the central nervous system (CNS). GFAP expression is altered in many human diseases and disorders, including multiple sclerosis, stroke, Alzheimer's disease and traumatic brain injury. There are 9 GFAP splice variants identified that differ from the originally described GFAPα (β, δ, γ, κ, Δ135, Δ164, Δexon6, Δexon7 and ζ). The majority of the splice variants differ in the tail region of the protein, an important domain in the assembly of the cytoskeleton. Differential expression of the GFAP splice variants may result in an unstable cytoskeleton, which will contribute to the astrocyte's morphology and function during development or recovery from CNS insults. We have generated polyclonal antibodies against GFAPα, GFAPδ, GFAPγ, GFAPκ, GFAPΔexon6 and GFAP+1. We examined the expression of GFAP isoforms using western blotting and immunohistochemistry in developing rat CNS tissues (n = 12 at each time point). Immunohistochemistry revealed that GFAP splice variants co-localized with pan-GFAP, detected using a commercial polyclonal antibody from DakoCytomation. GFAPα, GFAPδ and GFAPκ were detectable by western blotting in the majority of tissues examined (cortex, hippocampus, thalamus, brain stem, cerebellum, spinal cord and retina). Expression of GFAP splice variants increased during the first postnatal week. Changing the stability of the cytoskeleton may be an important mechanism for plasticity of the CNS during development. GFAP splice variant expression may also play an important role in the repair/recovery phase of CNS diseases or disorders including stroke, epilepsy, traumatic brain injury and neurodegenerative diseases.

WTH01-19

Sortilins and neuropathic pain following peripheral nerve injury

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CNS glial cells (astrocytes and microglia) are crucially involved in the initiation and maintenance of chronic pain. Much less is known about glial cells in peripheral sensory ganglia, however it is increasingly clear that Satellite Glial Cells (SGCs) are activated in response to peripheral nerve injury and play an important role in chronic pain mechanisms. SGCs show upregulation of GFAP and a dramatic increase in gap junctions between SGCs in newly formed processes. This increased coupling of SGCs is observed in several pain models, and blocking the gap junctions decrease spontaneous neuronal electrical activity and hyperexcitability. Further, gap junction blockers relieve pain symptoms in animal pain models without affecting control animals.

The mechanism by which nerve injury induces these changes remains speculative, however the involvement of neurotrophins and neurotrophic receptors has been proposed. The Sortilins comprising five type-I transmembrane receptors differentially expressed in neuronal tissues of the central and peripheral nervous system. Sortilins show multiple roles in cellular transport and signaling as a sorting receptor interacting with a wide range of ligands comprising neurotrophic factors and neuropeptides. We have previously demonstrated the involvement of the receptors sortilin and SorCS2 in neurotrophin signaling in by regulating Trk trafficking and by engaging in a complex with the neurotrophin receptor p75NTR.

Based on sensory phenotypes following peripheral nerve injury we wish to investigate the function of SorCS2 in the peripheral nervous system. More specifically we want to characterize the expression of SorCS2 in the DRG and subsequently resolve if SorCS2 affects neurotrophin signaling and neuron-SGC interaction following peripheral nerve injury.

WTH01-20

Role of microglia in mediating exercise-induced neurogenesis

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The hippocampus is one of the primary brain structures critical for learning and memory, where the continuous and regulated production of new neurons – adult neurogenesis – is believed to underpin some hippocampal-based cognitive functions (Vukovic *et al*., 2013). Newborn neurons are produced from a pool of neural precursor/stem cells. Many different factors regulate their activity. We have shown that exercise readily stimulates neural precursor cells and increases hippocampal neurogenesis (Vukovic *et al*., 2012). Moreover, we discovered that microglia (the brain's resident immune regulatory cells) have a direct positive influence on the activation of neural precursor cells in the adult brain following exercise. We took advantage of *Csf1r-GFP* transgenic mice to selectively remove (or add) microglia from hippocampal neurosphere preparations through FACS (n = 4–7 per experimental condition). We found that increase in NPC number by 37% observed with running was abolished when endogenous hippocampal microglia were removed ($p < 0.05$). Conversely, the addition of hippocampal microglia that were isolated from the brains of voluntary wheel running mice proved an effective way to activate latent neural precursor cells and thus to increase the neurosphere formation frequency by 36% in preparations from sedentary mice ($p < 0.05$). Furthermore, we provide *in vivo* evidence that the beneficial effects of exercise on hippocampal neurogenesis are mediated by microglia.

WTH01-21

Understanding "Stress X Microglial interactions" in stroke-induced secondary neurodegeneration: a major opportunity for the preser

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Stroke patients experience persistently high levels of psychological stress. As there is very little evidence to indicate that stress can modify longer-term recovery processes, we decided to utilize an experimental model of stroke to determine whether exposure to chronic stress could modify the development of secondary thalamic neurodegeneration (STND), a commonly reported complication associated with stroke induced cortical damage. We focused on the involvement of microglia-like cells, as several studies have linked microglial activation to the development of STND. One month following stroke, in male mice, we identified that numbers of microglial-like cells, as well as putative markers of microglial structural reorganization (Iba-1), complement processing (CD11b), phagocytosis (CD68), and antigen presentation (MHC-II) were all elevated in response to occlusion ($p < 0.05$). Occluded animals that were also exposed to chronic stress exhibited lower levels of Iba-1 positive cells and a reduced expression of Iba-1 and CD11b compared to the "occlusion-alone" group ($p < 0.05$). The dampened expression of microglial-like markers observed in stressed animals was associated with significant additional loss of neurons, indicating a potential neuroprotective role for microglia ($p < 0.05$). We will also present new findings concerning the effect of the microglial-modulator minocycline on these phenomena. Together, our results suggest that STND can be negatively modified, potentially in a microglial dependent manner, by exposure to chronic stress.

WTH02 Neurogenesis and Cel Differentiation (Part 2)

WTH02-01

Exposure to the antibiotic vancomycin modifies development of the enteric nervous system in early postnatal mice

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The trillions of microorganisms living in the bowel (microbiota) have recently been shown to be modulators of the nervous system. In the gut, these microbes influence the firing of enteric neurons and, as a result, gut motility and function. Establishment of microbiota and development of the enteric nervous system (ENS) both occur during early postnatal life. While it is likely that microbiota affect ENS development, this remains unproven. Gut microbiota are susceptible to external factors such as diet and antibiotics in infants and young children. We examined whether antibiotics ingested during early postnatal stages affects ENS development. Mouse pups were fed from birth with a daily dose of vancomycin (83.3 mg/kg/day) or water and their duodenums and colons were examined at postnatal day, P10/11. Motility patterns were examined using video imaging. The density of myenteric neurons marked by the pan-neuronal marker Hu, and proportion of neurons that expressed neuronal nitric oxide synthase (nNOS) and calbindin were examined immunohistochemically. Colonic motility was significantly increased in vancomycin-fed pups compared to their water-fed littermates, whereas no effect was observed in the duodenum (n = 12 mice). The colons of vancomycin-fed pups had a significantly lower neuron density (n = 4 mice). Moreover, there was a trend towards a lower proportion of nNOS and a higher proportion of calbindin-expressing neurons in the colons of vancomycin-fed pups. Thus, neonatal exposure to vancomycin alters development of enteric neurons and motility patterns in the colon, but not the duodenum.

WTH02-02

Rho kinase inhibition promotes functional improvement following traumatic brain injury

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While neurogenesis from subventricular zone (SVZ) neural progenitor cells is stimulated following traumatic brain injury, this potentially regenerative process is ultimately abortive, with most newborn neurons failing to survive. This is likely due to a lack of maturation and integration into circuitry in the injured cortex. Enhancing the survival of these new neurons may improve function following brain injury. Inhibition of Rho kinase is known to increase neurite outgrowth and regeneration and we have previously demonstrated that Rho kinase inhibition enhances survival of newborn neurons from the hippocampus under basal conditions. Here we examined the effect of Rho kinase inhibition on SVZ neurogenesis and behavioural outcome following controlled cortical impact injury. The Rho kinase inhibitor, Y27632 (20 μM), was infused in the ipsilateral lateral ventricle for 7 or 28 days after injury, with Edu administration for 7 days. Motor function was assessed at 2 and 33–35 days post-injury. Brains were taken at 1, 7 and 35 days for analysis. Both short and long-term infusion of Y27632 improved motor performance 33–35 days after injury. However, this beneficial effect of Rho kinase inhibition is independent of enhanced neurogenesis, since Y27632 did not affect the number of Dcx+ neuroblasts migrating to the pericontusional cortex at 7 days post-trauma, or the number of NeuN+/EdU+ new mature neurons at the injury site at 35 days. Rho kinase can contribute to inflammation and blood-brain barrier disruption in the injured brain. Therefore, we investigated the effect of Y27632 on gliosis and found that treatment had no effect on astrocyte and macrophage accumulation in the injured cortex at 35 days. Effects of Y27632 on early glial activation and on blood-brain-barrier integrity and angiogenesis are currently being examined as potential underlying mechanisms for the enhanced motor recovery promoted by Rho kinase inhibition.

WTH02-03

Microbats have adult neurogenesis which appears to vary with the different foraging ecologies and evolutionary history

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The mere existence of adult neurogenesis is undisputed today. Cognisant of the varying effect of selective pressures on the neuroanatomy and structural brain plasticity, adult neurogenesis in the speciose suborder of microchiroptera could provide insight to the extent of the effect of ecology and evolutionary history on the phenomenon. In this study we hypothesised that adult neurogenesis in the suborder of microchiroptera varies between the pteropodiformes and verspertilionformes given their different ecologies and evolutionary history. Using immunohistochemical techniques for the endogenous markers Ki-67 and DCX to demonstrate proliferating cells and immature neurons respectively, we performed immunohistochemistry on 18 brains from 9 microchiropteran species including the verspertilionformes such as *Miniopterus schreibersii*, *Chaerephon pumilus*, *Coleura afra*, and *Nycteris macrotis* and pteropodiformes

such as *Asellia tridens*, *Cardioderma cor*, *Hipposideros commersoni*, *Hipposideros fuliganosus* and *Triaenops persicus*. Our results indicate that hippocampal neurogenesis is a common feature in all the species of microchiroptera used in this investigation as we detected Ki-67-immunopositive and DCX-immunopositive cells in the dentate gyrus, subventricular zone, olfactory bulb, neocortex and piriform cortex. Pteropodiformes, which are closely related to pteropodidae with a larger relative brain size to body ratio and are mostly clutter-edge or clutter foragers, had high cell densities in most of these regions than verspertilionformes. The present study demonstrates that adult neurogenesis in the speciose suborder of microchiroptera varies with the ecologies and evolution.

WTH02-04

Differentiation of cortical neural precursor cells stimulated by direct current electrical field pulses

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Neural stem/progenitor cell (NPC) in the adult mammalian brain is one of the promising candidates for developing therapeutic strategies of neuroregeneration. The differentiation of NPCs depends on various *in vivo* environmental factors, such as nerve growth factor (EGF) and endogenous electrical field (EF). In this study, we investigated the morphologic and phenotypic changes of mouse neural stem and progenitor cell (mNPC) exposed to square-wave DC EF pulse with magnitude of 300 mV/mm at frequency of 100 Hz. The DC EF stimulation was lasted for 48 h and the morphology changes of mNPCs were monitored continuously. The length of primary processes and the amount of branching significantly increased during 24 to 48 h of the DC EF pulse stimulation. Our results showed that DC EF pulse enhanced the mNPC properties at 3 days *in vitro* (DIV). In addition, the DC EF pulse induced the mNPC differentiation into neurons, astrocytes and oligodendrocytes simultaneously in serum free medium with both EGF and basic fibroblast growth factor (bFGF). In another words, without chemical stimulation, the DC EF pulse indeed induces mNPC differentiation. With further studies, EF may be applied to control NPC differentiation and used for the development of therapeutic strategies that employs NPCs to treat nervous system disorders.

WTH02-05

The association of the vitamin D receptor and nuclear matrix in a neuroblastoma line

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Vitamin D (1α, 25 dihydroxy vitamin D_3) performs a critical role in brain development by modulating neuronal proliferation and differentiation. Vitamin D acts via the vitamin D receptor (VDR), a member of the nuclear receptor superfamily. Although VDR has been found in the cytoplasm and membrane, it is predominantly located within the nucleus. Little is known however, regarding the actual distribution of VDR within the nucleus. The aim of the current study is to investigate this *in vitro*, using a human SH-SY5Y cell line transfected with recombinant VDR. Using immunoblot, we showed that VDR is associated with the nucleus matrix. Furthermore, our immunofluorescence results indicate that VDR is found in the cytoplasm of mitotic cells, at all cell cycle phases (prophase, metaphase and anaphase). This cytoplasmic distribution of VDR in mitotic cells further suggests an interaction between VDR and the nuclear matrix, since the nuclear matrix disperses throughout the cytoplasm during mitosis. In addition, we observed a significant increase of VDR protein levels in mitotic cells. Taken together, this study shows for the first time that VDR is associated with the nuclear matrix in a neuroblastoma line. The nuclear matrix, as a component of higher-order nuclear architecture, contributes to the activity of genes and regulatory factors. Multiple co-activators of VDR, such as the VDR-interacting protein (DRIP205) and NcoA, are located in this structure. The VDR may bind to these co-repressors/co-activators in the nuclear matrix to modulate downstream genetic expression. Further experiments are warranted to examine whether the interaction of VDR and the nuclear matrix is ligand-dependent, and whether this interaction precedes vitamin D-induced transcriptional upregulation.

WTH02-06

FMRP and dendritic local translation of αCaMKII mRNA are required for the structural plasticity underlying olfactory learning

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In the adult brain, structural plasticity allowing gain or loss of synapses remodels circuits to support learning. In Fragile X Syndrom (FXS), plasticity defects leading to learning deficits originates in the absence of Fragile X Mental Retardation Protein (FMRP). FMRP is a master regulator of local translation but its implication in learning-induced structural plasticity is unknown. Using an olfactory learning task requiring adult-born neurons in the olfactory bulb (OB) and cell-specific ablation of FMRP, we investigated whether learning shapes adult-born neurons morphology during their synaptic integration into the pre-existing network and asked if these structural changes depend on FMRP. A local translation reporter was used to reveal local translation of the alpha subunit of the Calcium Calmodulin-dependent Kinase II (αCaMKII) mRNA, an FMRP target, during learning in presence or absence of FMRP. Finally, we used αCaMKII mutant mice with altered dendritic localization of αCaMKII mRNA to investigate the role of locally translated αCaMKII in learning-dependent structural plasticity.Learning induces profound changes in dendritic architecture of adult-born neurons. Learning and associated structural changes are

prevented by ablation of FMRP in adult-born neuronst. Moreover, learning triggers an FMRP-dependent increase of local dendritic mRNA translation of αCaMKII in adult-born neurons and dendritically translated αCaMKII is necessary for learning and associated structural modifications. Our results strongly suggest that FMRP mediates structural plasticity of OB adult-born neurons to support olfactory learning through αCaMKII local translation. This reveals a new role of dendritic local translation in learning-induced structural plasticity, necessary for dendrite morphogenesis and spine production. This might be of clinical relevance for the understanding of critical periods disruption in autism spectrum disorder patients, among which FXS is the primary monogenic cause.

WTH02-07

The response of endogenous neural progenitor cells throughout the neuroaxis after a rat spinal cord contusion injury

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Spinal cord injuries (SCI) result in the destruction of neuronal and glial cells causing complete/incomplete motor function within an individual. It has been reported that there is a niche of cells with long basal processes known as tanycytes exhibiting progenitor cell qualities in the ependymal layer of the central nervous system (CNS) and studies have shown that these endogenous neural progenitor cells (eNPC) proliferate and differentiate very early in response to a SCI. Little is known about the extent of the eNPC response distal to the injury site throughout the CNS. The response of eNPC 24 h after a mild thoracic SCI (NYU Impactor) was examined at 6 regions of the neuroaxis in adult rats ($n = 25$) using nestin and glial fibrillary acidic protein (GFAP) immunohistochemistry as markers for NPC and astrocytes respectively. The regions examined were the third ventricle of the brain, the cervical enlargement, caudal, central and rostral to the lesion, and the lumbar enlargement. GFAP intensity was significantly high only at the injury site (ANOVA; $p < 0.001$) indicating a localised response. In contrast, nestin activity at the central canal was high at all levels of the spinal cord in the injured group, indicating a systemic eNPC response throughout the spinal cord even distal to the lesion site. No significant difference in nestin immunoreactivity was found at the third ventricle of the brain between the injured and control groups (Bonferroni *post hoc*; $p < 0.05$). Future studies should determine the response of eNPC after a SCI in other ventricular systems of the brain such as the aqueduct, the fourth ventricle, the lateral ventricle and the brain stem. Determining the extent of nestin is important in considering eNPC manipulation in SCI repair and regeneration.

WTH02-08

A forward genetic screen for mice with defects in neuronal migration

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Neuronal migration is a complex cellular process that underlies the laminar architecture of the cortex and hippocampus in the vertebrate brain. It is reliant on a molecular network that includes extracellular guidance cues (REELIN), membrane-bound receptors (VLDLR), intracellular signaling molecules (DAB1), microtubule associated proteins (DCX) and components of the cytoskeleton (TUBA1A, TUBB2B). Mutations in these genes cause severe structural brain abnormalities and mental retardation associated with epilepsy in humans. To identify new genes involved in neuronal migration we established a forward genetic strategy in mice employing the mutagen ENU. This recessive screen analyzed a broad range of structural brain phenotypes (e.g. cortical thickness, hippocampal layering, ventricular volume) in 38 pedigrees. This resulted in the identification of 6 mouse lines with heritable phenotypes, including the Marble mouse line. This line presents with a fractured pyramidal cell layer in the hippocampus with an intact cortical architecture. Behavioral phenotyping has revealed abnormalities in sensory motor gating, and spatial working memory. Genetic, molecular and histological characterization of this mouse line will yield insight into neuronal migration.

WTH02-09

Rbfox1, an autism causal gene, plays an essential role in cortical development

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Rbfox1 (aka Fox1 or Ataxin-2-binding protein 1(A2BP1)) is an RNA-binding protein necessary for regulation of alternative splicing. Critical neurological functions for Rbfox1 have been approved by human mutations in RBFOX1 gene that cause neurodevelopmental disorders including autism spectrum disorder (ASD). To elucidate the pathophysiological relevance of Rbfox1, we here performed cell biological analyses of the neuron-dominant Rbfox1 isoform 1 (Rbfox1-iso1; A2BP1-A016) during mouse cerebral development. Knockdown of Rbfox1-iso1 by *in utero* electroporation method caused abnormal neuronal distribution during corticogenesis. Rbfox1-iso1 knockdown did not affect cell proliferation in the neural progenitor/stem cells. Confocal laser microscope-associated live-imaging analyses revealed that migration defects occurred during radial migration and terminal translocation. While Rbfox1-iso1-deficient neurons did not show any morphological abnormality during migration, they could not efficiently enter the cortical plate and were prevented from smooth migration in the cortical plate, perhaps due to impaired nucleokinesis. Indeed, the distance between nucleus to centrosome was abnormally elongated in

Rbfox1-iso1-deficient neurons during radial migration. Rbfox1 was also found to regulate neuronal network formation *in vivo* since interhemispheric axon extension and dendritic arborization were suppressed in Rbfox1-iso1-deficient neurons. Aberrant morphology was further confirmed in *in vitro* analyses; Rbfox1-iso1-silencing in primary cultured hippocampal neurons resulted in the reduction of primary axon length, total length of dendrites, spine density and mature spine number. Taken together, aberrant phenotypes observed in this study may relate to structural and functional defects of the cerebral cortex, leading to the emergence of the clinical symptoms of neurodevelopmental disorders.

WTH02-10

Neurodevelopmental disorders and the PI3K/PTEN pathway; how some proteins are more equal than others

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Neurodevelopmental disorders such as autism show large genetic heterogeneity, with each susceptibility locus accounting for only a small fraction of reported cases. There are however monogenic causes of autism that are associated to specific subsets of the disorder that occur with higher frequency. One such gene, PTEN, is linked specifically to autism with macrocephaly suggesting there are key pathways causal for the disorder. PTEN negatively regulates the PI3K/AKT/mTOR signalling pathway which is centrally important to both normal brain development and human neurological disorders. Recent evidence from our laboratory has highlighted that the location of PTEN in the cell is important for its function, with separate roles described for both cytoplasmic and nuclear PTEN[1]. However, little is known about the cellular transport mechanisms that control PTEN location during development. Here we report that Rab5 and the adaptor protein Ndfip1 can function to control the ubiquitin mediated localisation of PTEN during brain development, resulting in the regulation of brain size. Using immunohistochemistry, cell cycle analysis and neuronal fractionation studies we have found that nuclear PTEN can control the levels of two oncogenic proteins, Plk1 and cyclin D1. We have developed transgenic mice expressing Ndfip1 that promote the function of nuclear PTEN, these mice develop a phenotype of microcephaly. Interestingly, this is the opposite phenotype observed in PTEN knockout mice, highlighting the role of PTEN function in controlling brain growth and size. Together our data suggests an "Orwellian" view of protein hierarchy where PTEN is a central regulator of brain development that has a critical role in neurodevelopmental disorders such as autism.

[1]Howitt J et al., Ndfip1 represses cell proliferation by controlling Pten localization and signaling specificity. J. Mol. Cell. Biol, 2015 in press.

WTH02-11

Cell proliferation in adult brains of three prosimian primates: demidoff dwarf galago, potto and the ring-tailed lemur

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This study investigated pattern of adult neurogenesis in the subventricular zone (SVZ) of the lateral ventricle, the dentate gyrus (DG) of the hippocampus and potential neurogenic sites in three prosimian primates. While the two nocturnal species, demidoff dwarf galago and the potto were caught in the wild, the ring-tailed lemur was a zoo kept diurnal animal. Two brain specimens from each species, perfusion-fixed with 4% paraformaldehyde were cut at 50 μm thick frozen sections in sagittal and coronal planes. Using doublecortin (DCX) and Ki-67 antibodies, immature neurons and proliferating neurons were identified respectively in the SVZ and DG and in potential sites such as the striatum, corpus callosum, amygdala, and piriform cortex in all the three species. DCX positive cells were observed in the cerebellum of the ring-tailed lemur and demidoff dwarf galago but not in the potto. There were no Ki-67 positive neurons in the cerebellum in the three species. Interspecies analysis indicated that the estimated rate of Ki-67 proliferating cells in the potto was 1.9 times higher than that of lemur and 4.8 times higher than that of demidoff dwarf galago. There was no statistical significant variation in the number of estimated Ki-67 cells within the three species but a significant difference ($p \leq 0.05$) when comparing potto against the lemur and demidoff dwarf galago. There was no significant difference ($p \geq 0.05$) in the number of Ki-67 cells between the lemur and the demidoff dwarf galago. Variations do exist in the cell proliferation pattern among these three prosimian primates.

WTH02-12

Presence of distinct, stimulus-specific subpopulations of quiescent precursors in the adult mouse hippocampus

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New neurons are generated from resident populations of stem and precursor cells in the adult mammalian hippocampus, and have been shown to critically contribute to the regulation of spatial learning and memory, as well as mood. The activity of these hippocampal precursors is regulated by various stimuli; however, whether these stimuli regulate the same or different precursor populations remains unknown. Here, we developed a novel cell-sorting protocol that allows the purification to homogeneity of neurosphere-forming neural precursors from the adult mouse hippocampus and examined the responsiveness of individual precursors to various stimuli using a clonal assay. We show that within the Hes5-GFP$^+$/Nestin-GFP$^+$/EGFR$^+$ cell population, which comprises the majority of neurosphere-forming precursors, there are two distinct subpopulations of quiescent precursor cells, one directly activated by high KCl depolarization, and the other activated by norepinephrine (NE). We then demonstrate that these two populations are differentially distributed along the septo-temporal axis of the hippocampus, and show that the NE-responsive precursors are selectively regulated by

GABA, whereas the KCl-responsive precursors are selectively modulated by corticosterone. Finally, based on RNA-seq analysis by deep sequencing, we show that the progeny generated by activating NE- versus KCl-responsive quiescent precursors are molecularly different. These results demonstrate that the adult hippocampus contains phenotypically similar but stimulus-specific populations of quiescent precursors, which give rise to neural progeny with potentially different functional capacity.

WTH02-13

Stem cell activation and quiescence in the zebrafish brain

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It is well established that non-mammalian vertebrates such as zebrafish and amphibians display a robust potential to regenerate lost tissue after injury. Furthermore, across the evolutionary spectrum of organisms including mammals the juvenile phase has a superior capacity to repair tissues. A key question waiting for answer in stem cell and regenerative biology is how stem cell quiescence and activation is controlled. Recent studies suggest that an altered metabolic state can prime quiescent stem cells and improve regeneration of multiple adult mammalian tissues. This is at least in part regulated by growth factor signalling and the mTOR pathway. We have taken advantage of the highly regenerative genetic model zebrafish and identified modifiers and inducers of the genetic programme regulating metabolism and growth signals that are needed to activate quiescent stem cells and modulate tissue growth during homeostasis and after injury. These findings open up new avenues to improve tissue regeneration in vertebrates.

WTH02-14

Retinoic acid-induced neurodifferentiation of SH-SY5Y cells involves reactive species production and oxidative stress

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Introduction: Retinoic acid (RA) exerts a major role in neuronal differentiation and segmentation during development. The effects of RA are mediated by the nuclear retinoic acid receptors (RAR) and retinoid X receptors (RXR). The human neuroblastoma-derived SH-SY5Y cells may be differentiated into an adult neuronal phenotype by RA treatment for 5–10 days, presenting neurite extension, cell division inhibition and expression of synaptophysin, beta-III tubulin, neuron-specific enolase and tyrosine hydroxylase. Although it is known that RAR/RXR-mediated gene transcription exerts an important role in neuronal differentiation, the neccessity of high concentrations (5–10 μmol/L) of RA to induce SH-SY5Y differentiation suggests the participation of RAR/RXR-independent mechanisms.

Objective: To investigate the role of reactive species (RS) production and oxidative stress on RA-induced neuronal differentiation.

Results: SH-SY5Y differentiation by treatment with RA 10 μmol/L for 7 days was characterized through immunocitochemistry for tyrosine hydroxylase (TH) and β-III tubulin and western blot for enolase-2. Cells subjected to differentiation in the presence of antioxidant Trolox® 0.1 mmol/L demonstrated lower levels of TH immunostaining and increased number of cells per field, suggesting a higher number of cells. FACS analysis using TH and EdU® confirmed that Trolox® decreased the number of differentiated relative to proliferative (undifferentiated) cells induced by RA. Evaluation of intracellular RS production by DCFH oxidation assay and quantification of cell non-enzymatic antioxidant activity by TRAP demonstrated that RA increases RS production, and Trolox inhibits this effect.

Conclusions: The neurogenic effect of RA on SH-SY5Y cells seems to be partly mediated by RS production, indicating the presence of redox-dependent mechanisms in this process.

WTH02-15

The role of RYK/WNT signaling in the developing neocortex

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The mammalian neocortex is responsible for higher order functions such as sensory perception, generation of motor commands, language and consciousness. The six layers of the neocortex are comprised of various subtypes of excitatory pyramidal projection neurons that differ in morphology and connectivity. Neocortical neuron specification, elaboration of axonal processes and specific targeting of axons to their cortical or subcortical targets is governed by a pro-neurogenic transcription network. How extrinsic factors coordinate the differentiation of neural progenitors and the maturation of newborn neurons is poorly understood. The Wnt family of morphogenic factors are key regulators of neuronal specification and maturation within the embryonic cortex. However, the signaling pathways initiated by Wnt-receptor interactions in neural progenitors and newborn neurons have not been explored in detail. Our lab has identified the non-canonical Wnt receptor, RYK, as an important regulator of mouse corticogenesis. RYK is an atypical transmembrane receptor that plays multiple roles in development through the modulation of the planar cell polarity and Wnt/calcium signaling pathways. Previously our lab demonstrated that WNT5a/RYK interactions are essential for the chemorepulsive guidance of callosal axons across the corpus callosum into the contralateral hemisphere (Keeble *et al*., 2006). Therefore RYK signaling plays a central role in defining callosal neuron connectivity. We are currently investigating the molecular and cell biological consequences of RYK depletion in the embryonic cortex.

Keeble T.R., Halford M.M., Seaman C., et al., J Neurosci, 2006, 26: 5840–5848.

WTH02-16

Dual effect of the Wnt receptor Ryk in the regulation of dendritogenesis

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Dendritogenesis constitutes a key step in the formation of a functional neuronal network. Abnormalities in this process are observed in neurodevelopmental diseases such as autism and schizophrenia. Dendritogenesis involves remodelling of the actin cytoskeleton by the RhoGTPase Rac1. However, the signalling pathways controlling this process are not yet fully understood. The Wnt receptor Ryk has been identified as an axon guidance receptor in the developing cortex and is localized to growth cones. Our recent studies have shown that Ryk downregulation leads to modification of the dendritic tree complexity on cortical neurons. We thus hypothesize that Ryk could regulate neuronal network formation, in particular the processes controlling the dendritogenesis. Primary cultures of hippocampal and cortical neurons from *Ryk* knockout ($Ryk^{-/-}$) and WT embryos demonstrated that Ryk has opposing effects on dendrite morphogenesis in different neuronal subtypes. $Ryk^{-/-}$ cortical neurons exhibited an increased complexity of their dendritic arbor, with more branchings and an increased dendritic length compared to WT neurons. Conversely, hippocampal $Ryk^{-/-}$ neurons showed a significant decrease in these parameters compared to WT. This neuron-subtype-specific modulation of dendritogenesis suggests that Ryk may activate distinct signalling pathways in hippocampal versus cortical neurons. We are currently investigating this hypothesis by expressing Ryk constructs containing mutations in key motifs within its cytoplasmic domain in cultured neurons. These studies will provide a more in-depth understanding of the molecular signalling pathways controlling dendritogenesis and thus the formation of a functional neuronal network.

WTH02-17

Role of neuregulin-2 in synaptogenesis in newborn granule cells

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Neuregulin-2 (NRG2) was identified as a member of proteins containing EGF-like domain. NRG2 is expressed in a few restricted regions in the brain including the hippocampal dentate gyrus (DG) and subventricular zone, where neurogenesis persists during adulthood. Little is understood about the role of NRG2 in developments of newborn neurons. To study the role of NRG2 in synaptogenesis, we infected the newborn granule cells (GCs) in the *ex vivo* culture of hippocampus with retrovirus encoding microRNA against NRG2 (miNRG2). The miNRG2 was designed to be expressed under the control of Tet-On expression system. We recorded feed-forward GABAergic or glutamatergic postsynaptic current (GPSC or EPSC) evoked by stimulation of inner molecular layer of the DG. Depletion of endogenous NRG2 by treatment of doxycycline (dox) from 4 dpi (day-post-injection) suppressed GPSC amplitude. In contrast, dox-treatment from 7 dpi displayed no significant effect on GPSCs, suggesting NRG2 is essential in GABAergic synapse formation but not in its maintenance. Next, we studied the role of NRG2 in glutamatergic synapse formation by treating dox from 7 dpi. Whereas such dox treatment had no effect on GPSC, it lowered both amplitudes of AMPA- and NMDA-EPSCs, and abolished the normal increase in the ratio of AMPA- to NMDA-EPSC. In parallel, dendritic arborization of newborn GCs was reduced. These effects of knockdown were rescued by simultaneous overexpression of the intracellular domain of NRG2. Consistently, pharmacological inhibition of ErbB4, the receptor of Nrg2, suppressed the development of GABAergic synapses, but not glutamatergic synapses. These results suggest that the NRG2-mediated forward and reverse signalings participate in GABAergic and glutamatergic synaptogenesis, respectively.

WTH02-18

Nerve fibers infiltrate ovarian cancer and may be related to tumor aggressiveness

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Recent work suggests that the interaction between nerves and cancer cells goes well beyond the conventional concepts of perineural invasion and pain. Tumor cell growth and metastasis has recently been shown to increase in the presence of nerve infiltration in prostate and breast tumors and, conversely, neurotrophic growth factors produced by cancer cells can attract nerves in these tumors. Here we aimed to investigate potential nerve infiltration and the role of nerve growth factor (NGF) in ovarian cancer. Tissue microarrays (TMAs) of 202 human ovary cancers and 18 normal tissues were used to determine overall nerve density by immunohistochemistry against the pan-neuronal marker PGP9.5 and tyrosine hydroxylase as a marker for sympathetic nerves. We found PGP9.5 immunoreactive (IR) axons in 18/202 tumor biopsies. NGF –IR was observed in 57/202 tumor biopsies. TH-IR was observed in 16/18 normal ovary TMAs, but not found in tumor biopsies. Our data show that ovarian tumor cells express NGF and some tumors attract peripheral axons. Whether peripheral axons in tumors are derived from autonomic motor or sensory neurons remains to be determined. The role of NGF in attracting axons into tumors also remains to be tested. Finally, this data suggests that future therapies based on targeting NGF or peripheral nerves could be considered.

WTH02-19

Neogenin regulates progenitor polarity and function in the developing mouse cortex

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Within the developing CNS the neural tube is composed of polarised pseudostratified neuroepithelial cells (NECs). NECs are

the precursors of all CNS cell types. As development proceeds, NECs generate both apical progenitors (APs) and basal progenitors (BPs) which in turn undergo self renewing expansions as well as neurogenic divisions. This is achieved through tightly coordinated symmetric and asymmetric progenitor cell proliferation. One of the defining characteristics of APs is that they posses adherens junctions (AJs), the site of cell-cell adhesion at the apical surface of the neocortex. Conversely, BPs are delaminated from the apical surface. This highly ordered cytoarchitecture gives rise to the germinal zones of the neocortex, the ventricular zone (VZ) containing APs and the subventricular zone (SVZ) where BPs reside. The AJs of APs are situated at the border of the apical and lateral membrane adjacent to the ventricular surface. Assembly of AJs is required for the establishment of AP apico-basal polarity. Loss of AJs leads to cortical malformations such as cortical heterotopias and migration defects.

The axon guidance receptor, Neogenin (Neo), is expressed by APs at the apical surface and is coexpressed with Ncadherin at the AJs. In order to investigate if Neo is required for AJ assembly in the developing mouse neocortex, cDNA encoding Neo small hairpin RNAs (shRNAs) were electroporated *in utero* into the lateral ventricle of E12 CD1 embryos. Depletion of Neo resulted in a loss of AJ integrity in the APs compared to the control embryos. As a result, APs delaminated from the ventricular surface in the Neo knockdown cortex and a significant increase in Pax6+ progenitors was detected in the intermediate zone. Significantly higher numbers of dividing phosphohistone3+ (pH3) cells were also detected in the SVZ and cortical plate in the Neo knockdown brains. This data suggests that Neo is essential for the maintenance of AP apicobasal polarity and progenitor cell function in the developing neocortex.

WTH02-20

Investigating the role of the Netrin-1/RGMa receptor neogenin in adult hippocampal neurogenesis

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Adult neurogenesis occurs in two specific niches of the brain, the subgranular zone (SGZ) of the dentate gyrus in the hippocampus and the subventricular zone (SVZ) lining the lateral ventricles. Neurogenesis is a highly regulated multistep process that consists of three phases: (i) proliferation of neural stem cells and the subsequent expansion of intermediate progenitor cells, (ii) migration of newborn neuroblasts to their target region and (iii) functional integration of maturing neurons into the existing neuronal circuitry.

Neogenin is a bifunctional axon guidance receptor with two specific ligands, repulsive guidance molecule (RGMa) and Netrin-1, which illicit either chemorepulsive and chemoattractive responses respectively. Recent work from our laboratory unexpectedly revealed that neogenin also regulates cell cycle progression in neuroblasts derived from SVZ progenitors (O'Leary *et al*., 2015). However, it is not known what role neogenin plays in the hippocampal neurogenic niche.

In this project we investigate the role of neogenin in adult hippocampal neurogenesis. We have demonstrated that neogenin is expressed in neuroblasts and immature granule neurons within the mouse dentate gyrus. 5-bromo-2′-deoxyuridine (BrdU) chase experiments were carried out in *neogenin* loss-of-function ($Neo^{gt/gt}$) and wildtype ($Neo^{wt/wt}$) mice to investigate the how loss of neogenin affects adult born neuroblasts. BrdU was administered by intraperitioneal injection for 5 consecutive days, and the dentate gyri then analysed at 21 days post BrdU administration. Results show that there was a significant increase in the number of BrdU labelled neuroblasts in the $Neo^{gt/gt}$ compared to control mice. These data suggest that, as in SVZ neuroblasts, neogenin may regulate the ability of neuroblasts to exit the cell cycle in the dentate gyrus. To test this hypothesis we are currently investigating the cell cycle dynamics of neuroblasts in $Neo^{gt/gt}$ mice.

WTH02-21

Brain glycohydrolases: involvement of GBA2 in the neuronal differentiation

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Mammalian neurodevelopment is characterized by qualitative and quantitative changes in plasma membrane glycosphingolipid (GSL) composition. These changes are the result of a complex network of metabolic pathways involving GSL *de novo* synthesis and lysosomal catabolism as well as intracellular trafficking and exchanges between cell and extracellular environment. While the biosynthetic pathway is largely studied, scant is the information available on the catabolic one. For this reason, I studied the activity of the main glycohydrolases expressed in the central nervous system during neuronal differentiation. To this purpose the activities of the principal glycohydrolases involved in GSL catabolism have been evaluated in different experimental models such as brains and cerebella of mouse at different ages and neuronal cell cultures (immortalized mouse neuronal cell lines GN11 and GT1-7, primary cultures of mouse cerebellar granule cells and human neuroblastoma cells SH-SY5Y). The results obtained indicated that the process of neuronal differentiation is associated with a marked increase in the activities of all the glycohydrolases; in particular, the activity of the non-lysosomal β-glucosylceramidase GBA2 undergoes the most relevant increase representing the prevalent form of β-glucocerebrosidase in mature neurons.

In order to evaluate the possible role of GBA2 in the neuronal differentiation, SH-SY5Y cells have been stably trasfected for GBA2 overexpression (SH-SY5Y-GBA2). Cells overexpressing GBA2 acquired a neuronal phenotype and showed a significant increase in ceramide (Cer) levels. Therefore, it is possible to hypothesize that the hydrolysis of glucosylceramide to Cer, catalyzed by GBA2 at the PM level, has a functional role in the neuronal differentiation process. Moreover, in order to verify whether the increased activity of GBA2 influences the differentiation process induced by RA, SH-SY5Y-GBA2 cells were treated with retinoic acid (RA) for different times. As result, it was found that RA-treated clones showed a more marked neuronal phenotype when compared to control cells; this evidence suggests that GBA2-overexpression combined with RA treatment result in an additive/synergic effect on the neuronal differentiation processes. Collectively these findings suggest that GBA2 may represent a possible neuronal marker and demonstrate for the first time its direct involvement in the neuronal differentiation.

WTH02-22

Hippocampal-dependent effects of environmental enrichment versus exercise in the serotonin 1A receptor knock-out mouse

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Aim: Constitutive serotonin 1A receptor knock-out mice (5-$HT_{1A}^{-/-}$) have a well-established anxiety-like phenotype as well as hippocampal-dependent learning and memory (L&M) deficits. We aimed to assess whether activity-dependent effects of exercise (Ex) or environmental enrichment (EE) would rescue any of these deficits.

Background: The complex interactions of genetic and environmental factors predicting susceptibility to mental illnesses such as anxiety disorders are not well understood, nor are the L&M deficits that may underpin the aetiology of those disorders. In rodents, Ex or EE enhance aspects of L&M, as well as change emotionality-related behaviours. There is also a vast literature establishing the activity-dependent regulation of adult hippocampal neurogenesis (AHN) and hippocampal BDNF levels by these environmental stimuli.

Methods: 5-$HT_{1A}^{-/-}$ and wild-type (WT) littermate mice were either housed in environmentally enriched (EE) cages, exercise (Ex) cages, or under standard housing conditions. After 2 weeks, their anxiety-like behaviour was assessed on the elevated plus maze (EPM). At the 4 week point, their performance on the Morris water maze (MWM), a L&M task, was determined. Hippocampal BDNF protein levels and survival of adult-born cells in the dentate gyrus were both assessed.

Results: Interestingly, we saw a divergent rescue of different elements of the 5-$HT_{1A}^{-/-}$ phenotype. EE, but not Ex, rescued the anxiety-like behaviour on the EPM. However, Ex, but not EE, rescued the L&M impairment on the MWM retention probe. Intriguingly, increases in the rate of hippocampal adult-born cell survival and mature BDNF protein levels correlated with the rescue of L&M only.

Conclusions: The 5-$HT_{1A}^{-/-}$ L&M impairment is rescued by Ex and the anxiety-like behaviour is rescued by EE. Our data suggest that the 5-HT_{1A} receptor is not required for exercise-induced increases in adult-born hippocampal cell survival or mature hippocampal BDNF protein levels.

WTH02-23

Functional diversification of motor neuron-specific enhancers during evolution

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Functional diversification of motor neurons has occurred in order to selectively control the movements of different body parts including head, trunk and limbs. Here we report that transcription of *Isl1*, a major gene necessary for motor neuron identity, is controlled by two enhancers, CREST1 (E1) and CREST2 (E2) that allow selective gene expression of *Isl1* in motor neurons. Introduction of GFP reporters into the chick neural tube revealed that E1 is active in hindbrain motor neurons and spinal cord motor neurons, whereas E2 is active in the lateral motor column (LMC) of the spinal cord, which controls the limb muscles. Genome-wide ChIp-Seq analysis combined with reporter assays showed that major transcription factor complexes bind to E1 and drive hindbrain and spinal cord-specific expression of *Isl1*. Interestingly, the core region of E1 has been conserved in evolution, even in the lamprey, a jawless vertebrate with primitive motor neurons. All E1 sequences from lamprey to mouse responded equally well to transcription factors. Conversely, E2, the enhancer for limb-innervating motor neurons, was only found in tetrapod animals. This suggests that evolutionarily-conserved enhancers permit the diversification of motor neurons.

WTH02-24

Lactucopicrin potentiates neuritogenesis and neurotrophic factor secretion via regulating CaMKII/ATF1

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Neurotrophic factor depletion is a risk factor, established as well known therapeutic target for neuronal plasticity, transmission, and regenerative action in *in vitro* and *in vivo* studies for neurodegenerative disease (ND). The neuroprotection and neurorestoration are involved in the stimulation of neuronal connectivity and contribution of phosphotidylinositol-3 kinase (PI3K). We studied the lactucopicrin neurodifferentiation potentiality by altering the signalling in Neuro 2a neuroblastoma (N2a) cells and neurotrophic factors secretion potential in C6 glioma (C6) cells articulated with PI3K contribution. Lactucopicrin treatment in the presence and/or absence of LY294002 (PI3K inhibitor), soluble NGF, BDNF, and NT3 protein, and NGF mRNA expression in C6 cells were measured. And TrkA, ERK1/2, AKT, Ca^{2+}-Calmodulin Kinase (CaMKII), CREB and Activated Transcription Factor 1 (ATF1) with synaptopysin protein level along with neuritogenesis were measured in N2a cells. Beyond this observed for acetylcholinesterase inhibitor (AChEi) activity and Ca^{2+} secondary messenger in both cells. Lactucopicrin as AChEi functioning through calcium mediated activation of CaMKII and ATF1 induced neurite outgrowth of N2a cells along with the alteration of TrkA, Erk1/2, AKT, and synaptopysin level at dose dependently. Lactucopicrin increases soluble neurotrophic factor secretion in the absence of PI3K participation in C6 cells. These results disclose that lactucopicrin enhanced the acetylcholine level by an AChEi mediated-neuroprotection, neurorestoration, and neurodifferentiation in neuronal cells by Ca^{2+}-CaMKII/ATF1 with PI3K contribution. In non-neuronal cells by enhancing neurotrophic factor without PI3K participation, but PI3K mandatory for the NGF action. In conclusion, we propose that lactucopicrin act as an AChEi candidate for ND and further studies are needed to assess lactucopicrin effects on *in vivo* ND model.

WTH02-25

The amyloid β precursor protein influences the survival of immature neurons in the olfactory bulb and hippocampus *in vivo*

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The amyloid-β precursor protein (APP) is a transmembrane protein that is widely expressed within the central nervous system (CNS). The pathogenic dysfunction of this protein is intimately associated with Alzheimer's disease pathogenesis, yet the normal function of this protein is poorly understood. We examined the role of APP in regulating neurogenesis in the adult brain, specifically comparing neural stem and progenitor cell proliferation in the subventricular zone (SVZ) and hippocampal subgranular zone (SGZ) of 2-, 5- and 7-month APP-null and wild type mice. As expected the number of proliferating cells declined with age in both regions. However the number of proliferating EdU^+ cells as well as $PSANCAM^+$ neuroblasts were not influenced by APP, indicating that APP does not regulate the magnitude of neuroblast generation *in vivo*. However when the newly generated SVZ cells were traced to the olfactory bulb (OB), APP-null mice generated equal numbers of mature neurons in total but less calretinin cells ($p < 0.05$). This was contrary to our observation in the hippocampus where a larger number of EdU^+ neurons survived and integrated in the dentate gyrus of APP-null mice compared to wild-type. Furthermore the absence of APP expression significantly altered the timeframe over which the dendritic arbor developed: 7 days after birth neurons lacking APP had fewer branch points, however adult-born hippocampal neurons in APP knockout and wild-type mice were indistinguishable by 30 days. These data indicate that APP play a role in regulating neurogenesis in the adult brain, particularly by regulating neuron maturation and integration.

WTH02-26

Regulation of proliferative activity by PAR-1 in neural stem/progenitor cells derived from the adult hippocampal dentate gyrus

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It is now clear that there is a continual turnover of the mammalian hippocampal dentate gyrus neurons throughout life in adult. Large number of evidence demonstrated to promote endogenous neurogenesis in hippocampal dentate gyrus after various neurological injuries. Thrombin-activated/protease-activated receptor-1 (PAR-1) is known to regulate proliferation of neural cells following brain injury including intracellular hemorrhage. To elucidate involvement of PAR-1 in neurogenesis occurred in the adult hippocampus, we evaluated the effect of thrombin and PAR-related peptides on proliferation of the neural stem/progenitor cells (NPCs) derived from the hippocampal dentate gyrus of adult mouse. Immunostaining revealed that PAR-1 was co-localized with nestin, which is a marker for NPCs. Reverse transcription-PCR analysis showed the expression of mRNA encoding all subtypes of PAR in the NPCs. Exposure of the cells to thrombin significantly attenuated the cell proliferation without morphological change and cell damage. Moreover, thrombin had no effect on lactate dehydrogenase release, which is a monitor for cell death during the culture condition. In addition, the PAR-1 agonist peptide, $SFLLR\text{-}NH_2$, also attenuated the cell proliferation in a concentration-dependent manner. However, the cell proliferation was not affected by the PAR-1 negative control peptide, $RLLFT\text{-}NH_2$, which is an inactive peptide for PAR-1. These data suggest that PAR-1 negatively regulates proliferation of the NPCs in the adult hippocampus.

WTH02-27

Regulation of proliferation by nitric oxide in neural stem/progenitor cells generated after granule cell loss in the dentate gyrus

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Some endogenous factors released after neurodegeneration are known to produce both detrimental and favorable conditions for adult neurogenesis. Endogenous factors also affect the proliferation, migration, differentiation, and survival of the neural stem/progenitor cells (NPCs), with regulating the incorporation of newly generated neurons into the brain circuitry. Our previous studies demonstrated that the systemic treatment with trimethyltin chloride (TMT) causes the granule cell loss in the mouse dentate gyrus (DG), with being regenerated in the dentate granule cell after the neuronal loss. In this study, we evaluated the involvement of nitric oxide (NO)/cGMP pathway in proliferation of NPCs after dentate granule cell loss. NPCs were prepared from the mouse DG on day 3 post-TMT treatment by culturing in neurobasal A medium with B27 supplement, epidermal growth factor, and basic fibroblast growth factor for 14 days *in vitro* (DIV). After secondary replating, nestin-positive cells were cultured for 6 DIV under the same conditions in the absence or presence of NO synthase (NOS) inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME), 8-Bromoguanosine 3′,5′-cyclic monophosphate (8-Br-cGMP), protein kinase G inhibitor KT5823 or NO generator NOC for assessment of cell proliferation. Reverse transcription-PCR analysis showed the expression of mRNA encoding all subtypes of NOS in the NPCs during culture for 6 DIV after secondary replating. Exposure of the cells to L-NAME significantly attenuated the cell proliferation without morphological change and cell damage. However, the cell proliferation was not affected by 8-Br-cGMP, KT5823, and inactive NOC. By contrast, an exposure to NOC led to a significant increase in the proliferative activity. Taken together, our results support the possibility that NO enhances proliferative activity of the NPCs generated following neuronal loss in the DG independent of cGMP pathway.

WTH03 Brain Bioenergetics

WTH03-01

Xenoestrogen bisphenol-A induced DRP-1 dependent impaired mitochondrial dynamics and autophagy in the rat brain

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Mitochondria are crucial targets in Bisphenol-A (BPA) induced neurotoxicity. The human health hazards related to persisting use of xenoestrogen Bisphenol-A (BPA) are well documented. Mitochondria are considered dynamic cell organelles that constantly undergo fusion and fission process and maintain the mitochondrial homeostasis and further mitochondrial turnover by the process of mitophagy. Aberrant mitochondrial dynamics have been gradually incriminated in mitochondrial dysfunction in diseased conditions. We observed that BPA exposure enhances autophagy and disrupts the mitochondrial dynamics by increasing the fragmented mitochondria thereby disturbing the overall mitochondrial biogenesis in the hippocampal neural stem cells derived neurons. In, parallel with this we also observed that BPA exposure increased the levels of dynamin-related protein (Drp1) and also increased the co-localization of Drp-1 and pmKate mitochondrial resistance gene. Further, we found that BPA exposure decreases the expression and levels of PGC1-α, NRF1, NRF2, Tfam, and COX-IV and up-regulates Drp-1 suggesting that BPA decreases mitochondrial biogenesis by derangement in mitochondrial morphology and functions. In addition we found significant alteration in mitochondrial structure and increased Drp-1 levels after using shRNA mediated PGC1-α knock down followed with BPA treatment in neuronal cells. Contrarily, using PGC1-α AAV mediated over expression alleviate the mitochondrial derangement, Drp-1 levels after BPA exposure in neuronal cells. Conclusively, our study designated that BPA induced Drp1-dependent mitochondrial fragmentation by decreasing mitochondrial biogenesis and promoting neurotoxicity.

WTH03-02

Modulation of astrocytic glucose metabolism by the antidiabetic drug metformin

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Metformin is a widely used drug for the treatment of type 2 diabetes. While it increases peripheral glucose uptake and utilization in patients, its effects on the central nervous system are less clear. In the present study, we aimed to determine the effects of metformin on astrocytes, a cell type playing a key role in cerebral energy metabolism. Using primary cultures of mouse cortical astrocytes, we observed that metformin at 500 μM stimulates ^{3}H-2-deoxyglucose uptake (i.e. glucose utilization) in a time-dependent manner, with a maximal effect after 4 h of treatment (+239% vs. control values). This effect was protein synthesis-independent since it was not prevented in the presence of cycloheximide (10 μM). A more detailed characterization of metformin's effects showed that stimulation of glucose utilization is accompanied by an increase in lactate release (+160% vs. control values). Concentration-response curve analyses demonstrated that stimulation of glucose utilization and lactate release have similar EC_{50} values, 183 ± 20 and 211 ± 28 μM respectively. Interestingly, we observed that glycogen levels decrease in a time-dependent manner, concomitantly with the stimulation of glucose utilization, and reach 26% of control values after 9 h of treatment. In contrast to the observed increase of glycolytic metabolism, oxidative metabolism of glucose was not significantly modified by metformin as determined by measurement of total CO_2 production using ^{14}C(U)-glucose as substrate (93% of control values). Finally, it was shown that the increase in glucose utilization induced by metformin is not prevented in the presence of an inhibitor of the AMP kinase, compound C (20 μM), suggesting that metformin-mediated effects on glucose metabolism are independent of the activation of this kinase. As a whole, these results demonstrate that metformin strongly modifies glucose metabolism in astrocytes, turning them into a more glycolytic state.

WTH03-03

Bardoxolone methyl prevents high fat diet-induced impairments to hypothalamic leptin signalling in mice

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Leptin signalling in the hypothalamus plays a crucial role in the regulation of energy balance. A high fat (HF) diet is known to cause hypothalamic leptin resistance resulting in the impairment of neuroregulatory control of food intake. Recently, we have found that a derivative of oleanolic acid, bardoxolone methyl (BM), prevents HF diet-induced alterations in signalling molecules involved in recognition memory in the forebrain of mice fed a HF diet. Therefore, we tested the hypothesis that BM may have therapeutic effects in another brain region impaired by a HF diet, the hypothalamus. Twelve week old C57B1/6 male mice were fed a HF diet and administered BM (10 mg/kg a day, orally, $n = 14$) or saline (high-fat diet control, $n = 14$), or fed a lab-chow diet and saline treated (lab-chow diet control, $n = 14$) for 21 weeks. Body weights and food intake were measured weekly. Peripheral leptin sensitivity was assessed following 16 weeks of treatment. Leptin signalling molecules in mediobasal and paraventricular nuclei regions of the hypothalamus were investigated using immunoblotting. One and two way analysis of variance and the post-hoc Tukey-HSD test were used to determine statistically significant differences between groups. BM treatment in mice fed a HF diet for 21 weeks significantly prevented an increase in body

weight gain, food intake, and hyperleptinemia, and prevented a decrease in peripheral leptin sensitivity. In the mediobasal and paraventricular nuclei regions of the hypothalamus, BM administration ameliorated HF diet induced impairment of leptin signalling by modulating negative energy balance regulatory molecules involved in downstream protein kinase b (Akt)- forkhead box protein O1 (FOXO1) signalling. These results identify BM as a potential future novel pharmaceutical for preventing HF diet-induced obesity and associated hypothalamic leptin resistance.

WTH03-04

Impact of maternal smoking on brain mitophagy in new born male mice offspring

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Maternal cigarette smoke exposure (SE) during gestation can cause lifelong adverse effects on the brains of offspring. Mitochondria are critical for cell survival. To maintain the integrity, defective mitochondria are selectively degraded through autophagy. Maternal SE can increase oxidative stress in the newborn brain, which might in turn impact on mitochondrial function. Furthermore, the impact of maternal SE on brain mitophagy in offspring is unknown. Female Balb/c mice were exposed to cigarette SE for 6 weeks prior to mating, during pregnancy and lactation with control mice exposed to air. Male pups were sacrificed at postnatal day 1. Western blots were performed to analyse proteins of interest in brain.Autophagy markers phosphatase and tensin homolog-induced putative kinase (PINK) 1 and dynamin-1-like protein (Drp1) are increased in the SE offspring. The marker of autophagy conversion, light chain (LC) 3A/B-II to LC3A/B-I ratio was also increased in SE offspring. Protein levels of optic atrophy (OPA) 1 and autophagy-related protein (ATG) 16-L were reduced. Oxidative phosphorylation complexes I, III and V were reduced in SE offspring, although not significantly different from control.Maternal SE increased brain autophagy in SE offspring, which may reflect increased mitochondriaL damage. This adaptation is likely to maintain mitochondrial activity in the newborn to ensure normal brain function. Further studies are required to investigate the changes of brain autophagy and mitochondrial function in adult offspring, and whether this leads to any functional changes in the brain.

WTH03-05

Paraventricular NUCB2/Nesfatin-1 regulates feeding behavior and mediates anorexigenic effect of leptin

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Nesfatin-1, an anorectic peptide processed from nucleobindin-2 (NUCB2), is localized in the hypothalamus including paraventricular nucleus (PVN), the region serving as an integrative center for energy homeostasis. Despite substantial evidence supporting a role of NUCB2/nesfatin-1 in feeding and metabolism, the role of NUCB2/nesfatin-1 expressed in PVN is not clearly defined. We used AAV vector-mediated RNA interference to suppress nesfatin-1 expression specifically in the PVN and examined whether the NUCB2/nesfatin-1 expressing in PVN mediates anorectic action of leptin, a major hormone in feeding regulation. PVN-specific NUCB2 knockdown resulted increased in body weight gain and daily food intake, without affecting energy expenditure. AAV-NUCB2-shRNA injected mice also exhibited significant increases in mesenteric adipose tissue and liver mass. Furthermore, PVN NUCB2 knockdown impaired insulin sensitivity, indicating the role of the PVN NUCB2 in regulation of insulin action and glucose homeostasis. Furthermore, leptin increased $[Ca^{2+}]_i$ in PVN neurons, and 30 of 44 (60%) the leptin-responsive neurons were identified as nesftin-1 neurons showing leptin activate the PVN NUCB2/nesfatin-1 neurons. Central injection of leptin significantly increased NUCB2 mRNA expression *in vivo*, and treatment of isolated PVN slices with leptin in culture increased NUCB2 mRNA expression. In mice injected with AAV-NUCB2-shRNA, both central and peripheral leptin injection failed to inhibit food, indicating that PVN NUCB2 serves as a substantial mediator of leptin action in PVN. Our study suggest that the endogenous NUCB2/nesfatin-1in the PVN plays an essential role in the long-term regulation of energy balance at least party mediating the leptin signaling for inhibition of feeding.

WTH03-06

Brain energy metabolism alterations in experimental model of hepatic encephalopathy induced by partial hepatectomy

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Hepatic Encephalopathy (HE) is a highly lethal complication that is one of the main causes of death in patients with both acute and chronic liver failure. HE is characterized as a neuropsychiatric syndrome that ranges from mild cognitive impairment to coma and death through cerebral edema and brain herniation. It is known that ammonia (one of the main agents involved in HE's physiopathology) is responsible for many bioenergetic alterations in the brain. However, the literature brings controversial findings regarding the alterations of brain energy metabolism during HE. The purpose of the presenting study is to investigate energy metabolism alterations in cerebral cortex in an experimental model of HE. We used 90 days male Wistar rats which were submitted either to 92% partial hepatectomy (PH) or Sham Operation (SO). 24 h after the surgery, the animals were sacrificed by decapitation and the cerebral cortex was immediately collected for biochemical analysis. Compared to SO rats, we showed that PH rats have increased in mitochondrial oxygen consumption, tricarboxylic acid cycle enzymes activity and glutamate oxidation to CO_2 and decreased lactate and glucose oxidation. On the other hand, the complexes of the electron transport chain did not show any significant alterations between the two groups. Although bioenergetic in HE still need to be extensively studied, this study may provide new evidence of the alterations in brain energy metabolism that occurs during HE.

WTH03-07

Glutamate oxidation in cerebellar neurons requires the enzyme glutamate dehydrogenase

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Glutamate is the most abundant excitatory amino acid in the brain. The enzyme glutamate dehydrogenase (GDH) catalyses the oxidative deamination of glutamate to α-ketoglutarate or the reverse reaction. This reaction connects the major cellular pathways the tricarboxylic acid cycle, amino acid metabolism and neurotransmitter metabolism. To investigate the role of GDH in neuronal metabolism, glutamatergic neurons were cultured from mice with a central nervous system specific deletion of glutamate dehydrogenase 1 (GDH1-deficient neurons) or from control mice (control neurons). The neurons were incubated with 0.1 mM [U-^{13}C]glutamate and the occurrence of ^{13}C-labelled metabolites was investigated by gas chromatography mass spectrometry. GDH1-deficient neurons had a significantly higher cellular percent labelling of [U-^{13}C]glutamate compared to control neurons. The metabolites generated directly from [U-^{13}C]glutamate, the subsequent tricarboxylic acid cycle intermediates [U-^{13}C]succinate and [U-^{13}C]fumarate were significantly less labelled in GDH-deficient neurons suggesting a slower metabolism of [U-^{13}C]glutamate. Furthermore, in GDH-deficient neurons a significantly reduced labelling in the isotopomers generated via the first turn of the tricarboxylic acid cycle, i.e. triple labelled α-ketoglutarate and triple labelled glutamate, and double labelled succinate, double labelled fumarate and double labelled aspartate, were observed compared to control neurons. These data suggest that GDH plays an important role in oxidative catabolism of glutamate in glutamatergic neurons.

WTH03-08

Exercise and high-fat diet can recover altered levels of aging-related metabolites of the kynurenine pathway

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Tryptophan metabolites regulate a variety of physiological processes, and their downstream metabolites enter the kynurenine pathway. The metabolites and activities of associated enzymes in this pathway are potential aging-related biomarkers and intervention targets. Blood levels of serum tryptophan metabolites in C57BL/6 mice of different ages, ranging from 6 weeks to 10 months, were assessed using high-performance liquid chromatography, and the enzyme activities for each metabolic step were estimated using the ratio of appropriate metabolite levels. Mice were subjected to voluntary chronic aerobic exercise or high-fat diet to assess their ability to rescue age-related alterations in the kynurenine pathway. The ratio of serum kynurenine aminotransferase (KAT) to kynurenine 3-monooxygenase (KMO) decreased with advancing age. Voluntary chronic aerobic exercise and high-fat diet rescued the decreased KAT/KMO ratio in the middle- and old-aged groups. Tryptophan metabolites and their associated enzyme activities were significantly altered during aging, and the KAT/KMO ratio was a meaningful indicator of aging. Exercise and high-fat diet could potentially recover the reduction of the KAT/KMO ratio in the elderly.

WTH03-09

Wolfram syndrome: from er stress to mitophagy and delayed neuronal development

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Wolfram syndrome (DIDMOAD; WS) is a genetic disorder characterized by diabetes insipidus, diabetes mellitus, optical atrophy, deafness and brain atrophy that results in death in middle adulthood, typically due to brainstem atrophy-induced respiratory failure. The majority of WS cases are related to mutations in the gene Wolfram syndrome 1 (WFS1) that encodes a protein localized in the endoplasmic reticulum (ER) membrane. However, the clinical symptoms of WS resemble mitochondrial disease symptoms and suggest strong mitochondrial involvement in this disease. We therefore decided to examine the hypothesis that Wfs1 deficiency could disturb the mitochondrial dynamics and by that also affect neuronal function.

We demonstrate that Wfs1 deficiency-induced ER stress affects IP_3R receptor and by that supresses ER Ca^{2+} release. IP_3R-mediated Ca^{2+} release induced by photolysis of *caged IP$_3$* or by activating endogenous IP_3 production by the metabotropic glutamate receptor agonist DHPG was significantly lower in Wfs1-deficient neurons. We further demonstrate that low cytosolic Ca^{2+} levels disturb mitochondrial dynamics. Mitochondria in Wfs1-deficient neurons do not move properly, they do not fuse and split apart as frequently as their wt counterparts and they undergo more frequently mitophagy.

Our most important discovery is that Wfs1 deficiency delays neuronal development and axonal growth in primary neuronal culture. According to our data, the link between WFS1 deficiency and delayed neuronal development appears to be mediated by impaired mitochondrial dynamics because suppression of the Pink1-Parkin pathway corrected also the development delay.

Thus, our data suggest a causal relationship between ER stress, cytosolic Ca^{2+} disturbances, impaired mitochondrial dynamics and delayed neuronal development in Wfs1 deficient neurons.

WTH03-10

Circumventing the Crabtree effect: *in vitro* lactate utilization induces heightened sensitivity to the mitochondrial toxin rotenone

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Cells grown in standard culture conditions are exposed to supra-physiological glucose concentrations and generate almost all their ATP via glycolysis despite abundant oxygen supply and functional mitochondria, a circumstance known as the Crabtree effect. By contrast, cells within the body rely predominately on mitochondrial oxidative phosphorylation (OXPHOS) to generate the bulk of their energy supply. Thus, in order to utilize the accessibility of cell culture to elucidate the central role of mitochondrial dysfunction in diverse conditions deleterious to human health, such as neurodegenerative disease and advanced age, it is advantageous to better model the *in vitro* environment such that the cells have a greater reliance upon OXPHOS for the supply of their energy needs. Substituting galactose for glucose in the growth medium is one such model, but additional benefit can be gained from alternate *in vitro* models that more closely approximate the metabolic state of cells *in vivo*. In our model the depletion of medium glucose ultimately forces cells to utilize lactate via OXPHOS to supply their energy needs. Cells cultured in low glucose medium exhibited an initial glucose-consuming glycolytic phase followed by a lactate-consuming oxidative phase. Significantly increased mitochondrial superoxide dismutase (SOD2) levels ($n = 4$, $p < 0.05$) and increased MitoTracker intensity ($n = 3$, $p < 0.05$) indicated increased mitochondrial activity during the oxidative phase. Consistent with the lactate-consuming phase representing increased reliance upon OXPHOS for sustaining energy demand, cells in the oxidative phase were more sensitive to the OXPHOS inhibitor rotenone when compared to cells in the glycolytic phase ($n = 4$, $p < 0.05$). We propose this *in vitro* model is a closer approximation of the metabolic state of cells *in vivo* than current common practice of culturing cells in supra-physiological glucose conditions and that this model is applicable to the *in vitro* study of mitochondrial function and diseases involving mitochondrial dysfunction.

WTH03-11

Mitochondrial respiration of postnatal mice neocortex submitted to prenatal valproic acid treatment

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Mitochondrial abnormalities or dysfunctions have been reported in many neuropsychiatric conditions, including autism spectrum disorder (ASD), while growing evidence reveals a relationship between reduced ATP levels, electron transport chain protein expression and gene transduction in post-mortem autistic brains. Valproic acid (VPA) prescription to pregnant women is known to produce teratogenic effects, sensory-behavioral dysfunctions and may raise the risk of autism in children. Therefore, we investigated if a single embryonic exposure to VPA affects mitochondrial respiration in offspring measuring mitochondrial metabolism in neocortex homogenates from neonate, 1 and 2 weeks of age mice by high-resolution respirometry. Our preliminary results show that: (i) routine O_2 consumption does not change from birth to first and second weeks postnatal; (ii) O_2 usage for ATP synthesis tends to rise between these ages; (iii) maximal respiratory capacity also seems to improve in the same period; (iv) puppies exposed *in utero* to VPA did not present growth differences from their control counterparts; (v) VPA alters developmental metabolic patterns relative to O_2 flux and respiratory rates; (vi) reserve energetic capacity is significantly higher in PN15.5 animals from VPA litters than in control ones. According to these results, we conclude that: (i) morphological alterations in developing neocortex may cause or be followed by metabolic adaptation in the cellular level; (ii) gestational VPA single administration produces consequences in offspring metabolism, supporting the hypothesis of an environmental participation in autism; (iii) VPA may generate some of the mitochondrial metabolic alterations observed in human brains from autism subjects.

WTH03-12

NAD^+, NAD^+ recycling and brain metabolism

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NAD^+/NADH drives brain metabolism through its role as a carrier of reducing equivalents but availability of NAD^+ itself is also key to this process. NAD^+ is consumed in major cell reactions, such as those catalyzed by the silent information regulators (SIRTs), and it is recycled from nicotinamide back to NAD^+ via a recycling pathway whose activity decreases with age. While NAD^+ is a SIRT activator, nicotinamide is a known inhibitor. Here, we examined the effect of NAD^+ (0–500 μM) and nicotinamide (0–100 μM), as well as the recycling pathway intermediate nicotinamide mononucleotide (NMN, 0–300 μM) on brain metabolism of [1-^{13}C]D-glucose (5 mM) and [1,2-^{13}C]acetate (0.4 mM) in the Guinea pig brain cortical tissue slice and the effect of inhibitors of NAD^+ recycling and breakdown. We examined the resulting distribution of label using multinuclear NMR spectroscopy and multivariate statistics. All three compounds had similar effects on metabolism, albeit at different concentrations, with NMN having concentration dependent effects on lactate production from glucose. We interpret the similar outcomes as being due to the rapid interconversion of each metabolite via NAD^+ recycling, but also due to synergistic effects, such as nicotinamide inhibition of NAD^+ breakdown, or substitution of NAD^+ by the mononucleotide, thus sparing NAD^+. NAD^+ availability effects flux through the Krebs cycle and incorporation of label into GABA and glutamine. It shows similar effects on both glucose and acetate incorporation, suggesting the effect is not cell-type or compartment specific.

WTH03-13

Understanding brainpower: compartmentalized signaling controls astrocyte energy metabolism

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A fundamental requirement for any cell is the provision of metabolic energy in the form of ATP. While the brain as an organ relies on glucose from the blood, astrocytes contain a small, albeit metabolically active amount of glycogen. Contrary to common beliefs, glycogen is not a static molecule but highly dynamic being degraded and rebuild continuously – a process that seems to be perturbed in some pathologies including diabetes. If breakdown of glycogen is inhibited, astrocytes respond with an un-proportional increase in glycolysis fuelled by extracellular glucose, a phenomenon known as supercompensation. In astrocytes, two isozymes of glycogen phosphorylase, GPM and GPB, degrade glycogen. The differential regulation of these two isozymes has been somewhat enigmatic since the cAMP- and Ca^{2+}-mediated signaling pathways leading to activation of the two are same. Here, we hypothesize that compartmentalization of the signaling-metabolism coupling induced by distinct cellular cues, along with lateral interactions between Ca^{2+} and cAMP, underlies the differential regulation of these two isozymes. To begin to address this hypothesis, we have performed differential siRNA-mediated knock down of the two isozymes. Firstly, our data suggest that breakdown of glycogen is needed to support key astrocytic functions, including uptake of neurotransmitter glutamate and K^+. Secondly, the data suggest that the supercompensation phenomenon is linked to the presence of the GPB isozyme, since supercompensation was lower when GPM is functional, as compared to GPB. This is interesting from both a neurochemical viewpoint as well as from a disease perspective. In future studies, it will be interesting to investigate if faulty signaling-metabolism coupling plays a role in the neurological symptoms in diabetes.

WTH03-14

Silent information regulator 2 may significantly modulate acetate and glucose metabolism

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Acetylation is the attachment of acetate to a protein; this process can modify protein function and occurs on thousands of proteins in the brain, including those involved in critical metabolic pathways. The NAD^+ dependent Silent information regulators (SIRTs) 1,2 and 3 have the most deacetylation activity in brain. In this study we sought to identify how SIRT1, 2 and 3 affect brain metabolism. Guinea pig cortical brain slices were incubated in Krebs-Henseleit buffer with [1-^{13}C]glucose and [1,2-^{13}C]acetate and 200 μM NAD^+, added for optimum SIRT activity, in the absence (control) or presence of SIRT1, 2 and 3 inhibitors EX527, AGK2 and SRT1720. Carbon label patterns were analysed using [^{13}C, ^{1}H] nuclear magnetic resonance (NMR) spectroscopy. Inhibition of SIRT1 with EX527 increased labelling of Ala C3 and decreased labelling of aspartate from both glucose and acetate, consistent with the major interactions of SIRT1 being cytosolic [1]. Inhibition of SIRT2 with AGK2 caused a significant decrease in acetate incorporated into glutamate, glutamine and aspartate, increased labelling of lactate and alanine and also reduced incorporation of label from glucose into aspartate. Inhibition of SIRT3 with SRT1720 had milder effects with significant decreases in label incorporation from acetate into glutamine and aspartate and increased labelling of lactate and alanine. As SIRT2 is predominantly, if not exclusively, found in oligodendrocytes this suggests that oligodendrocyte metabolism may be dependent on the ability of SIRT2 to deacetylate/interact with proteins in this cell type. This work also shows significant effects of SIRT2 and 3 inhibition on the amount of label incorporated from [1,2-^{13}C]acetate consistent with the idea that acetate metabolism is strongly related to the acetylation status of acetylCo-A synthetase [2,3].

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WTH03-15

Energy metabolism in the rat cortex under deep thiopental anesthesia measured *in vivo* by 13C MRS at 14.1 T

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Coordinate interaction between neurons and astrocytes underlies the coupling between brain metabolism and neurotransmission. This study aimed at characterizing neuronal and glial pathways of energy metabolism in the rat cortex under deep thiopental anesthesia. ^{13}C magnetic resonance spectroscopy MRS was performed at 14.1T in the cortex (94 μL) of Sprague-Dawley rats infused with [1,6,-^{13}C] glucose under thiopental (100 mg/kg/h, $n = 6$) or α-chloralose anesthesia (27 mg/kg/h, $n = 8$) as previously (Front Neuroenergetics 3:3, 2011). A two compartment model of energy metabolism was used to analyze ^{13}C enrichment curves of aliphatic carbons of glutamate, glutamine and aspartate. A preliminary analysis resulted in glutamate-glutamine cycles of 0.07 ± 0.01 and 0.10 ± 0.02 μmol/g/min under thiopental and α-chloralose anesthesia, indicating reduced glutamatergic neurotransmission under thiopental. Thiopental anesthesia also reduced the rates of neuronal tricarboxylic acid (TCA) cycle (0.55 ± 0.03 vs. 0.59 ± 0.03 μmol/g/min), and the rates of glial TCA cycle (0.27 ± 0.04 vs. 0.34 ± 0.06 μmol/g/min), pyruvate carboxylase (0.03 ± 0.01 vs. 0.08 ± 0.01 μmol/g/min) and glutamine synthetase (0.11 ± 0.01 vs. 0.18 ± 0.02 μmol/g/min). Cerebral metabolic rate of glucose consumption were 0.43 ± 0.03 and 0.50 ± 0.03 μmol/g/min under thiopental and α-chloralose anesthesia, respectively. These findings

agreed well with the steady-state fractional enrichment measured in cortical extracts at the end of the MRS experiment *in vivo*: smaller rate of pyruvate carboxylase under thiopental can be inferred from smaller ratio of glutamine C2/C3 (0.96 ± 0.08 vs. 1.09 ± 0.12); slower glutamate-glutamine cycle is indicated by larger difference in the labeling of carbons from glutamate and glutamine, namely in C4 glutamine/glutamate that was 0.77 ± 0.12 for thiopental and 0.87 ± 0.15 for α-chloralose. We conclude that depressed brain activity under thiopental is associated with reduction of oxidative metabolism in both neurons and astrocytes.

WTH03-16

Defects in mitochondrial biogenesis at rostral ventrolateral medulla on neurogenic hypertension induced by systemic inflammation

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Systemic inflammation induces neuroinflammation and tissue oxidative stress in brain regions subserving neural regulation of blood pressure, leading to the development of neurogenic hypertension. Mitochondrial dysfunction is involved in the pathogenesis of neurogenic hypertension; however the underlying mechanism is elusive. We test the hypothesis that defects in mitochondrial biogenesis and bioenergetics in the rostral ventrolateral medulla (RVLM), location of the sympathetic premotor neurons, contribute to pathogenesis of neurogenic hypertension induced by systemic inflammation. In adult, normotensive Sprague-Dawley rats, intraperitoneal infusion of *E. coli* lipopolysaccharide (LPS, 1.2 mg/kg/day for 7 days) resulted in the decreases in mitochondrial DNA (mtDNA) copy number, phosphorylation of a potent bZIP transcription factor, nuclear factor (erythroid-derived 2)-like 2 (NFE2L2), protein expression of mitochondrial transcription factor A (TFAM), mitochondrial electron transport enzyme complexes (MtETC) I and III, and ATP production; alongside the increases in tissue interleukin-1β (IL-1β) and the reactive oxygen species (ROS) levels in RVLM. These cellular and molecular events were associated with increases in sympathetic vasomotor outflow and systemic arterial pressure. In the N2a cells, exposure to IL-1β suppressed NFE2L2 phosphorylation, down-regulated the TFAM expression, decreased mtDNA copy number, inhibited MtETC protein expression and enzyme activity. The chromatin immunoprecipitation assay further demonstrated that IL-1β suppressed the binding of the phosphorylated NFE2L2 to the TFAM promoter. All events could be reversed by the addition of MtETC electron carrier, coenzyme Q_{10} (CoQ_{10}), which preserves the MtETC function and ameliorates cellular oxidative stress. Functionally, intracisternal infusion of CoQ_{10}, IL-1 receptor antagonist or NFE2L2 inducer, tBHQ, abolished the LPS-induced suppression of TFAM and mtDNA copy number, as well as the pressor response induced by the systemic inflammation. Together, these data suggest that IL-1β-dependent depression of NFE2L2 phosphorylation and nuclear translocation, and its binding to the TFAM promoter may contribute to an impaired nuclear-mitochondrial interaction that leads to defects in mitochondrial biogenesis and bioenergetics, resulting in tissue oxidative stress in RVLM and the development of neurogenic hypertension induced by systemic inflammation.

WTH04 Neuroimmunology

WTH04-01

Nogo-receptor 1 expression on B-cell populations in the central nervous system during experimental autoimmune encephalomyelitis

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Although the fact that deletion of Nogo receptor 1 (NgR1) can protect against axonal degeneration and thus progression of disease, in the animal model of multiple sclerosis (MS), experimental autoimmune encephalomyelitis (EAE), the immunological role of this receptor is unclear. To further understand the function of NgR1 in regulating immune cells, flow cytometry-based phenotypic analysis was performed on isolates from spleens, lymph nodes and spinal cords at different clinically defined stages of EAE disease. The central nervous system (CNS)-infiltrating blood cells revealed an augmented response in the B-cell population, which expressed NgR, seeing in $ngr1^{+/+}$ mice with the onset and progression of the disease. This population of cells could not be demonstrated within the spinal cords of EAE-induced $ngr1^{-/-}$ mice or during the chronic stage of disease in $ngr1^{+/+}$ mice. At the disease onset, there was a significant increased in IgM-B-cells-expressing NgR in the spinal cord, when compared with the IgD populations. Remarkably, there was a cluster of B-cells expressing NgR present at the meninges of the spinal cords of $ngr1^{+/+}$ EAE-induced mice at clinical score 1.5 and these cells localised within small follicles in submeningeal regions. Furthermore, there was clustering of B-cell activating factor (BAFF) and NgR-positive immune cell infiltrates within the spinal cords of EAE-induced $ngr1^{+/+}$ mice at the disease onset. Collectively, these data indicate that there exists the inducible expression of NgR1 in specific immune lineage cells upon the induction of EAE, as well as, a strong correlation between the expression profiles of NgR1 and BAFF on neighbouring B-cells within spinal cord follicular structures.

WTH04-02

One or two bands? Question for IgM oligoclonal bands in confirmed multiple sclerosis

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Background: We previously reported specific IgM against *S*-nitrosylated proteins (anti-SNOcys) in the cerebrospinal fluid (CSF) of relapsing–remitting multiple sclerosis (RRMS) patients. By contrast, anti-SNOcys IgM were not detected in the CSF of patients with mild neurological conditions and normal IgM levels, whereas RRMS patient CSF had moderately elevated IgM levels. We formerly showed that CSF anti-SNOcys IgM inversely correlate with relapse onset, suggesting this specific IgM antibody is a potential biomarker from intrathecal synthesis. We aimed to (i) verify intrathecal synthesis by occurrence of IgM oligoclonal bands in CSF; (ii) compare these bands with established mathematical formulae including IgM index and Reibergram; and finally (iii) explore how well the presence of anti-SNOcys IgM in CSF measures to these factors, particularly IgM oligoclonal bands, for MS diagnosis accuracy.

Methods: CSF from 18 patients with RRMS and 14 patients with mild neurological symptoms were analysed for IgM levels, oligoclonal bands, 5 mathematical formulae of intrathecal synthesis and anti-SNOcys IgM. Sensitivity, specificity, and receiver operating characteristic curves were generated to examine the ability of these parameters to accurately distinguish RRMS patients from controls.

Results: The presence of only one extra IgM oligoclonal band in CSF gave the best accuracy for MS diagnosis. If the conventional cut-off of two bands was applied, several false negatives arose, though no false positives. The IgM index was found the most accurate of all mathematical formulae followed by anti-SNOcys IgM.

Conclusions: We conclude that the IgM index appears to be an old robust formula which can be more easily applied if the delicate detection of IgM oligoclonal bands is not feasible. The current standard of two extra IgM oligoclonal bands may be too stringent for a diagnosis of MS. The demonstration of at least one extra IgM oligoclonal band in CSF supports a prominent role of IgM in RRMS, and confirms anti-SNOcys IgM as potential biomarker. This work is supported by a grant from the Norwegian University of Science and Technology (NTNU) Faculty of Medicine.

WTH04-03

Antibodies to surface dopamine-2 receptor and *N*-methyl-D-aspartate receptor in the first episode of acute psychosis in children

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The dopamine and glutamate hypotheses are well known in psychosis. Recently, the detection of autoantibodies against proteins expressed on the surface of cells in the central nervous system has drawn attention to the possibility that specific immune-mediated mechanisms may define a biological subgroup within psychosis. Here, we describe the detection of serum antibodies to dopamine-2 receptor (D2R) and the NR1 subunit of the glutamatergic *N*-methyl-D-aspartate receptor (NMDAR) in paediatric patients presenting acutely with a first episode of psychosis. Serum taken during the acute presentation of 43 children with first episode of psychosis and serum from 43 pediatric controls was assessed for the presence of

IgG, IgM, or IgA antibodies against D2R and NR1 using a flow cytometry live cell-based assay. Using a cut-off of 3SD above the control mean, antibodies to D2R or NR1 were detected in 8/43 psychotic patients, but not detected in any of 43 control patients ($p < 0.001$). Positive immunoglobulin binding to D2R was found in 3/43 psychosis patients (3 IgG, 1 IgM, 0 IgA) and to NMDAR in 6/43 patients (5 IgG, 1 IgM, 1 IgA). Sera from patients with antibodies to D2R and NMDAR also immunolabelled murine primary neurons. We also demonstrated the specificity of antibody binding to D2R by immunoaffinity purification. This is the first report of antibodies to surface D2R in patients with presentations of isolated psychosis. Detection of these autoantibodies supports the hypothesis that a subgroup of patients may be immune-mediated and may benefit from immune therapies.

WTH04-04

Is the adaptive immune response detrimental in TBI?

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Traumatic brain injury (TBI) is a serious condition involving a cascade of neuroinflammatory and pathophysiological events that can result in long-lasting physical and cognitive disabilities. Neuroinflammation occurring after injury is essential for survival due to its importance in tissue repair as well as protection against invading pathogens. However, it has been speculated that this same response can be harmful if it is prolonged or intensified. It is not known why an estimated 60% of people with a sustained TBI experience moderate to severe long-term disability a year later. Whether the involvement of the chronic immune response could be responsible for these long term deficits has not been explored in detail. Hence, we sought to determine the role of the immune system following TBI using the cortical controlled impact model in mice. Interestingly, immunological investigation using flow cytometry in the injured mouse brain revealed a significant increase in different subsets of lymphocytes (key components of the adaptive immune system) at the acute and chronic phases of injury. In particular, activated effector cytotoxic CD8 T-cells as well as activated B cells were increased in the injured brain at the chronic phase of 8–32 weeks post-TBI. As expected, we found a rapid increase in inflammatory cells as well as CD4 T-cells within the first week post-TBI. Remarkably, circulating antibodies specific to myelin antigen were increased in TBI mice at the chronic phase, a typical feature of demyelinating diseases. Indeed, mice displayed neurological deficits that progressively deteriorated during the 32 week period. These included slow mobility, unilateral movement, uneven gait, tail weakness and spasms. Gait impairment was further confirmed using the DigiGait apparatus which measures changes in motor function and coordination. Our results indicate that the immune response is a key mediator of neurodegeneration after TBI.

WTH04-05

Novel mouse model of endometriosis permits identification of spinal glia and nociception characteristics involved in pelvic pain

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Background: Endometriosis is a female-specific chronic inflammatory condition that often manifests with severe pelvic pain. Recent studies have implicated glia and their interactions with the central nervous system ("neuro-immune communication") in facilitating pain development. This has not yet been studied in the context of endometriosis; thus, we sought to develop a minimally-invasive mouse model of disease that allows investigation of spinal glia reactivity and/or nociceptive nerve characteristics known to be involved in pain signalling.

Methods: Endometriosis was induced in 8–14 week-old C57BL/6 or Balb/C mice by intraperitoneal injection of syngeneic donor endometrium (15–40 mg) from either the proestrus (high estrogen) or estrus (low estrogen) phase of the estrous cycle. After 3 weeks development, the spinal cord was removed from recipient mice and dissected into segments spanning T13-S1. Spinal sections were then processed via fluorescent immunohistochemistry for glial fibrillary acidic protein (GFAP), isolectin B4 (IB4), substance P (SP) and calcitonin gene-related peptide (CGRP), and quantified using Image J software.

Results: Endometriosis lesions were successfully established; the extent of disease dependent on mouse strain, estrogen status and amount of donor material injected. Preliminary analysis of animals from robust disease conditions (proestrus C57BL/6 mice receiving 40 mg tissue; $n = 2$) indicates as much as an 85% increase in L4-5 GFAP-immunoreactive cells compared to saline controls ($n = 2$; 14.1 ± 2.5 vs. 7.5 ± 0.3% area fraction, respectively). Immunostaining for IB4, SP and CGRP remain unchanged.

Conclusions: The model described here has successfully recapitulated endometriosis in the mouse, and allowed for analysis of nociceptive signals in the spinal cord. Additionally, our early data suggests that altered glial reactivity may play an important role in facilitating pain development attributed to endometriotic disease.

WTH04-06

Does the plasminogen activating system contribute to immunosuppression after traumatic brain injury?

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Traumatic brain injury (TBI) is associated with severe complications such as coagulopathy resulting in enhanced bleeding, and immunosuppression leading to an increased incidence of infection. Sepsis, pneumonia, but also wound and device related infections are

a common cause of morbidity and death in such patients. CNS immunosuppression is a cause of infection after TBI. Our preliminary data suggest a possible contribution of plasmin, the final product in the cascade of fibrinolytic proteases via direct interaction with immune cells. Dendritic cells (DCs) are potent antigen presenting cells whose role in TBI related immunosuppression is largely unknown. Via presentation of antigens to lymphocytes they are able to induce T cell proliferation, and a T helper cell type-1 (Th1) response mediates the effective clearance of invading bacteria. *In vitro* findings generated in our laboratory showed that plasmin-treated DCs display a reduced capacity to induce an immune response. Accordingly, we are studying the spectrum of immunosuppressive responses in both, wild-type (WT) and plasminogen-deficient (PLG-KO) mice subjected to TBI. Interestingly, at both 24 h and 72 h post TBI we found a shift from CD8+ conventional (c)DCs in the cervical lymph nodes towards CD8- cDCs, which are less potent in inducing a Th1 response in WT, but not in PLG-KO mice. Moreover, we observed that baseline white blood cell count, neutrophil count and monocyte count as well as spleen weight are significantly increased in PLG-KO mice, suggesting that the absence of plasmin primes an inflammatory response. These results are consistent with the notion of a direct immunosuppressive role of plasmin following TBI. Further experiments are underway to better characterise this effect and also whether anti-fibrinolytic drugs commonly used to restore haemostasis in coagulopathic patients may also reduce infection rates following TBI.

WTH04-07

Metabolic connection of inflammatory pain: pivotal role of a pyruvate dehydrogenase kinase-lactic acid axis

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Pyruvate dehydrogenase kinases (PDK1–4) are mitochondrial metabolic regulators that serve as decision makers to convert pyruvate either aerobically to acetyl-CoA or anaerobically to lactate. Metabolic dysregulation and inflammatory processes are two sides of the same coin in several pathophysiological conditions. The lactate surge associated with the metabolic shift has been implicated in diverse painful states. In this study, we investigated the role of PDK2 and PDK4 in the pathogenesis of chronic inflammatory pain. Deficiency of *Pdk2* and/or *Pdk4* in mice attenuated complete Freund's adjuvant (CFA)-induced pain hypersensitivities and formalin-induced nociceptive behaviors. Likewise, *Pdk2/4*-deficiency attenuated the lactate surge and hallmarks of peripheral and central inflammation following intraplantar administration of CFA. *In vitro* studies supported the role of PDK2/4 and lactic acid as promoters of classical proinflammatory activation of macrophages. Moreover, the pharmacological inhibition of PDKs or lactate diminished CFA-induced inflammation and pain hypersensitivities. Thus, a PDK–lactic acid axis seems to mediate inflammatory pain, establishing a connection between metabolism and inflammation-driven persistent pain.

WTH04-08

Anti-musk myasthenia gravis: synapse loss due to blockade of tyrosine kinase signalling?

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Muscle specific kinase (MuSK) is the core of a signalling complex that stabilizes postsynaptic specializations at the developing neuromuscular junction (NMJ). A subset of myasthenia gravis patients express autoimmune antibodies against MuSK. Mice that received daily injections of IgG from such patients developed impaired neuromuscular transmission, progressing to weakness over a 14-day period. Since the MuSK autoantibodies are predominantly of the IgG4 subtype (which does not activate complement) we investigated their effects upon the physiological MuSK tyrosine kinase pathway. After injections of IgG from anti-MuSK myasthenia gravis patients, motor endplates revealed reduced densities of acetylcholine receptors. Reduced immunofluorescent staining for the phosphorylated forms of Src kinase (Y418) and acetylcholine receptors (Y390) was consistent with reduced tyrosine kinase activity in the postsynaptic membrane. To test whether a deficiency in MuSK function caused the reduction in acetylcholine receptor density, we injected muscles with adeno-associated viral vector encoding MuSK, which was fused to enhanced green fluorescent protein (MuSK-EGFP). Muscles expressing MuSK-EGFP were found to be resistant to the harmful effects of anti-MuSK IgG, retaining higher levels of acetylcholine receptors and with less synaptic impairment, compared to contralateral muscles that lacked MuSK-EGFP. The scaffolding protein, rapsyn, is normally recruited to bind phosphorylated acetylcholine receptors (downstream of MuSK activation). Rapsyn then stabilizes the acetylcholine receptor cluster. However, unlike MuSK-EGFP, rapsyn-EGFP did not protect motor endplates from loss of acetylcholine receptors in mice injected with anti-MuSK patient IgG. This suggests that the kinase activity of MuSK is critical to maintenance of stable postsynaptic acetylcholine receptor clusters at the neuromuscular junction. Thus, MuSK autoantibodies appear to cause the disassembly of postsynaptic specializations by blocking the tyrosine kinase activity of MuSK.

WTH04-09

Sickness responses following simulated "typical" or "atypical" central bacterial infection

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Comparatively little is known about fever and sickness behaviours resulting from "atypical" bacteria, including the *Mycoplasmas*, despite the prevalence of these pathogens. Apart from being causative agents for pneumonia, the *Mycoplasmas* have been associated with meningitis, encephalitis and other life-threatening

neurologic complications. To our knowledge, no one previously studied a suite of sickness responses, including fever, lethargy, anorexia and memory processes, following a simulated central *Mycoplasma* infection. Using a rat animal model to simulate infection, we measured and compared sickness responses following central administration of either FAM-20, a moiety derived from *Mycoplasma pneumoniae* or lipopolysaccharide (LPS), from Gram-negative bacteria. Male Sprague–Dawley rats were anaesthetized for intra-abdominal implantation of radiotransmitters, which recorded body core temperature and cage activity continuously. Rats then received an injection, via the cisterna magna, of either FAM-20 (100 μg/5 μl) or LPS (100 μg/5 μl) or vehicle (5 μl). Body mass and food intake were measured daily, and memory was assessed 7 days after the injection, using fear conditioning test. Central administration of either LPS or FAM-20 to rats induced fever for ~ 4–5 days, lethargy (~ 4–7 days), anorexia (~ 4–6 days) and body mass stunting (~ 4–7 days). Although central administration of FAM-20 seemed to have caused more exaggerated sickness responses compared to LPS, neither FAM-20 (from *M. pneumoniae*) nor LPS (from Gram-negative bacteria) impaired memory in rats. Our data suggest that memory may be spared in the face of other sickness responses, during a simulated "typical" or "atypical" central infection.

WTH04-10

Analysis of the binding specificity of antibody to dopamine-2 receptor in autoimmune movement and psychiatric disorders

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Several autoimmune movement and psychiatric disorders have been associated with autoantibodies directed against neuronal or synaptic proteins involved in neurotransmission. The identification of these autoantibodies is important as they can be used as biomarkers for diagnostic purposes. Recently, a novel antibody against the dopamine-2 receptor (D2R), an important receptor in the brain controlling movement and behaviour has been discovered. While these autoantibodies are known to target extracellular domains of D2R, the exact antigenic epitope is unknown. Defining the D2R antibody antigenic region could elucidate functional effects of these autoantibodies and offer novel treatment opportunities. To uncover the target epitope of autoantibodies, D2R mutants modified in their extracellular domains were subcloned and expressed in human embryonic kidney cells. The expression of D2R mutants at the cell surface was assessed by confocal microscopy and flow cytometry. A flow cytometry live cell-based assay was employed to determine the binding of human D2R antibodies from D2R antibody-positive patients to D2R mutants. Two D2R mutants were successfully expressed at the cell surface to levels comparable to WT D2R. These include D2R N23Q, which is a WT D2R with a mutation at the third N-Glycosylation site on D2R extracellular N-terminal domain, and the D2R short isoform construct. The D2R short isoform is identical to the D2R long isoform, except for the absence of 29 amino acids in the third intracellular loop. Next, surface expressed mutants were used to test the specificity of D2R antibodies from patient sera. One out of seven D2R antibody-positive patient sera bound to D2R N23Q mutant and 6 out of 7 bound to D2R short construct. These results suggest that the 3rd N-Glycosylation site of D2R is important for D2R autoantibody binding and D2R antibody-positive patients tested show similar immunoreactivity to both D2R isoforms.

WTH04-11

How multiple sclerosis patient's lymphocytes crosstalk with microglial cells impacts the remyelination process

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Myelin destruction leads to irreversible neurological disorders in patients suffering from multiple sclerosis (MS). An interesting feature of MS is that an endogenous remyelination process can be triggered. It involves the participation of oligodendrocyte precursor cells (OPCs) and sometimes Schwann cells (SC). The microglia cells (MIG) polarization state is essential for the success of remyelination and their polarization is dependant of their dialogue with T lymphocytes (LT). In this study we asked whether controls or MS patients LT influence differentially MIG polarization and how it impacts remyelination.

We determine LT composition in the blood of healthy donors and MS patients using flow cytometry and assess their secretory profile by luminex. We study *in vitro* the influence of LT supernatants on MIG polarization and its consequences on OPCs differentiation. Finally, we establish a new *in vivo* model by combining lysolecithine induced demyelination and human LT grafting to test the influence of controls and MS patients LT on the remyelination process.

While LT composition is similar in MS and controls blood, we evidence different molecular profiles in MS patients LT. When compared to controls, MS patients LT supernatants induce an increase of the M1/M2 microglia ratio which in turn results, *in vitro*, in a decrease of OPC differentiation into mature oligodendrocytes. Moreover, we clearly demonstrate that SC remyelination is significantly decreased in mice grafted with MS patients' LT.

In conclusion, we establish for the first time that MS patients LT can directly influence the lesionned environment by modulating MIG polarization and impede the repair process.

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WTH05 Cellular Mechanism of Alzheimer's Disease

WTH05-01

Propagation of tau phosphorylation following localised PP2A inhibition in wild-type mice

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In Alzheimer's disease the distribution and density of neurofibrillary tangles composed predominately of phosphorylated tau, follows a distinct pattern through anatomically connected brain regions. Investigations using inducible tau transgenic mice under cell-specific reporters demonstrated spreading of tau between synaptically connected neurons. Furthermore, tau pathology is accelerated and mature tangle formation seeded by injection of protein lysates isolated from tau transgenic mice and human tauopathies. Therefore, it has been proposed that spreading of tau occurs in a "prion-like" manner. However, as these studies have only been performed in tau transgenic mice it is unclear whether endogenous phospho-tau can act as a pathological seed in a wild-type mouse, or whether introduction of an exogenous mature protein seed is necessary to initiate the process.

We investigated this by performing a unilateral injection of 10 ng okadaic acid (OA), a protein phosphatase 2A (PP2A) inhibitor, into the amygdala of 8-month-old C57Bl/6 mice. Mice were left to recover for 30 min, 24 h and 7 days before sacrifice and harvesting of tissue. Tau phosphorylation and protein aggregation were investigated by soluble and insoluble protein fractionations, Western blotting, immunohistochemistry and Thioflavin-S fluorescence. To detect OA at the injection site we adapted a commercially available OA ELISA to detect OA in brain tissue for the first time, showing OA was restricted to the injected hemispheric quadrant and was detectable up to a week after injection. Phosphorylation of tau was observed not only at the injection site but in anatomically distinct areas throughout the brain, including cortex and hippocampus, and was abundant through white matter tracts. Furthermore, protein aggregation was detected by the β-pleated sheet sensitive dye Thioflavin-S at the injection site and in the cortex of both injected and contralateral hemispheres. An increase in insoluble tau was also observed in both hemispheres of injected brains by 7 days when compared to control. This study demonstrates that tau phosphorylation and aggregation can occur rapidly in wild-type mice and manifest in brain regions located distant to the primary site of OA exposure.

WTH05-02

Deferasirox attenuates iron induced amyloid beta accumulation and toxicity in aged rat brain: therapeutic implications in AD

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Both aging and AD (Alzheimer's disease) brains characteristically manifest iron dysregulation. Furthermore, the presence of iron-responsive element (IRE) in 5' UTR of amyloid precursor protein (APP) mRNA implies a direct link between iron metabolism and AD development. In the present study, we have attempted to explore how iron impacts the metabolism of amyloid beta (Aβ42) in the aging brain. We have shown that an increased accumulation of iron in aged brain may be attributed to oxidative stress induced over-expression of transferrin receptor 1 (TfR1), which in turn also elicits a compensatory mechanism in form of increase in ferritin level. We have also confirmed this transcriptional upregulation of TfR1 and ferritin could be due to nuclear translocation of ROS sensitive transcription factor, NFKB and its binding to TfR1 and ferritin mRNA. As a consequence of high redox active iron accumulation, we have reported increased production of APP, Aβ42, β secretase in the brain of aged (22–24 months) rats compared to young (4–6 months) animals and decreased activities of neprilysin, responsible for the proteolytic removal of Aβ42 in the aged rat brain. All these biochemical alterations in the aging brain are attenuated conspicuously by prolonged oral administration of the iron chelator, deferasirox, to aged rats, implying a therapeutic potential of this drug against AD. Hence, we suggest that, deferasirox in near future could emerge as iron chelating drug having amyloid beta peptide metabolism altering properties.

WTH05-03

Correlating membrane binding and toxicity of synthetically prepared amyloid beta peptide mutants (Q15A, K16A, K28A)

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The key protein causing Alzheimer's disease (AD) is called amyloid beta (Aβ). This protein can acquire a destructive nature to brain neurons and it is unclear why this occurs in a subset of the aging population. Therefore, to better understand what makes this protein neurotoxic, we hypothesise that its toxicity is correlated with binding to the lipid components of the plasma membrane. Further, we have identified the specific amino acids, glutamine (Q), at position 15 and lysine (K) at positions 16 and 28 in Aβ that may

have a critical role in mediating the binding to lipid membranes. We will present data showing the biophysical, cell binding and cell toxicity properties of the mutated Aβ peptides.

Methods: To study Aβ binding to lipid membrane surfaces, giant unilamellar vesicles will be prepared and treated with the Aβ peptides and the extent of binding will be quantitated. ThT aggregation assays will be performed. Primary cortical neuronal cultures will be used for cell toxicity and cell binding studies.

Results: All three mutant peptides have (i) diminished neurotoxic activity while the Q15A mutant peptide was not toxic at all (ii) diminished levels of peptide binding to cells in culture (iii) decreased rate of aggregation and fibril formation.

Conclusion: By understanding the role of these key residues within the Aβ peptide sequence in mediating cell binding and toxicity, this will assist us in future design of therapeutic drugs for the treatment of AD.

WTH05-04

Novel metallothionein (II)-based peptides as potential therapeutics for Alzheimer's disease

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Alzheimer's disease is a common neurodegenerative disease characterised by the extracellular accumulation of plaques, comprised predominantly of fragments of the β-amyloid peptide (Aβ). We have previously reported that metallothionein 2 (MT2) is protective against certain forms of Aβ such as copper-bound Aβ (Cu-Aβ), which is a known neurotoxic Aβ species. More recently we have investigated a synthetic peptide based on the MT2 peptide sequence, termed emtinB, and presented data showing that emtinB mimics MT2's ability to protect cultured neurons against Cu-Aβ *in vitro*. To further this study we investigated the *in vivo* action of emtinB in the APP/PS1 mouse model of AD. Mice for this study (APP/PS1) were treated with saline (ip $n = 15$), low dose emtinB (5 mg/kg ip, $n = 14$), or high dose emtinB (30 mg/kg, $n = 14$) five times weekly from 9 to 11 months if age. At the conclusion of treatment, animals were tested in a Y maze, with a significant improvement in performance for both high and low dose emtinB treated animals ($p < 0.05$). Brains were removed and processed for western blotting (WB) and immunohistochemistry (IHC). WB analysis of trial APP/PS1 and wild-type (WT) control brain revealed a decrease in Glial Fibrillary Acidic Protein (GFAP) in high dose treated mice (110.6% ± 11.4 of WT) and low dose emtinB treated mice (112% ± 17.3 of WT) compared with saline control mice (136% ± 8.55 of WT). Abeta levels by WB and IHC are currently under analysis.We report that in APP/PS1 mice, emtinB improves cognitive outcomes in the Y-maze paradigm and this correlates with a decrease in GFAP. More interesting is that this result was observed when treatment was commenced post symptom onset. We believe that emtinB may represent a novel potential therapeutic for the treatment of Alzheimer's disease.

WTH05-05

GSK-3 interacts with lysosomal networking and contributes to neurodegeneration

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Glycogen synthase kinase-3 (GSK-3) has been recently implicated in the etiological mechanisms of neurodegenerative disorders including Alzheimer's disease (AD). Inhibition of GSK-3 produces multiple therapeutic benefits in AD animal models including the ability to reduce amyloid-β deposition (Aβ), a major pathological hallmark of AD. This raises the possibility that GSK-3 interacts with pathways responsible for cellular clearance.

Lysosomes are acidic organelles responsible for clearance of damaged and obsolete proteins. Deficiency in lysosomal function has been implicated in enhance build-up of brain Ab deposits and reduced cognitive performance in the context of AD. Agents that repair defects in lysosomal function are thus considered a potential therapeutic intervention.

In a recent work we identified the lysosome as a GSK-3 target. We showed that hyperactive GSK-3 impairs lysosomal acidification and that inhibition of GSK-3 restores lysosomal acidification, reduces Ab loads, and improves cognitive performance in an AD mouse model, the 5XFAD mice. Further research using AD-like cell cultures or primary hippocampal neurons indicated that GSK-3 suppresses autophagic flux by impairing lysosomal acidification. In addition, GSK-3 interplays with mammalian target of rapamycin (mTOR) that inhibits autophagy and regulates de-novo lysosome biogenesis.

Altogether we suggest that the therapeutic activity achieved with GSK-3 inhibitors is mediated via enhanced lysosomal acidification hence accelerating degradation of Aβ deposits and other neurotoxic proteins. GSK-3 inhibitors are thus potential therapeutic agents targeting the lysosome and its degradation activity.

WTH05-06

Alzheimer's disease tau pathology found in cholinergic, but not dopaminergic neurons differentiated from patient stem cells

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Alzheimer's disease (AD) is a complex neurodegenerative disorder, characterized by extracellular plaques consisting primarily of amyloid β and intracellular tangles consisting primarily of tau. Recent work with transgenic AD animal models suggests that AD-characteristic tau pathology occurs prior to the onset of behavioural dysfunction. Tau pathology development appears to differ between brain regions in these AD mouse models. Analysis of post-mortem AD patient tissue indicates that cholinergic neurons in frontal cortex areas are affected early in the progression of AD, prior to dopaminergic neurons. However, the involvement and contribution of different neuronal cell types in the tau progression remains unclear.

Here we assessed whether expression levels of intracellular tau differ between cholinergic and dopaminergic neurons of individuals

with sporadic AD (SAD) in comparison to neurons from healthy individuals. We differentiated frontal cortex cholinergic and midbrain dopaminergic neurons from induced pluripotent stem cells and quantified total tau levels as well as AD-relevant pathogenic tau. We found that the ratio of total tau to AD-relevant pathogenic tau varies between the cell types and between diagnoses. Cholinergic neurons from SAD patients had a higher ratio of pathogenic tau (Alz50) versus total tau (Tau-13) compared to neurons from healthy individuals. Furthermore, the ratio of Alz50 versus Tau-13 was higher in cholinergic than dopaminergic neurons from SAD patients. These preliminary findings indicate that tau pathophysiology differs between neuronal subtypes. The heightened level of pathogenic tau in cholinergic neurons suggests an inherent difference in tau processing in this cell type. Furthermore, our results suggest that human iPSC-derived neural cultures provide a suitable system to study cell type specific AD mechanisms, specifically their contribution to the tau pathophysiology. In current experiments we are exploring the temporal progression of the variation in cell-type specific tau pathology and its consequence on dendrite morphology and cell viability.

WTH05-07

Neurofilament light gene deletion exacerbates Alzheimer's disease pathology in the APP/PS1 mice

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Alzheimer's disease (AD) is a neurodegenerative and progressive disease associated with the loss of cognitive function due to neuropathological changes in the brain. Neurofilaments (NFs), the major structural (intermediate filament) proteins of neurons, accumulate in dystrophic neurites (DNs) in the earliest stages of AD. Thus, we generated transgenic mice expressing familial AD genes (APPswe/PSEN1dE9) with the NF light (*NEFL*) gene knockout background to explore the role of NFL deletion in the context of AD. An F2 generation of mice from the APP/PS1 × NFL−/− cross were used for this study. APP/PS1/NFL−/− mice were compared to APP/PS1/NFL+/+ littermates, as well as C57/BL/6 wildtype mice (n = 5 animals per group) at 10 months of age.

We demonstrated that NFL deletion increased some of the pathological features of AD. There was a significantly ($p < 0.05$) increased cortical area occupied by Aß amyloid deposits in AD mice with NFL deletion relative to AD mice with normal NFL expression. Our results also demonstrated that NFL deletion altered the susceptibility of neurites to DN formation surrounding plaques. Quantitative analysis demonstrated a significant ($p < 0.05$) increase in the size of synaptophysin and α-internexin-labelled DNs in NFL knockout mice. Conversely, in NFL knockout mice, we observed a reduction the number of α-internexin-labelled and neurofilament triplet labelled DNs. Lack of NFL was associated with significantly increased ($p < 0.05$) loss of synaptophysin-immunolabelled presynaptic puncta surrounding amyloid plaques. Finally, lack of NFL significantly ($p < 0.05$) increased the density of microglia, but not astrocytes, compared with mice with normal NFL expression.We demonstrated that disrupting NF content resulted in increased AD pathology in APP/PS1 mice. NFs may have a role in regulating cellular pathways related to the generation of Aβ plaques and associated neuronal alterations.

WTH05-08

Regulation of the endolysosomal pathway by beclin-1 and its role in the proteolytic processing of the amyloid precursor protein

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Introduction: Beclin-1 is an essential gene that controls autophagy. It has been reported that the brain of patients with Alzheimer's disease express low levels of Beclin-1. Biochemically, Beclin-1 interacts with Vps34, Rubicon and UVRAG regulating the formation of PI3P, a crucial phospholipid in endosomes dynamics. In this regard recent observations indicate that Beclin-1 could also play autophagy-independent roles, such as in endocytosis and protein sorting. The aim of this study was to investigate the role of Beclin-1 in the delivery of cargoes to lysosomes and how is affected the turnover and proteolytic processing of APP.

Material and methods: to generate a stable cell line depleted in Beclin-1, H4 cells were infected with lentiviral particles encoding a specific shRNA to Beclin-1. These cells were used to analyze the levels of APP and its proteolytic fragments.

Results: We observed that depletion of Beclin-1 caused a significant reduction in the intracellular levels of the carboxy terminal fragment beta (also called C99) of APP, which is generated after proteolytic processing by BACE1. Importantly, western blot analysis revealed that Beclin-1 depletion causes a significant decrease in UVRAG and Rubicon protein levels strongly suggesting a change in endosome dynamics. Consistent with this phenotype we observed that depletion of Beclin-1 caused a significant increase in the rate of ligand-induced EGF receptor degradation.

Discussion: This study suggests a non-autophagic role for Beclin-1 in endocytosis connected to the Beclin-1-Vps34/PtdIns3KC3-Rubicon-UVRAG complex. Our results suggest that this complex regulates the proteolytic processing of APP in the endolysosomal system.

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WTH05-09

Does epothilone d protect against amyloid and axon pathology in a mouse model of Alzheimer's disease

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Objective: Microtubule stabilisation is a potential therapeutic target for Alzheimer's disease (AD), due to the link to tau pathology. Previously this therapeutic target has been explored in tau mouse models, however, no studies have investigated whether microtubule stabilization affects amyloid deposition and associated pathology. This study examines how microtubule stabilisation affects pathology in an AD mouse model.

Methods: 6-month-old APP/PS1 ($APP_{swe}/PSEN1_{dE9}$) mice were injected weekly with 2 mg/kg epothilone D or vehicle for 3 months. Coronal tissue (40 μm $n = 7$ per group) was examined for AD pathology. Amyloid plaques were stained with thioflavin S. Dystrophic neurites were immunolabelled with neurofilament marker SMI312 and synaptic vesicle marker synaptophysin. To assess any detrimental effects of epothilone D, motor function was also examined using footprint analysis.

Results: Epothilone D treatment resulted in a significant ($p < 0.05$) reduction in synaptophysin labeled dystrophic neurites surrounding amyloid plaques ($n = 10$) in APP/PS1 mice. Neurofilament immunolabelled dystrophic neurites and thioflavin S stained plaques were not significantly ($p > 0.05$) affected by treatment. Motor function was also not affected by treatment.

Conclusion: These data suggest that epothilone D has potential as a therapeutic agent in AD by reducing neurite dystrophy.

WTH05-10

Reduced CA1 action potential firing in the rTg4510 mouse model of tauopathy due to abnormal activity at the axon initial segment

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The hippocampus plays an important role in memory formation and is affected by early degenerative changes during the course of Alzheimer's Disease, suggesting that it has a role in the etiology of this disease. rTg4510 mice harboring a regulatable P301L human tau mutation recapitulates aspects of Alzheimer's Disease, including impaired spatial memory and hippocampal long-term potentiation. Histopathologically, hyperphosphorylated tau and neurofibrillary tangles are also observed in rTg4510 mice. However, little is known about how hippocampal neuron activity is affected in this model. We show here that from 1–2 months of age the levels of hyperphosphorylated tau are increased and CA1 pyramidal neuron activity in transgenic mice is reduced, with a decrease in action potential firing and action potential amplitude compared to wild-type. In addition, while after-hyperpolarization was not changed at 1–2 months of age, there was an age-dependent increase in transgenic mice compared to wild-type. Neuronal degeneration however only became evident at 12–14 months. Quantification revealed that the peak voltage acceleration from the axon initial segment was significantly reduced in transgenic neurons compared to wild-type, whereas the peak voltage acceleration from the soma was significantly increased. This suggests that the reduction in CA1 action potential firing results from abnormal activity at the AIS induced by hyperphosphorylated tau.

WTH05-11

Reduction of ROCK1 in human brain with Alzheimer's disease

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Neurofibrillary tanglesand senile plaques are the two key neuropathological hallmarks of Alzheimer's disease (AD). Intracellular hyperphosphorylated tau aggregates are the major component of NFTs, which seeds normal tau protein driving the pathogenesis. Tau phosphorylation and aggregation is affected by the tumor-suppressing protein PTEN. The ROCK1 protein kinase regulates PTEN phosphorylation and stability, but its role in AD has not been investigated. This study investigates the role of ROCK1 in the pathogenesis of AD.

Methods: Following approvals, human brain samples from 10 subjects affected by AD and 7 controls were obtained from the Sydney Brain Bank. Proteins (cytosolic and membrane-associated) were extracted from 500 mg of fresh inferior temporal cortex from each case and levels of ROCK1 assessed using western blotting. Single and double-immunolabelled ROCK1 and phosphorylated tau structures were identified in formalin-fixed, 10 μm thick sections of the inferior temporal cortex and counted in 10 standard images. SPSS-Spearman Rho analysis was performed to determine correlations between Braak NFT staging and ROCK1 protein levels or the numbers of tau-positive NFTs and ROCK-positive NFTs.

Results: The cytosolic levels of ROCK1 were significantly reduced in AD compared to control (80% deduction) and negatively correlated with Braak NFT staging ($p = 0.015$) but membrane associated ROCK1 levels remained unchanged. ROCK1 was present in a variable proportion of AT8 immunopositive NFTs, with the number of ROCK1 positive NFTs positively correlating with Braak NFT staging ($p = 0.001$, 36% of NFTs in AD were also ROCK1-positive). The levels of cytosolic ROCK1 protein negatively correlated with number of ROCK1 positive NFTs ($p = 0.011$).

Discussion: A reduction in soluble ROCK1 might lead to reduced phosphorylation and stability of PTEN in AD and hyperphosphorylation of tau due to the loss of the PTEN phosphatase. The strong association between ROCK1 changes and Braak NFT staging suggests an early involvement of ROCK1 in NFT formation.

WTH05-12

Histone deacetylase mediates drebrin loss from dendritic spines induced by amyloid beta oligomers

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Synaptic dysfunctions are found in a number of cognitive disorders, including Alzheimer's disease (AD). Amyloid beta (Aβ) toxicity is thought to be mediated by the soluble oligomers (Aβ-derived diffusible ligands, ADDLs). Drebrin is an actin-binding protein that is located at mature dendritic spines and forms a stable actin structure. Because drebrin expression is decreased in parallel with the severity of dementia, it is thought that drebrin is closely associated with cognitive functions. Recent studies show that

histone deacetylase (HDAC) activity is elevated in the AD mouse model, and that memory impairments in these animals can be ameliorated by the treatment of HDAC inhibitors. In addition, spine loss and memory impairment in HDAC2 over-expressing mice are also ameliorated by HDAC inhibitor treatment. Therefore, we hypothesized that the regulation of histone acetylation/deacetylation is critical to synaptic functions. In this study, we examined the relationship between HDAC activity and synaptic defects induced by ADDLs by drebrin-imaging-based evaluation of synaptic function. We prepared primary cultures of hippocampal neurons and either transfected them with GFP or immunostained them against drebrin. We show that ADDLs induce drebrin loss from dendritic spines without reducing drebrin expression. HDAC inhibitor, suberoylanilide hydroxamic acid (SAHA) increased the acetylation of histone proteins, and it simultaneously attenuated the ADDL-induced decrease in drebrin cluster density. In comparison, SAHA treatment did not affect the density of drebrin clusters or dendritic protrusions in control neurons. Therefore, SAHA likely inhibits ADDL-induced drebrin loss from dendritic spines by stabilizing drebrin in these structures, rather than by increasing the density of drebrin clusters or dendritic protrusions. Taken together, our findings suggest that HDAC is involved in ADDL-induced synaptic defects, and that the regulation of histone acetylation/deacetylation plays an important role in modulating actin cytoskeletal dynamics in dendritic spines under cellular stress conditions, such as ADDL exposure. Our findings provide insight into the mechanisms of amyloid toxicity and suggest that HDAC inhibitors may hold therapeutic potential in AD.

WTH05-13

Identifying the toxicity, biochemical and biophysical properties of purified Aβ oligomers

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Alzheimer's disease (AD) is the most common form of dementia. The major pathological hallmark of AD is the presence of amyloid beta (Aβ) plaques deposited in the AD brain. It is hypothesised that the process of Aβ peptide aggregation into oligomers, protofibrils, fibrils then deposited as Aβ plaques is a major contributing factor in the progression of AD pathogenesis. Lower order oligomers, including Aβ dimer, trimer and tetramer or a combination of these oligomers termed "amyloid-beta derived diffusible ligands" have been shown to be potently neurotoxic while the level of these soluble Aβ oligomers has been shown to correlate better with the development of AD compared to insoluble Aβ plaques in the AD brain. Nevertheless, the exact toxic Aβ species remains to be determined. Our previous studies showed that Aβ binding to neurons is critical for it to induce neurotoxicity. Therefore, we hypothesis that a specific oligomeric Aβ species binds to neurons and causes the neuronal dysfunction associated with AD. This study aims to identify the toxicity, biochemical and biophysical properties of purified low order Aβ oligomers. To address this, we generated Aβ40 oligomers (monomer to tetramer) using the photo-induced cross-linking of unmodified peptides technique and each oligomer was purified and exposed to cultures containing primary mouse cortical neurons to determine neuronal toxicity and Aβ binding. After 96 h treatment, only Aβ40 trimer and tetramer, not monomer and dimer, were significantly toxic to neurons, despite each oligomer binding to neurons. To determine the biophysical properties of these toxic oligomers, in particular their structural characteristics, we will employ an ion mobility spectrometry and mass spectrometry technique to provide us with a novel method to correlate the toxicity differences shown by the different oligomers to their conformational attributes. This work will provide important insights into the nature of the Aβ toxic species.

WTH05-14

Elevated glucose levels enhance pro-inflammatory responses of astrocytes and increase vulnerability of neurons to toxic insults

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Type 2 diabetes mellitus (T2DM) has been identified as a risk factor for Alzheimer's disease (AD). However, the cellular and molecular mechanisms linking these two pathologies still remain unclear. We hypothesized that high levels of glucose observed in T2DM could accelerate neuronal loss by increasing their sensitivity to toxic insults and by enhancing pro-inflammatory reactions of glial cells, which contribute to neuronal death in AD. *In vitro* experiments were conducted using primary human astrocytes and U-118 MG human astrocytic cells as their surrogates. Retinoic acid-differentiated human neuronal SH-SY5Y cells were used to model human neurons. Enhanced mRNA expression of the pro-inflammatory cytokine interleukin (IL)-6 and elevated secretion of both IL-6 and IL-8 was observed in human astrocytes and U-118 MG cells stimulated in the presence of high glucose (30.5 mM) compared to normal glucose (5.5 mM) levels. Data obtained indicated that increased activation of the p38 mitogen activated protein kinase (MAPK) might be mediating the effects of high glucose in astrocytic cells. High glucose increased the susceptibility of undifferentiated human SH-SY5Y neuronal cells and retinoic acid-differentiated SH-SY5Y cells to injury by hydrogen peroxide and fibrillar Alzheimer amyloid beta-42 protein (Aβ42). Our data indicate that high brain glucose levels observed in T2DM patients could contribute to an earlier appearance of AD-like symptoms by increasing glial cell-mediated inflammation and by making neurons more vulnerable to damage induced by oxidative stress and Alzheimer amyloid protein. This research highlights novel mechanisms that could be responsible for AD progression and could help identify new preventative and treatment strategies for AD.

WTH05-15

Effects of scanning focused ultrasound in an Alzheimer's mouse model

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Alzheimer's disease (AD) pathology is linked to the accumulation of assemblies of amyloid beta peptide. We report a reduction in plaque load, total amyloid levels, and improvements in memory and

learning following focused ultrasound (SUS) treatment in a transgenic mouse model of Alzheimer's disease (APP23 mice). Scanning focused ultrasound treatment combined with microbubbles led to a temporary increase in the permeability of the blood brain barrier. Microscopic analysis revealed increased internalization of amyloid beta within microglia. Nissl, hematoxylin and eosin and GFAP staining did not indicate damage and there was no increase in NF kappa B nuclear translocation. SUS Mice showed improved behavior in the Y maze, novel object test, and active place avoidance test. These findings indicate a potential of SUS to remove amyloid beta and may have therapeutic potential in AD.

WTH05-16

Effect of global brain ischeima after cardiac arrest oin producing the hallmarks of Alzheimer's disease

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Initial restoration of circulation and survival following cardiac arrest (CA) is often compromised by ischaemic brain damage with a significant impact on memory, cognitive and social behaviour of the victim. An increase of beta amyloid (Aβ) protein in the blood has been shown in patients suffering cardiac arrest (CA), suggesting that the brain ischemia associated with CA triggers the Aβ release. It is suggested that the extent of ischemia in some situations such as stroke can define if the subjects will go through a mild cognitive impairment (MCI) or will develop Alzheimer's disease (AD). The origin of Aβ increase in the blood after CA and the mechanism underlying the possible AD pathology in CA survivors however is not still clear. Aims: In this study we examined the short-term and long-term effect of global brain ischemia following CA on generating the AD pathological hallmarks in rats. Method: Various duration of CA (2, 4 and 8 min) was produced through our model of CA. An intra-oesophageal AC current of 24v has been applied for the first half of the time followed by an 18v current for the second half of the CA. Defibrillation (8j) was applied through external electrodes to defibrillate the heart along with adrenaline injection and mechanical ventilation, if it was necessary. The rats were kept alive for the periods of 1, 2 h (short-term) and 4 weeks (long-term). At the end of the recovery period, the brains have been evaluated for any alteration in Aβ, APP, phosphorylated tau (p-tau) and oxidative stress associated with ischemia, through western blot and immunohistochemistry. Results: P-tau levels decreased following 2, 4 min of CA with 1 and 2 h of recovery time, however it increased following 4 weeks of recovery. APP expression enhanced after 4 weeks. There was no significant change in Aβ 42 and Aβ 40. Conclusion: The level of tau phosphorylation showed an initial decrease possibly due to the lack of ATP within, followed by an increase in long-term survivors. That could suggest the long-term involvement of intracellular metabolic pathways and the alteration of their activities in producing AD hallmarks.

WTH05-17

Tau phosphorylation is enhanced following lysosomal acid ceramidase inhibition in rat hippocampal slices

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The risk for suffering of Alzheimer's disease has been recently correlated with accumulation of ceramide. We show here that rat hippocampal slices, preincubated with the acid ceramidase inhibitor (ACI) d-NMAPPD, exhibit increased *N*-methyl-D-aspartate (NMDA) receptor-mediated field excitatory postsynaptic potentials (fEPSPs) in CA1 synapses. The ACI by itself did not interfere with either paired pulse facilitation or alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptor-mediated fEPSPs, indicating that its influence on synaptic transmission is postsynaptic in origin and specific to the NMDA subtype of glutamate receptors. From a biochemical perspective, we observed that Tau phosphorylation at the Ser262 epitope was highly increased in hippocampal slices pre-incubated with the ACI, an effect totally prevented by the global NMDA receptor antagonist D/L(-)-2-amino-5-phosphonovaleric acid (AP-5), the calcium chelator 1,2-bis(o-aminophenoxy) ethane-*N*,*N*,*N*',*N*'-tetraacetic acid (BAPTA) and the GluN2B (but not the GluN2A) receptor antagonist RO25-6981. On the other hand, preincubation of hippocampal slices with the compound KN-62, an inhibitor known to interfere with calcium/calmodulin-dependent protein kinase II (CaMKII), totally abolished the effect of ACI on Tau phosphorylation at Ser262 epitopes. Collectively, these results provide the first experimental evidence that ceramide accumulation upregulates Tau phosphorylation in the hippocampus via a mechanism dependent on GluN2B receptor subunits and CaMKII activation.

WTH05-18

Amyloid precursor protein differentially regulates expression of neuronal genes involved in amyloid clearance

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Abnormal elevation of amyloid β-peptide (Aβ) levels in the brain is the primary trigger of neuronal cell death specific to Alzheimer's disease (AD). It is now evident that in the brain there is a functional equilibrium of Aβ levels between its production from the amyloid precursor protein (APP) and removal by amyloid clearance proteins. Clearance can be either enzymic or non-enzymic (binding/transport proteins). There are several amyloid-degrading enzymes in the brain, both membrane-bound and soluble, and of differing cellular location but the major role belongs to neprilysin (NEP), its homologues NEP2 and the endothelin converting enzymes (ECE-1 and -2), as well as to a distinct metallopeptidase, insulin-degrading enzyme (IDE). NEP plays an important role in brain function terminating neuropeptide signals and its decrease in specific brain areas with age or after hypoxia, ischaemia or stroke contribute significantly to the development of AD pathology. The recently

discovered mechanism of epigenetic regulation of NEP (and other genes) by the APP intracellular domain (AICD) and its dependence on the cell type and APP isoform expression suggest possibilities for selective manipulation of NEP gene expression in neuronal cells. We have also observed that another amyloid-clearing protein, namely transthyretin (TTR), is also regulated in the neuronal cell by a mechanism similar to NEP whereas IDE, ECE and NEP2 expression has a different response to over-expression of various APP isoforms and to different pharmacological agents including Gleevec and bexarotene. Selective dependence of NEP, TTR and IDE expression on histone deacetylases and the ability of HDAC inhibitors to up-regulate expression of some amyloid-clearing genes in the brain opens new avenues for developing preventive strategies in AD. *Supported by MRC UK, Alzheimer's Research UK, RFBR (13-04-00388).*

WTH05-19

Machine-learning alternative to thresholds for segmenting fluorescent amyloid-β images in an Alzheimer's disease mouse model

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The use of transgenic mouse models of amyloid-β deposition is well established for research into the basic mechanisms of Alzheimer's disease. However, amyloid-β pathology itself is difficult to measure precisely dye to the ill-defined boundaries produced by fluorescent dyes or antibodies when imaged. Widely-used thresholding methods to segment images into pathology and background are both subjective and sensitive to minor alterations in staining intensity and imaging parameters. Even when optimised on an image-by-image basis, standard thresholding methods are unable to accurately separate pathology from background as interpreted and labelled by human experts. By combining image derivatives with the random forests algorithm for supervised machine-learning, we present an open-source tool which segments fluorescent amyloid-β pathology images significantly more accurately and consistently than optimised thresholding. To evaluate the sensitivity of these methods to minor variation in standard pathology staining, we selected representative subregions of large confocal cortical images from three 7–12 month-old APPswe/PS1dE9 mice stained using thioflavin S and MOAB2, an antibody to amyloid-β. Experienced raters selected a threshold for each subregion to best represent their judgement of the plaque area. Applying each of these individual thresholds to the entire image produced major discrepancies (mean variations of ± 40% for thioflavin S, and ± 90% for MOAB2) across the range of measured plaque areas. Conversely, using the thresholded subregions as training input for our tool produced a model that more closely matched the rated areas in all subregions, when applied across all regions (mean variations of ± 15% for thioflavin S and ± 30% for MOAB2). The tool is available as a freely downloadable ImageJ plugin, along with training images for other laboratories to use in calibrating their imaging and staining setups. By offering these tools we hope to increase the consistency and comparability of pathology assays in the field of Alzheimer's disease research.

WTH05-20

Selective alteration of mitochondria membrane potential in glutamatergic terminals in an *in vitro* model of early Alzheimer's disease

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Alzheimer's disease (AD) is defined by a loss of cognitive function associated with an abnormal processing and accumulation of amyloid β-peptide (namely $A\beta_{1-42}$). With the aim to arrest the evolution of AD, particular attention has been devoted to synaptic dysfunction and loss. Mitochondria play a key role in the maintenance of adequate synaptic function and mitochondrial structural and functional abnormalities are well-characterized features of AD. On the other hand, a predominant susceptibility of glutamatergic synapses was already described, using an Aβ-based model of AD. This prompts us to post the hypothesis that mitochondria located in glutamatergic terminals are particularly affected in AD, leading to modifications of calcium balance and energy power supply that underlie the synaptic degeneration in AD.

Hippocampal nerve terminals obtained in a discontinuous Percoll gradient were plated and incubated with oligomeric $A\beta_{1-42}$ peptide (500 nM for 2 h). Qualitative analyses through live imaging experiments were done to measure the changes between final and initial baseline of the mitochondrial membrane potential ($\Delta\psi_m$), using a fluorescent probe, $TMRM^+$. Oligomycin and FCCP were used as stimuli.

We report a reduction of 23.0% ± 5.2% ($n = 6$) of $\Delta\psi_m$ after incubation of the nerve terminals with $A\beta_{1-42}$ peptide, without any modification of the plasma $\Delta\psi_m$. This reduction was mostly observed in the glutamatergic nerve terminals (immunopositive for vGluT1).

These results are in agreement with the contention that synaptic mitochondria are an important trigger of "synaptic apoptosis", contributing to synaptic dysfunction and degeneration in AD indicating glutamatergic terminals as primary targets of $A\beta_{1-42}$-induced toxicity. This prompts the correction of synaptic mitochondria dysfunction as an promising candidate to therapeutically alleviate early AD.

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WTH05-21

Protective effect of *N*-acetyl cysteine, a glutathione precursor against ICV streptozotocin-induced Alzheimer rat model

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Bachground: Growing evidences indicate that endogenous oxidants and antioxidant defense interact in a vicious cycle, which plays a critical role in the pathogenesis of cognitive dysfunction. In this study, we examined the effect of *N*-acetyl cysteine (NAC)

against the intracerebroventricular infusion of streptozotocin (ICV STZ)-induced cognitive impairment and mitochondrial oxidative damage in rats.

Methods: Male adult Wistar rats were injected with STZ (3 mg/kg) bilaterally through ICV. NAC (50 and 100 mg/kg) was administered for 3 weeks post-surgery. The rats were sacrificed on the 21st day following the last behavioral test, and cytoplasmic fractions of the hippocampus and cortex were prepared for the quantification of acetylcholinesterase, oxidative stress parameter, mitochondrial enzymes, inflammatory mediators and caspase-3 activity.

Results: ICV STZ resulted in poor retention of memory in Morris water maze. It also increased the mito-oxidative damage and tumor necrosis factor-α, interleukin 6 and caspase-3 levels in the hippocampus and cortex compared to sham animals. NAC significantly improved memory retention and attenuated oxidative damage parameters, inflammatory markers in STZ-treated rats.

Conclusions: The results of the present study strongly indicate the effectiveness of NAC in preventing cognitive impairment as well as mito-oxidative stress and may be considered as a potential agent in the management of cognitive-related disorders.

WTH05-22

Apolipoprotein E and amyloid β expression during differentiation of induced pluripotent stem cells in Alzheimer's disease

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Alzheimer's Disease is a neurodegenerative disease characterized by intracellular neurofibrillary tangles containing hyperphosphorylated tau and extracellular amyloid beta plaques containing high levels of Aβ42/40 peptides. Apolipoprotein E (APOE) genotype is the most significant genetic risk factor for sporadic disease.In humans the ε3 allele is the most common, whilst the ε2 allele confers neuroprotection, whereas the ε4 allele increases disease risk. How the APOE ε4 allele leads to an increased risk of Alzheimer's disease remains unknown. One hypothesis is that APOE ε4 increases the Aβ42/40 ratio, either by promoting the generation of Aβ42 or through a reduced ability to prevent its aggregation. In order to examine the effect of APOE on Aβ levels during neural development we have generated induced pluripotent stem cells lines from people with different APOE genotypes and assessed protein levels during neuronal differentiation. ELISAs were used to quantitate Aβ42 and Aβ40 secreted into cell media and APOE levels were measured by western blot in cell lysates and media.

Neurons from sporadic and familial Alzheimer's disease lines released higher levels of Aβ42 than those from healthy controls, with soluble Aβ42 present in the media of neuroprogenitors but not in pluripotent stem cells. APOE was expressed at high levels at pluripotency, with levels declining during neuronal differentiation in all APOE genotypes. We are currently undertaking further studies aimed at interrogating the underlying pathways linking APOE genotype and Aβ levels. Dissecting these pathways may lead to an understanding of the APOE4-induced risk for Alzheimer's disease.

WTH05-23

Induction of tau pathology in a P301S mutant tau transgenic mouse model

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Intracellular neurofibrillary tangles (NFT) are made up of pathologically hyperphosphorylated microtubule-associated protein tau and are a hallmark neuropathological feature in Alzheimer's disease (AD). Tau pathology follows an anatomically characteristic profile, starting from inner limbic regions of the brain and advancing to the neocortex. Recent evidence suggests that pathological tau derived from tau transgenic mouse brain extracts is capable of propagating pathology to neuroanatomically connected regions. Here we aimed to determine whether pathological tau in human brain tissue can prematurely propagate pathology in the P301S mutant tau transgenic mouse (TAU58/2) brain. 2 months old recipient TAU58/2 mice received injections into the hippocampus and cortex with brain extracts from: a donor human AD (iAD), a donor human control (iNC), a donor transgenic TAU58/2 mouse (iTAU58/2+), or a donor wild-type mouse (iTAU58/2−). Post injections, brains were histologically characterized using Gallyas silver impregnation and immunohistochemistry with various phosphorylation site-specific tau antibodies. Increased NFT numbers were detected in mice belonging to either the iAD or iTAU58/2+ treatment groups compared to the control human or wild type TAU58/2− treatment groups. NFT levels were higher in the injected hippocampus compared to the non-injected side. Together, the present findings indicate that injections of brain extracts containing pathological tau from human AD cases induce premature propagation of tau pathology in the TAU58/2 mouse model.

WTH05-24

Scanning ultrasound opens blood-brain barrier and improves pathologic abnormalities and behaviour in a mouse model of Alzheimer's

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Alzheimer's disease is a progressive brain disease that attacks the neurons in the brain, leading to cell death and degeneration. Early symptoms of the disease include synaptic loss and learning and memory impairment. The disease manifests as two different types of lesions in the brain: beta-amyloid plaques – clumps of protein that form extracellularly; and neurofibrillary tangles – composed of hyperphosphorylated tau protein, which build up inside the neurons. Preclinical research is exploring the use of scanning ultrasound (SUS) in the presence of microbubbles, as a non-invasively way to induce reversible blood-brain barrier (BBB) opening. In our study we investigated the effects of focused ultrasound treatment in an tau

based mouse model by using in-house prepared single chain antibody fragments (scFv). To evaluate the preventative potential of ultrasound treatment, tau mice with an early tau pathology were used in this study, and each mouse was retro-orbitally injected with scFv once a week for a total of 4 weeks. Immediately after administration, mice were subjected to focused ultrasound. BBB opening was confirmed using the indicator dye Evans blue. We will present a histological and behavioural analysis that will inform whether ultrasound treatment can be used for prevention of an Alzheimer's pathology.

WTH06 Therapeutic Approaches of Parkinson's Disease

WTH06-01

Differences in number of human midbrain dopamine neurons associated with summer and winter photoperiods

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Brain plasticity underlies adaptive behavior and brain repair following injury or disease. In adult rodents we have evidence of a new form of brain plasticity wherein hypothalamic and midbrain neurons switch dopamine (DA) neurotransmission on or off in response to environmental cues (including photoperiod), leading to alterations in behavior. This may have implications for treating brain diseases and disorders associated with imbalances in brain DA, if it occurs also in humans. To test this we examined markers of DA neurons in post-mortem midbrain of people who died in summer (long-day photoperiod, $n = 5$) or winter (short-day photoperiod, $n = 5$). Tyrosine hydroxylase (TH, the rate-limiting enzyme in DA synthesis) and DA transporter (DAT) immunoreactivity were qualitatively higher in summer compared with winter. The density of TH^+ cells was ~6-fold higher ($p = 0.009$, t-test), and the density of TH- cells was ~2.5-fold lower ($p = 0.04$, t-test) in summer compared with winter. Midbrain DA nuclei volume was the same in both seasons. The density of DAT+ and DAT- cells exhibited the same trends, although with smaller magnitudes and the seasonal differences were not statistically significant. TUNEL staining was the same in summer and winter, indicating apoptosis did not underlie these differences in cell number. These are the first data suggesting that environment (photoperiod) alters the number of DA neurons in the adult human brain via neurotransmitter switching.

WTH06-02

Neuroprotection against Parkinson's disease with near infrared light

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Low-level near infrared light (NIr) is emerging as a viable neuroprotection intervention for Parkinson's disease (PD). Our team has demonstrated the efficacy of NIr, delivered transcranially, in mitigating dopaminergic cell loss, abnormal neuronal firing and behavioural deficits in mice treated with the parkinsonian toxin MPTP, and in mitigating dopaminergic cell loss and oxidative stress in the K369I tau transgenic model of parkinsonism.

However a major barrier to transcranial NIr treatment of Parkinson's disease patients remains the delivery of sufficient light energy to midbrain. As one strategy to overcome this barrier, we developed a NIr-emitting fibre optic device for intracranial surgical implantation. Our proof-of-principal studies in rodents support the feasibility of intracranial NIr delivery and demonstrate its neuroprotective efficacy. In our recent pre-clinical trials in MPTP-treated monkeys, intracranial NIr was associated with significantly improvement in clinical motor activity scores and significant protection of midbrain dopaminergic neurons.

Perhaps most fascinating is that NIr need not be targeted to the site of damage. In pursuing a non-invasive delivery strategy, we have shown that NIr targeted specifically at the dorsum of MPTP-treated mice significantly mitigates midbrain dopaminergic cell loss. Potential mechanisms are under investigation. For example, flow cytometry studies have revealed that NIr targeted at the body significantly increases the proportion of bone marrow $Sca\text{-}1^+PDGFR\alpha^+$ cells (markers of mesenchymal stem cells; >90% increase, $p = 0.008$). Microarray studies of the midbrain of MPTP-treated mice receiving body-targeted NIr indicate a significant enrichment of upregulated genes in the "chemokine signalling pathway" (fold-enrichment = 9.0, $p = 0.007$), suggesting that NIr stimulates chemokine signalling activity within damaged regions of the brain, possibly facilitating the recruitment of MSCs and/or other reparative cell types.

In conclusion, barriers precluding the clinical use of NIr in PD patients may be overcome by delivering NIr intracranially or targeting NIr at a remote tissue (e.g. bone marrow).

WTH06-03

Effective delivery of dopamine to brain using pegylated immunoliposome in Parkinson's disease animal model

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The blood-brain barrier (BBB) restricts the brain uptake of many important hydrophilic drugs and limits their efficacy in the treatment of brain disease, such as Parkinson's disease (PD) because of the presence of tight junctions. Dopamine that has been widely used for the treatment of PD has limitation that is difficult to transport on the brain. Thus we examined the effectiveness of brain transport of dopamine through BBB, using the OX26 mAb and liposomes. Dopamine was encapsulated in PEGylated liposome (PL) using the thin-film hydration method and PEGylated immunoliposome (PIL) is PL which was conjugated with OX26 mAb. The average diameter and zeta potential of the dopamine, PLs and PILs were measured by a laser-scattering technique. Pharmacokinetics of free dopamine, PLs and PILs after i.v. injection to the normal and PD rat induced MFB transaction and brain uptakes of free dopamine, PLs and PILs using internal carotid artery perfusion method (ICAP) in rats were determined. We used about 46 ± 2 nm as mean diameter of PLs that were made using the extruder and exhibited zeta potentials (6 ± 1 mV). PILs were ranging in size from 42 to 50 nm. PLs and PILs contained dopamine were cleared slowly from plasma compartment, compared to the free dopamine. The V_{dss} and CL of

PILs in normal and PD rat were decreased and AUC of that was increased compared with free dopamine. Brain uptake of PILs contained dopamine was increased 7-fold compared with PLs in PD rats using ICAP method. In this study, dopamine-loaded PILs formulation showed a significantly longer stability and maintained good systematic effects to treat PD symptoms by performing an *in vivo* brain uptake and pharmacokinetic studies in PD rat models. Additionally, to transport of dopamine to the brain, it will be expect that PILs are effective system for relieving the PD symptoms.

WTH06-04

Small molecule PKD1 activator protects progressive nigral dopaminergic neuronal degeneration in the MitoPark animal model of PD

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Oxidative stress has been associated with many neurological diseases including Parkinson's disease (PD). Therefore, identifying cell signaling mechanisms associated with oxidative stress is critically important to the development of new treatment strategies for PD. We have recently identified a novel oxidative stress-associated signaling pathway in which protein kinase D1 (PKD1) plays a major compensatory survival role in dopaminergic neurons. Therefore, we adopted a rationale based pharmacological screening approach and identified several small molecule PKD1 activators in our cell based screening assay. Herein, we report that quercetin, a natural flavonoid widely found in vegetables and fruits can effectively activate PKD1 protective signaling. Western blotting analysis revealed that quercetin treatment significantly induced the phosphorylation and activation of PKD1 as well as CREB and Akt phosphorylations in MN9D dopaminergic neuronal cells. Activation of Akt, however, was inhibited by siRNA knockdown of PKD1, suggesting that Akt acts as a downstream target of PKD1 signaling during quercetin treatment. Results from qRT-PCR, Western blot analysis, and other biochemical assays revealed that quercetin can effectively protect dopaminergic neuronal cell death. Quercetin treatment protected against 6-OHDA-induced neurotoxicity in dopaminergic cell models. Next, we evaluated the neuroprotective efficacy of quercetin against the progressive neurodegenerative process by using MitoPark mouse model of PD. Administering quercetin (25 mg/kg) once daily to 12-week-old Mitopark mice via oral gavage for 6 weeks significantly reversed behavioral motor deficits in this chronic animal model of PD. Notably, quercetin also protected against striatal dopamine depletion, and TH positive neuronal cell loss in MitoPark mice. Our findings suggest that quercetin, by virtue of its ability to activate the PKD1-mediated neuroprotective signaling, is a promising neuroprotective drug candidate for the treatment of PD (NS 074443 and ES 10586).

WTH06-05

New therapeutic strategies in Parkinson's disease: the transcription factor NRF2 as a target for dimethyl fumarate

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Parkinson's disease (PD) is one of the most common disabling neurodegenerative disorders with treatments based on the clinical symptomatic aspects. Therefore it is very important to find a preventive or disease modifying therapy for PD. Dimethylfumarate (DMF), the main ingredient of an oral formulation of fumaric acid esters, has been found to have therapeutic efficacy in relapsing-remitting multiple sclerosis and psoriasis, diseases where inflammation has an essential role. DMF targets the transcription factor NRF2, a master regulator of antioxidant response and inflammation, which are two major hallmarks of PD. Therefore, in this study we investigated whether DMF administration could prevent or ameliorate the neurodegeneration/neuroinflammation caused by overexpression of α-synuclein (α-SYN) and thus provide a new therapeutic strategy for PD. Treatment of microglial BV2 cells with DMF showed a time-dependent increased in mRNA and protein levels of phase II detoxificantion enzymes like heme oxygenase 1 (HO-1), NAD(P)H quinone oxidoreductase 1 (NQO1) and Osgin-1 as well as autophagy related protein p62. Interestingly, DMF treatment decreased the mRNA and protein levels of IL-1b and iNOS induced by α-SYN, indicating that DMF could modulate the inflammatory response induced by α-SYN. To further investigate the effect of DMF in a preclinical setting, we used recombinant viral vectors to overexpress human α-SYN protein in the substantia nigra of $Nrf2^{+/+}$ and $Nrf2^{-/-}$mice, which provides a relevant model that recapitulates many cardinal features of the human disease. We found that, in $Nrf2^{+/+}$ mice, daily oral gavage of 100 mg/kg DMF provided a significant protection of nigral dopaminergic neurons against α-SYN toxicity after 3 and 8 weeks following injection with AAV6-α-SYN and at the same time reduced astrogliosis and microgliosis. This protective effect was not observed in the $Nrf2^{-/-}$ mice. These experiments provide compelling rationale for targeting NRF2 with DMF as a therapeutic strategy in synucleinopathies.

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WTH06-06

Pharmacological inhibition of LRRK2 blocks alpha-synuclein induced neurodegeneration in rats

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Genetic studies prominently implicate alpha-synuclein and leucine-rich repeat kinase 2 (LRRK2) genes in the pathogenesis of late-onset Parkinson disease (PD). The G2019S mutation in the LRRK2 gene is the most common known genetic cause of PD and

increases LRRK2 kinase activity in model systems. Additionally, alpha-synuclein gene-multiplications trigger PD in rare forms of familial disease, and over-expression of alpha- synuclein causes neurotoxicity in model systems. Some evidence demonstrates that these two genes may act in concert in neurodegenerative processes. Here we show that rats expressing human G2019S- LRRK2 have exacerbated dopaminergic neurodegeneration in response to virally-expressed human alpha-synuclein. The effects of G2019S-LRRK2 expression on alpha-synuclein induced neurodegeneration and inflammation can be mitigated using a highly potent and selective orally-available LRRK2 kinase inhibitor. Further, neurodegeneration in wild-type rats can also be prevented by LRRK2 kinase inhibition. These results demonstrate that pharmacological inhibition of LRRK2 kinase activity can be used to counteract dopaminergic neurodegeneration caused by alpha-synuclein and provide further rationale for the development of LRRK2 kinase inhibitors for neuroprotection in PD.Genetic studies prominently implicate alpha-synuclein and leucine-rich repeat kinase 2 (LRRK2) genes in the pathogenesis of late-onset Parkinson disease (PD). The G2019S mutation in the LRRK2 gene is the most common known genetic cause of PD and increases LRRK2 kinase activity in model systems. Additionally, alpha-synuclein gene-multiplications trigger PD in rare forms of familial disease, and over-expression of alpha-synuclein causes neurotoxicity in model systems. Some evidence demonstrates that these two genes may act in concert in neurodegenerative processes. Here we show that rats expressing human G2019S- LRRK2 have exacerbated dopaminergic neurodegeneration in response to virally-expressed human alpha-synuclein. The effects of G2019S-LRRK2 expression on alpha-synuclein induced neurodegeneration and inflammation can be mitigated using a highly potent and selective orally-available LRRK2 kinase inhibitor. Further, neurodegeneration in wild-type rats can also be prevented by LRRK2 kinase inhibition. These results demonstrate that pharmacological inhibition of LRRK2 kinase activity can be used to counteract dopaminergic neurodegeneration caused by alpha-synuclein and provide further rationale for the development of LRRK2 kinase inhibitors for neuroprotection in PD.

WTH06-08

Dopamine loaded PLGA nanoparticles ameliorate the functional recovery in parkinsonian rats

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Parkinson's disease (PD) is characterized by the degeneration of midbrain nigrostriatal dopaminergic neurons resulting in severe motor symptoms. The loss of dopaminergic neurons obstructs the supply of dopamine (DA) to the brain striatum. Currently, clinical management of PD is done by DA precursor levodopa, the gold standard. But as the disease progresses, the patients become less responsive to levodopa and begin to develop peripheral side-effects. This encourages the development of more effective strategies for the therapeutic management of PD, but the constraint of the blood brain barrier (BBB) limits the choice of therapeutics. Additionally, DA is unable to cross the BBB. Nanotechnology provides a possible solution in overcoming these challenges by affording targeted drug delivery. In line with this, we designed dopamine loaded poly-lactide-co-glycolide (PLGA) nanoparticles (DA NPs) utilising double emulsion solvent evaporation technique. These particles released dopamine in a continuous and sustained manner, thereby enhancing the bioavailability at the desired site. Entrapment of DA in polymeric matrix prevented its oxidation and related toxicity in the peripheral nervous system. Safety and therapeutic efficacy of DA NPs was tested *in vivo* using 6-OHDA induced rat model of PD and *in vitro* using SH-SY5Y cell line. TEM showed that these particles were internalized in the SH-SY5Y cells, and traversed the BBB to reach striatum and substantia nigra of the rat brain. DA NPs didn't exhibit significant toxicity under *in vivo* or *in vitro* conditions. Further, the particles displayed neurorestorative potential following systemic intravenous administration in rat model of PD where they reversed lesion induced impairment in locomotor activity, loss in DA levels and dopamine D2 receptor mediated supersensitivity. Thus, the nanoformulation may provide a novel therapeutic approach for delivery of DA to the brain for the treatment of PD.

WTH06-09

Thiol repletion therapy in animal and human studies of Parkinson's disease

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Oxidative stress causes cell damage and ultimately cell death in conditions such as stroke and Parkinson's disease (PD). Glutathione is the most abundant thiol antioxidant in neurons. In PD, glutathione depletion precedes cell death in dopaminergic neurons of the substantia nigra, which may contribute to subsequent neuronal death. Pharmacological repletion of glutathione or its precursors is, therefore, a potential approach for slowing disease progression in PD. We developed a method for evaluating pharmacological approaches for restoring glutathione content in CNS neurons. The method involves use of EAAC1$^{-/-}$ transgenic mouse, which has reduced glutathione content selectively in neurons due to a defect in neuronal cysteine transport. Neuronal glutathione content in these mice was evaluated by immunohistochemical quantification of neuronal GSH in brain sections. In parallel, the capacity of the neurons to scavenge reactive oxygen species (ROS) was assessed in *ex-vivo* brain sections by monitoring the formation of nitrotyrosine. Neurons in the EAAC1$^{-/-}$ mice showed substantially reduced glutathione content and reduced ROS scavenging capacity relative to wild-type mice. EAAC1$^{-/-}$ mice treated orally with a membrane-permeable analogue of cysteine, *N*-acetyl cysteine (NAC), showed near-normalization of both neuronal glutathione content and ROS scavenging capacity. The CSF NAC concentration associated with this biological effect was 150 nM. In a parallel study, human subjects with Parkinson's disease were given NAC at doses ranging from 7 mg/kg to 70 mg/kg twice daily. Doses above 35 mg/kg produced CSF concentrations well above 150 nM, thus establishing a dosing target for clinical trials. Ongoing studies are comparing the effects of NAC with other cysteine analogues that may have better bioavailability and neuronal penetration. These studies aim to provide a foundation for clinical trials of thiol-repleting agents in PD.

WTH06-10

Nimodipine attenuates mitochondrial dysfunctions and altered calcium dynamics to protect against experimental parkinsonism in mice

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The most common motor neurodegenerative disorder Parkinson's disease (PD) is caused by loss of dopaminergic neurons of substantia nigra (SN), which use calcium channels for pacemaking activity. Interestingly the ventral tegmental area (VTA) dopaminergic neurons that use Na^+ channels for pacemaking activity are relatively spared in PD. This suggest a possible involvement of voltage gated calcium channels (VGCC) in the pathogenesis of PD. It is reported that expression of a Ca^{2+} buffering protein, calbindin is inversely related to vulnerability of the neurons in PD. We investigated the neuroprotective mechanisms underlying an L-type calcium channel blocker, nimodipine in animal and cellular models of PD, respectively caused by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in mice and its active metabolite, MPP^+ in SH-SY5Y cell line. In mice, MPTP-induced behavioral dysfunctions (reduced swimming ability, akinesia and catalepsy), striatal dopamine depletion, increases in mitochondrial reactive oxygen species generation and loss in mitochondrial respiration were significantly attenuated by nimodipine (5, 10, 15 mg/kg i.p.) administration. In SH-SY5Y cells, pretreatment with nimodipine attenuated MPP^+-mediated increase in intracellular calcium as monitored employing Fura-2AM, reduction in cell viability studied by MTT assay, decrease in mitochondrial membrane potential as revealed by TMRM staining and corrected the aberrations in mitochondrial morphology examined by Mitotracker Green® fluorescence. Additionally, the aberrant expression of calbindin, calpain and calcineurin, a series of proteins regulated by calcium were also corrected by nimodipine in the striata of mice treated with MPTP or in SH-SY5Y cells treated with MPP^+. These results suggest that VGCC activity could be targeted to control dopaminergic neuronal death.

WTH06-11

Preclinical evaluation of tecfidera (dimethylfumarate) in Parkinson's disease: are we ready for repurposing?

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Targeting oxidative stress either by providing exogenous antioxidants or by enhancing the endogenous antioxidative capacity has been intensely investigated for PD therapies. The latter includes the activation of nuclear-factor-E2-related factor 2 (Nrf2)/antioxidant response element (ARE) signaling pathway which regulates the expression of a battery of genes encoding anti-oxidative, anti-inflammatory, and cytoprotective genes. Tecfidera is an oral formulation of dimethylfumarate (DMF) approved for Multiple sclerosis based on its promising beneficial effects. Fumaric acid esters such as dimethyl and mono-methylfumarate have been found to exert neuroprotective effects by activating the Nrf2/ARE signaling pathway. We investigated *in vivo* pharmacokinetics, effects on Nrf2/ARE signaling both *in vitro* and *in vivo* and its ability to block 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxicity and associated oxidative damage, mitochondrial dysfunctions, and neuroinflammation in mice. We found that dimethyl and mono-methylfumarate activate Nrf2 pathway using Neh2-luciferase reporter *in vitro*, upregulated mRNA and protein levels of several cytoprotective and antioxidative genes in discrete mouse brain regions commensurate with its pharmacologic levels *in vivo*, and in wild type mouse embryonic fibroblasts but not in Nrf2 null fibroblasts *in vitro*. Oral administration of both dimethyl and mono-methylfumarate at (10, 25, 50, and 100 mg/kg) dose dependently protected against acute MPTP neurotoxicity assessed by stereological cell counts of total and tyrosine hydroxylase positive neurons of substantia nigra and striatal levels of catecholamines employing HPLC electrochemistry in wild type but not Nrf2 null mice. Fumrate esters blocked against MPTP-induced oxidative damage assessed by 3-nitrotyrosine and inflammation determined CD68 immunoreactivity and expression of pro-inflammatory cytokines in the midbrains. Monomethylfumarate enhanced mitochondrial bioenergetics assessed by oxygen consumption rate in mouse embryonic fibroblasts in an Nrf2 dependent fashion. Our results suggest that fumaric acid esters protect against nigrostriatal dopaminergic neurotoxicity and associated oxidative damage, neuroinflammation, and mitochondrial dysfunction by virtue of its ability to activate neuroprotective Nrf2/ARE genetic program.

WTH07 Myelination and Demyelination

WTH07-01

Autoantibody mediated CNS myelin morphology in the acute phase of experimental autoimmune encephalomyelitis

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Multiple sclerosis (MS) is the most common chronic inflammatory demyelinating disease of the CNS. Demyelination and axonal damage are responsible for neurological deficits in MS. However, the mechanisms of demyelination and axonal damage have not been fully understood. To clarify the mechanism of demyelination in experimental autoimmune encephalomyelitis (EAE), we examined myelin morphology during the course of MOG35-55-induced EAE in the C57BL/6 mice. Osmium-maceration scanning electron microscopic (SEM) analysis displayed ultrastructural abnormalities of myelin structure in the white matter of the EAE spinal cord. In addition, abnormal morphology of myelin was observed at early stages of EAE. While infiltrating immune cells into the CNS were not observed in the spinal cord, anti-MOG autoantibody was observed in the CNS at this point. These observations suggest that anti-MOG antibody plays an important role in the pathogenesis at the acute stages of EAE.

WTH07-02

Role of astroglial methyl-CpG-binding protein 2 in central nervous system myelination

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Astrocytes are important regulators of neuronal function and also maintain Central Nervous System (CNS) homeostasis. Reports suggest role of astrocytes in regulating myelination by promoting neuronal survival and secreting growth factors like neurotrophins [Brain Derived Neurotrophic Factor (BDNF) and Nerve Growth Factor (NGF)]; however, the underlying mechanism is still unclear. BDNF promotes CNS myelination and is also known to be a downstream target gene of methyl-CpG-binding protein 2 (MeCP2), an epigenetic regulator. However influence of glial MeCP2 on myelination is still unclear. The present study thus focuses on the ability of astrocytes to support myelination by employing a co-culture of Dorsal Root Ganglion neurons and oligodendrocytes, where these dissociated cells were plated on a monolayer of normal and MeCP2 siRNA transfected astrocytes, and myelination was followed over time using semi-quantitative PCR, Immunocytochemistry and Western Blot. Our preliminary results indicate astrocytic MeCP2 is involved in CNS myelination by altering the transcript levels of myelin proteins [Myelin Basic Protein (MBP) and Proteolipid Protein (PLP)]. The results from the current study provide a significant understanding of role of astrocytes and astrocytic MeCP2 in CNS myelination.

WTH07-03

Role of bone morphogenic protein signalling in oligodendrocyte differentiation and myelination

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Multiple sclerosis (MS) is a demyelinating disease of the Central Nervous System (CNS) wherein the insulating myelin sheath of the neuron is degraded, severely affecting neuronal signal transmission. Myelin is generated by oligodendrocytes in the CNS, which are derived from oligodendrocyte progenitor cells (OPCs) present in the brain throughout life. In MS lesions, OPCs fail to differentiate, and myelin repair fails. Bone Morphogenic Proteins (BMPs) are factors present in MS lesions that inhibit oligodendrocyte differentiation and remyelination.

Here, we investigated whether modulating BMP signaling in OPCs can increase oligodendrocyte differentiation and enhance myelin repair. We initially adopted a pharmacological approach, to assess whether blocking BMP signaling effects OPC differentiation *in vitro*. BMP4-treated OPC monocultures showed near complete astrocyte differentiation. This effect was significantly reduced using LDN-193189 (LDN), a potent inhibitor of BMP signaling ($p < 0.0001$, $n = 4$), resulting in a significant increase in differentiation into mature oligodendrocytes ($p < 0.0001$, $n = 4$). Thus, LDN potently blocks BMP signaling to promote oligodendrocyte differentiation. We next assessed whether LDN affects oligodendrocyte myelination, utilizing the *in vitro* myelination assay. LDN+BMP4 treatment significantly increased ($p < 0.0001$, $n = 3$) the number of myelinated axonal segments compared to BMP4-treatment alone, indicating a positive effect of LDN on myelination.

To assess whether this was a direct effect mediated via OPCs, we cultured OPCs derived from BMP receptor Ia (BMPRIa) inducible conditional knockout mice. No change in OPC proliferation was observed; however, differentiated BMPRIa-KO OPCs displayed an altered morphology compared to controls. Finally, utilizing *in vitro* myelination assays, we found that BMPRIa deficient OPCs exhibited a significant increase ($p < 0.05$, $n = 3$) in the number of myelinated axonal segments compared to control OPCs.

These results suggest that reducing BMP signaling is a potential strategy to enhance oligodendrocyte differentiation and promote myelin repair in demyelinated lesions. We are planning to further test the effect of both transgenic and pharmacological disruption of BMP receptors on myelination *in vitro* and *in vivo*.

WTH07-04

Hfe genotype and a formulated diet controlling for iron deficiency attenuate cerebral malaria in mice

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Plasmodium falciparum infects about 500 million individuals each year. A small but significant number of infections lead to complications such as cerebral malaria (CM). CM is associated with myelin damage and neurological deficits in survivors, and iron status is thought to impact the outcome of infection. We evaluated whether *Plasmodium berghei* ANKA-induced CM was altered by dietary iron deficiency (ID) or genetic iron overload (H67D HFE). We found that H67D mice had increased survival over H67H (wildtype) mice. Moreover, a customized diet increased survival regardless of whether the diet was iron deficient or iron adequate. To determine potential mechanisms underlying demyelination in CM, we measured Semaphorin4A (Sema4A) levels in the brain because we previously reported it to be cytotoxic to oligodendrocytes. Sema4A was increased in wildtype mice that developed CM while consuming standard rodent chow, consistent with a decrease in myelin basic protein (MBP); an indicator of myelin integrity. The brains of ID and H67D mice had lower levels of Sema4A. In the ID brains, MBP was decreased, but this was likely due to the ID diet. We also examined erythropoietin (Epo), which is under consideration for treatment of CM, and IL-6, which is known to increase during infection. We found that plasma Epo was elevated and IL-6 was low in H67D mice and in the recipients of the customized diets. These data reveal a paradigm-shifting concept that elevated iron reserves may not increase the mortality associated with malaria as long as there is a strategy to limit inflammation which should include decreased Sema4A in brain, elevated Epo, and lower IL-6 in plasma.

WTH07-05

Polymorphism in IL7-Ra gene in asoociation with mutiple sclerosis in Slovak population

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Interleukin-7 receptor-alpha (IL7-Ra) is involved in homeostasis of autoreactive T-cells in multiple sclerosis (MS). Several studies confirmed an association of the gene polymorphism rs6897932 C/T in *IL-7Ra* gene with MS susceptibility. In our study, we tried to identify possible association of this polymorphism with the risk of MS and the rate of disease disability progression in Slovaks. Gene polymorphism rs6897932 was detected in 219 clinically diagnosed MS patients and 218 healthy control subjects. We ascertained an Multiple Sclerosis Severity Score (MSSS) of each patient. According to the MSSS score we chose 53 patients with rapidly progressing and 57 patients with slow progressing disability progression. DNA samples were extracted from peripheral white blood cells and genotyped by PCR and restriction analysis. We found significantly higher frequency of ancestral allele C in MS patients compared to controls ($p = 0.033$). We did not find any significant differences in genotype or allele frequencies of SNP rs6897932 between the subgroups of slow progressing and rapidly progressing MS patients. Allele C of rs6897932 seems to increase the risk of MS development in Slovak population. We did not confirm any association of this gene polymorphism with the rate of MS disability progression.This work was supported by the grants MZ SR No 2012/30-UKMA-7 *Biological and molecular markers of MS and* MZ SR No 2012/31-UKMA-8.

WTH07-06

Central nervous system immune cell profiling in a model of chronic experimental autoimmune encephalomyelitis

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Multiple Sclerosis (MS) is a demyelinating disease of the central nervous system (CNS) believed to result from the infiltration of pro-inflammatory myelin-specific T cell subtypes. Experimental autoimmune encephalomyelitis (EAE) is an animal model of MS, and serves as a useful platform for studying the influence of specific T cell subsets in disease pathogenesis. This study aimed to characterise the infiltration of pro-inflammatory type 1 T helper cells (Th1; CD4+IFN-g+), type 17 T helper cells (Th17; CD4+IL-17+) and immunosuppressive regulatory T cells (Treg; CD4+CD25+FoxP3+) into the CNS at the clinical peak (day 16) and chronic phases (day 32) of EAE. EAE was induced in C57BL/6 mice by immunisation with a MOG_{35-55}/CFA emulsion and the infiltration of T cell subsets into the brain and spinal cord was measured using flow cytometry. Results showed that the EAE clinical peak corresponded with significantly increased Th1 and Th17 cell infiltration into the brain compared to the chronic phase. Conversely, Treg cell infiltration was significantly increased in the chronic phase compared to the clinical peak. Next, we tested the effects of conditional Treg cell depletion at different time points in a model of mild EAE using application of diphtheria toxin in transgenic DEREG (DEpletion of REGulatory T cells) mice. We confirmed the efficient and selective ablation of Treg cells in DEREG mice. We found that Treg cell depletion prior to immunisation didn't affect the development of EAE. However, post-immunisation Treg cell depletion at days 8 and 9; 18 and 19; 28 and 29; or 33 and 34 post-immunisation resulted in induction of clinically severe EAE. Overall, these findings indicate a central pathogenic role for Th1 and Th17 cells in the pathogenesis of EAE, and a time-dependant suppressive influence of Treg cells in EAE. Thus, the *in vivo* role of Treg cells in EAE may form an invaluable basis for the development of novel therapeutics for the treatment of MS in humans.

WTH07-07

Modeling and treatment of the novel white-matter disorder HBSL

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It is becoming increasingly apparent that mutations in genes encoding both cytoplasmic and mitochondrial tRNA synthetases (ARSs) can cause a wide range of neurological and multisystem disorders.

The recently diagnosed leukodystrophy HBSL (Hypomyelination with brain stem and spinal cord involvement and leg spasticity)

groups amongst these disorders and is caused by compound-heterozygous and homozygous mutations of the cytoplasmic aspartyl-tRNA synthetase gene *DARS*, leading to changes of highly conserved amino acids located within or adjacent to the active site of the enzyme. Clinically, all subjects develop nystagmus in the first year of life and have severe leg spasticity and mild cerebellar dysfunction. So far the underlying disease mechanisms are poorly understood and there is no efficient treatment available.

In this study we established *Dars*-null mice as a model for HBSL and subjected them to specialized behavioral tests as well as molecular, biochemical and histological analyses. The *Dars*-KO was created using the pU-17 gene trap vector containing a splicing acceptor with three stop codons in frame with a β-galactosidase/neomycin-resistance fusion gene. The gene trap cassette integrated into intron 4 of the *Dars* locus resulting in a disruption of the DARS expression but allows for reporter gene expression under the endogenous *Dars* promoter. These mice will help to elucidate the underlying pathomechanisms of the disease and will be instrumental to test new therapeutic strategies. In order to develop a treatment for HBSL, we created and tested adeno-associated virus (AAV) vectors for the *in vivo* delivery of the human *DARS* coding region to the brain of *DARS*-KO mice. Our results suggest that HBSL is a target for gene therapy.

WTH07-08

A novel small molecule neurotrophin-based strategy for treating demyelinating diseases

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Developing novel neurotrophin-based therapeutic strategies could promote repair in a number of neurodegenerative diseases. We have generated cyclo-dPAKKR, a small peptide that structurally mimics the region of BDNF that binds $p75^{NTR}$, and shown that it selectively targets $p75^{NTR}$ to uniformly promote peripheral myelination during development. We aim to investigate whether cyclo-dPAKKR can reduce myelin loss and promote myelin repair in experimental autoimmune neuritis (EAN), in two independent rodent models of peripheral demyelinating neuropathy. In rats, cyclo-dPAKKR administration significantly reduced the clinical disease severity in EAN, significantly abrogated the reduction in myelin protein expression, and significantly reduced the loss of myelinated axons in EAN-induced mice compared to vehicle controls. Furthermore, expression of APP+ (a marker for acute axonal damage) was also reduced in the peripheral nerves of cyclo-dPAKKR-treated animals, suggesting that cyclo-dPAKKR not only reduces the extent of demyelination but also inhibits axonal damage in EAN. In addition, rat EAN animals that were continuously administrated cyclo-dPAKKR after disease peak exhibited significantly faster recovery compared to controls, suggesting that cyclo-dPAKKR also promotes repair following EAN. These findings were further confirmed using a murine model of EAN, in which cyclo-dPAKKR significantly reduced the EAN disease severity in mice compared with mice administered a vehicle control. To determine whether cyclo-dPAKKR exerts these neuroprotective effects via selectively targeting $p75^{NTR}$, EAN was induced in $p75^{NTR}$ heterozygote (p75 + /−) and wild type (p75 + /+) mice. Interestingly, cyclo-dPAKKR failed to ameliorate disease severity in p75 + /− mice, compared to WT littermates controls. Our preliminary results suggest that cyclo-dPAKKR ameliorate EAN *in vivo* via $p75^{NTR}$. Collectively, our data from both rat and mouse EAN models argues that cyclo-dPAKKR reduces the extent of demyelination against EAN in a $p75^{NTR}$ dependant manner, and that cyclo-dPAKKR ameliorates peripheral demyelinating neuropathy by protecting against demyelination and by inhibiting axonal damage.

WTH07-09

Oligodendrocyte generation in the normal and injured forebrain of adult zebrafish

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Oligodendrocytes are glial cells which myelinate axons in the central nervous system. Olig2 is the basic helix-loop-helix (bHLH) transcription factor and is required for the generation of both motor neurons and oligodendrocyte progenitor cells (OPCs) in the olig2-expressing pMN progenitor domain of ventral spinal cord. Previously we have shown that *olig2*:$EGFP^+$ precursors adopt radial glial morphologies and proliferate continuously during post-embryonic stages in the spinal cord of the Tg(olig2:EGFP) zebrafish. The soma of these cells lies near the central canal and they extend radial processes to the pial surface. Immunostaining with antibody specific to an atypical protein kinase C (aPKC) showed that *olig2*:$EGFP^+$ radial glia has apical-basal polarity, suggesting that *olig2*:$EGFP^+$ radial glial cells function as stem cells to give rise to multiple oligodendrocytes in post-embryonic zebrafish.

In the present study, we investigated oligodendrocyte formation in the adult forebrain of the Tg(olig2:EGFP) zebrafish. olig2:EGFP expresses continuously in the oligodendrocyte lineage cells in the adult CNS, and *olig2*-expressing radial glial precursor cells exist in the forebrain of adult zebrafish. We next hypothesized that *olig2*-expressing radial glia are responsible for the regeneration of oligodendrocytes in the injured forebrain. However, we found that oligodendrocytes are actively generated from the proliferating OPCs instead of *olig2*-expressing radial glia, indicating that pre-existing OPCs are main source for the generation of oligodendrocytes in the injured forebrain. We next examined the role of Notch signaling and found that the number of proliferating OPCs was dramatically reduced in the presence of active Notch signaling, suggesting the possibility that Notch signaling has a inhibitory function for the proliferation of OPCs or promotes differentiation of OPCs into mature oligodendrocytes in the injured forebrain of adult zebrafish. In the previous report, F3/Notch signaling (Non-canonical pathway) is responsible for the differentiation of OPC into mature oligodendrocyte. So we found that F3 expression is slightly increased under injured situation. We are currently investigating Notch function in oligodendrocyte regeneration in the injured forebrain of adult zebrafish.

WTH07-10

The role of myelinating glia in zebrafish model of amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by progressive loss of motor neurons in the brain and spinal cord. Although ALS is a motor neuron disease accompanied by motor neuron death, non-neuronal cells such as astrocytes, microglia and oligodendrocytes have been shown to contribute to ALS pathogenesis. Myelin is a multilayer sheath, which is produced by oligodendrocytes in the CNS and Schwann cells in the PNS, for rapid axonal conduction and metabolic supports to axon. To understand the role of myelinating glia in the pathogenesis of ALS, we first developed zebrafish model of ALS by injecting mRNAs for ALS-related genes such as mutant Sod1 and TDP-43, into Tg(olig2:dsred2) zebrafish, where the spinal motor axons and NMJs were readily detectable by the expression of Dsred Fluorescent protein. By analyzing the myelination of zebrafish model of ALS, we found that ectopic expression of ALS-related genes trigger myelin defects accompanied by axonal disorganization in the CNS and PNS of zebrafish embryo. Interestingly, we also found that specific expression of ALS-related genes in oligodendrocytes and schwann cells caused myelin sheath abnormalities in cell-autonomous manner. Altogether, our data indicate that myelinating glial cells play a important role in the pathogenesis of ALS.

WTH07-11

Analysis of neuronal responses against disruption of neuro-glial interaction and its effect on brain functions

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Myelinated axons are composed of four distinct regions: the node of Ranvier, paranode, juxtaparanode and internode, characterized by the presence of specific component proteins. The major components of paranodal junction are neurofascin155, Caspr and contactin. Although myelin sheaths are thought to have crucial roles in cognition and motor function, the significance of paranodal junction in exerting higher brain function and in neurological diseases remains unclear.

In the previous study, PLP Cre-ERT recombinase-inducible ablation of neurofascin155 in oligodendrocytes led to disorganization of paranodal junction and reduction in the nerve conduction velocity. In the present study, we analyzed the myelinated optic nerve of the tamoxifen-injected mice by immunofluorescence for observing alterations in neurofascin155 and Caspr expression. We found that the number and length of these immunoreactive signals at the paranode were significantly decreased at 60 days after tamoxifen injection. To examine whether disruption of paranodal junction affects the neuronal gene expression, we prepared total RNA from the retina of oligodendrocyte-specific conditional neurofascin155 knockout mouse and wild-type mice, and proceeded to microarray analysis. Interestingly, we found that expression level of many neuronal genes dramatically changed in response to the ablation of the paranodal junction, and some of the identified gene expression can be primarily observed in neurons by *in situ* hybridization.

These results suggest that neurons are sensing an early change in the myelin-axon interaction during demyelination, such as paranodal opening.

WTH07-12

The consequence of dysfunctional myelin on neural processing

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In the current study, we have generated mutant mice that lack Claudin 11 (Cldn11) tight junctions in CNS myelin sheaths. In myelin sheaths, Cldn11 forms tight junctions located along the outer and inner edges of the membrane spiral, preventing ions and small molecules from entering the intramyelinic space. The function of Cldn11 tight junctions is to improve the passive properties of the myelin membrane, increasing membrane resistance and reducing capacitance, thereby improving the speed of saltatory conduction. In the absence of Cldn11, conduction velocity is slowed, most dramatically in small diameter myelinated fibers, analogous to reducing myelin thickness. Notably, the absence of Cldn11 is without degenerative myelin pathology, enabling direct study on the impact of dysfunctional myelin on neural processing.

Undoubtedly, slowed conduction velocity along myelinated axons increases temporal dispersion and, consequently, degrades information transfer between neural circuits to the detriment of the integration of sensory information and overall neural processing. Herein, we explore the impact of dysfunctional myelin on neural processing in an integration circuit of the auditory brainstem between the cochlear nucleus and the superior olivary complex (SOC). This circuit serves as a model system because its integration properties are hardwired during development and well characterized. Using psychoacoustics, we have demonstrated that *Cldn11*$^{-/-}$ mice have altered neural processing, suggesting an inability to lateralize sound sources on the azimuth plane. Now, we are functionally characterizing this behavioral deficit using auditory operant chambers. Further investigation into neural circuitry of the SOC reveals increased steady state levels of the neurotransmitters, glutamate and glutamine, which may exacerbate neural processing deficits or may be an attempt by neurons in the SOC to compensate for increased temporal dispersion. In either case, our data are significant in two ways. First, they represent the first demonstration that non-degenerative changes in myelin membrane passive properties can lead to neurochemistry changes that perturb behavior/perception. Second, they have important implications for the etiology of behavioral disorders in general, and more specifically for the behavioral components of hypomyelinating and demyelinating diseases like multiple sclerosis.

WTH07-13

Therapeutic use of microrna mimics to promote remyelination in viral encephalomyelitis

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MicroRNAs (miRs) are small (~22 nt) non-coding RNA molecules controlling gene expression by posttranscriptional regulation. miRs regulate the proliferation, survival and differentiation of oligodendrocytes (OLs). miRs also have functional roles in the pathogenesis of demyelinating diseases such as Multiple Sclerosis (MS). Therefore, there is an intrinsic potential for the therapeutic use of miR-related biology, especially considering that the regenerative capacity of the CNS (remyelination) in demyelinating diseases is generally impaired. The goal of this study is to examine the potential use of artificial miRs to promote remyelination by stimulating the number of functional OL precursor cells.

We used an experimental model of demyelination, Theiler's Murine Encephalomyelitis Virus (TMEV), in susceptible SJL mice (*in vivo*) and its susceptible neuroglial progenitor cultures (*in vitro*). We found that miR-17, -19b, and -138 (involved in oligodendrogenesis) were decreased in TMEV-infected cells and TMEV-infected mice. These results were correlated with an increase in caspase-1 mediated death of OL progenitor cells. To test our hypothesis that complementation with miR mimics protects OLs, miR-17 and miR-19a were transfected in TMEV-infected cells. Our results showed that complementation of glial cultures with miR-17 exerted a protective effect on OL progenitor cells.

Our results showed that levels of OL-related miRs were significantly decreased during demyelination and these molecules have the potential to exert therapeutic effects by *in vivo* complementation. This study has an intrinsic clinical value for improving therapies in the treatment of myelin diseases such as MS and highlights the importance of miRs biology in myelinating cells.

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WTH07-14

IPS-derived neural precursors outcompete endogenous cells for cns remyelination

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Induced pluripotent stem cell-derived neural precursors may represent the ideal autologous cell source for cell-based therapy to promote remyelination and neuroprotection in myelin diseases. However, the repair efficacy and safety of these cells in demyelinating conditions remain to be well addressed. We fully characterized mouse iPS-NPCs (miPS-NPCs) *in vitro* and *in vivo* and compared side-by-side their repair efficiency to embryonic mouse NPCs (mE-NPCs) which are physiologically committed inside the brain. We used the $Sox2^{\beta geo/\beta geo}$ knock-in mice strategy to safely select iPS-NPCs. We demonstrate that miPS-NPCs expressed immature markers of naturally committed NPCs *in vitro* at the protein and transcriptional levels. Single injections of miPS-NPCs in two models of spinal cord induced demyelination revealed their prominent capacity of survival, migration and timely differentiation into mature oligodendrocytes. Grafted miPS-NPCs generated extensive and compact myelin around host axons, restoring nodes of Ranvier and conduction velocity. miPS-NPCs were safe as tumor following engraftment was never observed. Thus miPS-NPCs differentiated successfully into bona fide remyelinating oligodendrocytes outcompeting endogenous cells for myelin repair and behaved as efficiently as naturally committed NPCs. These novel insights into the biology of iPS-derived NPCs should help establishing the pertinence of their use for regenerative biomedicine of CNS myelin diseases.

WTH07-15

Nogo receptor 1 regulates axo-glial units in the central nervous system

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The axo-glial unit is a highly organized microstructure that establishes myelination and facilitates saltatory conduction of action potentials. This unit is fundamentally disrupted during inflammatory demyelination as exhibited in multiple sclerosis (MS). In previous studies, mice lacking the Nogo receptor 1 (NgR1) allele showed diminished axonal pathology following the induction of the mouse model of MS, experimental autoimmune encephalomyelitis (EAE). Although the reduction in Nogo-A-NgR1 signal transduction may be central for neuroprotection, possible micro-structural differences in the central nervous system (CNS) architecture of $ngr1^{-/-}$ mice may also contribute to the resistance of axonal damage observed following inflammatory challenge. In the current study, we examined the microstructure of axo-glial junctions in the CNS of $ngr1^{-/-}$ and $ngr1^{+/+}$ littermates in the context of established myelination. Electron microscopic analysis revealed that the internode, paranode, and node of Ranvier are significantly longer in $ngr1^{-/-}$ mice compared to $ngr1^{+/+}$ littermates in both the optic nerves and spinal cords. Furthermore, axonal caliber and thickness of the myelin sheath were thinner in $ngr1^{-/-}$ mice. We have identified elongated Caspr-positive paranodes in $ngr1^{-/-}$ mice compared with $ngr1^{+/+}$ spinal cords. Electrophysiological recordings from naïve $ngr1^{-/-}$ mice exhibited delayed and reduced compound action potentials in the optic nerves spinal cord white matter segments. Immunostaining in EAE-induced $ngr1^{+/+}$ mice spinal cord also exhibited elongated Caspr-positive paranodes near inflammatory lesions when compared to non-lesion areas that were analogous to $ngr1^{-/-}$ mice. Our results suggest that NgR1 may partially regulate axo-glial unit formation and that this altered molecular and ultrastructural axo-glial unit arrangement exhibited in the CNS of $ngr1^{-/-}$ mice may establish a survival advantage for axons and myelin of this genetic mutant during neuroinflammatory insults. This biologically facilitated axonal protection may therefore be a result of the altered molecular

arrangements and/or energy demands present at the glial-axonal interface in the *ngr1*$^{-/-}$ mice.

WTH07-16

The role of the transient receptor potential Ankyrin 1 (TRPA1) receptors in the cuprizone-induced demyelinization model

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Multiple sclerosis is a chronic inflammation with demyelinization and neurodegeration in the CNS. Lately, the neuro-immune crosstalk between the neurons and glial system has come to the centre of the interest. Transient Receptor Potential Ankyrin 1 (TRPA1) receptor expressing cells are localized in brain. The copper-chelator cuprizone induces glial degeneration with subsequent demyelinization. It acts mainly in the corpus callosum and triggers the destruction of the oligodendrocytes with microglial invasion and astrocyte reaction. Our aim was to investigate the role of TRPA1 receptors in the cuprizone-induced model. Demyelinization was induced in TRPA1 receptor KO and WT mice. Animals got 0.2% cuprizone containing diet for 6 weeks. Their behaviour was investigated by open field test, mechanical hyperalgesia was measured by dynamic plantar aesthesiometry. Demyelinization was shown by Luxol Fast Blue and myelin basic protein immunohistochemistry. Expression of TRPA1 receptors was visualized by antibodies. Glial markers were also used. Spontaneous activity was changed during the cuprizone-treatment. Animals became more excited with increased locomotor activity. Mechanical hyperalgesia did not differ in the cuprizone-treated groups compared to the controls. Significant differences were not shown between the WT and TRPA1 KO mice. Cuprizone elicited only moderate demyelinization in the corpus callosum of KO but induced more severe degeneration in the WT animals. The treatment strongly decreased the number of oligodendrocytes in both groups, but more mature cells were identified in the TRPA1 KO mice. Infiltration of astrocytes and microglial cells were also decreased in the KO mice. It is concluded that genetic lack of TRPA1 receptors is protective in the cuprizone-induced demyelinization model. Supported by the Hungarian Brain Research Program KTIA_NAP_13-1-2013-0001.

WTH07-17

Neuroanatomical and neurodevelopmental white matter differences between rat strains with differing vulnerability to epileptogenes

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The neurobiological factors that predispose to developing epilepsy (epileptogenesis) following a brain insult are poorly understood. FAST rat strain has been selectively bred to have enhanced vulnerability to acquired epileptogenesis, while SLOW rat strain has been bred to be resistant. FAST rats also exhibit behavioral traits that are reminiscent of those observed in developmental disorders comorbid with epilepsy. We hypothesized that white matter structure and development may differ between these strains.

T2-weighted MRI images were acquired in 6 month old male FAST and SLOW rats and volumes of different brain regions were measured. The rate of myelination during development was examined by mRNA expression profile of myelin proteins at postnatal day (PND) 5, 11, 17 and 23. Neurodevelopment was assessed by locomotor activity, reflex response and eye opening.

MRI analysis revealed significant structural differences between FAST and SLOW brains, including enlarged cerebrum volume (SLOW 1344 ± 19.41 mm^3, $n = 8$; FAST 1411 ± 17.04 mm^3, $n = 12$; $p < 0.05$) and reduced hippocampus (SLOW 7.411 ± 0.047 mm^3, $n = 8$; FAST 7.068 ± 0.115 mm^3, $n = 12$; $p < 0.05$) and anterior cerebellar vermis (SLOW 34.95 ± 1.078 mm^3, $n = 9$; FAST 31.52 ± 0.815 mm^3, $n = 14$; $p < 0.05$) in FAST rats. Compared to SLOW rats ($n = 8$), FAST rats ($n = 8$) had significantly reduced mRNA expression of myelin proteins at PND 5 and 11 in brainstem ($p < 0.05$), cerebellum ($p < 0.05$) and cerebral hemisphere ($p < 0.01$) indicating slower rate of myelination. Behavioural developmental delay was observed in FAST rats ($n = 11$) with only 9% of pups compared to 81% of SLOW rats ($n = 11$) having opened their eyes by PND 13, with less locomotor activity between PND 12–16 ($p < 0.05$) and delayed reflex response between PND 5–10 ($p < 0.01$).

The FAST rat strain has enlarged white matter volumes and a slower rate of myelination relative to the resistant SLOW rat strain. These changes may be related to heightened seizure susceptibility and behavioral features that these rats manifest.

WTH07-18

Signaling pathways controlling cns myelin compaction in gain of function rasopathies

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In human Rasopathies, activating mutations in Ras-MAPK pathway genes result in compromised brain function. Loss of *Nf1*, encoding the negative regulator of Ras signaling neurofibromin, or hyper activation of HRas GTPase in murine models causes myelin decompaction. Here we found that myelin decompaction in the corpus callosum occurs within 1 month of selective *Nf1* loss or *HRasG12V* mutation in adult oligodendrocytes. *Nf1* and *HRas* mutant precursors expressed high levels of the *Hes5* gene, an effector of canonical Notch signaling. Hyper-activation of Notch in oligodendrocytes partially recapitulated the decompaction phenotype, while loss of aberrant canonical Notch signaling, through loss of the RBPJ DNA binding protein or treatment with gamma secretase inhibitor, partially rescued decompaction in *Nf1* and *HRas* mutant mice. Flow cytometry analysis revealed that *Nf1* and *HRas* mutant oligodendrocytes have elevated levels of nitric oxide (NO). Inhibition of nitric oxide synthase, NO production, with L-NAME partially rescued decompaction in *Nf1* and *HRas* mutants. Moreover, simultaneous inhibition of nitric oxide synthases with L-NAME and canonical Notch signaling with loss of *Rbpj* completely rescued

decompaction in *Nf1* and *HRas* mutants, indicating that both pathways contribute to the myelin decompaction. We conclude that canonical Notch signaling down stream of hyperactive Ras GTPase contributes to myelin decompaction. NO and Notch pathway activation may additively contribute to brain pathology and we identify these pathways as potential therapeutic targets to ameliorate myelin abnormalities in NF1 and Costello Syndrome patients.
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WTH07-19

Investigating the role of amyloid precursor protein in axonal myelination and remyelination using the cuprizone model

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Myelination is the process of enwrapping myelin around axons with the resulting myelin sheaths acting as insulators that ensure proper and faster transmission of electrical impulses along axons. The importance of myelination for neuronal function is illustrated by demyelinating diseases such as multiple sclerosis (MS). The amyloid precursor protein (APP) family are type I transmembrane glycoproteins that are expressed in the brain as well as in most other tissues. BACE1, a protease involved in the cleavage of APP into the amyloid beta peptide that implicated in Alzheimer's disease, is important in regulating axonal myelination both in the central and peripheral nervous system. Since APP is a substrate for BACE1, we hypothesised that APP could have a role in myelination. To test our hypothesis we analysed myelination in knockout (KO) mice for APP and its homologue, APLP2 in both the peripheral and central nervous system. Electron micrographs showed that myelination is altered in the peripheral and central nervous system in APP-KO and APLP2-KO, both young and adult mice. We then tested the role of APP and APLP2 on axonal demyelination and remyelination using the cuprizone model. We found that APP is important for axon myelination while APLP2 regulates the extent of myelination. APP altered the extent of demyelination and impaired the process of remyelination. Moreover, APP deficiency reduced the extent of demyelination and caused a delay in myelin repair, indicating that APP is necessary for normal remyelination to occur. This study identifies novel roles for both APP and APLP2 in myelination and shows they regulate axonal myelination and remyelination in both the central and peripheral nervous systems. This data is pertinent given the Alzheimer drugs being developed that target APP metabolism, and their effects on myelination should be considered.

WTH07-20

Targeting oligodendrocytes in rAAV mediated gene replacement therapy for Canavan disease

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Loss of function mutations in the aspartoacylase gene (*Aspa*) cause Canavan Disease (CD). This devastating childhood leukodystrophy is characterized by hypomyelination and spongiform degeneration particular of the white matter in the central nervous system. ASPA is a somatic enzyme restricted to oligodendrocytes. Enrichment of *N*-acetyl aspartate (NAA) caused by the lack of its degrading enzyme ASPA in oligodendrocytes is a diagnostic biomarker for CD. As the monogenic cause and the progressive severe pathology are well understood the current absence of a treatment options make CD a *bona fide* target for a gene replacement therapy. Recombinant adeno-associated viral (rAAV) vectors are safe and efficient tools for somatic gene transfer to the brain. We have recently shown that myelin basic protein (*Mbp*) promoter selectively targets rAAV-mediated GFP reporter gene expression to oligodendrocytes in juvenile rodents. Here we have exploited this approach in a murine CD model by direct CNS delivery of rAAVcy5-*Mbp-Aspa* to symptomatic *Aspa*-null mice. Robust oligodendroglial specificity and vector spread were confirmed 8 weeks after therapeutic vector infusion. Striking improvement of motor-deficits depending on the test even to wild-type levels was observed in ASPA-deficient animals that received rAAVcy5-*Mbp-Aspa* compared with rAAVcy5-*Mbp-Gfp* controls. Key histopathological and metabolic hallmarks of CD including vacuolisation, ventricle size, astrocytosis, and NAA accumulation, were significantly ameliorated in the treated cohort. Taken together our data underscores the therapeutic potential of rAAV mediated gene therapies for leukodystrophies or, more broadly, for conditions that could be ameliorated by expression of nucleic acids in oligodendrocytes.

WTH07-21

Highly efficient conditional ablation of oligodendrocyte progenitor cells (NG2 GLIA) in mice

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Remyelination is primarily achieved by generating new myelin-forming oligodendrocytes, which can be derived from both parenchymal oligodendrocyte progenitor cells (OPCs/NG2 glia) and neural precursor cells (NPCs) that reside in the adult subventricular zone (SVZ). Our recent studies have demonstrated that NPCs are major contributors to oligodendrogenesis and remyelination following cuprizone-induced demyelination, but on a regionally restricted basis. We demonstrated that NPC-derived oligodendrocytes predominantly remyelinate regions of the demyelinated corpus callosum (CC) that are adjacent to the SVZ whereas OPC-derived oligodendrocytes occupy regions of the CC that are distal to the SVZ. To explore whether the regionally restricted migration of NPCs reflects spatial competition with OPCs for oligodendrogenesis and remyelination, an approach to conditionally ablate OPCs while sparing the NPC population is required. To this end, we have established a methodology to conditionally ablate OPCs by comprising both genetic and pharmacological techniques. Here we show that, by crossing *Pdgfra-CreER^{T2}* mice with the conditional oligodendroglial-specific ablation line *Sox10-lox-GFP-STOP-lox-DTA,* tamoxifen-mediated Cre recombination resulted in the deletion of GFP-STOP cassette in recombined PDGFRa^{+} OPCs and the expression of a suicide gene (diphtheria toxin fragment A, DTA) rendering OPCs specifically sensitive to DTA-mediated apoptosis.

Subsequent intracisternal infusion of the antimitotic drug cytosine-beta-D-arabinofuranoside (AraC), resulted in efficient ablation of the entire OPC population without OPC regeneration. Importantly, AraC treatment resulted in only transient ablation of NPCs, which were replenished to control levels within 10 days post AraC infusion due to the activation of quiescent neural stem cells, which repopulated the SVZ with newly generated NPCs. In the future, this protocol of highly efficient selective OPC ablation could be adopted to establish the influence of OPC ablation upon the regenerative capacity and function of NPC-derived oligodendrocytes in demyelinating disease. Additionally, the model provides a long sought after methodology to investigate the function of OPCs/NG2 glia in the normal healthy brain.

WTH07-22

Adhesion properties mediated by PKC-dependent phosphorylation are different between myelin P0 and its readthrough isoform L-MPZ

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Large myelin protein zero (L-MPZ) is a novel isoform of PNS myelin protein zero (MPZ/P0) that contains an additional domain at the cytoplasmic C terminus. L-MPZ is synthesized from the P0 mRNA by translational readthrough of the regular stop codon. Homophilic interaction of P0 between the extracellular Ig domain leads to tight adhesion between each layers in PNS myelin. This adhesion is affected by the protein kinase C (PKC) –dependent phosphorylation site in the cytoplasmic region of P0. L-MPZ has the same PKC–dependent phosphorylation site as well as an additional putative PKC phosphorylation site in the extra L-MPZ specific domain. Since L-MPZ is localized in the PNS compact myelin and cell-cell adhesion sites in the L-MPZ transfected cells, L-MPZ may be potentially involved in cell adhesion and myelination. However, adhesion activity of L-MPZ and role of PKC-mediated phosphorylation are still unknown. To elucidate PKC-mediated phosphorylation of L-MPZ, we performed Western blot analysis of rat sciatic nerve homogenate using phospho-(Ser) PKC substrate antibody. PKC- phosphorylation of L-MPZ was detected in the unique two-dimensional electrophoresis system using cationic detergent. The increase of phosphorylated L-MPZ was observed during early postnatal development of sciatic nerve. Additionally, two states of phosphorylation in L-MPZ were demonstrated by Western blotting using Phos-tag. Next, to clarify adhesion activity of L-MPZ, we performed the adhesion assay using HeLa cells which semipermanently expressed P0, L-MPZ, or phosphorylation site mutants. L-MPZ exhibited a cell adhesion activity which was clearly weaker than P0. This binding activity was affected by mutation of phosphorylation sites. Further, heterophilic binding of L-MPZ to P0 was demonstrated by fluorescence-labeled cells. These results suggest that content of L-MPZ in P0-rich myelin membrane may be related to flexibility of myelin structure which affects myelin function.

WTH07-23

GlcNAc6ST-1 regulates sulfation of N-glycans and myelination in the peripheral nervous system

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Myelin is a multilamellar, tightly compacted membrane that surrounds axons in the peripheral nervous system (PNS) and central nervous system (CNS). Because glycoproteins are prominent components of plasma membranes, a growing number of glycoproteins have been identified and characterized in myelin. In this study, we found that PNS myelin had many anionic N-glycans, especially sulfated N-glycans, harbored on glycoproteins in pigs and mice at a much higher rate than CNS myelin. Major sulfated N-glycans in porcine and mouse PNS myelin were identified. The sulfation at the 6-O-GlcNAc position on glycoproteins was highly conserved in PNS myelin between these species. P0 protein, the most abundant glycoprotein involved in PNS myelin compaction, had 6-O-sulfated N-glycans abundantly. Mice deficient in N-Acetylglucosamine 6-O-Sulfotransferase-1 (GlcNAc6ST-1) were impaired in the elaboration of 6-O-sulfated N-glycans in PNS myelin. Further, GlcNAc6ST-1 deficiency in mice caused hypomyelination and axonal degeneration. Taken together, these results indicate that GlcNAc6ST-1 plays critical roles in PNS myelination through the elaboration of 6-O-sulfated N-glycans.

WTH08 Ischemia and Oxidative Stress

WTH08-01

Effect of mild traumatic brain injury on brain functional outcome in rats

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Background: Traumatic Brain Injury (TBI) remains one of the leading causes of death and disability, worldwide. Mild TBI may lead to neurocognitive sequelae, including memory impairment and motor impairment. It is evident that mitochondrial dysfunction and oxidative stress have a contributory role in several neurological disorders; however, their association with TBI is unclear.

Aim: To determine the acute effects of mild TBI on motor, cognitive and brain mitochondrial functional changes in rats.

Methods: Mild TBI was induced in female Sprague Dawley (SD) rats using a New York University Impactor. Novel Object Recognition and Error Ladder tests were performed before and 24 h post-injury. Mitochondria Manganese Superoxide Dismutase (MnSOD), Translocase of Outer Mitochondrial Membranes 20 (TOM20), and the Oxidative Phosphorylation (OXPHOS) complexes I-V (CI-CV) from the lesion site were measured by western blot.

Results: Sham rats spent similar times exploring the two identical objects, but spent longer with the novel object when it was introduced; while TBI rats devoted similar time with the new object and the old one. TBI rats also made more errors while walking on the ladder, compared with the Sham rats. MnSOD, OXPHOS CI and CIII protein levels were significantly lower in the TBI group compared with the Sham group, whereas TOM20 was not different between the groups.

Conclusion: Mild TBI caused immediate cognitive and motor functional deficits in the rats. Reduced antioxidative capacity and possibly compromised mitochondrial function may affect the long-term functional recovery.

WTH08-02

Dynamic alterations in VEGF receptor 3 expression following hypoxic-ischaemic injury in neonatal rat brain

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Vascular endothelial growth factor receptor 3 (VEGFR3) is expressed in developing rat forebrain and has been implicated in adult subventricular zone (SVZ) neurogenesis. It is upregulated in SVZ and peri-infarct zones in adult rodent forebrain after hypoxic ischaemic (HI) injury. We recently reported that VEGFR3 protein is expressed by choroid plexus epithelium, pigmented retinal epithelium, retinal ganglion cells, neural progenitors, neurones and astrocytes in developing rat brain. Our objective in this study was to examine changes in VEGFR3 expression in the neonatal rat CNS after HI injury. Post-natal day (P) 7 Sprague-Dawley rat pups underwent right common carotid artery occlusion, followed by exposure to 8% oxygen for 3 h, with sham-operated and no treatment controls. Euthanasia was performed on P10, P14 and P21, pups were perfused, brain tissue harvested and post-fixed. Brains were paraffin-embedded and 4 μm sections collected. Double-labelling immunohistochemistry was performed using an antibody for VEGFR3 combined with antibodies to nestin, GFAP, BT3, NeuN and aquaporin 1, and images obtained using fluorescent digital microscopy. Following HI injury, we found increased VEGFR3 expression in the SVZ, midline cortex and dentate gyrus of the hippocampus on neural progenitors, which peaked at P10, and increased astrocytic expression of VEGFR3 in the SVZ and peri-infarct zone. We also found disruption to the choroid plexus epithelium, retinal ganglion cell layer and pigmented retinal epithelium following HI at P14, with some recovery in evidence by P21. VEGFR3 expression was significantly diminished in these regions compared to controls. In conclusion, our findings support a role for VEGFR3 in promoting neurogenesis and gliosis following ischaemic insult in the neonatal brain. The loss of VEGFR3 expression after HI, in critical blood-CSF interfaces, may have importance for CNS fluid homeostasis. The expression of VEGFR3 in the retina and its distinct changes after HI, raise the possibility that augmentation of VEGFR3 signalling might provide therapeutic benefit for these vulnerable cell populations.

WTH08-03

Minocycline treatment increases peri-infarct astrocytic responses and functional recovery following photothrombotic stroke

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Astrocytes in peri-infarct tissue produce responses that can potentially promote local neuronal plasticity but also release proteoglycans and are a major contributor to the development of a glial scar that impairs plasticity and recovery. Minocycline can modify inflammatory responses associated with stroke. Long-term treatment with this drug has been found to improve functional recovery in rodent models of stroke but the mechanism underlying these effects is not understood. In this study, we investigated whether short-term treatment with minocycline treatment restricted to the initial 2 days after stroke can influence downstream astrocytic responses and improve neurological function. Photothrombotic stroke was induced in the region of the forelimb motor cortex in Sprague-Dawley rats. The rats were injected intraperitoneally with 90 mg/kg minocycline at 1 h after stroke induction and then 45 mg/kg minocycline at 12, 24, 36 and 48 h. Neurological function was evaluated by investigators blinded to the treatments. This treatment did not alter the lesion size assessed at 3 days but improved

neurological function at 7 days as assessed from a forelimb placing test. Unexpectedly, this improvement was associated with increased expression of the astrocytic cytoskeletal proteins, vimentin and GFAP, as assessed from immunoblots and immunohistochemistry but no change in the proteoglycan, neurocan. The increases in the astrocytic protein expression contrasted with the consequences of early treatment with the cell cycle inhibitor, olomoucine, which reduced the content of GFAP and vimentin. Delaying the minocycline treatment by 24 h resulted in no significant changes in vimentin or GFAP expression and no significant improvement in neurological function. These findings suggest that early modulation of inflammatory responses can improve functional recovery following stroke. However, in contrast to some other treatments improving outcome, this was associated with an increase rather than a decrease in peri-infarct astrocytic responses.

WTH08-04

Spatiotemporal delineation of a cerebral photothrombotic infarct in mouse using darkfield microscopy

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Ischemic brain injury from thrombotic strokes is characterized by a primary ischaemic core infarct and a lesion that subsequently extends from the region of hypoperfused tissue known as the penumbra. Previous studies have shown apoptotic-associated damage alters the appearance of tissue visualised under darkfield microscopy. Here we examined the utility of darkfield microscopic images as an efficient means of quantifying infarct volume following photothrombosis in mice. Photothrombosis in the cerebrum was achieved by tail vein injection of rose bengal dye (50 μg/g) followed by illumination with green laser light (532 nm, ~ 3 mW) for 6 min, in an isoflurane anaesthetised mouse (C129SvEv). Skull thinning was performed over the region of interest (2 mm^2) prior to irradiation. Mice were euthanized at 2 h, 1, 4, and 14 days post injury (DPI; $n = 3$). The tissue was fixed in paraformaldehyde (4%) and cryosectioned coronally. Integration of infarct areas from serial darkfield images enabled measurement of infarct volume. Result: The lesion was evident as an increase in tissue opacity at the site of irradiation. Relative to the lesion core at 2 h post injury (2.54 ± 0.73 mm^3), at 1DPI, the lesion volume doubled (6.64 ± 1.58 mm^3), and by 4DPI the lesion volume as ~ 6× baseline (17.83 ± 3.68 mm^3, $p = 0.005$ vs. 2 h post-injury and $p = 0.019$ vs. 1DPI, one way ANOVA). However, by 14DPI, dark field opacity in the sections was reduced, and the volume was equivalent to 2 h post injury; likely reflecting tissue remodelling. Conclusion: Darkfield microscopy is a useful tool for quantifying infarct volume arising from photothrombotic infarcts. Infarct volumes are spatiotemporally predictable, providing an opportunity to use the model to study neuroprotection. Further histological studies will be undertaken to determine progressive neuronal damage and glial influx into the infarct area.

WTH08-05

LIF haplodeficient mice are more vulnerable to injury and sustain greater sensorimotor deficits after perinatal brain injury

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When the nervous system sustains injury, the daily activities of glial cells are reprogramed by the actions of extracellular signals that are released in response to damage. Their responses are sophisticated and matched to the nature and severity of histopathology. Leukemia inhibitory factor (LIF) is rapidly induced in response to a spectrum of CNS and PNS injuries whereupon it stimulates receptors present on neurons, macroglia and microglia. Curiously, LIF has been shown to be either beneficial or detrimental for functional recovery depending upon the injury model and region affected. Here we compared the extent of neocortical and subcortical white matter damage sustained by LIF haplodeficient mice vs. wild type mice using a mouse model of birth asphyxia. Our studies reveal that astrogliosis but not microgliosis was diminished in the LIF haplodeficient mice acutely after injury (48 h). Moreover, degenerating astrocytes were evident in the affected striatum of LIF haplodeficient mice. Fluoro-Jade staining revealed increased numbers of degenerating neurons in the neocortex, striatum, thalamus and hippocampus of LIF haplodeficient mice. At 14 days of recovery after injury, LIF haplodeficient mice showed increased infarct volume and they performed poorly on a battery of motor, sensory, reflex and balance tests as compared to WT mice. Altogether, these data demonstrate that LIF is an essential timing signal for astrogliosis after perinatal brain injury, and that a 50% reduction in LIF expression is sufficient to cause a second wave of neurodegeneration and more severe neurological deficits. Supported by NIH R01 HD052064 and a grant from the Leducq Foundation awarded to SWL.

WTH08-06

Decreased orexin (HYPOCRETIN) in the hypothalamus/pons after hypoxic insults in a piglet model and sudden infant death syndrome

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Orexin is an important regulator of sleep and arousal. Under hypoxic conditions, orexin expression is decreased. Intermittent hypercapnic hypoxia (IHH) is experienced in infants who suffer from obstructive sleep apnea and who prone sleep (a risk factor of Sudden Infant Death Syndrome [SIDS]), both of which are associated with dysfunctional sleep and poor arousal thresholds. This study examined the immunoreactivity of orexin (OxA and OxB) neuropeptides in the hypothalamus and pons of a piglet model of postnatal IHH exposure and in infants that had died of SIDS. The percentage of orexin immunoreactive neurons and the total number of neurons were quantified in the dorsomedial, perifornical and lateral hypothalamus. In the pons, the density of orexin immunoreactive fibres were quantified in the locus coeruleus (LC), dorsal raphe (DR), laterodorsal tegmental (LDT), medial parabrachial

(MPB), dorsal tegmental (DTg) and pontine nuclei (Pn) using automated methods. OxA and OxB were co-expressed in all hypothalamic and pontine nuclei examined in both human and pigs. IHH exposed piglets demonstrated a 25% decrease in OxA and OxB immunoreactivity in the hypothalamus, no changes in total hypothalamic neuronal numbers and a 50% decrease in immunoreactivity in all pontine nuclei ($p \leq 0.01$). In SIDS infants, orexin immunoreactivity was decreased by 21% in the hypothalamus ($p \leq 0.050$) and no significant changes in total neuron numbers were observed. In the pons, a 40-50% decrease in OxA occurred in the all pontine nuclei, while a similar decrease in OxB immunoreactivity was observed in the LC, LDT, DTg and Pn ($p \leq 0.025$). These findings show that an IHH exposure in the early postnatal period decreases orexin immunoreactivity and given that the reduction was similarly observed in SIDS infants, supports the hypothesis that SIDS infants may have experienced IHH prior to death.

WTH08-07

Progranulin: a vascular stabilising agent with therapeutic potential for the treatment of stroke

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Introduction: Progranulin is a secreted growth factor previously shown to play an important role in protecting the brain against frontotemporal dementia (Baker et al., *Nature*, 2006). In our earlier study we demonstrated benefits of this protein in the ischaemic brain. Progranulin-deficient mice had decreased blood brain barrier (BBB) integrity that predisposed to greater brain injury after stroke (Jackman *et al*., *J Neurosci*, 2013). These findings suggested that progranulin directly promotes BBB integrity & may represent an attractive therapy for the treatment of stroke.

Study Aims/Hypotheses: (i) characterise the BBB stabilising effects of progranulin *in vitro* and (ii) explore its therapeutic potential for treatment of stroke. We hypothesise that progranulin is a powerful vascular stabilising agent that can protect against ischaemic brain injury when given after reperfusion.

Methods: *BBB integrity:* An *in vitro* human co-culture model of the BBB (Niego *et al.*, *Blood*, 2012) was exposed to tissue plasminogen activator (tPA, 25 nM)/plasminogen (plgn, 100 nM) or oxygen glucose deprivation (OGD, 6 h) in the presence and absecnce of progranulin (200 – 800 ng/ml). Permeability was assessed using fluorescent albumin. *Stroke:* Mice (male C57Bl/6) were subjected to middle cerebral artery occlusion (35 min) followed by reperfusion (Jackman *et al.*, *Meth Mol Biol*, 2010). Mice were treated with progranulin (100–200 ng, i.p.) at reperfusion & infarct volume and motor function assessed at 3 days.

Results: Progranulin attenuated BBB permeability in response to tPA/plgn ($n = 3$, $p < 0.05$, 1 way ANOVA), suggesting that it directly promotes vascular integrity. Furthermore, there was a trend for progranulin to attenuate permeability in response to exposure to OGD ($n = 3$, $p > 0.05$). In the setting of stroke, preliminary data demonstrated that treatment with progranulin improved functional outcome (hanging-wire; $n = 5$–6, $p < 0.05$) and tended to decrease total brain injury ($p > 0.05$).

Conclusions/Future Directions: This study provides the first direct evidence that progranulin acutely promotes BBB integrity & demonstrates its ability to improve stroke outcome when given at reperfusion. Ongoing experiments in the laboratory are exploring mechanism of progranulin's vascular stabilising effects.

WTH08-08

Hypoxic postconditioning protects against long-term deficits in a neonatal rat model of hypoxic-ischemic brain injury

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Objective: Hypoxic-ischemic (HI) brain injury in newborns is associated with high morbidity and mortality rates, with many babies suffering neurological deficits. Recently, we have shown that hypoxic postconditioning (PostC) can protect against HI injury in the neonatal rat up to 7 days after the insult. Here, we aimed to examine whether these neuroprotective effects were sustained, and whether functional deficits were also improved by PostC.

Methods: Sprague-Dawley rat pups were assigned to control or HI group on postnatal day 7 (P7). The HI group underwent unilateral carotid artery occlusion followed by hypoxia (7% oxygen, 3 h). Pups were then assigned to normoxia or PostC groups. The PostC group were exposed to 8% oxygen (1 h/day for 5 days post-injury), and normoxic animals were kept under ambient conditions. Righting reflex and negative geotaxis tests were performed on P8 and P14. On P42, the rats underwent further behavioural tests of motor function and memory (forelimb grip strength, grid walking and novel object recognition tasks). Brain injury was assessed using histological scoring of fixed brain sections stained with cresyl violet. Data was analysed using ANOVA.

Results: When righting reflex was tested in pups on P14, PostC significantly reduced the deficit compared to HI alone ($p < 0.05$). Long term behavioural deficits (P42) were observed in grid walking and novel object recognition tests after HI alone and both of these were improved in pups exposed to PostC ($p < 0.05$). No deficits in negative geotaxis or grip traction tests were observed after HI ($p > 0.05$). In addition, following HI, there was a significant increase in the extent of brain injury assessed by histological scoring (median score = 2; $n = 16$) when compared to control (median score = 0; $n = 14$), and this damage was reduced by PostC (median score = 1; $n = 15$) ($p < 0.05$).

Conclusions: This novel finding of the long-term histological neuroprotection accompanied by functional improvements by PostC further demonstrates the clinical potential of mild hypoxia for the treatment of HI brain injury.

WTH08-09

Plasma lipid peroxides level in Schizophrenia patients

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Objective: Oxidative stress is a state of imbalance between the generation of reactive oxygen species and antioxidant defence capacity of the body. Oxidative stress causes altered phospholipid metabolism which have been reported in Schizophrenia.

Malondialdehyde (MDA) is a lipid peroxidation product. This study was conducted with the aim to compare plasma Malondialdehyde (MDA) among schizophrenia patients, their first degree relatives and healthy controls.

Method: This was a hospital based cross sectional study, the subjects (23 schizophrenia patients, 23 first degree relative and 20 healthy controls) were recruited by purposive sampling method as per the inclusion and exclusion criteria. Tools applied were-Sociodemographic datasheet, Positive & Negative Syndrome Scale for Schizophrenia, General Health Questionnaire-60 item version, High performance liquid chromatography for accessing levels of MDA. Written informed consent was taken from every participant of the study. The study was passed by the ethical committee of the institute.

Results: Average level of MDA in patients was 11.05 ± 7.67 mcg/L. One way-ANOVA analysis and *post hoc* analysis for group comparison of MDA level between patients, FDRs and control group was done. No significant difference in MDA levels was noted. There was a significant ($p = 0.005$) difference with respect to family history, with the patients having a positive family history of psychiatric disorder have a significant elevated MDA Level. There is a significant ($p = 0.435$) correlation (Pearsons r) between MDA level & total leucocyte count of the patients. The correlation between MDA level and thyroid harmone level, liver enzyme level, lipid profile status and blood sugar level were also not significantly different.

Conclusion: The study provides an indirect evidence of oxidative stress in patients suffering from psychosis.

WTH08-10

NRF2 is required for pyrrolidine dithiocarbamate-mediated defensive action in astrocytes

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Endogenous defense against oxidative stress is controlled by nuclear factor erythroid 2-related factor 2 (Nrf2). The normal compensatory mechanisms to combat oxidative stress appear to be insufficient to protect against the prolonged exposure to reactive oxygen species during disease. Counterbalancing the effects of oxidative stress by up-regulation of Nrf2 signaling has been shown to be effective in various disease models where oxidative stress is implicated, including Alzheimer's disease. Stimulation of Nrf2 signaling by small-molecule activators is an appealing strategy to up-regulate the endogenous defence mechanisms of cells. We show here that the metal chelator pyrrolidine dithiocarbamate (PDTC) is a potent inducer of Nrf2 signaling specifically in astrocytes, upregulating antioxidant targets including heme oxygenase 1 (HO-1), glutamate cysteine ligase, glutathione and NAD(P)H:quinone oxidoreductase-1. We show these effects are dependent on kinase phosphorylation and demonstrate the critical role of Nrf2 in PDTC-mediated protection against oxidative stress. The presence of amyloid-beta magnifies PDTC-mediated induction of endogenous protective mechanisms, therefore suggesting that PDTC may be an effective Nrf2 inducer in the context of Alzheimer's disease. Finally we demonstrate that PDTC increases brain copper content and expression of glial HO-1, and decreases lipid peroxidation *in vivo*. These effects may contribute to the neuroprotection we and others have observed for PDTC in models of Alzheimer's disease.

WTH08-11

Lead exposure in early life and its long term effects on retinal degeneration in mouse model of retinal ischemia

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Objective: Early life environment can influence the life of an organism in long run. The present study was carried out to determine the effect of early life lead exposure on the later life of Swiss albino mice. The study was designed on the basis of LEARn (Latent early life associated regulation).

Methodology: Mice were first exposed to lead which act as first hit and second hit was provided in form of Pterygopalatine artery (PPA) occlusion. The animals were exposed at very early life i.e. immediately after birth from postnatal day 1 to postnatal day 20 to lead acetate through mother's milk. PPA intervention to the animals was given at either 10 week or 20 weeks of age. Animals were subjected to retinal ischemia by ligation of PPA and external carotid artery (ECA) using sutures. After 2 h of ischemia, sutures were removed to allow reperfusion for 5 days after which the animals were sacrificed. The establishment of PPA ligation as retinal ischemia was validated by fundus angiography, laser Doppler blood flow assessment and FITC staining. Animals were sacrificed after day 5 and eyes were enucleated for various histological and molecular analysis.

Summary: PPA ligation resulted in reduced flow of blood and thinning of retinal blood vessels supplying retinal tissue. The study thus presented PPA as a good model of retinal ischemia. The imunofluorescence and molecular analysis using real time PCR (BDNF, GFAP, Thy-1) showed a possible effect of early life exposure of lead on later life retinal degeneration.

Conclusion: The study revealed a positive relation between the early life environment and later life diseases. It provides a possible mechanism to prevent the onset or progression of disease by consciously modulating or controlling the exposure to different environment factors during early age.

WTH08-12

The effects of hypoxic postconditioning in a rat model of endothelin-1 induced middle cerebral ischemia

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Background and purpose: Hypoxic postconditioning (HPC) is defined as an exposure to mild hypoxia following an injury.

Previous studies have shown that HPC can activate beneficial pathways in *in vivo* models of brain injury. The purpose of this study is to investigate the neuroprotective and behavioural outcomes of HPC in an endothelin-1 (ET-1) induced middle cerebral artery occlusion (MCAO) in rats.

Methods: Conscious male Sprague Dawley rats were subjected to MCAO by perivascular microinjection of ET-1 (120 pmol) or saline (sham) via a previously implanted cannula. Animals were divided into four groups: sham normoxia ($n = 3$), sham HPC ($n = 3$), stroke normoxia ($n = 9$) and stroke HPC ($n = 13$). One day post stroke, HPC groups were exposed to 8% oxygen 1 h/day for 5 days; normoxic groups were kept in room air. Functional outcomes were tested 6 days after stroke using foot fault and sensory hemineglect tests. Rats were sacrificed 6 days post stroke and histopathological assessments using cresyl violet and neuronal staining of brain sections were performed.

Results: ET-1 induced MCAO resulted in functional deficits and neuronal loss compared to sham animals. Histopathological scoring of injury severity revealed that HPC did not reduce cortical or striatal damage when applied 24 h post stroke ($p > 0.05$). Quantification of neuronal staining showed an estimated 16% loss in the hemisphere ipsilateral to injury of stroke normoxia group. HPC did not rescue this neuronal loss ($p > 0.05$). There was a functional deficit in the front left foot placing 6d post stroke and this was improved in the stroke HPC group ($p < 0.05$). There was a small deficit in the sensory hemineglect test, but HPC did not improve this deficit.

Conclusion: This study was the first to examine the effects of HPC in an ET-1 induced MCAO in conscious rats. This study demonstrated that HPC performed 24 h after stroke did not reduce the overall extent of brain injury. However, HPC improved motor coordination, suggesting that protection may be location specific. Further studies need to examine the specific regions protected by HPC and the mechanisms involved.

WTH08-13

Mouse photothrombotic cerebellar infarct evaluated using magnetic resonance imaging and histology

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Objectives: Cerebellar strokes represent 2–3% of acute strokes worldwide, with a mortality rate 23% greater than strokes in other brain region. The current hindbrain stroke models are highly invasive and not temporally or spatially confined. Here we present a photothrombotic mouse model of cerebellar ischaemia to study neuroprotection for hindbrain strokes. We aim to determine the validity of the photothrombotic stroke model, producing a site-specific ischaemic brain injury in the cerebellum, and examining the progression of the cerebellar infarct via magnetic resonance imaging (MRI), histology and immunohistochemistry.

Methods: All experiments were approved by the UNSW animal care and ethics committee. Photothrombosis in the cerebellum was achieved by intravenous administration of rose bengal dye followed by illumination at 561 nm of the vermis region of the cerebellum in an anaesthetised mouse (129SvEv). Infarcts were examined by T2 weighted MRI at days 1, 4, and 7 post-ischaemia using a Bruker 9.4T MRI scanner, following which animals were euthanised and the fixed tissue was paraffin embedded, sectioned, stained with haematoxylin and eosin (H&E) and immunostainedwith anti-glial fibrillary acidic protein antibody and anti-calbindin antibody to detect astrocytes and assess damage to Purkinje neurons respectively.

Results: The thrombus formation was determined in real-time using intravital multiphoton LSM to detect aggregation of fluorophore-conjugated anti-CD42 immunolabelled platelets. MRI scans, in the days following the infarct, showed development of oedema within the area delineated by the platelet labelling. Greater resolution of tissue damage was evident in the histological sections stained with H&E, with clear demarcation of infarcted area, which increased over the course of several days. Immunohistochemistry against glial fibrilary acidic protein and calbindin further illustrated an increase in astrocytes, a loss of Bergmann astrocyte processes, and a loss of Purkinje neurons at the site of lesion.

Conclusion: This model was found to be a robust model of focal ischaemia, delivering thrombi to a specific and constrained area and hence holds great potential to aid investigations into novel therapeutic options targeting the cerebellum.

WTH08-14

Complement peptide c3a promotes astrocyte survival in response to ischemic stress

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Astrocytes are the most numerous cells in the central nervous system with a range of homeostatic and regulatory functions. Under normal conditions as well as after ischemia, astrocytes promote neuronal survival. We have previously reported that the complement-derived peptide C3a stimulates neuronal differentiation of neural progenitor cells and protects the immature brain tissue against hypoxic-ischemic injury. Here, we studied the effects of C3a on the response of mouse cortical astrocytes to ischemia. We have found that chemical ischemia, induced by combined inhibition of oxidative phosphorylation and glycolysis, up-regulates the expression of C3a receptor in cultured primary astrocytes. C3a treatment protected wild-type but not C3a receptor deficient astrocytes from cell death induced by chemical ischemia or oxygen-glucose deprivation by reducing ERK signaling and caspase-3 activation. C3a attenuated ischemia-induced upregulation of glial fibrillary acidic protein, however the protective effects of C3a were not dependent on the presence of the astrocyte intermediate filament system. Pre-treatment of astrocytes with C3a during recovery abrogated the ischemia-induced neuroprotective phenotype of astrocytes. Jointly, these results provide the first evidence that the complement peptide C3a modulates the response of astrocytes to ischemia and increases their ability to cope with ischemic stress.

WTH08-15

Ischaemia-induced neuronal cell death is mediated by molecular targeting of CaMKII phosphorylated at T253

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Purpose: Ischaemia/excitotoxicity produces a persistent activation of CaMKII (Calcium-calmodulin stimulated protein kinase II) and inhibiting this persistent activity post-injury is strongly neuroprotective *in vitro* and *in vivo* showing that CaMKII is a major mediator of ischaemia/excitotoxicity-induced cell death. CaMKII is regulated by multi-site autophosphorylation (e.g. at T253, T286) and molecular targeting (binding to particular protein partners). As CaMKII phosphorylation at T286 is known to persistently activate CaMKII, it has been assumed that the ischaemia-induced persistent CaMKII activity is mediated by T286 phosphorylation. However, we have shown that brain regions with enhanced sensitivity to ischaemic damage show enhanced ischaemia/excitotoxicty-induced phosphorylation of CaMKII at T253 but not T286. We have directly tested the role of phosphorylation at T286 and T253 in ischaemic cell death.

Methods: Middle cerebral artery occlusion (MCAo) for 60 min was performed in T286A-αCaMKII transgenic mice that cannot phosphorylate CaMKII at T286, and normal littermate controls. Infarct volume was measured 24 h later. Differentiated neuroblastoma cells (SHSY5Y) transfected with inducible recombinant αCaMKII bearing phosphomimic or phosphonull mutations at T286 or T253 were treated with 1 mM glutamate and cell death measured 24 h later.

Results and Conclusion: There was no significant difference in infarct volume between T286A and control mice. Differentiated SHSY5Y cells showed dose and time dependent glutamate-induced excitotoxic cell death. Induced expression of T253D αCaMKII (mimics T253 phosphorylation) significantly enhanced the glutamate-induced cell death. Expression of T253V αCaMKII (prevents T253 phosphorylation) prevented the enhanced cell death and expression of T286D αCaMKII (mimics T286 phosphorylation) had no significant effect. These results suggest that the ischaemia-induced persistent activation of CaMKII is produced by phosphorylation of CaMKII at T253 and allosteric changes induced by interactions with specific binding proteins. Hence, the interaction between pT253-CaMKII and its binding proteins may provide a therapeutic drug target in stroke.

WTH08-16

Neuroprotective effect of a combination of baicalin and catechin: evidences from rat model of transient global cerebral ischemia

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Inflammation, one of the key pathological factors that precipitate the deteriorating effect of cessation of blood supply to brain during stroke. Targeting the inflammatory pathway by naturally occurring flavanoids provides a potential approach for neuroprotection.Flavocoxid, a prescription medical food used in osteoarthritis, containing natural flavonoids, baicalin and catechin was evaluated in experimental transient global cerebral ischemia in rats. *Methods:* Transient global cerebral ischemia induced by bilaterally common carotid artery occlusion (BCCAO) for 15 min. Flavocoxid (50, 100, 200 mg/kg, p.o.) was administered 6 days before BCCAO. Fluoxetine (10 mg/kg, p.o.) and rivastigmine (2 mg/kg, p.o.) were administered 1 day after BCCAO as standard treatment for post-stroke depression and cognition loss respectively. Neurobehavioral tests, biochemical and neurochemical markers in brain, inflammatory markers (TNF-α, interleukin-1β, nuclear factor-κB) in serum and ischemic infract size using triphenyltetrazolium chloride were estimated.Transient occlusion of carotid artery precipitated hallmark characteristics of ischemic stroke as depicted by disturbed neurological function, depressive stature and loss of long and short term cognition in behaviour outcomes. BCCAO significantly enhanced the Malondialdehyde and total nitrite concentration along with reduced endogenous antioxidants viz. reduced glutathione and superoxide dismutase along with increased infract size. It also increased serum TNF-α, interleukin-1β and nuclear factor-κB concentration. All these deteriorating effect of cessation of blood flow to brain were prevented by flavocoxid depicted its strong antioxidant and anti-inflammatory potential.Flavocoxid, a reported dual cyclooxygenase-1 and -2 along with 5-lipoxygenase activity has a potential to be used as a neuroprotectent in ischemic stroke and post-stroke anomalies by virtue of their antioxidant and anti-inflammatory properties.

WTH08-17

Neonatal hypoxic-ischaemic brain injury is more severe in offspring of obese versus lean female rats

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Objective: In humans, maternal obesity is associated with an increase in the incidence of birth related difficulties. However, the impact of maternal obesity on the severity of brain injury in offspring is not known. Recent studies have found evidence of increased glial response and inflammatory mediators in the brains of obese humans and rodents. We hypothesised that hypoxic-ischaemic (HI) brain injury is greater in neonatal offspring from obese rat mothers compared to lean controls.

Methods: Female Sprague Dawley rats were randomly allocated to high fat (HFD, $n = 8$) or chow ($n = 4$) diet and mated with lean male rats. On postnatal day 7 (P7), male and female pups were randomly assigned to HI injury or control (C) groups. HI injury was induced by occlusion of the right carotid artery followed by 3 h exposure to 8% oxygen, at 37°C. Control pups were removed from the mother for the same duration under ambient conditions. Righting behaviour was measured on day 1 and 7 following HI. The extent of brain injury was quantified in brain sections from P14 pups using cresyl violet staining and the difference in volume between brain hemispheres was measured.

Results: Before mating, HFD mothers were 11% heavier than chow mothers ($p < 0.05$, *t*-test). The Chow-HI pups ($n = 31$) showed an 8.9 ± 3.3% loss in ipsilateral brain tissue, while the HFD-HI group had significantly greater loss (20.5 ± 3.2% $n = 44$, $p < 0.05$, 2 way ANOVA). No significant difference was detected in brain volume between the HFD-C ($n = 25$) and Chow-C ($n = 16$)

pups. When analysed on a per litter basis, the size of the injury was significantly correlated with maternal weight ($r = 0.89$; $p < 0.05$).

Conclusions: Our data clearly demonstrate that maternal obesity can exacerbate the severity of brain damage caused by HI in neonatal offspring. Given that previous studies have shown enhanced inflammatory responses in offspring of obese mothers, these factors including gliosis and microglial infiltration, may contribute to the enhanced brain injury.

WTH08-18

Ephrin-A1 through EphA4 is responsible for reduced astrogliosis in the infant primate neocortex following ischemic stroke

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EphA4 is a key modulator of astrogliosis after CNS injury. Astrogliosis and glial scarring after ischemic strokes is a major impediment to functional recovery although the specific ephrin ligands responsible for EphA4 activation remain unknown. Previous studies have suggested that glial scarring in the infant neocortex is less severe and more permissive towards regeneration after an injury. Therefore, a systematic comparison of the mechanisms regulating astrogliosis and glial scarring after infant and adulthood CNS injuries is of great clinical significance. We utilised a clinically translatable nonhuman primate model of ischemic stroke in the infant and adult visual cortex. We identified the ephrin ligands involved in EphA4-mediated astrogliosis in the post-ischemic infant and adult and their effects on glial scarring. In infants, ephrin-A1 was increased on reactive astrocytes proximal to the ischemic core. In contrast, the onset of stroke triggered the expression of ephrin-A2/-A5, but not ephrin-A1 on reactive astrocytes juxtaposing the ischemic core. In functional assays, ephrin-A1 induced astrocyte repulsion, supressed proliferation and prevented wound closure. Conversely, ephrin-A2 and -A5 elicited cell attraction, increased proliferation and induced rapid wound closure. Ephrin-A1 treatment was able to attenuate astrocyte wound closure induced by ephrin-A2/-A5 forward signalling. We conclude that ephrin-A1 signalling in infants limit astrogliosis resulting in a less prolific, discrete scar formation that may be more permissive to regeneration. In contrast, ephrin-A2/-A5 signalling through EphA4 in adults promotes astrogliosis, contributing to a more widespread and denser scar formation that likely leads to the significant inhibition of neural regeneration. Finally, the exogenous administration of ephrin-A1 competitively inhibits astrocyte reactivity induced by ephrin-A2/-A5. We demonstrate for the first time that the primate brain undergoes differential Eph/ephrin mediated astrogliotic responses depending on the age at which ischemic stroke occurred. We suggest the reintroduction of ephrin-A1 signalling after a neocortical ischemic stroke in adults as a novel therapeutic strategy to attenuate the severe reactive astrogliosis and glial scarring induced by ephrin-A2/-A5 signalling through EphA4.

WTH08-19

Measuring glutathione in the human brain: a comparison of methods for 3T mrs

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Glutathione (GSH) is an important antioxidant implicated in the pathology of neurodegenerative diseases and schizophrenia. Magnetic resonance spectroscopy (MRS) has been used to measure brain GSH, but low concentration and overlap with other metabolite signals make accurate quantification difficult. To mitigate this J-difference edited 1H MRS pulse sequences such as MEGA-PRESS have been used to measure GSH, though several studies report quantification using short-echo PRESS pulse sequences. Here we compare GSH quantification using these methods in phantoms and human brain. We prepared two brain-mimetic phantoms containing the major metabolites in human brain. We added 1 mM glutathione to one phantom. We also acquired data from the anterior cingulate cortex (ACC) in 7 healthy volunteers. Spectra were acquired on a 3T Philips scanner using MEGA-PRESS and short-echo PRESS. Spectra were fitted in jMRUI and GSH concentrations calculated for both sequences. GSH concentration calculated from PRESS was not significantly different in the phantoms (P1:1.19 mM; P2:1.05 mM), despite the absence of GSH in P1. In contrast, in MEGA-PRESS no resonance from GSH appeared in the spectrum from P1: concentrations were P1 = 0 mM, P2 = 1.2 mM. In the human study, GSH could be clearly identified using MEGA-PRESS, while no particular peak can be assigned to GSH using PRESS. In the ACC, GSH concentration was 0.7 ± 0.16 mmol/kg from MEGA-PRESS, compared to a significantly different value of 2.0 ± 0.42 mmol/kg from PRESS. Due to the uncertainties in GSH quantification raised by the phantom and human study, normal physiological concentrations of GSH cannot be reliably quantified using PRESS at 3T, whereas in MEGA-PRESS the GSH signal is visually detectable and more accurate quantification can be performed.

WTH08-20

Disruption of ion-trafficking system in the cochlear spiral ligament fibrocytes prior to permanent hearing loss induced by intense

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Most of sensorineural hearing impairment, which causes the inner ear dysfunction, is irreversible and it has become a grobal social problem because there are no treatment of sensorineural hearing impairment. The endocochlear potential (EP), which is maintained by various K^+-transport apparatuses including Na^+, K^+-ATPase and gap junction in the lateral wall structures, is important for the functional expression of the hair cells in the cochlea. Noise-induced hearing loss is at least in part due to disruption of the EP and excess oxidative stress. In this study, we examined the changes in the ion trafficking-related proteins in the cochlear spiral ligament fibrocytes (SLFs) following *in vivo* acoustic overstimulation or *in vitro* exposure of cultured SLFs to 4-hydroxy-2-nonenal, which is a mediator of oxidative stress. Connexin (Cx)26 and Cx30 were

ubiquitously expressed throughout the spiral ligament, whereas Na^+, K^+-ATPase α1 was predominantly detected in the stria vascularis and spiral prominence (type 2 SLFs). One-hour exposure of mice to 8 kHz octave band noise at a 110 dB sound pressure level produced an immediate and prolonged decrease in the Cx26 level and in Na^+, K^+-ATPase activity, as well as a delayed decrease in Cx30 level in the SLFs. The noise-induced hearing loss and decrease in the Cx26 protein level and Na^+, K^+-ATPase activity were abolished by a systemic treatment with a free radical-scavenging agent, 4-hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl or with a nitric oxide synthase inhibitor, N^{ω}-nitro-L-arginine methyl ester hydrochloride. *In vitro* exposure of SLFs in primary culture to 4-hydroxy-2-nonenal produced a decrease in protein levels of Cxs and Na^+, K^+-ATPase α1, as well as in Na^+, K^+-ATPase activity, and resulted in dysfunction of the ion-trafficking system between the SLFs. Taken together, our data suggest that disruption of the ion-trafficking system in the SLFs is caused by an oxidative stress-induced decrease in the levels of Cxs and Na^+, K^+-ATPase, and is at least in part involved in permanent hearing loss induced by intense noise.

WTH08-21

Human placenta stem cells secrete IL-6 and vegf and prevent death in PC12 cells after glucose- and oxygen deprivation

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The neuroprotective effects of human placental mesenchymal-like adherent stromal cells (PLX) were studied in nerve growth factor (NGF)-differentiated pheochromocytoma PC12 cells after a 4 h episode of both oxygen and glucose deprivation (OGD) followed by 24 h reperfusion (ischemia). Under optimal conditions, $2x10^5$ PLX cells, added in a trans-well system, conferred 30–60% neuroprotection to PC12 cells subjected to OGD insult. PC12 cell death, measured by LDH release was reduced by PLX cells or by a conditioned medium derived from PLX cells previously exposed to ischemia, suggesting active release of factorial components. In search for such biofactorial signals, levels of cytokine IL-6 and angiogenic factor $VEGF_{165}$, were determined under the ischemia-like stress conditions using selective ELISA. The results indicate that both IL-6 and $VEGF_{165}$ are actively secreted by PLX cells in co-cultures with PC12 cells after ischemic conditions compared to control co-cultures. Furthermore exogenous supplementation of 10 ng/ml each of IL-6 and $VEGF_{165}$ to ischemia-stressed PC12 cells confer neuroprotection reminiscent of the beneficial effect noticed in the presence of PLX cells or aliquots of their conditioned medium. Growth factors as well as co-cultures conditioned medium effects were reduced by 70% and 20% upon pretreatment with 240 ng/ml Semaxanib (anti $VEGF_{165}$) and/or 400 ng/ml neutralizing anti IL-6 antibody, respectively. Therefore, PLX-driven neuroprotection of the stressed PC12 cells by IL-6 and $VEGF_{165}$ release may be part of a wider mechanism of beneficial consequences recently reported in clinical trials of stroke patients after administration of these cells. Whether additional factors are secreted and the specificity of the targeted tissue to activate this secretion remain to be investigated.

WTH08-22

Early post-stroke treatment with minocycline promotes functional recovery without modulating key aspects of microglial activation

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Glial cell responses in peri-infarct tissue following stroke contribute to changes, including the development of an astrocytic scar, that influence the extent of functional recovery. Microglial activation that is initiated soon after infarct formation is thought to be a key step in the development of peri-infarct glial cell changes. However, there is limited understanding of the consequences of modulating these early microglial responses. Based on previous reports that minocycline can reduce microglial activation, we tested the effects of early post-stroke minocycline treatment on peri-infarct cellular changes and functional recovery following stroke. Photothrombotic stroke was induced in the region of the forelimb motor cortex in Sprague-Dawley rats. Minocycline was injected intraperitoneally at either a lower dose (45 mg/kg at 1 and 24 h after stroke induction) or a higher dose (90 mg/kg at 1 h, 45 mg/kg at 12, 24, 36 and 48 h). Control rats received vehicle injections. Forelimb function and microglial properties in brain sections were evaluated by investigators blinded to the treatments. Neither of the minocycline treatment protocols altered lesion size. Interestingly, these treatments also did not alter key aspects of the microglial response assessed at 3 days after stroke induction. This included measures of microglial responses based on immunolabelling with Iba1 that assessed morphological changes and proliferation and migration of these cells in the peri-infarct tissue. The higher dose minocycline improved functional recovery as assessed from a forepaw placing test at 7 and 14 days after stroke induction. These results provide evidence that early treatment with minocycline following stroke can promote functional recovery but this does not require changes in key microglial responses in the peri-infarct tissue. The effects of minocycline could have arisen via subtle changes in microglial gene expression that did not influence the properties tested or via modulation of responses of other cells including those in the circulation.

WTH08-24

Abnormal glutathione pathway in postmortem dorsolateral prefrontal cortex from people with Schizophrenia

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The glutathione (GSH) pathway is the most important antioxidant system to protect against oxidative stress in human brain. GSH is biologically synthesized by the rate-limiting enzyme glutamyl-cysteine ligase (GCL); and then GSH detoxifies ROS via GSH peroxidase (GPx). In this study, I examined the GSH antioxidant system in post-mortem brain from patients with schizophrenia. The quantities of glutamyl-cysteine ligase subunit C (GCLC), glutathione peroxidases (GPx) and GSH in the pathway were measured. It is

hypothesized that an abnormal GSH pathway is associated with schizophrenia.

Dorsolateral prefrontal cortex from 37 schizophrenia cases and 37 matched controls were studied. Western blotting and slot blotting analysis was used to examine GCLC and GPx protein levels. The protein level of each individual schizophrenia case was divided by the level of each matched individual control subject. By one-sample *t*-test analysis, the ratios was compared to 1.00 (1 refers to no change) to determine the difference of the protein levels between schizophrenia and controls. The levels of total GSH was determined and analysed by spectrophotometry.

We found that the levels of GCLC/β-actin for people with schizophrenia relative to control subjects (mean ± SD: 1.1739 ± 0.3831) were significantly increased [$t(35)2.719$, $p = 0.01$]. GPx levels of people with schizophrenia relative to control subjects (1.2407 ± 0.49) was also significant increased [$t(32)2.821$, $p = 0.008$]. Moreover, we found that levels of total GSH in schizophrenia (1.0120 ± 0.17) were significantly less than in controls (1.1471 ± 0.20) [$t(61)2.875$, $p = 0.006$].

In our study, people with schizophrenia had significantly more GCLC and GPx, but less total GSH than matched controls. A decrease in total GSH indicates impaired antioxidant defences in the prefrontal cortex of people with schizophrenia. Upregulation of GCLC is consistent with attempts at increased production of total GSH. Upregulation of GPx is consistent with increased levels of oxidative stress in schizophrenia.

WTH08-25

Hemodynamics and vascular plasticity but neuronal degeneration after chronic cerebral hypoperfusion

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Chronic cerebral hypoperfusion (CCH) induces cognitive impairment, but the compensative mechanism of cerebral blood flow (CBF) is not fully understood. The present study mainly investigated dynamic changes in CBF, angiogenesis and cellular pathology in the cortex, striatum and the cerebellum, and also studied cognitive impairment of rats induced by bilateral common carotid artery occlusion (BCCAO). MRI techniques, immunochemistry and Morris water maze were employed to the study. The CBF of the cortex, striatum and cerebellum dramatically decreased after right common carotid artery occlusion (RCCAO), and remained lower level at 2 wk after BCCAO. It returned to the sham level from 3 to 6 wk companied by the dilation of vertebral arteries following BCCAO. The number of micro-vessels declined at 2, 3 and 4 wk but increased at 6 wk after BCCAO. Neuronal degeneration occurred in the cortex and striatum from 2 to 6 wk, but the number of glial cells dramatically increased at 4 after BCCAO. Cognitive impairment of ischemic rats was directly related to ischemic duration. Our results suggest that CCH induces a compensative mechanism attempting to maintain optimal CBF to the brain. However, this limited compensation can not prevent neuronal loss and cognitive impairment after permanent ischemia.

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WTH08-26

Long-lasting motor improvement following neural transplantation in athymic rats with ischemic brain injury

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Human pluripotent stem cells have the capacity for directed differentiation into a wide range of neuronal subtypes that may be useful for brain repair in neurological conditions including ischemic stroke. While a substantial body of research has led to a detailed understanding of the ability of neurons in fetal tissue grafts to structurally and functionally integrate after intra-cerebral transplantation, we are only just beginning to understand the in vivo properties of neurons derived from human pluripotent stem cells and their true potential in terms of its therapeutic effect. In a previous study, we showed that cortical progenitors generated from human ES cells showed a remarkable degree of integration after transplantation into the neonatal rat brain. In the current study we aimed to assess the therapeutic potential of cortical cell replacement in a model of cortical ischemia. Our model used vasoconstrictive peptide endothelin- 1 (ET-1; 400 pmol/Al in saline) which was injected into the frontal motor cortex of athymic rats. The staircase test was used in this study to provide a sensitive measure of skilled forepaw motor function of the lesion rats. Serial coronal sections were obtained for assessment of cortical volume. We have found that transplantation of cortical progenitors in animals with ischemic damage to the motor cortex provides significant improvement in the resulting motor deficit. This is likely to be due, at least in part, to neuroprotective effects of the grafted cells, which prevents some degree of cortical atrophy that follows the primary ischemic event.

WTH09 Synaptic Plasticity

WTH09-02

BDNF signaling recruits metabolic and plasticity signals to counteract the pathobiology of TBI: a study of trkb agonist 7,8-DHF

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Traumatic brain injury (TBI) is followed by a state of metabolic dysfunction, compromises the capacity of neurons to metabolize energy and support brain plasticity. There are no effective therapies to counteract the pathobiology of TBI based on multifactorial nature of the pathology. Brain-derived neurotrophic factor (BDNF) has an exceptional capacity to support metabolism and plasticity, which highly contrasts with its poor pharmacological profile. We evaluated the action of a flavonoid derivative 7,8-dihydroxyflavone (7,8-DHF), a TrkB receptor agonist with the pharmacological profile congruent for potential human therapies. Results showed that treatment with 7,8-DHF (5 mg/kg, ip, daily for 7 days) was effective to ameliorate the effects of TBI on plasticity markers (CREB phosphorylation, GAP-43 and syntaxin-3 levels) and memory functions observed in Barnes maze test. Treatment with 7,8-DHF restored a decrease in activation and phenotypic expression of TrkB after TBI. In turn, intrahippocampal injection of K252a, a TrkB antagonist, counteracted the 7,8-DHF induced TrkB signaling activation and memory improvement in TBI, suggesting the pivotal role of TrkB signaling in cognitive performance after brain injury. A potential action of 7,8-DHF on energy homeostasis was corroborated by the normalization in levels of PGC-1α, TFAM, COII, AMPK and SIRT1 in animals subjected to TBI. The studies in N2a-neuroblastoma cells showed that 7,8-DHF (200 nM) elevates the levels of PGC-1α, CREB phosphorylation and mitochondrial respiratory capacity, suggesting that activation of BDNF-TrkB signaling is crucial for engaging signals related to energy homeostasis and brain plasticity. Results suggest a potential mechanism by which brain plasticity is affected in conjunction with energy homeostasis during TBI, and 7,8-DHF likely counteracts the TBI pathology by recruiting signals of metabolism and plasticity. Taken together, this study highlights the role of BDNF-TrkB signaling for building neuronal resilience and functional recovery following brain trauma, thus provide the preclinical evidence for the therapeutic role of 7,8-DHF in TBI.

WTH09-03

Mechanisms of action of repetitive transcranial magnetic stimulation upon motor learning - an *in vivo* imaging approach

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Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive method for modulating cortical plasticity by applying extrinsic pulsed magnetic fields. rTMS has generated significant interest, as it can induce long-lasting changes in cortical plasticity in both humans and rodents. In particular, complex pattern TMS such as intermittent theta burst stimulation (iTBS) has been shown to enhance motor learning tasks when applied to the motor cortex of human subjects. However, the biological mechanisms underlying these long-lasting effects remain poorly understood.

This study examined the effect of rTMS on the motor cortex of mice with both behavioural testing (skilled motor learning) and 2-photon *in vivo* imaging of cortical circuitry.

We used a rodent-specific TMS circular coil to deliver iTBS (600 pulses) over the M1 motor cortex of awake, restrained mice. For skilled motor learning, male C57Bl6/J mice received daily iTBS immediately prior to testing in a pellet reaching task for 10 consecutive days. Analysis with a mixed model showed a modest but significant increase in accuracy (but not speed) associated with the iTBS treatment, compared to sham controls.

In a separate group, we used Thy1-GFP-M mice which express EGFP in ~5% of excitatory neurons in the cortex. These underwent cranial window implantation and subsequent imaging to enable visualisation of axons and dendritic arbors in L1/2 of the cortex. Using a multiphoton microscope equipped with a MaiTai DeepSee Ti-sapphire laser, a timecourse of images of synaptic structures were collected at regular intervals before and after iTBS and analysed for alterations in connectivity associated with stimulation.

These results help address the biological mechanisms underlying rTMS, which will undoubtedly pave the way forward in the therapeutic applications of non-invasive brain stimulation in health and disease.

WTH09-04

Reverberating cell assemblies in the amygdala

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Neural cell assemblies are one of the hypotheses for working memory and associative learning in the brain first proposed by Donald O Hebb. Hebb suggested cell assemblies encode information by reverberation through recurrent feedback excitation. Many *in vivo* field recordings support existence of cell assemblies in the

brain, yet direct evidence at neuronal level is lacking. The properties of neurons that form cell assemblies; their temporal characteristics and synaptic mechanisms involved in generation of reverberation are not known. We investigated presence of cell assemblies in mouse basal amygdala, an important structure for associative learning. We show that the basal amygdala contains networks of neurons that can generate reverberating oscillations with time locked recurrent feedback excitation. These networks can be triggered by activation of axo-axonic neurons through GABA mediated excitation at the axon initial segments of glutamatergic neurons that generates recurrent feedback excitation.

WTH09-05

Functional interplay between 5-HT$_7$R, MMP-9 and CD44 in the regulation of neuronal plasticity

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Changes in dendritic spine morphology are important for synaptic plasticity. Among other factors, neuronal morphology can be influenced by 5-HT$_7$ receptor (5-HT$_7$R)-mediated signaling and the proteolytic activity of extracellular matrix modifiers. Here, we provide evidence that 5-HT$_7$R, matrix metalloproteinase-9 (MMP-9), the hyaluronan receptor CD44, and the small GTPase Cdc42 belong to the same signaling pathway, and their interplay is involved in regulating neuronal morphology. We observed a physical interaction between 5-HT7R and CD44. We also identified CD44 as a novel MMP-9 substrate in neurons and found that 5-HT$_7$R stimulation increased MMP-9 activity at dendritic spines, initiating spines remodeling. This effect depended on the expression and function of 5-HT$_7$R, MMP-9, and CD44, since no changes in spine morphology were observed in 5-HT$_7$R, MMP-9-, or CD44- knockout neurons. Mechanistic insights into this signaling pathway were obtained by applying a novel CD44 shedding reporter, demonstrating that the 5-HT$_7$R-mediated activation of MMP-9 leads to CD44 cleavage followed by Cdc42 activation. Our results reveal unknown link between 5-HT$_7$R-mediated signaling and the MMP-9-mediated cleavage of CD44 in regulation of neuronal plasticity.

WTH09-06

Translational control of mglur-dependent long-term depression and object-place learning by eIF2α

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At hippocampal synapses, activation of group-I metabotropic glutamate receptors (mGluRs) induces long-term depression (LTD), which requires new protein synthesis. However, the underlying mechanism remains elusive. Here we describe the translational program that underlies mGluR-LTD and identify the translation factor eIF2α as its master effector. Genetically reducing eIF2α phosphorylation, or specifically blocking the translation controlled by eIF2α phosphorylation, prevented mGluR-LTD and the internalization of surface AMPA receptors (AMPARs). Conversely, direct phosphorylation of eIF2α, bypassing mGluR activation, triggered a sustained LTD and removal of AMPARs. Combining polysome-profiling and RNA-sequencing, we identified the mRNAs translationally up-regulated during mGluR-LTD. Translation of one of these mRNAs, oligophrenin-1, mediates the LTD induced by eIF2α phosphorylation. Mice deficient in phospho-eIF2α–mediated translation are impaired in object-place learning, a behavioral task that induces hippocampal mGluR-LTD *in vivo*. Our findings identify a new model of mGluR-LTD, which promises to be of value in the treatment of mGluR-LTD-linked cognitive disorders.

WTH09-07

Neurons specifically activated in auditory fear learning

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Objectives: Memory formation is thought to occur via enhanced synaptic connectivity between particular populations of neurons in the brain. However, the neurons which are involved in any particular memory have not been defined. The objective of this study was to identify the neurons which are activated by a particular form of learning and memory.

Method: *Fos-tau-LacZ* (FTL) mice enable the identification of functionally activated neuronal circuits. Six groups of FTL mice were habituated to a fear chamber and exposed to an enriched environment daily over 3 weeks. One group then received a single paired presentation of an auditory tone and footshock. Control mice

included Unpaired, Tone only, Context only, Home cage only and Fear recall mice. Four hours after training, mice were tested for fear memory and then perfused. Brain sections were stained for FTL activity and neurons were identified and counted using light microscopy.

Results: Only paired mice froze to tone, and insignificant freezing to context was observed. Distinct populations of learning specific neurons were identified in lateral amygdala, amygdalostriatal transition area, medial amygdala, capsular central amygdala, ventromedial hypothalamus, lateral hypothalamus, and intermediate lateral septum. There were significantly more FTL neurons in paired mice compared to other groups ($p < 0.05$).

Conclusions: Identification of neurons that are specifically activated by the association of tone to shock suggests that these neurons are involved in formation of the auditory fear memory. Further aspects of the fear learning process may be possible following this identification.

WTH09-08

Chronic intermittent toluene inhalation during adolescence results in glutamatergic dysfunction in corticostriatal processing

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The abuse of inhaled vapours form petrol and glues is a growing problem for societies world-wide. This is especially so for adolescent populations who constitute over 60% of the user population. Consequently, inhalants are often the first "drug" than many youngsters experience with exposure often coinciding with key elements of brain maturation. We have shown in rodents that adolescent exposure to inhalants impairs instrumental learning implicating corticostriatal dysfunction (Dick *et al*., 2013). AIMS: To determine the underlying processes mediating inhalant-induced changes in cognition, focusing on *N*-methyl-D-aspartate (NMDA) and neuronal nicotinic acetylcholine (nACh) receptors both of which are sensitive to inhalants. METHODS: Male adolescent Wistar rats (postnatal day 27) were exposed to air (n = 6–8/cohort) or chronic intermittent toluene (the volatile solvent in preferentially inhaled products; CIT-10 000 ppm, n = 6–8/cohort) for 1 h, 3 xweek for up to 4 weeks. RESULTS: After 4 weeks exposure qPCR revealed no differences in mRNA levels for subunits of both NMDA and nACh receptors. Binding to nACh receptors using tissue autoradiography for [^{3}H]-epibatidine was unaltered, however there was a significant decrease ($p < 0.05$) of [^{3}H]-ifenprodil binding, presumably to NMDA receptors containing N2B subunits, in the dorsal anterior cingulate and striatum specifically; binding in other regions including the hippocampus was unaltered. Together these data suggest i.e. reduced membrane bound availability or trafficking of receptors, as opposed to changes in receptor expression, following CIT specifically to NMDA receptors containing 2B subunits. When tested in adulthood, the NMDA receptor antagonist MK801 (0.5 mg/kg, ip) caused exaggerated psychomotor responses in CIT-exposed rats ($p < 0.05$) compared to controls suggesting long-term dysregulation of glutamatergic signalling. CONCLUSIONS: This study suggests that CIT during adolescence results in sustained effects on NMDA receptor signaling within the corticostriatal pathway which may contribute to toluene-induced alterations in information processing during complex behaviours.

WTH09-09

Secreted amyloid precursor protein alpha regulates protein synthesis in primary hippocampal neuronal cultures

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The secreted fragment of the amyloid precursor protein, sAPPα, has recently been shown to both enhance spatial memory as well as long-term potentiation, an experimental model of memory. Surprisingly little is known about how sAPPα induces changes in nerve cell activity. Since the persistence of memory and LTP is dependent on the synthesis of proteins by activated hippocampal neurons we hypothesized that sAPPα may affect protein synthesis and in early work showed that sAPPα stimulated protein synthesis in isolated nerve cell synapses. As LTP is underpinned by regulation of the AMPA-subtype of glutamate receptors, in the current study we explored whether application of sAPPα to primary hippocampal neuronal cultures regulates the synthesis of the AMPA receptor subunits GluA1 or GluA2. Following incubation with 1 nmol sAPPα, newly synthesized proteins were labelled using fluorescent non-canonical amino acid tagging plus proximity ligation assays (PLA-FUNCAT). This method incorporates a methionine analogue bound to an azide group into newly synthesized proteins. Click chemistry attaches a biotin-bound alkyne group to the newly synthesized protein, which can then be recognized with antibodies and PLA probes to determine the identity and location of newly synthesized proteins. Using PLA-FUNCAT, we found that sAPPα upregulated protein synthesis ($p < 0.05$, Kruskal–Wallis test), and specifically, levels of newly synthesized GluR1 were increased threefold ($p < 0.0005$) while GluR2 levels were unaffected compared to baseline. By focusing specifically on newly synthesized proteins in cell soma we found a 2-fold increase in GluR1 synthesis compared to baseline ($p < 0.05$). These results indicate that a sAPPα-mediated increase in protein synthesis, including the GluR1 subunit of AMPA receptor, may be one mechanism through which it enhances LTP and memory.

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WTH09-10

Does depolarization-induced reprogramming of the presynaptic phosphoproteome mediate changes in neurotransmitter release?

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Learning and memory result from synaptic plasticity - the ability of synaptic transmission to be modulated over time in response to stimuli. Compared to the more well-studied postsynaptic changes, presynaptic change in neurotransmitter release (exocytosis) from synaptic vesicles (SVs) is a mechanistically less well-defined form of synaptic plasticity. It is known that the phosphorylation of several exocytic proteins affects transmitter release. Exocytosis is followed by synaptic vesicle endocytosis which is initiated by rapid dephosphorylation of endocytic proteins upon depolarisation. Phosphorylation of a few key presynaptic proteins has thus been shown to play an essential role in neurotransmitter release and synaptic vesicle regeneration. However, changes in pre-synaptic protein phosphorylation in response to depolarization and recovery has previously not been characterised globally, in spite of the highly likely role of phosphorylation in pre-synaptic plasticity. We have used quantitative mass spectrometry-based phosphoproteomics to study protein phosphorylation in isolated rat nerve terminals (synaptosomes) that were at rest, depolarised for 10 s using KCl stimulation, or depolarised and allowed to recover for up to 15 min after depolarisation. The quantitative results show a prolonged, widespread reprogramming of the synaptic phosphoproteome, with an overrepresentation of exo- and endocytic proteins displaying long-term (15 min.) altered phosphorylation levels after high intensity depolarization. Kinase-substrate analysis indicated long-term per-synaptic effects of specific kinases after high, but not low intensity depolarisation. Additionally, key phosphatase inhibitor proteins showed differential phosphorylation upon depolarisation/recovery, offering another potential mechanism for the global phosphoproteome reprogramming. We therefore hypothesise that the initial short depolarisation changes the potential for subsequent neurotransmitter release on a longer time-scale via global changes in the presynaptic phosphoproteome. Exo- and endocytosis assays from rat synaptosomes as well as electrophysiological experiments in cultured hippocampal neurons are currently being performed to elucidate the functional effects of the global phosphorylation changes.

WTH09-12

Dorsal medial prefrontal cortex contributes to conditioned taste aversion memory consolidation and retrieval

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The medial prefrontal cortex (mPFC) is known for its role in decision making and memory processing, including the participation in the formation of extinction memories. However, little is known regarding its contribution to aversive memory consolidation. Here we demonstrate that the dorsal mPFC is involved in the retrieval of recent and remote long-term memory of a conditional taste aversion (CTA) task, and that neural activity and protein synthesis are required in this region to establish CTA memory. In addition, both NMDA receptor and CamKII activity in dorsal mPFC are needed for CTA memory consolidation, highlighting the complexity of mPFC functions.

WTH09-13

Does memory retrieval depend on precise synaptic organization?

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To investigate whether synaptic re-organization is required for memory trace, we developed a virus-based approach for inducible silencing and unsilencing of synaptic transmission. Our system is based on a novel destabilized tetanus toxin light chain variant (dsTeTxLC) with a half-life time of 3 min instead of 6 days. We targeted the dentate gyrus (DG) granule cells of rabbits to investigate the role of DG-CA3 circuit in trace classical eyeblink conditioning. Electrophysiological recording of DG-CA3 synapses during conditioning showed learning-dependent increases in field excitatory post-synaptic potentials (fEPSPs). After conditioning, we silenced synaptic transmission (ST) of the DG mossy fiber (DG-MF) input onto CA3 neurons. Conditioned responses were impaired after silencing of ST, and there was a decrease in fEPSP amplitudes. However, after un-silencing of ST, both fEPSP and memory retrieval were recovered. In spite of prolonged silencing of the ST, the previously formed memory trace was not lost. TeTxLC expression induced a reduction in the pre- and postsynaptic markers of the DG-CA3 synapse; VGlut and PSD95 puncta corresponding to the DG-MF boutons and CA3 spines were both reduced $\geq$ 20%. Imaging of DG-MF boutons in organotypic cultures before and after silencing of ST showed that their distribution remained stable throughout a period of several days. With large reduction in VGlut and PSD95 puncta after silencing of ST, it is possible that there is a strong reduction in the synaptic weight parameters. Thus, after silencing of ST, memory traces can be re-assessed in a new assembly of reorganized synaptic weights.

WTH09-14

Role of RyR2-mediated calcium release in synaptic plasticity, learning and memory in control and AD-model rats

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Introduction: Ryanodine receptors (RyR) are present in many cell types as components of intracellular Ca^{2+} storage organelles. By mediating intracellular Ca^{2+} release, RyR channels play key roles in

cellular Ca^{2+} signaling processes. The three mammalian RyR isoforms (RyR1-3) are present in the endoplasmic reticulum of hippocampal neurons. We reported previously that the Alzheimer's disease (AD) associated amyloid beta oligomers (AβOs) down regulate RyR2 expression and decrease structural plasticity in primary hippocampal neurons. Here, we determined the associated changes in RyR mRNA and protein levels by q-PCR and Western blot analysis, respectively, in rat hippocampal slices after theta burst stimulation (TBS) to induce LTP. We investigated as well whether *in vivo* hippocampal injection of antisense anti-RyR2 oligonucleotides or AβOs down regulates RyR2 protein content and disrupt spatial learning and memory.

Results: RyR inhibition prevented LTP induction by TBS. A significant increase in RyR1/RyR2 mRNA levels and RyR2 protein content occurred 1 h after LTP induction, while RyR3 mRNA and protein levels remained unchanged. These findings suggest that LTP induction requires functional RyR and that LTP-associated neuronal activity promotes fast increases in RyR2 expression. In addition, rats injected intra-hippocampus with AβOs or anti-RyR2 oligonucleotides exhibited decreased RyR2 protein content, without alteration in RyR3 protein levels, and displayed impaired performance in learning and memory tasks. We interpret these results as an indication that RyR2-mediated Ca^{2+} release is crucial for LTP induction and spatial memory processes, and suggest that deficient RyR2-mediated Ca^{2+} signaling contributes to AβOs-induced learning and memory deficits.

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WTH09-15

The role of action potential firing in metaplasticity

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Distinct patterns of inter-neuronal communication, encoded via action potential (AP) firing, can drive synaptic plasticity such as long-term potentiation (LTP), a neurobiological substrate of at least some types of memory. Recent evidence demonstrated that postsynaptic action potential firing alone facilitates subsequent LTP induction, suggesting a role of AP firing in regulating future plasticity, i.e., metaplasticity. However, cell firing has also been shown to inhibit later LTP. With the aim of unravelling this inconsistency, we recorded *in vitro* field potentials from CA1 stratum radiatum of 400 μm slices taken from 6 to 8 week-old male Sprague-Dawley rats. LTP was induced with 1 train (10 bursts), or a 0.5 train (5 bursts) of theta-burst stimulation (TBS). In an attempt to specifically activate multiple CA1 pyramidal cells postsynaptically without synaptic activation, antidromic priming stimulation (3 trains of TBS, 3 × 3 trains of TBS, or 2 trains of 100 Hz) was delivered to the alveus, 30 min prior to LTP induction. LTP induced with 1 TBS was significantly greater in slices receiving 3 × 3 TBS priming stimulation ((n = 9): 28.9 ± 3.6%, p = 0.02) compared to unprimed control slices ((n = 9): 17.1 ± 4.2%), measured 1 h following LTP induction. Other priming protocols were not sufficient to significantly facilitate LTP (3 TBS (n = 4): 27 ± 5.2%, p = 0.25; 100 Hz (n = 7): 22.6 ± 4.1%, p = 0.48). However, slices primed with 3 trains of TBS displayed a trend towards facilitated LTP maintenance, suggesting the possibility of an enhanced late phase of LTP. Surprisingly, LTP induced with 0.5 TBS was not significantly affected by the 3 × 3 TBS priming protocol (Primed (n = 7): 21.3 ± 5.9%; Control (n = 6): 14.2 ± 4.9%, p = 0.35. Overall, these data provide support for a role of AP firing in generating metaplasticity which appears to be highly sensitive to the pattern of cell firing and the strength of the LTP induction protocol.

WTH09-16

Serotonin - more than a neurotransmitter: impact of serotonylation of extracellular matrix proteins on neuronal plasticity

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Serotonin (5-HT, 5-hydroxytryptamine) is synthesized in enterochromaffin cells in the gastrointestinal tract and taken up into blood platelets. Contact of platelets to a site of vascular injury leads to release of 5-HT followed by platelet adhesion and aggregation. Therefore, a subpopulation of platelets, so-called "coated-platelets", retain high levels of procoagulant proteins such as fibrinogen, von Willebrand factor, factor V and thrombospondin on the cell surface. This process requires transglutaminase-mediated incorporation of 5-HT into specific acceptor proteins and is termed "serotonylation". Tight interaction of serotonylated proteins with specific 5-HT binding sites on fibrinogen and thrombospondin contribute to thrombus formation.

Considering the non-synaptic release of 5-HT in the brain and the role of 5-HT in thrombus formation, one may hypothesize that also in the CNS transglutaminase (TGases) transamidate extracellular serotonin to neuronal and glial cell surface exposed proteins and/or proteins of the extracellular matrix. Tight interactions between serotonylated proteins and 5-HT binding proteins may be important for the formation of proteinaceous networks. Such networks may then contribute to cell–cell contact formation and the generation of new synapses or the consolidation of already present synapses.

With respect to this hypothesis we provide data that TGases also covalently incorporate extracellular serotonin to neural proteins thereby enhancing extracellular protein expression. Moreover, we show that the catecholamines dopamine (DA) and noradrenaline (NA) inhibit serotonylation and that DA and NA themselves can be selectively transamidated into neural proteins by TGase. All three biogenic monoamines also block TGase-mediated transamidation of another monoamine, monodansylacadaverine, suggesting a general mechanism of TGase-mediated "monoaminylation" of neural proteins.

WTH09-17

Activity-dependent nuclear import and association of jacob with nuclear compartments

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Jacob is a protein that encodes and transduces the synaptic and extrasynaptic origin of the GluN2B-containing NMDARs to the nucleus and couples NMDAR activity to CREB-dependent gene expression (Karpova *et al*., 2013). The precise mechanisms of Jacob's nucleocytoplasmic shuttling, association with particular nuclear loci and subcompartments as well as nuclear residing-time remains unresolved. Here we show that the nuclear residing-time of Jacob is tightly regulated by nuclear calcium levels. Moreover, Jacob harbours a functional nuclear export signal which is involved in the active export of Jacob from the nucleus via a canonical CRM1- and RanGTP-dependent mechanism. Using pre-embedding immunogold and cryosection labeling methods for electron microscopy we detect Jacob-silver enhanced gold particles in association with the inner nuclear membrane (INM). Quantitative immunocytochemical experiments revealed that Jacob's association with the INM is dependent on NMDAR activity. Enhanced synaptic activity results in increased association of Jacob with nuclear splicing compartments. Immunogold EM revealed an association of Jacob with nucleoli and Cajal bodies. Double immunofluorescence stainings with the fibrillarin (a nucleoli marker) and p80-coilin (a Cajal body marker) confirmed an association of Jacob with both structures. In a yeast-two hybrid screen we identified AIDA-1D as a binding partner of Jacob. AIDA-1D shuttles to the nucleus in activity and NMDAR-dependent manner where it induces Cajal body-nucleolar association. We found that both Jacob and AIDA-1D are highly enriched and co-localized in dendritic spines and at Cajal bodies. Using in-situ 5-fluorouridine incorporation assay we found that Jacobs presence at the Cajal bodies correlates with enhanced levels of non-coding RNA biogenesis. We hypothesize that the AIDA-1D/Jacob interaction is involved in activity-dependent formation of Cajal body-nucleolar association and non-coding RNA metabolism.

WTH09-18

Early BDNF/c-Fos cascade in the retrosplenial cortex is required for the persistence of a long-lasting aversive memory

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During the past few years there has been growing interest in the role of the cortex in memory processing. In the present work, we studied the early posttraining participation of retrosplenial cortex (RSC) in the formation and storage of a long-lasting memory, and the molecular changes that take place in this brain region during memory storage. We found an increase in c-Fos levels in the anterior part of the RSC (aRSC) after inhibitory avoidance (IA) training. Interestingly, this increase was associated with memory durability, since blocking c-Fos expression using specific antisense-oligonucleotides (ASO) impaired long-lasting retention 7 days after training without affecting memory expression 2 days after training. In addition, we showed that BDNF is one of the upstream signals for c-Fos expression required for memory persistence. We found that injection of BDNF around IA training into aRSC was sufficient to establish a persistent memory and that this effect was prevented by *c-fos* ASO infusion into the same structure. These findings reveal an early posttraining involvement of aRSC in the processing of a long-lasting aversive memory and also some of its key molecular components necessary for this process.

WTH09-19

Regulation of HDAC1 and HDAC2 following long-term potentiation

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Long-term potentiation (LTP) is an enhancement of synaptic transmission, thought to underlie memory. LTP persists up to a year *in vivo*, and this persistence critically requires gene expression. Histone deacetylases (HDACs) regulate gene expression and are implicated in LTP and memory. HDAC2 binding is enhanced at the promoter region of LTP related genes. Further, inhibiting HDAC activity around the time of LTP induction enhances LTP persistence. However, such HDAC activity has only been investigated *in vitro* and thus only during early-phase LTP. Additionally, HDAC inhibitor studies mainly utilise class I inhibitors which affect HDAC1 *and* HDAC2. Previously we reported that HDAC1 and HDAC2 centrally regulate gene networks generated 5 h and 24 h post-LTP induction, and have temporally distinct mRNA expression profiles. This suggests these HDACs act in discrete phases after LTP induction, potentially controlling specific gene expression profiles and stabilisation of LTP. We investigated the activity and protein expression of HDAC1 and HDAC2 in dorsal dentate gyrus (DG) of male Sprague-Dawley rats. We compared sham-stimulated controls with the stimulated and control hemispheres of animals in which LTP had been induced in the DG. HDAC immunoprecipitation/fluorometric activity assays showed HDAC1 (fold change (FC)=2.6) and HDAC2 (FC=2) activity was increased in stimulated *and* control hemispheres of test animals 20 min after LTP induction relative to control animals ($p < 0.01$), while western blotting showed protein levels were unchanged. At 12 h after LTP induction, HDAC1 protein was increased in stimulated compared to control hemisphere of test animals (FC=2.36; $p = 0.01$) and activity levels were increased in stimulated hemispheres compared to control animals (FC=1.3; $p = 0.04$). These results identify bi-hemispheric changes in HDAC activity following LTP induction, and support reports of a second wave of protein synthesis and gene expression occurring around this time after learning. Our results suggest temporally distinct, subtype-specific phases of HDAC activity after LTP induction.

WTH09-20

Stem cell-derived serotonergic neurons: an in-vitro model for volume transmission and maintenance of serotonergic neuroplasticity

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Serotonergic neurons communicate with their neighbouring cells by volume transmission and release serotonin predominantly from extrasynaptic release sites. Among other important functions, serotonin has been shown to be involved in cognitive processes such as learning and memory formation. In the recent years we have applied stem cell-derived serotonergic neurons to gain insight into regulation of serotonergic neurotransmission as well as its role in mental diseases. In combination with fluorescent dyes such as fluorescent false neurotransmitters or fluorescent analogs of the neurotoxin MPP^+, these cellular models enable us to image and analyze serotonin release and re-uptake in live cell imaging experiments.

With regard to self-maintenance of the monoaminergic neurotransmitter systems in the context of mental diseases, the loss of serotonergic neuroplasticity is implied to play an important role in the pathogenesis of depression. Here, the neurotrophin Brain-Derived Neurotrophic Factor (BDNF) has been shown to be involved in the regulation of the serotonergic phenotype as well as outgrowth of serotonergic neurons. Therefore we applied stem cell-derived serotonergic neurons to gain insight into BDNF's role in differentiation of serotonergic neurons as well as BDNF-dependent signaling in the context of antidepressant treatment. First experiments showed that (a) endogenous and exogenous BDNF differentially effect neurite outgrowth from stem cell-derived serotonergic neurons, and (b) antidepressant treatment enhances BDNF-dependent signaling cascades in stem cell-derived serotonergic neurons.

WTH09-21

Role of 3′UTR polymorphism of MMP-9 in its regulation and local translation

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Matrix metalloproteinase 9 (MMP-9) plays pivotal role in synaptic plasticity underlying both physiological and pathological processes. Recent studies from our lab have shown that MMP-9 is locally translated at dendrites and synapses in response to synaptic stimulation and this process is controlled by Fragile X mental retardation protein (FMRP). FMRP is also involved in MMP-9 mRNA transport to the dendrites and spines. Since 3′UTR plays an essential role in mRNA transport to the dendrites and in its local translation, any polymorphisms in this region may affect synaptic availability of MMP-9, which in turn can influence the key processes in which MMP-9 is involved. Our preliminary results from rat neuronal culture indicate that there is a difference in MMP-9 activity levels depending on the rs20544 3′UTR polymorphism. Moreover, predicted for this SNP *in silico* 2D and 3D models of MMP-9 3′UTR demonstrated high probability of structural changes depending on the polymorphism, potentially altering interactions with RNA-binding proteins and short non-coding miRNA molecules targeting MMP-9 3′UTR. Preliminary RNA structure analysis suggests altered structure in the predicted fragile X mental retardation protein (FMRP) binding site. Currently we are probing MMP-9 3′UTR structures to verify the *in silico* models, as well as performing gel shift assay with purified FMRP and the two studied polymorphic MMP-9 3′UTR variants to study the impact of the SNP on FMRP binding. We are also using dye-quenched gelatin to measure MMP-9 activity at the synapse after stimulation depending on the SNP. Studying MMP-9 genetic variants can help us understand better MMP-9 regulation and mechanism of action and hopefully thanks to this – to have a better insight into the pathogenesis of many diseases in which the protein plays a role.

WTH09-22

Mild traumatic brain injury results in impairments of hippocampal synaptic plasticity and acute cognitive deficits in mice

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Traumatic brain injury has been recognized as a major health issue and receives therefore increasing attention. Traumatic brain injury has devastating acute effects and seems to initiate long-term neurodegeneration. Concussion or mild traumatic brain injury (mTBI) results in transient cognitive impairments in humans. Although not directly injured by the impact, the hippocampus has been found to be highly vulnerable to mTBI. In the days, weeks and months following mTBI, the hippocampus undergoes atrophy and severe changes in synaptic plasticity. Here we subjected adult mice to a single mild impact on the intact skull, just above the midline suture. Three days after trauma, we measured long-term potentiation (LTP) and long-term depression (LTD), the two most established cellular correlates of learning & memory and best investigated types of synaptic plasticity. Using *ex vivo* long-term field recordings in hippocampal CA1-region, we found that mTBI mice show an impaired LTP and slight changes in LTD as compared with sham-treated mice. In agreement with these deficits in synaptic plasticity, mTBI mice exhibited transient deficits in spatial memory as measured in the Morris water maze. These findings support a substantial impairment of hippocampal synaptic plasticity and cognition in response to even mTBI despite the lack of gross pathological changes in the hippocampus. Our data advocate LTP and LTD as sensitive tools for the development of new lead compounds and therapeutic targets for mTBI.

WTH09-23

HtrA1 - novel stress-regulated protease in mouse limbic system

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Psychological stress and fear evolved as an instinctive reaction, improving chances of survival in a changing environment. None-

theless, prolonged exposure to frightening stimuli may be the source of anxiety and mood disorders such as post-traumatic stress disorder and depression.

Disorders arising from adverse experiences result from improper forms of plasticity in specialized brain areas such as the hippocampus and amygdala. Such long-lasting changes require permanent alteration of neuronal properties including gene expression patterns and extracellular matrix (ECM) remodelling.

To understand the molecular mechanisms underlying stress-related neuroplasticity, we performed microarray analysis to search for novel genes regulated by stress in mouse amygdalae. One of the transcripts affected by stress is a gene which codes for the extracellular serine protease HtrA1. Proteases regulate neuronal morphology and function in a multilevel manner including ECM rearrangements and dendritic spine formation.

Taking advantage of immunohistochemistry, we characterized the expression of HtrA1 in the limbic system. In the hippocampus, we found HtrA1 to be present mostly in the extracellular space with localization reminiscent of perineuronal nets (PNN), whilst in the amygdala HtrA1 is present mostly in neuronal cell bodies. Detailed characterisation revealed that HtrA1 is expressed in ~60% of amygdala neurons. Among them ~75% consist of the glutamatergic projection neurons. We also found that HtrA1 co-localizes with its substrate aggrecan, the ECM and PNN component involved in regulation of neuroplasticity. Upon psychological stress cFOS positive, activated neurons showed significantly reduced amounts of HtrA1 protein suggesting its release into extracellular space.

Distinct localisation of HtrA1 in the hippocampus and amygdala seems to be related to their various functions. Short-term memory processed by the hippocampus requires high rates of neuroplasticity associated with a relaxed ECM, allowing spine/synapse formation, while long-term memory storage in the amygdala requires activation of small subpopulations of neurons, triggering only local rearrangement of the ECM.

We hypothesise that HtrA1-positive amygdala cells containing PNN represent neurons kept in the poised state for long-term memory storage. Activity-dependent release of HtrA1 may trigger relaxation of PNNs, promoting spinogenesis and novel memory formation.

WTH09-24

Seizure-related gene 6 (SEZ6) family proteins regulate excitatory synapses with effects on learning and memory

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Mice lacking Sez6 throughout development display dendritic branching abnormalities and fewer spines on pyramidal neurons. The persistence of Sez6 expression, and that of related family members Sez6L and Sez6L2, suggests an ongoing role for these proteins in the mature brain including the cortex and hippocampus. Transmembrane isoforms of Sez6, Sez6L and Sez6L2 are cleaved by β-secretase to produce a shed ectodomain which may be important for their function. AIMS: Determine (i) if Sez6 expression is required for normal synaptic function in adulthood, (ii) the effect of Sez6 family (Sez6/Sez6L/Sez6L2) knockout on synapse function and behaviour, and (iii) whether β-secretase processing is necessary for the synapse-promoting actions of Sez6 family proteins. METHODS: To investigate the role of Sez6 in adulthood we used a tamoxifen-inducible conditional KO (Sez-6 flox/KO x CaMKIIαCreER^{T2}) in which Sez6 is deleted in pyramidal neurons. Sez6 family triple KO mice underwent behavioural testing and provided neurons for β-secretase inhibitor studies in culture. Behavioural tests included rotarod, Y maze, open field, and context fear conditioning. Excitatory synapses were counted when punctate immunostaining for pre- and post-synaptic markers overlapped. RESULTS: Sez6 cKO and control mice performed similarly in the Y maze with visual cues, but differed in their response to context fear conditioning. Sez6 cKOs had decreased PSD-95 labelled puncta in hippoampal CA1. Sez6 family KO mice display motor coordination deficits as previously reported and decreased movement in the open field. When a β-secretase inhibitor was applied to cultured neurons (after the development of dendrites), preventing the production of shed Sez6 family ectodomains, a decrease in synapse number was observed in wild-type but not Sez6 family knockout neurons. CONCLUSIONS: Sez6 family proteins play important roles in synapse formation, maintenance and behaviour.

WTH09-25

Transient effect of X-irradiation and carbon ion-irradiation on synaptic function

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Neurons are known to be radio-resistant because they are post-mitotic. Carbon-ion therapy becomes widely known and is used as the advanced therapy for cancer. Although carbon-ion irradiation is thought to show a higher biological effectiveness compared to the X-irradiation, the acute immediate effect on the brain is poorly understood. In this study, we compared the acute effects of X-ray and carbon-ion irradiation on fear memory formation and drebrin accumulation. We used 10–12 week old male mice, and administered single dose of 10 Gy of either X-ray or carbon-ion beam to whole brains. Then fear conditioning test was conducted at 7 and 24 h after the irradiation. We found that the mice irradiated by either X-rays or carbon-ion beam at 7 h before training did not retrieve the contextual and auditory memories, but those irradiated at 24 h before training did retrieved the both memories. We analyzed drebrin intensities on neuropil of dentate gyrus (DG) of hippocampus and the number of doublecortin in the DG and we found there were significant decreases of drebrin intensities at 2 and 8 h and it returned to the normal level at 24 h after the irradiation, while the number of doublecortin were decreased at 8 h and still remained decreased at 24 h after the X-irradiation. Interestingly, the drebrin clusters were also decreased with a similar time course *in vitro* study. When we analyzed the number of drebrin clusters after we irradiated the mature primary hippocampal neurons, the number of drebrin clusters significantly decreased at 2 and 8 h after X-irradiation and returned to the normal level after 24 h. Similarly, the number of drebrin clusters significantly decreased at 2 h after carbon-ion irradiation. These results suggest that there are transient effects on the synaptic function of both X-irradiation and carbon-ion irradiation and these may cause fear memory deficits.

WTH09-26

The spinal cord that changes itself: spontaneous recovery of interneurons after incomplete spinal cord injury

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An injury to the spinal cord leads to devastating dysfunctions in sensory and motor systems. Regardless of the degree of damage, however, some recovery of function nearly always occurs, even in the absence of applied interventions. The specific cellular mechanisms underlying this spontaneous recovery of function, in the transition from acute to chronic stages of injury, are unknown. Here we use whole-cell patch clamp electrophysiology in a hemisection model of SCI in adult mice to characterize the changes in intrinsic and synaptic properties of deep dorsal horn (DDH) interneurons at two time points after SCI. Male mice (C57Bl/6; ~P63) received a spinal hemisection (T9-10) and were allocated to short-term (4 wks recovery) and long-term (10 wks recovery) SCI groups. Age matched mice served as uninjured controls. After the recovery period, mice were sacrificed and horizontal spinal cord slices (T6-T12, 250 μm thick) were prepared for whole cell patch clamp electrophysiology. Important passive membrane properties (rheobase current and resting membrane potential) are altered in the short-term after SCI, but recover to uninjured control levels in long-term SCI. The expression of several voltage-gated subthreshold currents, and the AP discharge patterns they shape, are also reorganized in short- and long-term SCI. Recordings of spontaneous excitatory postsynaptic currents (sEPSCs) show excitatory synaptic inputs onto DDH interneurons are significantly restructured in long-term SCI mice. sEPSC amplitude vs. rise-time plots suggest considerable dendritic expansion or synaptic reorganization occurs in DDH neurons during long-term recovery from SCI. Together these data demonstrate that substantial plasticity in the intrinsic and synaptic properties of DDH neurons occurs after SCI, which appears to stabilize by 10 wks after the initial injury.

WTH09-27

The extracellular matrix affects surface expression of GluN2B containing NMDA receptors

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In late postsynaptic development when neuronal networks are established, a drop in structural plasticity can be observed. On the molecular level, neurotransmitter receptors are recruited and stabilized at synaptic contacts, which determine the mature properties of synaptic transmission. Key events are changes in the subunit composition of NMDA receptors from heterodimeres containing mainly GluN1/GluN2B subunits to heterodimeres containing predominantly GluN1/GluN2A subunits. This switch influences the plasticity of the synapses. At the same time the brain-specific form of the extracellular matrix (ECM), the so-called perineuronal net (PNN) is formed, which marks the end of the so-called juvenile plasticity. The PNNs stabilize synaptic contacts and alter receptor mobility on the neuronal surface. With regard to these results and the overlap in timing of these two developmental changes, we hypothesize an influence of the ECM on the characteristics of the GluN2B containing NMDARs. We first tested whether enzymatic digestion of the ECM by hyaluronidase changes the surface expression of GluN2B. We could show that there is an up regulation of the GluN2B subunits on the surface of cultured hippocampal neurons after removal of the ECM. This increase in surface expression rises from an altered phosphorylation state of GluN2B followed by a decreased endocytosis. Further experiments demonstrated that this effect is β1-integrin dependent. Taken together, these results suggest that the subunit switch of NMDA receptors may be the basis of the observed increase in neuronal plasticity after ECM removal.

WTH09-28

G9A governs mGluR-LTD by regulating NSF/GluR2 dependent trafficking of postsynaptic AMPA receptors

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Synaptic plasticity mechanisms such as long -term potentiation (LTP) and long -term depression (LTD) are the cellular correlates of learning and memory. *N*-methyl-D-aspartate receptor dependent LTD (NMDAR-LTD) and metabotropic glutamate receptor dependent LTD (mGluR LTD) are the two forms of LTD that contribute to memory mechanisms at cellular level. The role of translation and transcription in LTP and LTD is well studied but the role of transcriptional regulators in memory is less well understood. G9a, a methyltransferase, has been reported to regulate the gene transcription in the hippocampus during memory consolidation. G9a controls a prominent Histone H3 lysine 9 dimethylation (H3K9me2). In the present study, we have investigated the role of G9a on mGluR-LTD in CA1 region of acute rat hippocampal slices using long-term functional plasticity methods and elucidated the mechanism of regulation of mGluR-LTD. Our preliminary results show that G9a inhibition prevents mGluR-LTD and increases the levels of protein kinase M zeta (PKMζ), a critical plasticity protein (PRP) necessary for the maintenance of LTP. Associated with the increased level of PKMζ, the phosphorylated GluR2 subunits of AMPA receptors were also high. Our results suggest that G9a is a negative regulator of plasticity that act as a molecular switch for mGluR-LTD induction and maintenance by inhibiting PKMζ, thereby regulating the *N*-ethylmaleimide-sensitive factor/GluR2 (NSF-GluR2) -dependent trafficking of postsynaptic AMPA receptors.

WTH09-29

Functional deficit of IQSEC2, a known intellectual disability gene, disrupts normal dendritic spine morphogenesis

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There is considerable genetic and phenotypic heterogeneity associated with intellectual disability, specific learning disabilities, ADHD, autism and epilepsy. Our laboratory has been involved in

identifying genetic causes of ID, focusing on genes of the X-chromosome including the IQ motif and SEC7 domain containing Protein 2 (*IQSEC2*) gene. The disease spectrum due to mutations in *IQSEC2* continues to expand with more than 15 distinct mutations contributing to non-syndromic ID though to early onset seizure phenotypes in affected male, and in some cases, female patients. The pathogenesis underpinning these mutations is not known. Here we report our investigations on the role of *IQSEC2* on the plasticity of dendritic spines. A lentiviral shRNA approach achieved a 57% ablation of *Iqsec2* expression in primary hippocampal cell cultures from mice, modeling partial loss-of-function mutations. Investigating gross morphological parameters after 8 days of *in vitro* culture (8 DIV) identified a ~ 32% reduction in primary axon length, and 27% increase in the number and 31% increase in complexity of dendrites protruding from the cell body. Focusing on the development of dendritic spine structures at 15DIV there was an increase of 34% in the number of protrusions per dendritic segment compared to control with the proportion of immature filopodia to mature spines similar across all treatments. By 21DIV the number of dendritic spines had normalised between the controls and ablation groups but showed a concomitant reduction in the number of immature spines with Iqsec2 ablation. In contrast to this increased complexity and spread of dendritic neurons with ablation of Iqsec2, overexpression of IQSEC2 WT leads to neurons with shorter axons that are more compact and display simpler dendritic branching. These observations provide evidence of dosage sensitivity for this X-chromosome gene that normally escapes X-inactivation in females and links these disturbances in expression with alterations in the morphology of developing neurons.

WTH09-30

EGF downregulates presynaptic maturation and suppresses synapse formation *in vitro* and *in vivo*

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Neuronal differentiation and synapse formation are regulated by various growth factors. Here, we show that epidermal growth factor (EGF) negatively regulates presynaptic maturation and synapse formation. EGF maintained axon elongation by enhancing actin dynamics at the growth cones. EGF decreased the levels of presynaptic molecules and presynaptic puncta in culture. The attenuation of functional synapses due to the presynaptic actions was confirmed by electrophysiology. *In vivo* analysis revealed that EGF downregulated presynaptic maturation in the neocortex. Further, ErbB1 inhibitors upregulated presynaptic molecules, suggesting endogenous EGF suppresses presynaptic functions. Morphological analysis by confocal and electron microscope indicated EGF decreased the numbers of synaptic structures but not their sizes. These findings suggest that EGF enhances growth cone motility and thus maintains continuous axon elongation and suppress appropriate synapse formation.

*Equal Contribution.

WTH09-31

The activity of caspase-3 is essential for development of brain cortex in rats exposed to prenatal stress

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The present work was designed to analyze the changes in caspase activity, brain plasticity and memory both in control rats and in rats subjected to prenatal stress (hypoxia on E14; 7%O_2; 3 h). We have found that prenatal hypoxia leads to overexpression of active form of caspase-3 and its enhanced activity in the neocortex of P20-30 pups. Synaptopodin is a protein localized in the spine apparatus of dendritic spines. It is known to be essential for the rearrangement of spine cytoskeleton, LTP and memory. We found that in adult animals, subjected to hypoxia, there was a reduced level of synaptopodin protein in the brain structures. However, there were no changes in the amount of a postsynaptic protein PSD95, or of presynaptic protein synaptophysin, suggesting that prenatal hypoxia had no significant changes in the number of postsynaptic terminals or in the total synaptic activity.The decrease in the number of labile synaptopodin-positive dendritic spines in the cortical areas of adult hypoxia-exposed rats was accompanied by disruption of memory. We have analysed the effect of *i.v.* injection of caspase-3 inhibitor to normal pups on P18-23 and found that it resulted in an overproduction of this enzyme and was accompanied by degradation of postsynaptic proteins and memory deficit in adult rats. However, injections of these inhibitors on P18-23 to hypoxic rats led to the decrease in caspase-3 activity, restoration of postsynaptic proteins expression as well as of distribution of the labile spines and restoration of memory in adult rats. The data obtained suggest involvement of caspase-3 in normal development of the postsynaptic terminals in the brain. Our data also suggests that alterations in caspase activity in early postnatal development of the brain might affect neuronal plasticity and cognitive functions in adulthood.

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WTH09-32

Paternal environmental enrichment has no transgenerational effect on offspring spatial memory

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Recent studies have demonstrated that paternal stress can result in modification of their offspring depression and anxiety-related phenotypes. Environmental enrichment is an experimental paradigm which reduces stress-response in animals. Enrichment is also associated with enhancement of cognitive performance of rodents. Maternal environmental enrichment results in offspring with improved stress response and enhanced learning and memory abilities. This project aimed to investigate the transgenerational influence of paternal environmental enrichment. We hypothesized that offspring born to enriched sires would show improved cognitive performance in tests for spatial working memory. Environmental

enrichment comprised of adult male mice housing in bigger cages supplemented with toys, ladders and shredded paper for 4 weeks. Controls were housed in standard laboratory cages. After 4 weeks, mice were mated with naïve females. When offspring were 8–12 weeks of age, they were tested on Y-maze and Morris Water Maze (MWM). In the Y-maze, both female and male mice show no differences between paternal enrichment and controls in the novel arm preference, entries and latency to enter the novel arm. Similarly, in the MWM, male mice displayed similar results in both the short-term probe and the long-term probe for time spent in the platform quadrant. Thus, in contrast to maternal enrichment, paternal enrichment exerts no transgenerational benefit on offspring spatial learning and memory. However, broadening the range of tests could yield differences.

WTH09-33

Teasaponin improves leptin sensitivity in the prefrontal cortex of obese mice

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Purpose: Obesity impairs cognition, and leptin-induced increases in neurogenesis and expression of brain-derived neurotrophic factor (BDNF). Tea consumption improves cognition and increases brain activation in the prefrontal cortex. This study examined whether teasaponin, an active ingredient in tea, could improve memory and central leptin effects on neurogenesis in the prefrontal cortex.

Methods: C57/Bl mice were divided into lab chow fed control (LC) and high fat diet-induced obesity (DIO) groups. A subgroup of DIO mice was treated orally with teasaponin to examine recognition memory with novel object recognition (NOR) tests. Another subgroup of DIO mice was injected intraperitoneally with teasaponin to test its effects on leptin signaling and leptin-induced neurogenesis in the prefrontal cortex. Cultured prefrontal cortical neurons pre-treated with leptin to induce BDNF expression and neurogenesis were treated with either palmitic acid or a combination of teasaponin and palmitic acid, and then examined by IHC and RT-PCR.

Results: Oral teasaponin treatment significantly improved the memory of DIO mice ($p = 0.027$) in the NOR test. Intraperitoneal teasaponin improved downstream leptin signaling in the JAK2 and STAT3 pathways, and leptin-induced BDNF expression in the prefrontal cortex of DIO mice. Cultured prefrontal cortical neurons pre-treated with leptin showed increased neurite outgrowth, and expression of post-synaptic density protein 95 (PSD-95) and BDNF. Treatment with palmitic acid abrogated the leptin-induced effects. However, treatment with teasaponin significantly increased the leptin effects on neurite outgrowth ($p < 0.05$), and PSD-95 ($p < 0.05$) and BDNF expression ($p < 0.05$).

Conclusion: Teasaponin improves obesity associated memory deficit and central leptin effects in the prefrontal cortex *in vivo*. Furthermore, *in vitro* observations show that teasaponin sensitizes leptin activity within the prefrontal cortex. Therefore, teasaponin supplementation may be useful for treating obesity-associated neurodegenerative disorders by improving prefrontal cortical function.

WTH09-34

Plastic changes of the early spinal cord in mice with the genetic absence of corticospinal tract

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Spinal cord maturation is followed with the corticospinal inputs. In order to understand how much the early events in the spinal cord are influenced by the arrival of corticopsinal axons, we studied the monosynaptic elimination, the expression of activity-related genes, neurotrophins and their receptors from postnatal day (P) 0 to 21, using *Celsr3|Emx1* mice, in which corticospinal axons never reach the spinal cord. The corticospinal tract was specifically labeled by anti-PKCγ antibody in the dorsal funiculus, showing gradually increased from P0 to P21 in the control, but never visible in the mutant. Using anti-parvalbumin and ChAT double immunostaining, close contacts of proprioceptive fibers and spinal monotoneurons appeared at P0 and were gradually eliminated thereafter, showing no difference between control and mutant mice. The number of parvalbumin-positive interneurons behaved an increased trend from P7 to P21 and an elevation of c-Jun protein was seen at P7, but there was no significant difference at each time-point between two groups. In control samples, CNTF protein was increased at P7 and gradually decreased after P14, which fluctuation from P0 to P14 was not found in mutant samples, and reached comparable levels at P14 and P21 between two groups. The expression patterns of NT3, truncated and full-length TrkC were similar in the control: increasing at P7 and decreasing after P14. In contrast, a significant increase of these neurotrophins was not found at P7 in the mutant. The similar fluctuation of BDNF, TrkB, GDNF and $p75^{NTR}$ proteins was present in two genotypes, such as BDNF increased at P7 and decreased at P14, the ratio of truncated to full-length TrkB increased after P14, GDNF increased at P14, and $p75^{NTR}$ gradually decreased after P7. In conclusion, the elimination of monosynaptic contacts and the changes of the expression of activity-related genes, neurotrophins and their receptors happen during the critical period, but these events are not highly dependent on the corticospinal inputs, except for the expression of CNTF, NT3 and TrkC.

WTH10 Neuronal Polarity

WTH10-01

Manipulation of the actin cytoskeleton promotes neurite outgrowth on inhibitory substrates

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Neurite outgrowth is highly dependent on the underlying actin cytoskeleton. Actin filaments are polymers built through the controlled dynamic assembly of monomeric actin. Most actin filaments contain a second polymer composed of tropomyosins which controls the access and activity of other actin-associated proteins, thereby determining assembly and disassembly rates, cross-linking and stability of the filaments. In neurons products from three tropomyosin genes are found (TPM1, 3 and 4). Previously, we have found that that the overexpression of tropomyosins is sufficient to induce the formation of neurites in rat B35 neuroblastoma cells and that this is associated with an upregulation of the neuronal differentiation marker MAP2c. Furthermore, tropomyosins differentially control the extension and branching of neurites in dibutyryl cyclic AMP stimulated B35 cells.

We have now carried out RNAseq of B35 neuroblastoma cells, overexpressing the major TPM1, 3 or 4 gene products (TPM1.12, TPM3.1 and TPM4.2. respectively) to identify tropomyosin induced changes in the transcriptome. Cluster analysis of the data revealed significant changes in genes involved in the regulation of cell morphogenesis and axonogenesis. We have further tested the potential of tropomyosin overexpression to overcome inhibitory cues for neurite outgrowth in primary cortical neurons. We found that the overexpression of TPM3.1 promotes neurite outgrowth on the inhibitory substrate Nogo66. Our data suggest a central role for tropomyosin in neuronal differentiation and neurite outgrowth and that the manipulation of actin filament populations through a modulation of tropomyosin expression levels may overcome inhibitory cues in the central nervous system.

WTH10-02

Contribution of NADPH oxidase (NOX) to the establishment of hippocampal neuronal polarity

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Basal production of Reactive Oxygen Species (ROS) by the NADPH (NOX) complex contribute to LTP, memory consolidation and neuronal actin cytoskeleton dynamics. Actin dynamics is regulated by members of the Rho family of GTPases, such as Rac1 and Cdc-42. Neuronal polarity establishment is a fundamental process during nervous system development which is intimately related with neuronal functions. However, the contribution of ROS during neuronal polarity has not been explored. In this work, we showthat physiological ROS produced by NOX complex contributes to the acquisition of neuronal polarity and axonal growth. We evaluated ROS production in primary cultured neurons and its involment in the establishment of neuronal polarity. Both ROS content and actin dynamics were visualized using genetically encoded biosensors. In addition, we used FRET biosensors to measure local activity of Rac1 and Cdc-42 after NOX inhibition. Our results suggest that ROS produced by NOX complex contributes to the acquisition of neuronal polarity by regulating actin cytoskeleton. We propose that physiological levels of ROS may be necessary for polarization and maturation of hippocampal neurons.

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WTH10-03

Slow axonal growth of human iPSCs-derived neurons

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Recent advances in human induced pluripotent stem cells (hiPSCs) offer new possibilities for biomedical research and clinical applications. However, the detail processes of neuronal development from hiPSCs have not been known. In this study we analyzed development of hiPSCs-derived neurons, particularly focusing on their early developmental stages. We cultured iCell Neuron (Cellular Dynamics International) and compared their development with that of the primary cultured neurons derived from rat hippocampus. In 2 days *in vitro* (DIV) culture of the rat neurons, according to the classification of Dotti *et al*. (1988), stage 3 neurons are characterized by one long neurite which is supposed to become an axon. In iCell Neuron, however, we hardly observed cells with one long neurite. To investigate if the axonal differentiation had occurred even though iCell Neuron did not have obvious longer neurite, we used anti-phosphorylated neurofilament (p-NF) antibody which is an axon marker. We found a few neurons with p-NF positive neuritis in DIV 2 iCell Neuron and this indicates that although the axonal growth speed of iCell Neuron is slower compare to that of rat neurons, the polarity of iCell Neuron is normal. We thus classified stage 3 neurons as the neurons which has p-NF positive neurites and calculated the ratio of each stage. The results showed that iCell Neuron had significantly smaller amount of stage 3 neurons compared to the rat neurons. The length of the axon was also measured and iCell Neuron had significantly shorter axons. Next, the speed of axonal elongation was measured and we found significantly slower elongation speed of axon in iCell Neurons. Finally we examined if this slow axonal elongation in iCell Neuron is because of the abnormality of the growth cones, and found no differences. Together, our study shows the growth of iCell Neuron axon is slower but its differentiation is normal compare to rat neurons.

WTH10-04

The interaction of calcium signalling and the cytoskeleton in navigating growth cones

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The coordination of calcium signalling at the neuronal growth cone is essential for axon guidance during development. Directed axon extension is regulated by the activity of calcium within the growth cone, which indirectly regulates the reorganisation of cytoskeletal structures. While the endoplasmic reticulum (ER) is an internal store of calcium, its role in the calcium dynamics of motile growth cones remains poorly understood. Stromal interaction molecule 1 (STIM1) is an ER calcium-sensing protein that is able to form highly selective calcium channels. The processes by which the ER is remodelled in navigating growth cones are unknown. It has been proposed that STIM1 interacts with microtubules in immune cells (Smyth *et al*., 2007). Previously, we have shown that STIM1 is necessary for the regulation of SOCE in turning growth cones in response to the calcium-dependent and independent guidance cues (Mitchell *et al*., 2012). The ability of STIM1 to regulate growth cone turning in response to diverse guidance cues might reflect a direct signalling mechanism with the cytoskeleton. Therefore we asked whether STIM1 interacts with the microtubule cytoskeleton. Our data demonstrate that reduced STIM1 expression, using STIM1-specific morpholinos, significantly reduces polymerisation of microtubules in rat dorsal root ganglia sensory neurons ($p < 0.001$). Recruitment of polymerised microtubules to the motile side of turning growth cones in response to guidance cues BDNF and Sema3a was also significantly perturbed when STIM1 expression was reduced ($p < 0.001$). Our data suggest that STIM1 is necessary for microtubule polymerisation and recruitment. We propose a model in which dynamic ER remodelling to the motile side of the growth cone requires STIM1-microtubule interaction. In this mechanism, asymmetric ER translocation would regulate the spatiotemporal release of calcium and the distribution of instructive calcium signalling in navigating growth cones.

WTH10-05

Regulation of alcadeinα association with kinesin-1 by phosphorylation of the cytoplasmic region

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Alcadeins/Calsyntenins constitute a family of neural type I membrane proteins, designated Alcα, Alcβ and Alcγ, which express predominantly or exclusively in neuron (*J. Biol. Chem.* [2003] 278, 49448), and subject to proteolysis primarily by ADAM10/17 at jaxtamembrane region, and secondly cleaved by γ-secretase at intramembrane region (*J. Biol. Chem*. [2004] 279, 24343). These cleavages secrete the large (sAlc) and small (p3-Alc) fragments into extracellular milieu along with the generation of intracellular cytoplasmic domain (ICD) fragment (*J. Biol. Chem.*[2009] 284, 36024).

Alcα among Alcs family functions as a cargo receptor of kinesin-1 which is composed of two heavy chains (KHC) and two light chains (KLC). Cytoplasmic region of Alcα binds KLC of kinesin-1, and this binding activates kinesin-1 to transport Alcα vesicles from cell body to nerve end (*EMBO J.* [2007] 26, 1475; *Traffic* [2012] 13, 834). However, it remains unclear how Alcα association with kinesin-1 is regulated and what function can Alcα ICD express in Alcα vesicular transport. We, herein, focused our analysis on the regulation of Alcα association with kinesin-1.

We found that the cytoplasmic domain of Alcα has many consensus sequence of phosphorylation, especially by casein kinase I and/or II. We demonstrated that Alcα cytoplasmic domain is phosphorylated in HEK293 cells expressing Alcα or Alcα cytoplasmic domain with [^{32}P] orthophosphate. Next we investigated the function of Alcα phosphorylation in the interaction with kinesin-1. Alcα-FLAG expressed in N2a cell are recovered with anti-FLAG antibody and subjected to dephosphorylation with λPPase. The dephosphorylated Alcα-FLAG was mixed with N2a cell lysate expressing HA-KLC1 and a binding ability was examined. The dephosphorylation of Alcα-FLAG decreased the binding with HA-KLC1. We explored phospholable amino acids, which regulate the interaction with KLC using procedures with deletion constructs and alanine-scanning mutagenesis into the candidate sites. Association of Alcα with kinesin-1 is regulated by phosphorylation, and the transport of Alcα vesicles may be regulated by phosphorylation and the function of Alcα ICD.

WTH10-06

Regulation of dendritogenesis by IMP1 depends on its phosphorylation at SER181

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IGF2 mRNA Binding Protein 1 (IMP1) is mRNA binding protein that controls ß-actin mRNA transport and translation in dendrites and acts as a translational repressor until phosphorylated by Src kinase [1].

We have previously shown that IMP1 regulates dendritic arbor formation during development of hippocampal neurons. For proper dendritogenesis, the following appeared crucial: accumulation of IMP1 at branching points, mRNA binding and release capacity [2].

It is known that mRNA release is regulated by Src kinase [3]. Is it the only kinase regulating IMP1? Here we demonstrate that phosphorylation of IMP1 at Ser 181 is mTORC2-dependent, which is in agreement with previously published data describing RD cells [3]. Based on this finding, we constructed non-phosphorable (S181A) and phosphomimicking (S181E) mutants of IMP1. We have observed that S181E but not S181A reversed morphological deficits caused by IMP1 knockdown. Therefore we showed that phosphorylation of IMP1 at Ser181 is needed for dendritic arborization of developing hippocampal neurons.

Next, we have investigated if Ser181 phosphorylation affects IMP1 distribution. We have shown that S181E, but not S181A mutant is distributed similarly to WT IMP1, which accumulates in dendritic branching points. Also, initial live cell imaging experiments indicate that this phosphorylation is important for targeting IMP1 anterogradely. Whether this phosphorylation is important for mRNA binding is yet to be investigated.

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WTH11 Animal Model of Neuropsychiatric Disorders (Part 2)

WTH11-01

Developmental vitamin D deficient rat model of autism spectrum disorder

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Background: Autism Spectrum Disorder (ASD) is a heterogeneous group of neurodevelopmental disorders with unknown etiology. ASD is characterized by behavioural abnormalities such as impaired social interaction, language difficulties and stereotyped repetitive behaviour. We are exploring the hypothesis that Developmental Vitamin-D (DVD) deficiency is a risk factor for ASD. A meta-analysis of 12 studies clearly shows the incidence of autism is higher in children of vitamin D deficient pregnant mothers. Two prominent epidemiological clues in ASD literature are that ASD is higher in mothers who experienced infection during pregnancy. Vitamin D is a potent anti-inflammatory agent. Vitamin D also has regulatory effects on steroidogenic enzymes such as aromatase that is involved in estrogen synthesis which has well known neuroprotective effects.

Method: To explore the neurobiology behind DVD-deficiency and ASD we have developed developmental vitamin D (DVD) deficient rat model of autism. We are examining the effect of low vitamin D on gene expression levels of all cytochrome P450 (CYP450) and non CYP450 enzymes involved in steroid hormone synthesis in brain and placental tissues of the DVD- deficinet rat model. Amniotic fluid was also collected at day 18 of gestation and is being subjected to quantitative analysis for steroid hormones by LC/MS/MS. Inflammatory cytokine profiles are being established in placenta and foetal brain.

Result: We studied gene expression of short and long form of aromatase in DVD pup brain and we found no difference in the gene expression of all studied groups. Inflammatory cytokines such as IL-6, IL-1β, TNF-α and IL-10 were also examined in the brain and placental tissues. IL-10 was not detectable and IL-6, IL-1β and TNF- α were not changed by maternal diet.

Conclusion: These results showed that maternal absence of vitamin D does not appear to affect one important steroidogenic pathway or produce a pro-inflammatory cytokine profile. We continue to examine other metabolic and steroidogenic pathways relevant to autism.

WTH11-02

The influence of pharmacogenetic excitation of vlPAG on pavlovian fear conditioning

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The ventrolateral periaqueductal gray (vlPAG) has been implicated in predictive fear learning, specifically in encoding fear prediction errors. How this role is achieved remains poorly understood. We investigated the role of the vlPAG during Pavlovian fear conditioning using an excitatory DREADD with a conditioned suppression measure of fear. Rats were microinjected with AAV5-SYN-hM3Dq-eYFP or AAV5-SYN-eYFP into vlPAG 3 weeks prior to fear conditioning. Experiment 1A tested the effects of pharmacogenetic excitation of vlPAG on acquisition of fear. Rats were injected intraperitoneally (i.p.) with 3 mg/kg clozapine-*N*-oxide (CNO) or vehicle 30 min prior to conditioning sessions. During conditioning rats were presented with a visual conditioned stimulus (CS) paired with a footshock unconditioned stimulus (US). Rats were then tested drug-free in extinction settings. Results: hM3Dq mediated excitation of vlPAG, impaired the acquisition of fear learning. Rats in hM3Dq-CNO group show reduced fear learning to the visual CS when compared to remaining groups. Experiment 1B tested the effects of pharmacogenetic excitation on fear extinction. Rats received fear conditioning of an auditory CS paired with a footshock US. CNO or vehicle injections were administered i.p. 30 min prior to extinction sessions. Results: hM3Dq mediated excitation of vlPAG augmented the extinction of fear learning. Rats in hM3Dq-CNO group showed enhanced extinction compared to the remaining groups. These findings show that hM3Dq-mediated excitation of vlPAG neurons impairs the acquisition but augments the extinction of fear learning and are consistent with vlPAG neurons signalling the expected outcome of a conditioning trial.

WTH11-03

Differential cognitive and behavioural impacts of lps and PolyI:C prenatal infections on adult female offspring

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Purpose: Maternal infection during pregnancy is a risk factor for offspring developing schizophrenia. Behavioural changes reported in adult offspring in animal studies are inconsistent due to variations in type, dose and timing of immunogen administration during gestation, and postnatal age examined. In this study, two different prenatal infection models, bacterial (lipopolysaccharide; LPS) and viral (polyinosinic-polycytidylic acid; PolyI:C), were investigated to determine the impacts on cognitive functioning of adult rat offspring.

Methods: Maternal Sprague-Dawley rats were injected intraperitoneally with saline solution, LPS, or PolyI:C at embryonic days 15 and 16, to approximate the second trimester in human pregnancy; a critical period for immunogen exposure in schizophrenia aetiology. Adult female offspring ($n = 12$) were treated with saline or olanzapine from postnatal day (PN) 90 for 5 weeks. From PN 118, cognition and behaviour of adult offspring were examined by open field (OF) and novel object recognition (NOR) tests. Cognitive functioning investigated in NOR tests was expressed as a discrimination index, reflecting recognition memory.

Results: Adult prenatal LPS offspring showed a 74% decrease in discrimination index compared to saline ($p = 0.011$) in the NOR test. Prenatal PolyI:C had no significant effect on discrimination index. Prenatal injection of PolyI:C significantly increased peripheral rearing activity of adult offspring in the OF test ($p = 0.034$). However, prenatal LPS had no significant effect on peripheral rearing activity. Olanzapine administration significantly decreased peripheral rearing activity during the OF test for prenatal saline ($p < 0.001$), LPS ($p < 0.001$), and PolyI:C ($p < 0.001$) offspring.

Conclusion: Bacterial and viral prenatal infections produce differing cognitive and behavioural effects in offspring. Cognitive deficit observed in prenatal LPS offspring suggests a risk of disrupted neurodevelopment. Prenatal PolyI:C-induced hyperlocomotor activity is reversed by chronic olanzapine treatment. Though the outcomes differ, both bacterial and viral prenatal infections appear relevant to schizophrenia aetiology in offspring.

WTH11-04

Methylmercury exposure induces oxidative stress, impairs social interaction and affects the behavior of drosophila melanogaster

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Our recent studies have suggested the use of Drosophila melanogaster (flies) as a model for studying neurobehavioral developmental disorders such as autism. Although the cause of autism is not known, the roles of prenatal/postnatal exposure to environmental factors and increased vulnerability to oxidative stress have been implicated in autism. Among all metals, the role of exposure to mercury from consumption of contaminated seafood during pregnancy, dental amalgams and thimerosal in vaccines remains a controversial issue in autism. In the flies, exposure to methylmercury increased free radicals generation in a concentration-dependent manner. It also decreased the total thiol content. These results suggest that methylmercury induces oxidative stress in flies. Methylmercury exposure also affected the negative geotaxis in a dose-dependent manner. In a T-Maze assay, when a choice was given to flies to move towards a dark arm (covered with aluminum foil) or to uncovered arm, the exposure to methylmercury increased the percentage of flies moving towards a dark arm in a dose - dependent manner. In a social space assay, distance between an individual fly and its closest neighboring fly was used as a measure of social interaction within the group. Mercury-treated flies were significantly apart from each other in a dose-dependent manner as compared to vehicle-treated control flies, indicating impaired social interaction of the flies with mercury exposure. It is suggested that environmental factors may act as trigger for interaction of genetically susceptible alleles in autism, and oxidative stress may serve as a common link between genes and environmental factors leading to behavioral abnormalities.

WTH11-05

Modelling white matter neuron pathology in schizophrenia using maternal immune activation

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Most neurons in the brain are located in the cortex and subcortical nuclei. A small percentage exist within the white matter of the brain. White matter neurons (WMNs) are less well characterised and consist of excitatory and GABAergic inhibitory neurons. Schizophrenia is underpinned by deficits in cortical GABAergic interneurons. Interestingly post mortem studies have identified increased WMN density underneath the cortex in schizophrenia. Modelling this pathology in rodents will be critical to understanding their functional significance. In this study we determined the effect of maternal immune activation (MIA), a risk factor for schizophrenia development on WMN density. MIA was induced in pregnant Wistar rats using an injection of Polyinosinic: polycytidylic acid (PolyI:C) at either gestational day (GD) 10 or GD19. WMN density was determined using immunohistochemistry and manual counting. Dual-label immunofluorescence was used to examine co-localisation of GABAergic markers. $NeuN^+$ WMNs were observed in the corpus callosum of untreated rats; some of which co-expressed known GABAergic markers; neuropeptide-Y, somatostatin (SST), parvalbumin and calretinin. The density of NeuN+ WMNs in the corpus callosum of GD19-PolyI:C treated rats was increased by 17% ($p = 0.07$) compared to sham controls. The density of SST^+ WMNs was increased by 25% ($p = 0.006$) in GD19-PolyI:C and GD10-PolyI:C treated rats compared to sham controls. These findings mirror those observed in the brain in schizophrenia. This study showed that MIA during prenatal development may have profound effects on WMN density, but how this relates to findings in schizophrenia requires further investigation.

WTH11-06

Rapid modulation of neuronal voltage-gated calcium channels by vitamin D

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Introduction: The secosteroid vitamin D [$1,25(OH)_2D_3$] drives *genomic* changes in the brain and body via classical steroid hormone pathways. While $1,25(OH)_2D_3$ is also known to drive *non-genomic* effects in some peripheral tissues, principally the rapid modulation of L-type voltage-gated calcium channels (VGCC), its non-genomic effects within the brain remain unexplored. Since developmental vitamin D deficiency is a risk factor for schizophrenia, and accumulating evidence links common L-VGCC genetic variants with neuropsychiatric disorders, we are investigating the non-genomic effects of $1,25(OH)_2D_3$ on VGCCs in the developing brain.

Methods: All methods were performed on acute coronal slices (300 μm), within the prefrontal cortex (PFC), collected from p8-12 BALB/C mice. Wide-field calcium imaging was time-locked to local fiber electrical stimulation, and utilised the Ca^{2+} indicator CAL-520. Nucleated patch recordings were visualised under IRDIC microscopy; where Ba^{2+} replaced Ca^{2+} as the charge carrier; and VGCC currents were isolated pharmacologically using TTX (1 μm) and 4AP (5 mM).

Results: Using two different methods, we show that 1,25 $(OH)_2D_3$ (0.1 nM) increases VGCC current amplitudes in a subset of neurons in the PFC, termed *vitamin D responsive neurons* (*VDRNs*). Bath application of $1,25(OH)_2D_3$ significantly increased somatic Ca^{2+} fluorescence levels in a subset of layer 1–3 cells (n = 28 cells, n = 8 slices, n = 5 animals), which was blocked by nifedipine. Nucleated patch recordings revealed that $1,25(OH)_2D_3$ significantly increased VGCC current amplitudes in a subset of layer 2/3 cells (n = 4/14). For both methods, the changes were rapidly induced (< 1 min) and long lasting (> 10 min).

Conclusion: These findings demonstrate that physiologically relevant levels of $1,25(OH)_2D_3$ rapidly modulate VGCCs in a subset of PFC neurons during development. Future experiments will investigate the molecular mechanism underlying this effect, as well as the identity, proportions, and distribution of these VDRNs. Since developmental L-VGCC activity is required for critical processes such as neuronal maturation and gene transcription, we will also investigate the consequences of vitamin D deficiency for VDRNs.

WTH11-07

Examination of mismatch negativity, oscillatory activity and related neurochemistry in a developmental rat model of Schizophrenia

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Subjects with schizophrenia exhibit alterations in mismatch negativity (MMN) and gamma oscillations. These features have been related to impairments in NMDAR signalling and inhibitory interneurons. In the current study, we investigated these and expression of markers of NMDAR and GABAergic neurotransmission in rats exposed to a risk factor for schizophrenia, maternal immune activation (MIA). MIA was induced in Wistar rats by administering pregnant rats with Poly (I:C), a viral mimic (or saline), at gestational day 19. In adulthood, oscillatory activity and MMN were assessed using wireless telemetric recordings in control and MIA offspring and brain tissue was collected from separate cohorts of adult animals to examine expression of NMDAR subunits and markers of GABAergic neurotransmission. Rats exposed to MIA did not exhibit changes in MMN, but exhibited reduced 50 Hz (gamma) power. MIA-exposed rats had increased ratio between NR2A and NR2B receptors in the hippocampus and auditory cortex. MIA-exposed rats had decreased mRNA for the interneuron marker and GABA-synthesis enzyme, GAD67 and the interneuron marker somatostatin. Similar reductions in GABAergic markers GAD67 and somatostatin also found consistently in post-mortem investigations in schizophrenia. These findings indicate that the late gestational MIA model is an excellent model for further investigation of the role of inhibitory neurotransmission in gamma activity.

WTH11-08

Neurobiological mechanisms linking obesity and depression

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Accumulating evidence suggests that inflammation and oxidative stress contribute to the pathophysiology of depression and may explain the increased frequency with which depressive illness occurs in individuals with somatic inflammatory disorders, such as obesity. To explore the common underlying mechanisms of these disorders, we used an animal model of diet-induced obesity (DIO) and examined the effects of high fat feeding and the development of obesity on behaviour. C57BL/6 male mice were fed a high-fat diet (45%kcal) or standard chow for 13 weeks followed by 4 weeks of drug administration (agents with known anti-inflammatory and antioxidant activity) treatment by oral gavage. Behavioural tests to measure depressive behaviour were conducted, and brain and peripheral tissues were collected for analysis. Initial analyses of diet-induced obese mice showed reduced consumption of sucrose in the sucrose preference test, a correlate of anhedonic behaviour common to depression. In addition, these obese mice demonstrated increased duration of immobility in the Porsolt Forced Swim Test, indicative of behavioural despair. These data indicate that a high fat diet is associated with in the induction of depression-like behaviour. The next phase of this experiment will be to examine the effects of novel agents which regulate inflammatory and oxidative stress pathways in this model, and have been shown to have clinical efficacy in depression, and to examine the biological pathways modulated by these agents in brain and other tissues.

WTH11-09

Broadband local field potential characteristics in rat cingulate cortex are predictive of high-effort, goal-directed behaviour

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Converging work on the anterior cingulate cortex (ACC) suggests that this region plays a critical role in motivating goal-directed behaviours, particularly by biasing actions towards goals

that present optimal effort:outcome payouts. ACC activity may therefore help prompt an individual to invest effort towards a goal, when the outcome is worth that effort. However it is not known whether small but significant variations in basal ACC activity predict individual variations in effortful goal-directed behaviour. Here we tested the hypothesis that "high effort" and "low effort" laboratory rats could be delineated prior to actual effort:outcome testing tasks, based on broadband ACC local field potential (LFP) characteristics. Sprague Dawley rats ($n = 6$) were chronically implanted with adjustable electrode arrays in ACC; bandpass filtered LFP activity is presented here. Each recording session consisted of an initial open field assessment, followed by one of three tasks: an effortful persistence task, a cost-benefit decision-making task, or an open field foraging task. Power spectral density analysis of ACC LFPs revealed significant power differences between "high effort" and "low effort" rats over multiple frequency bands both during the tasks, and during initial open field assessments. These preliminary findings suggest that "high effort" individuals can be identified via broadband LFP characteristics in ACC, prior to actual observable high-effort goal-directed behaviour.

WTH11-10

Maternal separation alters glucocorticoid signaling in the nucleus accumbens of female mice

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Early life stress (ELS) has been identified as a significant contributing factor in numerous neuropsychiatric disorders. In fact exposure to multiple traumatic early life events (4 +) increases the risk of developing alcohol use disorders 7 fold and post-traumatic stress disorders 2 fold. Women are particularly vulnerable; they are twice as likely to develop a disorder following ELS than men. Maternal separation is commonly used to model ELS in rodents. We adapted the maternal separation model to develop a method for identifying mice with abnormal basal plasma corticosterone levels. We then applied this protocol to examine the effects of maternal separation on glucocorticoid signalling in the nucleus accumbens (NAc) and amygdala. We found that maternal separation increased basal plasma corticosterone levels in female but not male mice. We then categorized female mice that had been exposed to ELS as having either high, normal and low basal plasma corticosterone levels and compared them to control mice. Plasma corticosterone levels were significantly elevated in high ELS and reduced in low ELS mice compared to control and normal ELS mic respectively. There was no difference between plasma corticosterone levels in normal ELS and control mice. We then measured glucocorticoid receptor (GR) and corticosterone levels in the NAc and amygdala. ELS mice with high basal plasma corticosterone also had elevated NAc corticosterone levels. No differences were identified in the NAc between low ELS, normal ELS or control groups. There was a trend for reduced NAc GR expression in both high and low ELS mice compared to normal ELS and control groups. The data suggest that maternal separation alters glucocorticoid signaling in the NAc in females and not males, which supports findings in human studies. Future studies will explore the effects of stress on glucocorticoid signalling following ELS.

WTH11-11

Prenatal infection promotes olanzapine-induced obesity in rats: implications for antipsychotic-induced obesity in Schizophrenia

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Purpose: Atypical antipsychotic drugs such as olanzapine and clozapine induce significant weight gain and obesity in schizophrenia and allied disorders. Prenatal infection is considered to be a severe impact to brain development which can lead to offspring developing late onset behavioural abnormalities. These abnormalities resemble some aspects of schizophrenia. This study aims to develop a rodent model mimicking patients of schizophrenia with metabolic syndrome after chronic treatment with olanzapine.

Methods: Pregnant SD rats were injected with saline, PolyI:C (polyinosinic-polycytidylic acid, 8 mg/kg), or LPS (lipopolysaccharide, 100 mg/kg) on gestation days 15 and 16. Each group was subdivided into a control and olanzapine group, and treated for 5 weeks from 13 weeks of age. All rats throughout the experiment were fed lab chow *ad libitum*. Body weight and food and water intake were measured twice a week.

Results: Prenatal PolyI:C and LPS rats gained weight quickly and developed obesity after olanzapine administration. The final body weight of the prenatal LPS group with olanzapine treatment was higher than the saline ($p = 0.002$) and LPS control groups ($p = 0.004$) respectively. Prenatal infection with LPS in addition to olanzapine significantly increased peri-ovarian adipose weight ($p = 0.001$). Analysis of hypothalamic neuropeptides relevant to body weight control and metabolism is currently ongoing.

Conclusion: For the first time, the viral and bacterial prenatal infection models successfully replicate antipsychotic drug-induced obesity and metabolic syndrome in SD rats. This model represents the diseased condition in humans brought on by prenatal viral or bacterial infection. This animal model will be used to study the mechanism of antipsychotic drug-induced obesity and metabolic side effects, and to identify therapeutic targets for treatment.

WTH11-12

Predatory stress elicits anxiety- and depressive-like behaviours in mice

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Animal models of chronic stress are frequently used to elucidate the mechanisms underlying the relationship between stress and affective disorders such as anxiety and depression. The current study expands upon a novel chronic psychological stress paradigm for mice (1): predatory stress, where the natural predator-prey association that exists among rats and mice produces an ethologically relevant stressful experience without risk of harm. Mice (male, C57Bl6, singularly housed, $n = 9$) were placed inside a clear plastic

hamster ball and then placed into the home cage of a large Sprague Dawley male rat for 30 min/day for 28 days. To avoid familiarity and habituation, chronically stressed mice were paired with a different rat on successive days. Control mice ($n = 10$) were singularly housed and kept in the same room as the stressed mice, but had no exposure to rats. Chronically predator-stressed mice showed impaired weight gain over the 4 week period ($p < 0.01$). Anxiety-like behaviour was exhibited in the elevated plus maze test with chronically-stressed mice spending significantly less time in open arms ($p < 0.05$). Stressed mice also expressed an increase in depressive-like behavior in the two-bottle sucrose preference test, consuming significantly less sucrose than controls on the second day of a 48 h preference test ($p < 0.05$); and the forced swim test with increased immobility times ($p < 0.05$). No significant differences were observed in a tail-suspension test ($p = 0.50$). These results demonstrate that predatory stress can elicit anxiety- and depressive-like behavioural changes comparable to other rodent models of chronic stress without long periods of restraint or chance of injury by social defeat. We suggest that this predatory stress model has strong validity as an animal model of depression.

(1) Barnum et al. Journal of Neuroinflammation 2012, 9:9.

WTH11-13

Effects of optogenetic stimulation of lateral versus ventrolateral periaqueductal gray on fear expression and fear learning

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The midbrain periaqueductal gray (PAG) plays a critical role in the regulation of fear and defensive behaviour. Here we used adenoassociated viral vectors (AAV) to express ChR2 or eNpHr3.0 in neurons of the lateral or ventrolateral columns of the PAG to examine the effects of optogenetic excitation versus inhibition on fear behaviour and fear learning. ChR2 stimulation of lPAG resulted in short-latency (< 25 ms) ballistic locomotor activity characteristic of flight responses that was bound to the stimulation period. This flight behaviour was observed independently of the frequency of stimulation (1–20 Hz) but the intensity of this behaviour was linked to stimulation frequency. eNpHr3.0 inhibition of lPAG had no effect on behaviour. ChR2 stimulation of vlPAG evoked a passive immobility - identical in appearance to "freezing" - which was slow (2–4 s) in latency to onset and not stimulation bound. eNpHr3.0 inhibition of vlPAG had no effect on behaviour. To determine the effects of these manipulations on fear learning we activated (via ChR2) or silenced (via eNpHr3.0) lPAG or vlPAG during Pavlovian fear conditioning. Specifically, rats received pairings of an auditory conditioned stimulus (CS) with a footshock unconditioned stimulus (US) and optical stimulation was provided during the US only. Preliminary results showed that eNpHr3.0 silencing of lPAG impaired, whereas eNpHr3.0 silencing of vlPAG augmented, the acquisition of fear to the CS. These results show complementary roles for lPAG and vlPAG in regulation of both fear responding and fear learning.

WTH11-14

Maternal immune activation at two gestational time-points: examination of schizophrenia-related behavioural phenotypes in the rat

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Maternal immune activation (MIA) is a risk factor for schizophrenia. Evidence from a mouse model suggestes late-gestation MIA is associated with schizophrenia-related cognitive dysfunction, whereas early-gestation MIA alters dopamine-related behaviours. The current study investigates if MIA at different gestational stages preferentially alters either dopaminergic or NMDA-related behaviours. Wistar rats were administered with 4.0 mg/kg of PolyI:C or saline on gestational day 10 (early) or 19 (late). Prepulse inhibition (PPI), working memory (WM) and locomotion in response to MK-801 and amphetamine were examined in offspring. Females exposed to late MIA had impaired WM in a 1-s delayed non-match to position task. A similar non-significant trend was seen in males. No differences were seen at longer delays indicating that WM deficits are not delay-dependent. Early MIA males had significantly reduced PPI in comparison to controls and late-gestation animals. MIA at either time-point did not produce effects on MK-801- or amphetamine-induced locomotion. Reduced PPI in early, but not late MIA males, suggests dopamine neurotransmission may be perturbed. However, the lack of changes in amphetamine-induced locomotion at either dose indicate this rat model does not fully replicate the perturbed dopaminergic phenotype previously seen in the mouse, and other rat models in which MIA was induced at different time-points. The presence of non-delay dependent WM deficits suggests that late MIA, in females, produces a model of schizophrenia-related cognitive impairments.

WTH11-15

Proteomic analysis of rat saliva proteins for restraint stress biomarkers

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Saliva is a useful sample non-invasively collected from body fluid. Our objective in the present study is to search for saliva biomarkers for the differentiation between physical and mental stress. First, we analyzed candidates of saliva biomarker proteins for mental stress by proteome after restraint stress loading to rats and without stress. After separation of rat saliva proteins by Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), the proteins increased or decreased by restraint stress were observed in Coomassie Brilliant Blue-stained polyacrylamide gel, and then liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) were used for the analysis. We detected known several enzymes and secretary proteins in the MW range with 20–70 kDa. Whether these candidates are stress biomarkers is under way. This study (No.25350824) is supported by Grant-in-Aid for Scientific Research (KAKENHI); Grant-in-Aid for Scientific Research (C) in 2013, 2014 and 2015.

WTH11-17

Chronic cannula implantation into the cerebral lateral ventricle does not impair spatial or recognition memory

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Many nootropic agents are administered directly into the brain via chronic indwelling cannulas with the lateral ventricles being the most common target of delivery. This raises the question as to whether these agents are enhancing normal memory or merely rescuing impaired memory resulting from damage caused by the cannula implantation. Hence the current study sought to assess the behavioural and cellular responses to this form of surgery. 7 week old male Sprague–Dawley rats were implanted with chronic indwelling 22-guage cannula into the right lateral ventricle while under isoflurane anaesthesia and allowed to recover for 5 days. Control animals received no treatment. Rats were then tested sequentially on the rotorod, spontaneous alternation and novel object recognition tasks before being culled 8 days post-surgery. While a slight reduction in fine motor control was found as determined by the rotorod task (likely due to the cannula's path through the motor cortex), no impairment was found in either spatial or recognition memory. To support this work immunohistochemistry was performed to assess the immune cell response 1 week post-surgery. While a thin scar formed along the tract of damage caused by the cannula and a clustering of astrocytes and microglia was observed at the base of the cannula, there was little lateral spread of the inflammation and little neuronal death was observed. Congruent with this levels of inflammatory markers, as assessed by RT-PCR, were not found to be significantly elevated compared to controls in the contralateral cortex and also the hippocampus. This work demonstrates that memory, as assessed by two of the most common behavioural tasks, the novel object recognition and spontaneous alternation task, is not adversely affected by implantation of a chronic indwelling cannula into the lateral ventricle, unsurprising given the minimal inflammatory response. These results lend support to the notion that nootropic agents delivered by this route are improving normal memory rather than rescuing a damage state.

WTH11-18

Analysis of mGlu5/homer signalling complex in rodent neurons knock out for shank3 and in shank3 Exon11 ko mice

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Shank proteins are the major scaffold proteins that organize the postsynaptic density at the excitatory synapses. Shank proteins are associated with type I mGluRs via an interaction with Homer. Phelan-McDermid (PMS) syndrome is characterized by intellectual disability and autistic-like behavior. Loss of Shank3 is considered to major cause of the neurobehavioral symptoms of PMS.

We have recently demonstrated that in rat cultures, mGlu5 receptor expression is reduced in absence of Shank3, leading to impairment of mGlu5 signaling and alteration of DHPG-dependent LTD mediated by mGlu5.

Considering that the Shank3-Homer complex is essential to link the mGlu5 to its downstream signal pathways we measured calcium signaling after DHPG stimulation in neurons KO for Shank3 and we found a reduction of calcium release. Morphologically this defect is associated with alteration of Homer 1b/c cluster formation.

To test our hypothesis *in vivo* we measured mGlu5 and Homer protein expression in different brain areas of Shank3 Exon11 deleted mice and we found a reduction in the expression of both mGlu5 and of Homer1/b protein expression specifically in the striatum.

Our data strongly suggest that alterations in mGlu5 signaling pathways, due to disassembly of the mGlu5-Homer-Shank complex, are involved in the pathogenesis of PMS.

We behaviorally characterized Shank3 KO mice with the aim of rescue the phenotype by mGlu5 positive modulation. Shank3 KO mice showed an increase in self-injurious repetitive grooming that may be rescued by the mGlu5 positive modulator CDPPB.

Thus we think that mGlu5 modulation may represent a new way to ameliorate cognitive impairment found in patients affected by PMS.

WTH11-19

Effect of a novel cognitive enhancer on decreased CaMKII activity in Schizophrenia model rats

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Background: Development of novel cognitive enhancer is essential to improve quality of life for schizophrenia patients. Atypical antipsychotics improve prepulse inhibition (PPI) deficits observed in neonatal ventral hippocampus (NVH)-lesioned rats as schizophrenia model animal. Recently, we reported that Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) activity was reduced in medial prefrontal cortex (mPFC) and hippocampus in NVH-lesioned rats (Yabuki *et al*., Neuroscience 2013;234:103–115). In the present study, we investigated the effect of a novel cognitive enhancer ST101 (piro[imidazo[1,2-a]pyridine-3,2-indan]-2(3H)-one) (Moriguchi *et al*., J Neurochem 2012;121:44–53) on cognitive impairment of NVH-lesioned rats and elicits its mechanism.

Methods: To prepare NVH-lesioned rats, ibotenic acid was injected into bilateral ventral hippocampus on the postnatal day (PD) 7. On and after PD 70, male rats were subjected to schizophrenia-related behavioral tests. We also investigated whether risperidone (0.3 mg/kg, i.p.) or ST101 (0.01, 0.1, or 0.5 mg/kg, p.o.) administration improves those behavioral deficits. After behavioral tests, CaMKII activity was measured using immunohistochemical and western blotting analyses in order to define mechanism underlying cognitive improvement in NVH-lesioned rats.

Results: Risperidone treatment (0.3 mg/kg, i.p.) improved PPI deficits but not cognitive impairment in NVH-lesioned rats. Risperidone treatment significantly restored the abnormal dopaminergic signaling but not the decreased CaMKII activity in mPFC and hippocampal CA1 region of NVH-lesioned rats. On the otyher hand, ST101 elicited a potential for promoting CaMKII activity, thereby improving cognitive impairment in NVH-lesioned rats. In parallel, the decreased CaMKII activity in the mPFC and hippocampal CA1 regions was restored by ST101 administration.

Conclusion: NVH-lesioned rats are potential animal model of schizophrenia because the animals show schizophrenia-like behaviors including cognitive impairment and PPI deficits only after post-pubertal. Risperidone treatment can improve the PPI deficits but not cognitive impairment. On the other hand, a novel cognitive enhancer ST101 restores cognitive impairment by restoring decreased CaMKII activity in mPFC and hippocampus. Taken together, ST101 is attractive candidate therapeutics for cognitive impairment in schizophrenia patients.

WTH11-20

Role of probdnf in the rodent model of depression

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Brain derived neurotrophic factor (BDNF) plays roles in the depression of patients and animal models, but the function of its precursor (proBDNF) in depression is not known. Our previous studies show that proBDNF and its receptors are upregulated in the blood of subjects with major depression, suggesting proBDNF may be involved in pathogenesis of depression. In the present study we have built the depression model in rats and mice by chronic unpredictable mild stress (CUMS), examined the expression of BDNF and proBDNF and their receptors P75NTR, sortilin and trkB in the brain by Western blots and RT-PCR, and investigated whether BDNF and its precursors play any roles in the development of depression. By the method of CUMS, we have successfully established the depression model in rats and mice. The levels of proBDNF and its receptor P75 and Sortilin were significantly upregulated where the levels of mature BDNF and trkB were down regulated compared with the control ($p < 0.05$). Golgi staining showed the length of spines in the depression group was significantly reduced compared with the control group ($p < 0.05$), which was reversed by the injection of anti-proBDNF to the lateral ventricle or intraperitonium ($p < 0.05$). The treatment of anti-proBDNF also reversed the depression behaviors including the increase in immobility in force swimming test and less consumption of sucrose. Intramuscular AVV-BDNF in the depression rats can significantly increase the length of dendrites and improve the depression symptoms. Our data suggest that the balance between mature BDNF and proBDNF signaling in the model of depression was broken and restoration of the balance can have beneficial effect on depression.

WTH12 Molecular Mechanism of Alzheimer's Disease

WTH12-01

Quantifying the impact of iron in Alzheimer's disease

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Brain-iron elevation is implicated in Alzheimer's disease (AD) pathogenesis, but the impact of iron on AD outcomes has not previously been investigated in a longitudinal study. Ferritin is the major iron storage protein of the body; by using cerebrospinal fluid (CSF) levels of ferritin as an index, we explored whether brain iron status impacts on longitudinal outcomes in the Alzheimer's Disease Neuroimaging Initiative (ADNI) ADNI cohort. We show that baseline CSF ferritin levels were negatively associated with cognitive performance over 7 years (ADAS-Cog13: $p < 0.001$) in cognitively normal ($n = 91$), mild cognitive impairment (MCI; $n = 144$) and AD ($n = 67$) subjects, and predicted MCI conversion to AD (OR 2.32 [1.86–2.90], $p < 0.001$). We calculated that drugs that lower iron (previously shown to be beneficial in Phase II studies of Alzheimer's and Parkinson's disease) could delay AD onset by as much as 3 years. The magnitude impact of CSF ferritin on longitudinal cognition and brain atrophy (MRI) was comparable to the CSF tau/Aβ ratio. It is not known how iron is elevated in Alzheimer's disease. We showed that CSF ferritin was strongly associated with CSF apolipoprotein E protein levels ($R^2 = 0.34$, $p < 0.001$) and was elevated by the Alzheimer's risk allele, *APOE e4* (+22%, $p < 0.001$). These findings reveal that elevated brain iron adversely impacts on AD progression, and introduce brain-iron elevation as a possible mechanism for *APOE-e4* being the major genetic risk factor for AD.

WTH12-02

Intrahippocampal delivery of lineage negative stem cells in reversal of memory impairment in amyloid-β injected mice

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After several decades of research in Alzheimer's disease (AD) there is no gold standard achieved in disease diagnosis and its suitable treatment. Hence there is a pressing need to understand the disease pathology more precisely at the molecular and functional level. Cell based therapeutics have shown promise in preclinical AD models altering the underlying disease pathology compared to symptomatic relieves offered by current treatment regimes. Very few studies have examined the effect of human umbilical cord blood (hUCB) derived stem cells in neurodegeneration despite growing number of cord blood banks worldwide. Here we have examined the therapeutic efficacy of intrahippocampally transplanted cord blood derived lineage negative stem cells in augmenting the behavioral improvement by masking amyloid-β induced memory deficits in a mouse model. Lin-ve cells were transplanted at two doses (50 000 and 100 000) at the site of injury and evaluated at 10 and 60 days post transplantation. These cells were found to ameliorate cognitive impairment in 50 000-60 days and 100 000-10 days groups whereas, 50 000-10 days and 100 000-60 days groups could not exert any significant improvement. This constitutes the first ever evidence in the world showing that the lineage negative stem cells from human umbilical cord blood ameliorate learning in mouse model of amyloid-β induced cognitive impairment mediated by Fas-L which was further supported by up-regulation of neurotrophic factors such as BDNF and its associated transcription factor, CREB. The transplanted cells were found in the host tissue up to 60 days without expressing markers of neuronal differentiation or reducing Aβ burden in mouse brain. Our study shows the therapeutic efficacy of Lin-ve stem cells exerting neuroprotection at the site of injury. The study outcome will also impact cord blood banking and translational research in the field of Alzheimer's disease.

WTH12-03

Energy metabolism and the progression to dementia in Down syndrome and Alzheimer's disease

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Deficits in mitochondrial function and oxidative stress play major roles in Down syndrome (DS) and Alzheimer's disease (AD) and functional alterations in energy metabolism in both conditions occur systemically. To determine the extent of the metabolic changes actually present in peripheral cells, we performed a comprehensive analysis of energy metabolism and proliferation in lymphoblastic cell lines (LCLs) derived from 1- subjects with DS with AD dementia (DSAD), 2- without AD dementia (DS), 3- subjects with sporadic AD, and 4- age-matched controls. LCLs were maintained under regular or minimal feeding regimes with either galactose or glucose to assess energy metabolism under glycolysis and oxidative phosphorylation respectively. DS and DSAD LCLs both showed slower growth than control cells with minimal feeding. Under oxidative conditions, DS LCLs grew slower and exhibited higher cellular and mitochondrial free radical levels. The response to pro-oxidant stimuli was particularly exacerbated in DSAD LCLs. OXPHOS inhibitors significantly reduced ATP levels in DSAD and AD LCLs, which also exhibited reduced mitochondrial Ca^{++} uptake capacity. In contrast, mitochondrial membrane potential was reduced in DS, DSAD and AD LCLs. Finally, we found that changes in protein levels of phosphofructokinase 1 liver isoform (PFK1L) and LC3 can further discriminate between the different LCLs groups. Together, these results indicate that there are significant mitochondrial functional changes in LCLs derived from subjects with DS, DSAD and AD. Several parameters analyzed were consistently different between DS, DSAD, and AD lines suggesting that differential/transitional metabolic states between LCL groups may be utilized as biomarkers of disease progression and/or treatment outcome.

WTH12-04

Enhancing TRK mediated trophic signalling to augment cognitive processes

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Neurotrophic growth factors essential for neuronal survival during nervous system development and regulate neuronal function in the adult brain. Brain-derived neurotrophic factor (BDNF) is a major positive modulator of cortical synaptic plasticity, underpinning long-term potentiation. Nerve growth factor (NGF), on the other hand is also expressed throughout the brain and has more restricted effects, being required for optimal function of a discrete population of neurons in the basal forebrain that provide acetylcholine to the cortex. Low levels of BDNF are associated with mood disorders and have been observed in the serum and plasma of Alzheimer's disease patients, where it is associated with faster ongoing cognitive decline. Similarly, reduced expression of NGF results in degeneration of cholinergic basal forebrain neurons, a feature of Alzheimer's disease. Enhancing neurotrophic signaling is a validated therapeutic strategy for treating this condition.

NGF and BDNF signal via two classes of neurotrophin receptors, the p75 neurotrophin receptor ($p75^{NTR}$) and TrkA and TrkB respectively. Together $p75^{NTR}$ and Trk receptors form a high-affinity receptor complex. We have previously shown that a cell-permeable peptide c29, derived from a sequence of the $p75^{NTR}$ intracellular domain can promote neuronal survival and neurite outgrowth in low neurotrophin concentrations *in vitro* and *in vivo*. As a first step to determining whether c29 can also act as a cognitive enhancer, we asked whether c29 treatment affected learning. Using a contextual and cue-dependent fear memory in a fear-conditioning paradigm we obtained preliminary evidence that c29, when given systemically during training, can facilitate memory. A novel transgenic mouse that can be induced to express the c29 peptide sequence is also currently being analysed.

WTH12-05

Hippocampal adenosine A2A receptor up-regulation is necessary and sufficient to trigger memory dysfunction in Alzheimer's disease

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Recent epidemiological and animal studies have converged to conclude that caffeine consumption attenuates cognitive deficits in Alzheimer's disease (AD), dementia and aging. These beneficial effects of caffeine involve the antagonism of G-protein coupled adenosine receptors, namely adenosine A_{2A} receptors (A2AR) since AD and aging enhance A2AR density and the selective pharmacological or genetic blockade of forebrain A2AR mimic the robust neuroprotection and the control of abnormal synaptic plasticity afforded by caffeine. However, it is unknown if the abnormal activation of forebrain A2AR is sufficient to trigger memory impairment. We have developed a chimeric rhodopsin-A2AR protein (optoA2AR), which fused the intracellular loops of the A2AR into the rhodopsin backbone, allowing light stimulation to trigger A2AR transducing system (increase of cAMP levels and MAPK phosphorylation). Two weeks after optoA2AR expression in dorso-hippocampal neurons using a Ca^{2+}/calmodulin-dependent kinase IIα (CaMKIIα) promoter, light stimulation increased the levels of p-CREB and c-Fos specifically in optoA2AR-expressing neurons, which was recapitulated by intra-hippocampal administration of CGS21680 (A2AR agonist). OptoA2AR reached glutamatergic nerve terminals, where A2AR are located, and light stimulation modulated long-term potentiation, as did CGS21680. Notably, light activation of optoA2AR in the hippocampus during the 5 min testing period in a modified Y maze, reduced about 2-fold the time spent in the novel arm in comparison with non-light stimulated mice, without changes in locomotion as assayed by the total distance travelled in the Y maze. Thus, transient activation of optoA2AR signaling and intracellular recruitment of the CREB pathway in the hippocampus was sufficient to trigger memory impairments. (Supported by Santa Casa da Misericórdia and DARPA).

WTH12-06

Loss of ceramide synthase 2, an essential enzyme for myelin lipid biosynthesis, drives myelin degeneration in Alzheimer's disease

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Myelin is essential for the electrical conductivity of neurons, and as a source of trophic support that maintains axon integrity and neuronal viability. Myelin is comprised 80% of lipids, and consequently, disruption of myelin lipid biosynthesis destabilises myelin, leading to neurological decline. There is strong evidence to indicate that deterioration of myelin is a key pathophysiological driver of Alzheimer's Disease (AD). Firstly, the greatest genetic risk factor for AD is the E4 allele of the *APOE* gene, encoding the major lipid transport protein of the brain. Secondly, the defined anatomical progression of neuronal pathology over the course of AD pathogenesis runs inverse to the pattern of developmental myelination, and the same brain regions are heavily affected by age-related myelin loss.

In a lipidomic analysis of post-mortem brain tissue from subjects with different stages of pre-clinical or clinical AD pathology, we observed a pronounced loss of the prototypical myelin lipids galactosylceramide and sulfatide in AD brains (reduced 70% and 60%, respectively, in frontal grey matter). This was traced metabolically to an even more pronounced depletion of myelin-enriched, very long chain (VLC) forms of the lipid ceramide, which is the biosynthetic precursor to galactosylceramide and sulfatide. The synthesis of VLC ceramide is catalysed by ceramide synthase 2 (CERS2), an enzyme that is known to be essential for myelin stability and function. A robust deficit in CERS2 activity and expression was detected at the preclinical stages of AD pathogenesis (Braak stages I–IV), reaching statistical significance at Braak stage I/II in temporal grey matter, and stage III/IV in the hippocampus and frontal grey matter. Loss of CERS2 therefore precedes hallmark neurofibrillary tangle pathology in these regions.

We therefore demonstrate, for the first time, a loss of myelin biosynthetic capacity early in AD pathogenesis. We posit that, over time, this defect causes loss of myelin, precipitating deterioration of axons and neurological decline.

WTH12-07

The influence of amyloid-β precursor protein proteolytic processing on neuronal iron homeostasis

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Iron is an integral cofactor in many metabolic processes involved in transcriptional signalling, synapse formation and neuroplasticity, in all of which a function for amyloid-b precursor protein (APP) has also been heavily implicated but remains unclear. Intraneuronal iron imbalance is a predominant catalyst for reactive oxygen species production, particularly within iron accumulating neurodegenerative diseases such as Alzheimer's disease (AD). In AD, APP has historically been associated with amyloid-β (Aβ) derived neurotoxicity, but we recently discovered that APP also has a role in neuronal iron homeostasis by, in part, promoting iron efflux through cell surface stabilization of the iron pore ferroportin.

Detailed cell surface characterization confirms that the location of ferroportin on the neuron surface is increased upon iron incubation and is dependent upon APP. By altering the proteolytic processing of APP at the cell surface by suppressing secretase expression or activity, consequential changes in neuronal iron homeostasis arise. Enhancing the amyloidogenic pathway of APP processing leads to intracellular iron accumulation. With increased amyloidogenic processing of APP being a major contributor to sporadic AD, these studies increase our understanding as to why iron accumulation and increased susceptibility to reactive oxygen species neurotoxicity are prevalent with the disease.

WTH12-08

PP2A methylation plays a critical role in cAMP/PKA-dependent regulation of tau and neurite outgrowth

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The cAMP/Protein kinase A (PKA) signalling cascade, which plays an essential role in the regulation of synaptic plasticity, memory and neurite outgrowth, becomes dysregulated in ageing and Alzheimer's disease (AD). Notably, PKA-mediated phosphorylation of tau at Ser-214 is increased in AD and may render neurons susceptible to neurodegeneration. Here, we show in cultured neuronal cells that the activation of cAMP/PKA signalling induces alterations in protein phosphatase 2A (PP2A) methylation and subcellular distribution in a time-dependent manner. Notably, methylation of PP2A catalytic subunit at Leucine-309 modulates the substrate specificity of PP2A, the primary brain tau Ser/Thr phosphatase. We have reported that PP2A methylation becomes down-regulated in AD and following alterations in folate and homocysteine metabolism, resulting in enhanced accumulation of AD-like phosphorylated tau. Here, we further show that PP2A methylation plays a critical role in cAMP/PKA-dependent regulation of tau, the transcription factor CREB, and neurite outgrowth. Conversely, inhibition of cAMP/PKA signalling alters PP2A methylation-dependent neuritogenesis. Our findings establish a novel mechanistic link between neuronal cAMP/PKA dependent signal transduction pathways and the regulation of PP2A methylation, which likely contributes to AD pathogenic processes.

WTH12-09

P38 MAP kinase-mediated NMDA receptor-dependent suppression of hypersynchronicity in a mouse model of Alzheimer's disease

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Hypersynchronicity of neuronal brain circuits is a feature of Alzheimer's disease (AD). Mouse models of AD expressing mutated forms of the amyloid-ß precursor protein (APP), a central protein involved in AD pathology, show cortical hypersynchronicity. We studied hippocampal circuitry in APP23 transgenic mice using telemetric electroencephalography (EEG), at the age of onset of memory deficits. APP23 mice display spontaneous hypersynchronicity in the hippocampus including epileptiform spike trains. Furthermore, spectral contributions of hippocampal theta and gamma oscillations are compromised in APP23 mice, compared to non-transgenic controls. Using cross-frequency coupling analysis, we show that hippocampal gamma amplitude modulation by theta phase is markedly impaired in APP23 mice. Hippocampal hypersynchronicity and waveforms are differentially modulated by injection of riluzole and the non-competitive *N*-methyl-D-aspartate (NMDA) receptor inhibitor MK801, suggesting specific involvement of voltage-gated sodium channels and NMDA receptors in hypersynchronicity thresholds in APP23 mice. Furthermore, APP23 mice show marked activation of p38 mitogen-activated protein (MAP) kinase in hippocampus, and injection of MK801 but not riluzole reduces activation of p38 in the hippocampus. A p38 inhibitor induces hypersynchronicity in APP23 mice to a similar extent as MK801, thus supporting that suppression of hypersynchronicity involves NMDA receptors-mediated p38 activity. In summary, we characterize components of hippocampal hypersynchronicity, waveform patterns and cross-frequency coupling in the APP23 mouse model by pharmacological modulation, furthering the understanding of epileptiform brain activity in AD.

WTH12-10

Short chain ceramides, associated with insulin resistance, increase with age in the human hippocampus

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Alzheimer's disease (AD) affects a third of Australians over the age of 85, making age the major risk factor. The main genetic risk factor for AD is the E4 allele of the *APOE* gene, encoding the major lipid transport protein of the brain, apolipoprotein E (ApoE). Ceramide, from a class of lipids known as sphingolipids, has previously been shown to be higher in AD brain tissue compared to controls. Ceramides with different carbon chain lengths have different physiological functions. For example, C16:0 ceramide has been shown to promote insulin resistance in the brain, whilst C24:0 ceramide is a major constituent of myelin and is protective in the context of insulin resistance. To examine the relationship between age, *APOE* genotype and levels of different chain length ceramides, a lipid analysis was carried out using post mortem human brain tissue from neurologically normal subjects aged 65 years or older. This involved lipid extraction from 81 hippocampal brain tissue samples from the NSW and Queensland Brain Banks, followed by quantification of ceramide levels using liquid chromatography tandem mass spectrometry (LC-MS/MS). Levels of C16:0 ceramide increased with age, a relationship that was highly significant by Spearman correlation analysis ($r = 0.3019$, $p = 0.0065$). No significant association was observed for ceramides of other chain lengths. Furthermore, there was no association between *APOE* genotype and hippocampal ceramides. Recent findings demonstrate that AD progression is associated with a decline in cerebral glucose utilisation, potentially caused by loss of insulin receptors at synaptic membranes of the cerebral cortex and hippocampus. We have now identified an age-dependent increase in C16:0 ceramide, likely to be an important factor driving age-dependent insulin resistance in the brain. This may be a significant influence in AD pathogenesis.

WTH12-11

A molecular mechanism of shifting the cleavage site of APP by BACE1

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Objectives: Alzheimer's disease is a progressive neurodegenerative disease with dementia. Aβ, the major component of the senile plaque, is generated via sequential proteolysis by BACE1 and γ-secretase. BACE1 cleaves APP at β-site (Asp1 of Aβ) and β'-site (Glu11 of Aβ). The molecular mechanism how BACE1 determins which site cleaves is unknown. Recent studies suggest that the disruption of balance between β-site and β'-site cleave is a cause of Alzheimer's disease (*EMBO Mol Med.* [2011] 3, 291–302). We analyzed how BACE1 determine the cleavage site of APP to clarify a molecular mechanism of Alzheimer's disease onset.

Methods: We expressed mouse APP (mAPP), human APP (hAPP) or hAPP mutants in N2a cells, and analysed Aβ species in the conditione media with immunoprecipitation-MALDI-TOF/MS. To analyze localization of APP-CTFs, which is the C-terminal fragment of APP truncated by α-secretase or BACE1, we prepared DRM (detergent resistant membrane) and non-DRM fractions of mice brain and respective fractions were analyzed by Western blotting.

Results: N2a cells expressing mAPP secreted lower levels of Aβ1–40 and higher levels of Aβ11–40. By contrast, cells expressing hAPP secreted higher levels of Aβ1–40 and lower levels of Aβ11–40. These results indicate that a major cleavage site of mAPP is Glu11 site and that of hAPP is Asp1 site. DRM fractionation study with mice brain shows that APP-CTFβ localized differently on membrane depending on cleavage sites.

Conclusions: We demonstrate that the cleavage site of APP is a difference between mAPP and hAPP, and identify the amino acid sequence to cause a different cleavages. DRM fractionation suggests that the cleavage site of APP is determined by a membrane environment in which localize APP and proteases.

WTH12-12

Transcriptome analyses using human and zebrafish brain data reveal hypoxia as an important element in Alzheimer's disease

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A wide variety of observations point to the importance of hypoxia in Alzheimer's disease pathology. These include that hypoxia increases γ-secretase activity, *BACE1*, *PSEN1*, *PSEN2* and *APP* transcript levels and production of Aβ peptides. Hypoxia also stimulates inflammatory responses, inhibits correct protein folding and increases oxidative stress by stimulating production of reactive oxygen species (ROS). Since brain microvasculature is sensitive to ROS and Aβ the potential exists for damaging positive feedback loops to arise where hypoxia leads to vascular damage that further reduces the supply of oxygen. We have performed gene regulatory network analysis of publicly available Alzheimer's disease brain transcriptome data and have compared this to analysis of data from the brains of zebrafish exposed to hypoxia. This reveals remarkable overlap in the patterns of gene transcription in these systems supporting that hypoxia is an important element in Alzheimer's disease pathology. In related work we are analysing the brain transcriptomes of young adult zebrafish (recently sexually mature) into which we have engineered familial Alzheimer's disease-like mutations. This is indicating distinct changes in gene regulation that may mirror changes in familial Alzheimer's disease brains before development of overt histopathology. Hypoxia response signatures are also evident. We hope that this work will reveal the initial stresses caused by familial Alzheimer's disease mutations that ultimately result in the disease.

WTH12-13

The PI3K/AKT/GSK3β pathway is not involved in early Alzheimer's disease

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Alzheimer's disease (AD) and diabetes are two common diseases that have reached epidemic proportions in Australia. Epidemiological studies suggest that type 2 diabetes (T2D) is also a risk factor for AD. The AD brain is pathologically characterised by plaques and tangles, with the latter being made up of hyperphosphorylated forms of the protein, tau. T2D results in a paradoxical decrease in insulin signalling in the brain, that is predicted to decrease signalling through the PI3K/Akt pathway and lead to increased activation of the major tau kinase, glycogen synthase 3beta (GSK3b). Studies using post-mortem brain tissue are inherently retrospective with the most susceptible areas of the AD brain suffering major neuronal loss and compensatory changes. In contrast, areas such as the superior temporal gyrus display plaques but few tangles and retain nearly all their neurons. These regions may be equivalent to severely affected regions earlier in the disease. Here we explored the levels of AKT and GSK3b in the superior temporal gyrus of 20 AD cases and 20 age-, gender- and APOE e4 genotype-matched controls. There were no differences in the levels of active AKT or GSK3b between cases and controls. As a prospective humanized model of AD we also explored insulin signalling in patient-derived neurons from four AD cases and four controls. There were no group differences in baseline levels of active GSK3b. Our results suggest that there is no linear relationship between decreased insulin signalling, GSK3b and increased tau hyperphosphorylation in early phases of clinical AD.

WTH12-14

D-serine levels in Alzheimer's disease: implications for novel biomarker development

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Alzheimer's disease (AD) is a severe neurodegenerative disorder still in search of effective methods of diagnosis. Altered levels of the NMDA receptor co-agonist, D-serine, have been associated with neurological disorders, including schizophrenia and epilepsy. However, whether D-serine levels are deregulated in AD remains elusive. Here, we first measured D-serine levels in postmortem hippocampal and cortical samples from non-demented subjects ($n = 8$) and AD patients ($n = 14$). We next determined D-serine levels in experimental models of AD, including wild type rats and mice that received intracerebroventricular injections of amyloid-β oligomers, and APP/PS1 transgenic mice. Finally, we assessed D-serine levels in the cerebrospinal fluid (CSF) of 21 patients with a diagnosis of probable AD, as compared to patients with normal pressure hydrocephalus ($n = 9$), major depression ($n = 9$) and healthy controls ($n = 10$), and results were contrasted with CSF amyloid-β/tau AD biomarkers. D-serine levels were higher in the hippocampus and parietal cortex of AD patients than in control subjects. Levels of both D-serine and serine racemase, the enzyme responsible for D-serine production, were elevated in experimental models of AD. Significantly, D-serine levels were higher in the CSF of probable AD patients than in non-cognitively impaired subject groups. Combining D-serine levels to the amyloid/tau index remarkably increased the sensitivity and specificity of diagnosis of probable AD in our cohort. Our results show that increased brain and CSF D-serine levels are associated with Alzheimer's disease. CSF D-serine levels discriminated between non-demented and AD patients in our cohort and might constitute a novel candidate biomarker for early AD diagnosis.

WTH12-15

Developing an animal model of global brain ischemia through cardiac arrest

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Understanding the complications of cardiac arrest (CA) and its effect on rest of the body including the brain is necessary for development of further management approaches. Most of the current animal models of CA are complicated and invasive with little or no similarity with the actual situation of CA in human.

Aims: We developed a model of whole-brain ischemia through ventricular fibrillation (VF)/ventricular standstill followed by defibrillation, mechanical ventilation and standard resuscitation through injecting adrenaline and cardiac compression. This model mimics the cardiopulmonary arrest situation in human and allows testing of the effectiveness of experimental interventions to restore/preserve brain function.

Methods: Our model of CA is based on producing cardiac standstill and VF under anaesthesia, by two phase electrical stimulation via an oesophageal electrode, placed behind the right atrium. The first phase (AC; 24V, 50 Hz) is followed by the second phase of electrical stimulation (AC; 18V, 50 Hz) immediately after the first phase. The animals will be subjected to a specified period of CA based on their groups (4 and 8 min). At the end of the CA, if the rhythm of heart is VF or VT, the rats will be defibrillated (6–8 joules) via external electrodes, attached to defibrillator. Standard resuscitation procedures was performed using i.v. adrenaline, cardiac compressions and ventilation. Ventilation was provided through intra-tracheal intubation, connected to a specific rat ventilator. Throughout the experiment cardiac rhythm and oxygen saturation were monitored via echocardiograph machine (Philips HeartStart MRx Defibrillator) and Pulse-oximeter, respectively. Results: 24 v followed by 18 v AC current produced effective CA. Animals resucitated from CA via defibrilation, or via adrenalin injection and mechanical resuscitation. The animals survided during recovery periods(1 h to 4 weeks).

Conclusion: This model to achieve global brain ischemia, followed by cardiopulmonary resuscitation is more accessible, easier, less traumatic and closer to actual CA in human.

WTH12-16

Uperin 3.5: a membrane active, amyloid forming, antimicrobial peptide

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In Alzheimer's Disease (AD) amyloid is linked to the neurodegeneration process. The morphology of the extracellular "amyloid" deposits is primarily, highly ordered, b-sheet rich fibrils of Ab, in AD. These "fibrillar" amyloid species are implicated as the "toxic agents". Recently, focus has shifted to the investigation of the soluble, pre-fibrillar Ab and their role in amyloid-diseases. Importantly, an understanding of the molecular properties of these soluble pre-fibrillar peptides is needed and the macroscopic effect on cells and tissues in the brain. Arguably the most challenging aspects of studying amyloid, lies in the high propensity of these peptides to aggregate!

The 17-amino acid, antimicrobial peptide (uperin 3.5, U3.5) is derived from a small frog, *Uperoleia mjobergii*, and aggregates to form amyloid in a buffer solution. Unlike Ab, the U3.5 is stable to aggregation in aqueous solution. Thus, aggregation only begins after the addition of salts. So, U3.5 is a good peptide in which to investigate the molecular events along the path to amyloid. U3.5 is also an antibacterial peptide and acts towards gram-positive bacteria with a wide spectrum of activity. It is unusual among the host-defence peptides secreted by frogs to aggregate.

The effect of the U3.5 peptide and several mutant U3.5 peptides on a lipid membrane layer was studied using a quartz crystal microbalance (QCM). These data revealed that; (i) that the soluble and aggregated U3.5 disrupts a lipid membrane layer (DMPC or DMPC:PG); (ii) removal of a lysine residue at position 7, eliminated membrane interaction; (iii) the presence of cholesterol in a DMPC lipid membrane showed pore formation in the membrane and (iv) a higher cholesterol ratio (up to 50%) showed no difference in the membrane effect. These data were primarily obtained using a quartz crystal microbalance and were supported by Molecular Dynamics simulations AFM and fluorescence. Together these data provide a molecular basis and knowledge needed to establish novel strategies for new treatments for amyloidogenic diseases, such as, AD.

WTH12-17

Synaptosomal bioenergetic defects are associated with cognitive impairments in a transgenic rat model of early Alzheimer's disease

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Accumulation of amyloid β, mitochondrial dysfunction and bioenergetic defects in nerve terminals have been linked to cognitive impairment that precedes Alzheimer's disease (AD) onset. However, the hypothesis that synaptic bioenergetic deficiencies are associated with the progression of AD has not been proven in transgenic (Tg) mice models with AD-like phenotypes. To further explore this concept we performed a time-course analysis to assess brain mitochondrial function and synaptosomes[P1] bioenergetics in an animal model of early AD, the hemizygous (+/−) TgMcGill-R-Thy1-APP rat. Decrements in oxygen consumption rate and ATP synthesis along with oxidative damage were observed in hippocampal mitochondria of Tg(+/−) with aging. In addition, a decrease in the respiratory control ratio and spare respiratory capacity was found in mitochondria from synaptosomes of 6 month-old Tg(+/−) as compared to aged-matched control (WT) rats, that are associated with a decrease in complex I enzymatic activity. However, stereologic electron-microscopy did not show synaptic or mitochondrial changes in CA1 hippocampal region of Tg(+/−) as compared to WT. Cognitive impairments were prevented and bioenergetic deficits partially reverted when Tg(+/−) rats were fed a nutritionally complete diet from weaning to 6 month-old supplemented with pyrroloquinoline quinone, an antioxidant with cardio and neuroprotective effects. These results provide evidence that mitochondrial bioenergetic capacities of synaptosomes are not conserved in the Tg(+/−)McGill-R-Thy1-APP rats in a manner similar to that described in pathological human brain and reinforce the utility of this animal model as a platform for developing insights into the biological basis of early AD.

WTH12-18

The influence of chronic nicotine treatment on proteins expressed in the mouse hippocampus and cortex

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Some epidemiological and clinical studies have shown that cigarette smoking is linked with both increased and decreased risk for Alzheimer's disease (AD). Nicotine, a major component of cigarette smoke, has been shown to have protective effects against amyloid β-induced neurotoxicity in the brains of AD transgenic mice, although the precise mechanisms are still unclear. In this study, we performed proteomic analysis of the hippocampus and cortex of chronic nicotine-treated mice using two-dimensional gel electrophoresis (2-DE) followed by mass spectrometry. Sixteen proteins and one phosphoprotein were identified to be significantly changed in the hippocampus of chronic nicotine-treated mice: profilin-2, tubulin β-5 chain, atp5b, cytosolic malate dehydrogenase, cytochrome b-c1 complex subunit 1, heat shock protein 70 cognate, vacuolar adenosine triphosphatase subunit B, calpain small subunit 1, dynamin, partial and 4-aminobutyrate aminotransferase, mitochondrial isoform 1 precursor were increased, and γ-actin, NADH dehydrogenase 1 α subcomplex subunit 10, mitochondrial precursor and Glucose-6-phosphate 1-dehydrogenase X were decreased. Twenty-two proteins and two phosphoproteins were identified to be significantly changed in the cortex of chronic nicotine-treated mice: spectrin α chain, non-erythrocytic 1 isoform 1, creatine kinase B-type, 14-3-3 γ, phosphatidylethanolamine-binding protein 1, ubiquitin carboxy-terminal hydrolase L1, isoform CRA a, L-lactate

dehydrogenase B chain, type II peroxiredoxin 1 and secernin-1 were increased, and NADH dehydrogenase (ubiquinone) Fe-S protein 1, heterogeneous nuclear ribonucleoprotein H2, protein disulfide-isomerase A3, α-internexin, dihydropyrimidinase-related protein 2, γ-enolase isoform 1, vacuolar adenosine triphosphatase subunit A, translationally-controlled tumor protein, pyruvate dehydrogenase E1 component subunit β, vacuolar adenosine triphosphatase subunit B and Atp5b were decreased. Additionally, showed the changes in dynamin1, heat shock protein 70, NADH dehydrogenase 1 α subcomplex subunit 10, α-internexin, tubulin β-5 chain and secernin-1 levels were validated by western blot analysis. We propose that nicotine might have a certain influence of AD by changing the levels of proteins and phosphoproteins in the brain.

WTH12-19

The amyloid-beta-dependent phosphorylation of CRMP-2 dissociates kinesin in Alzheimer's disease

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the accumulation of amyloid plaques and neurofibrillary tangles. Before the development of these two characteristic features, impairments in anterograde axonal transport develop. However, molecules that initiate these impairments are still unknown. Collapsin response mediator protein-2 (CRMP-2) plays an integral role in kinesin-1-dependent axonal transport and there is evidence that phosphorylation of CRMP-2 releases kinesin-1. However, the molecular trigger regulating CRMP-2 phosphorylation is not known. Here, we tested the hypothesis that amyloid beta (Ab)-dependent phosphorylation of CRMP-2 regulates disruption from the kinesin-1 axonal transport motor protein in Alzheimer's disease. We found enhanced Ab-dependent phosphorylation of CRMP-2 at the T555 site and reduced CRMP-2 association with kinesin-1, while the overexpression of an unphosphorylatable form of CRMP-2 in neurons promoted the re-establishment of CRMP-2-kinesin association and axon elongation. Additionally, in the transgenic Tg2576 mouse model of familial AD (FAD), that carries the Swedish mutation in amyloid precursor protein (APP) enhancing Ab overproduction, we found substantial staining with pT555CRMP-2 and axonal dystrophy. Consistent with these findings, brain lysates from AD patients demonstrated phosphorylation of CRMP-2 at T555 site and dissociation of CRMP-2 from kinesin-1. These data suggest that Aβ-dependent phosphorylation of CRMP-2 at the T555 site may directly impair anterograde axonal transport and is sufficient to lead to axonal defects.

WTH12-20

Impact of the transcription factor NRF2 on tau and β-amyloid pathology in a combined mouse model of Alzheimer's disease

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There is an urgent need to find new molecular targets that could modify the progression of Alzheimer's disease (AD). Here, we identified the transcription factor NRF2 as a novel therapeutic target for this disease. NRF2 is considered the master regulator of redox homeostasis and regulates the expression of more than 200 antioxidant genes. We have generated a new AD mouse model consisting on expression of human APP^{V717I} and TAU^{P301L} in the wild type background (biAT) and in *Nrf2*-knockout mice (triAT). NRF2-deficiency aggravated the long term potentiation deficiency and impaired spatial memory (Morris water maze). The levels of pro-inflammatory markers as well as astrogliosis and microgliosis were exacerbated in the *Nrf2*−/− animals compared to age-matched *Nrf2*+/+ mice. Intracellular insoluble TAU aggregates were more evident in hippocampus of *Nrf2*−/− mice, what correlated with an exacerbated synaptic spine dysfunction. All mice developed Aβ plaques after 13–14 months, preferentially in subicullum and cortex, but *Nrf2*−/− mice developed fewer plaques and a larger number of intracellular APP-positive granules. Immuno-colocalization analyses showed that these Aβ-aggregates were contained in autophagic vesicles (p62, Beclin-1, NDP-52, etc.) which could be related to the unconventional secretory pathway (Rab8a), but *Nrf2*−/− mice showed a lower colocalization of Aβ with these markers indicating alterations of the autophagy flux and Aβ secretion. These animals will be an excellent tool to analyze the role of oxidative stress, neuroinflammation and proteinopathy in the onset of AD and assess the relevance of NRF2 as a drug target in this and other proteinopathies.

WTH12-21

Melatonin regulates the expression of amyloid precursor protein (APP) secretases in human neuroblastoma SH-SY5Y cell line

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Amyloid (Aβ) peptide is initiated by β-secretase (BACE1) and γ-secretase (PS1), cleaving at β-site and γ/ε-site of amyloid precursor protein (APP), respectively. The cumulative Aβ peptides further aggregate in the brain and is believed to be the etiology of Alzheimer's disease (AD). However, Aβ production could be occluded by cleaving at α-site (at the middle of Aβ sequences) of APP by α-secretase (ADAM10). Melatonin, the hormone secreted by pineal gland, is known to decrease with progressing ages and melatonin loss is co-incident with AD progression. Substantial studies *in vitro* and *in vivo* reported that melatonin treatment could

decrease Aβ burden and its toxicity, but which of the underlying mechanism is still elusive. The present results show that melatonin modulates transcriptional level of the secretases and their related product. Melatonin at 0.1 μM, 1 μM, and 10 μM significantly attenuated BACE1 and PS1 but augmented ADAM10 expression level. The mechanism of melatonin on altered level of the secretases was mediated via melatonin membrane-bound receptor signaling pathway, since pretreatment with luzindole (MT1 and MT2 melatonin receptor antagonist) prior to melatonin could abolish the effect of melatonin on the secretase expression. Moreover, Pertussis Toxin (PTX), which inactivates Gi/o type G proteins, could also block the effect of melatonin on regulating the secretase level. These data suggest the beneficial roles of melatonin on regulating the secretases in AD and according to a co-incident of AD progression and reduction in melatonin level, melatonin may become a promising therapeutic target for prevention and treatment of AD.

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WTH12-22

Imaging mass spectrometry (IMS) of cortical lipids from preclinical to severe stages of Alzheimer's disease

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Alzheimer's disease (AD) is a progressive neurodegenerative disease affecting millions of patients worldwide. Previous studies have demonstrated alterations in the lipid composition of lipid extracts from plasma and brain samples of AD patients. However, there is no consensus regarding the qualitative and quantitative abnormalities of lipids in brains from AD patients. In addition, the recent developments on the imaging mass spectrometry methods are reaching a new step in the *in situ* analysis of lipid species on brain tissue slices from human *postmortem* samples. The present study employs the matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS), enabling direct anatomical analysis of lipids in the *postmortem* sections of brain of AD patients and are compared with those levels obtained in matched cases with no neurological diseases. The laser scanning of the frontal cortex samples from AD patients classified attending to histochemical Braak's criteria, allowed us to compare from non-cognitively impaired status to severely affected patients. The main results indicate a depletion of different sulfatide lipid species from the earliest stages of the disease in both white and gray matter areas of the prefrontal cortex. Therefore, the decrease on sulfatides at cortical areas could not only be a marker of the disease but also is showing neurochemical modifications that might be involved in the pathogenesis of the disease.

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WTH12-23

Distinct microrna expression in a transgenic mouse model of Alzheimer's disease

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MicroRNA are small, highly conserved non-coding RNA that function as critical negative regulators of gene expression. They are inextricably linked to many cellular processes and have emerged as potential therapeutic targets as evidenced by their roles in a number of neurological diseases including Alzheimer's disease. In light of these studies, we sought to investigate if microRNA are dysregulated in a transgenic mouse model of Alzheimer's disease (APPswe/presenilin-1dE9). To do this, we profiled hippocampal microRNA expression prior to and when amyloid-β build-up is advanced (4 and 15 months respectively; $n = 4$) using Next Generation sequencing (Illumina). A total of 730 mature mouse miRNA were identified from the miRBASE database. Following statistical analysis using the Bioconductor/R software package EdgR, we identified little microRNA expression alterations at 4 months of age, however by 15 months, 6 microRNA were significantly down-regulated ($p < 0.05$). This included a novel candidate Alzheimer-associated microRNA, linked to the Glycogen Synthase Kinase 3 Beta signaling, which mediates many critical factors associated with Alzheimer's disease such as tau hyperphosphorylation, amyloid-β production and neuronal death.

WTH12-24

Kinesin-1 cargo receptor APP and Alcadeinα transport different type of vesicular cargo to the axonal terminal

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Kinesin-1 is a most common molecular motor to perform axonal anterograde transport in neuron. The kinesin-1 is composed of two heavy chains (KHC) and two light chains (KLC). Kinesin-1 transports various cargos through the binding to distinct adapter proteins or cargo receptors mediated by TPR motifs in the C-terminal of KLCs. Identification and characterization of cargo receptors and/or adaptor proteins, which connect a cargo to motor, have progressed. Alzheimer's β-amyloid precursor protein (APP) and Alcadeinα (Alcα)/Calsyntenin-1 were identified as a cargo receptor proteins for kinesin-1-mediated axonal transport of vesicular cargos along axons (*EMBO J.* [2007] 26, 1475), and JIP1b protein was found as an adaptor protein to connect kinesin-1 with APP (*Mol. Biol, Cell* [2014] 25, 3569). However, cargos transported by APP or Alcα are still controversial. In this study, we isolated APP and Alcα vesicles from mouse brain homogenates with specific antibodies and performed a proteome analysis with MS. We found that APP and Alcα transport distinct types of components as transport vesicles. We previously revealed that the anterograde transport velocity of APP cargo is almost 1.5- to 2-fold greater than that of Alcα when an adaptor molecule JIP1b is mediated the connection between APP and kinesin-1 (*Mol. Biol, Cell* [2014] 25,

3569). Taken together with these observations, present results strongly support the conclusions that APP and Alcα transport a distinct type of vesicular cargo under the different regulation even if they use a same kinesin-1 motor.

WTH12-25

Zinc modulates tau phosphorylation, APP activity and cognitive dysfunction in aluminium induced neurodegeneration

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Metals are considered as important components of a physiologically active cell and imbalance in their levels may lead to various neurological disorders. Aluminium (Al) is one of the most abundant elements in the biosphere, which is etiologically and epidemiologically related to several neurodegenerative ailments including Alzheimer's disease. On the other hand, zinc (Zn), an essential micronutrient, has been regarded as a vital metal for the normal brain functioning. The aim of the current study was to determine the protective role of Zinc in attenuating the neurodegenerative changes induced by Aluminium in rat brain. Male Sprague Dawley rats weighing 140–160 g were divided into four different groups viz: Normal control, Aluminium treated (100 mg/kg b.wt./day via oral gavage), Zinc treated (227 mg/l in drinking water) and combined aluminium and zinc treated for a total duration of 8 weeks. Al treatment resulted in statistically significant alterations in the cognitive behavior, anxiety and motor activities of rats, whereas zinc supplementation caused an improvement in various neurobehavior parameters. Further, Al exposure decreased ($p \leq 0.001$) the levels of neurotransmitters (dopamine, serotonin), but increased ($p \leq 0.001$) the levels of L-citrulline as well as activities of nitric oxide and monoamine oxidase in brain. However, zinc administration to Al-treated animals regulated the altered levels and activities of brain markers. Western blot of tau, amyloid precursor protein, glial fibrillary acidic protein, ubiquitin, alpha-synuclein and heat shock protein 70 were also found to be elevated after Al exposure, which however were reversed following Zn treatment. Al treatment also revealed alterations in neuro-histoarchitecture in the form of loss of pyramidal and purkinje cells, which were reversed with zinc co-administration. Thus, the present study demonstrated that zinc plays an important role in regulating the activities of various proteins and neurotransmitters that are involved in aluminium induced neurodegeneration.

WTH12-26

Novel mechanisms of C-SRC kinase-dependent regulation of PP2A: implications for tauopathies

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Accumulation of highly phosphorylated tau species is a hallmark of tauopathies, including Alzheimer's disease (AD). Significantly, Protein phosphatase 2A (PP2A) can bind to tau and is the primary neuronal tau Ser/Thr phosphatase. Alterations in PP2A occur in AD-affected brain regions. PP2A dysfunction is associated with the accumulation of phosphorylated tau in cultured cells and *in vivo,* and deregulation of tau function in microtubule assembly. While PP2A has gained momentum as a potential therapeutic target for AD, its regulation remains poorly understood. It has previously been reported that PP2A is inactivated by phosphorylation of its catalytic C subunit on the Tyr307 site by the Src family kinase member, c-Src. Moreover, phosphorylation of PP2A at Tyr307 was shown to be increased in AD brain tissue. Notably, c-Src plays a key role in neuronal signalling, developmental pathways, axonal growth, and tau phosphorylation on Tyr18. Conversely, by acting as a protein scaffold, tau can increase c-Src tyrosine activity, thereby facilitating c-Src-mediated actin rearrangements. Here, using site-specific PP2A phosphomutants, we show that Src tyrosine kinase can phosphorylate PP2A on novel tyrosine phosphorylation sites, but not on the previously reported Tyr307 site. Indeed, we show that Tyr phosphorylated PP2A is not specifically recognised by commercial anti-pTyr307 antibodies used in many previous studies assessing PP2A phosphorylation state. We also show that PP2A-Src protein interactions are modulated by Src and PP2A activities, and regulate the tau phosphatase activity of PP2A. Our findings suggest that deregulation of Src signalling in AD could indirectly lead to the deregulation of both PP2A and tau, and subsequent alterations in microtubule and actin cytoskeletal dynamics.

WTH12-27

Regulation of axonal transport of app cargos by kinesin-1

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Kinasin-1 is a common molecular motor to transport vesicles and organella anterogradely in neuron. Kinesin-1 is composed of two light chains (KLC) and two heavy chains (KHC). Amyloid b-protein precursor (APP) and Alcadeina/Calsyntennin1, type I membrane proteins, function as a cargo-receptor of kinesin-1. APP associates with KLC via JNK-binding protein (JIP1) while Alcadeina (Alca) directly binds KLC with its WD motif in the cytoplasmic domain. Both APP and Alca are transported almost independently by kinesin-1 in axon with different velocities. Alca is transported with a velocity of conventional kinesin-1, while the anterograde transport velocity of APP cargos is almost 1.5- to 2-fold greater in the presence of JIP1 even when an identical type of kinesin-1 motor works. We explored the role of JIP1 in APP cargo anterograde transport by kinesin-1.

In primary cultured neurons of JIP1-KO mice, we expressed APP-GFP with various types of JIP1b, and performed a quantitative analysis to understand the role of JIP1 in APP axonal transport. We also analyzed the detailed interaction between JIP1b and KLC using many JIP1b and KLC mutant/deletion constructs with immunocoprecipitation study. Relationships between the JIP1 roles in velocity and efficiency and the JIP1 binding ability to KLC were analyzed.

We identified two novel elements linked to JIP1 function, located in the central region of JIP1b, that interact with N-terminal half of KLC, which is essential for a high efficiency of APP anterograde transport. We also found that the conventional interaction of the JIP1b C-terminal with TPR motifs regulates the enhanced velocity of APP cargo transport.

References:
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WTH12-28

Delineating the specific binding pattern of exogenously administered abeta42 to organotypic whole brain slices

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Alzheimer's disease (AD) is the most common form of dementia that is characterized by the clinical symptoms of memory, behavioural and cognitive impairment. Pathologically, the AD brain undergoes atrophy due to substantial synaptic dysfunction and neuronal losses, particularly in the hippocampus and cortical brain regions where abnormal deposits of extracellular beta amyloid (Aβ) plaques and intraneuronal neurofibrillary tangles are found. Although the exact aetiology of AD remains unclear, the onset of this disease is associated with Ab deposition that spreads in a spatiotemporal manner over time until the majority of the brain is affected: implying that there is a pattern of region specific binding of Aβ in the aging AD brain. Therefore, the aim of this project was to determine if Aβ has a specific binding pattern to certain brain regions using adult mice organotypic brain slice cultures. 400 mm thick serial whole brain slices were treated with Ab42 for 1, 2, and 4 h at RT. Aβ42 binding to the treated brain slices increased in a time dependent manner and only the monomeric Ab42 species was detected by western blot. Aβ binding was primarily surface bound and restricted to neuronal cell bodies and structures. Analysis of the Aβ42 binding pattern revealed 3-fold higher binding in the hippocampus compared to cortical region ($p < 0.05$), similar levels between entorhinal and perirhinal cortex regions, while within the hippocampal region, the dentate gyrus had the highest level of binding and was significantly higher compared to the cornu ammonis (CA3, $p < 0.05$) region. These regions play a key role in memory function via the perforant pathway and are one of the earliest circuits to demonstrate pathology in AD. Future studies will be done to identify the specific mechanisms of vulnerability to Ab in these areas and can be used as a future therapeutic target(s) for the treatment of AD.

WTH12-29

Characterisation of a novel P301S mutant tau transgenic mouse model

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Introduction: Alzheimer's disease and frontotemporal lobar degeneration are two of the most common causes of dementia. Although the behavioural and pathological features of these two disorders vary greatly, tau pathology is commonly observed in both dementia types. Transgenic mice expressing mutant human tau in neurons of the brain are commonly used to study the tau pathology characteristic of Alzheimer's disease and frontotemporal lobar degeneration. In this study we aimed to characterise the behavioural and pathological features of a novel P301S mutant tau transgenic mouse model, the TAU58/2 strain.

Methods: Both young and aged mice underwent motor testing after which the brains were removed and analysed using immunohistochemistry, silver staining, and Western blotting.

Results: TAU58/2 mice presented with early-onset motor impairments which became more pronounced with age. Furthermore, male mice showed greater impairments then female mice. Throughout the brains of these mice, tau became increasingly phosphorylated with age, resulting in the formation of tau tangles, and the accumulation of pathologically insoluble tau. Male mice were found to display greater pathology burden than female mice. Finally, increased numbers of glial cells were observed in areas displaying signficant amounts of tau pathology.

Conclusions: TAU58/2 mice develop early tau pathology, motor impairments and marked gliosis. These mice therefore recapitulate a number of features of Alzheimer's disease and frontotemporal lobar degeneration, making these mice an ideal model for studying underlying disease mechanisms and for the development of new treatments for these disorders.

WTH13 Neurodegenerative Disease (Part 2)

WTH13-01

Does nogo-receptor 1 (NGR1) play a role in microglial activity within neuroinflammatory lesions?

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Multiple sclerosis (MS) is a neurodegenerative disease comprising of axonal damage and demyelination as the main pathological hallmarks. As the disease progresses, substantial central nervous system (CNS) degeneration can be correlated histopathologically with amoeboid microglia and numerous studies report this activity as central to the disease course of MS. In this study we focused on identifying whether there exists a role for nogo-receptor (NgR) in regulating microglial activity during experimental autoimmune encephalomyelitis (EAE). Immunohistochemistry was initially utilised to identify and enumerate microglial cells, which populate lesion and non-lesion areas of the spinal cord following EAE-induction. EAE spinal cord tissues were immunostained using two specific markers to these reactive cells, which are the anti-CD11b and anti-Iba-1 antibodies, along with an anti-NgR antibody to localise any co-labelled cells. In addition, we mapped the populations of activated microglia that were expressing NgR in spinal cord grey and white matter to illustrate their relevance during the disease progression. Immunopanning was subsequently utilised to isolate spinal cord microglia from *ngr-1*$^{-/-}$ and *ngr-1*$^{+/+}$ mice following EAE-induction, performed by using Iba-1 (lectin) as the capture antibody and the population of these cells were examined by flow cytometry. Based upon both immunohistochemistry and flow cytometry analysis, we detected similar numbers of NgR positive microglial cells in our *ngr-1*$^{-/-}$ mice both with and without EAE induction; thus, we suggest that there may not be a direct signalling role for NgR1 in the activation of microglial cells during the neuroinflammatory and neurodegenerative processes. However, increases in the number of NgR-positive microglial cells within the EAE progression suggest that other NgR homologues (NgR2 or/and NgR3) produce a response in the chronic stage of disease observed in *ngr-1*$^{+/+}$ mice. We now aim to define the type and mechanism of NgR being expressed and activated in these microglial cells.

WTH13-02

Effect of gene polymorphism of interleukin 6 in patients with Alzheimer's disease

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Introduction: Ageing is the main risk factor of neurodegenerative disorders. Although the cause or causes of Alzheimer's disease (AD) are not yet known, most experts agree that AD, like other common chronic conditions, probably develops as a result of multiple factors rather than a single cause. Interleukin 6 (IL-6) may be a candidate gene for AD because increase concentration of IL-6 may be involved in neurodegeneration, cognitive deficits, and accumulation of amyloid precursor protein. Interleukin 6 is a pleiotropic inflammatory cytokine involved in the inflammatory response and in the modulation of immune responses, including B-cell and T-cell differentiation. There are the several known polymorphisms in its promoter region, two polymorphisms, namely −174 G/C(rs 1800795) and −572 G/C(rs 1800797), are known to be associated with inter-individual variation IL-6 production.

Methods: We analysed healthy subjects and patients with AD. Genotyping of healthy and affected individuals was realized using PCR-restriction fragment length polymorphism technique. Restriction enzyme NlaIII and BsrBI was used to digest the PCR fragment of the IL-6 genes.

Results: There was no significance difference between in the genotype and allele of the polymorphisms IL-6 −572 G/C among asthmatic and healthy subjects. The frequency of recessive homozygote of IL-6 −174 G/C was significantly lower in the AD patients (18.20%) than in healthy subjects (27.90%) (OR = 0.334; $p = 0.01$). The C allele was less frequent in AD patients compared to healthy individuals (OR = 0.52; $p < 0.02$).

Conclusion: Alzheimer`s disease is a multifactorial disease and genetic as well as environmental factors are included in disease pathology. Interleukin 6 gene polymorphism in promoter −174 G/C may be associated with AD. Moreover multiple genotype analyses are necessary because a single gene polymorphism can be without relationship to increased risk of neurodegenerative disorders but the combination of gene polymorphisms may have significant effect, positive or negative. *This work was supported by grant MZ SR 2012/29-UKMA-6.*

WTH13-03

Low-dose enzyme replacement therapy reduces brain pathology in a neurodegenerative lysosomal disorder

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Repeated replacement of sulphamidase via cerebrospinal fluid injection is an effective treatment for neurodegenerative changes in the brain in mice and dogs with the lysosomal storage disorder, mucopolysaccharidosis type IIIA (MPS IIIA). Trials of this approach are underway in children with this condition, however the infusions require attendance at a specialist medical facility. Aim: we sought to extend our preliminary proof-of-principle studies that demonstrated the effectiveness of sustained-release (osmotic pump-delivered) enzyme replacement therapy in murine MPS IIIA, as this method, if applied to humans, would require only subcutaneous administration of enzyme once the pump was installed. Methods: 6-week old MPS IIIA and unaffected mice were implanted with subcutaneous mini-osmotic pumps connected to an infusion cannula directed at the right lateral ventricle. Either recombinant human sulphamidase or vehicle were infused over the course of 7 weeks, with pumps replaced part-way through the experimental period.

Results: we observed near-normalisation of primarily-stored substrate (heparan sulphate) in both hemispheres of the MPS IIIA brain and the cervical spinal cord, as determined using tandem mass spectrometry. Immunohistochemistry indicated a reduction in secondarily-stored GM3 ganglioside and neuroinflammatory markers. A bias toward the infusion side was seen in some, but not all, outcomes. The recombinant enzyme appears stable under pump-like conditions for at least 1 month. Conclusions: given that infusion pumps are in clinical use in other nervous system disorders e.g. for treatment of spasticity or brain tumours, this treatment method warrants consideration for testing in MPS IIIA patients and potentially other neurodegenerative disorders.

WTH13-04

Pharmacological chaperones for dopamine transporter deficiency syndrome

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Hereditary dopamine transporter deficiency syndrome (DDS) is a recently described rare genetic pediatric condition that is caused by loss-of-function mutations in the dopamine transporter (DAT). The disorder is characterized by parkinsonism-dystonia and raised CSF levels of dopamine metabolites. Patients generally do not survive past adolescence and no treatment is currently available. When expressed *in vitro,* the DAT missense mutations prevent DAT protein maturation and result in reduction or elimination of dopamine uptake. Pharmacological chaperoning, the use of small molecules to selectively bind and improve folding/maturation of a protein of interest, is an approach that has been used previously to rescue protein misfolding due to mutations causing cystic fibrosis and diabetes. We propose to identify pharmacological chaperones of DAT to rescue DDS mutations as a potential treatment for this condition.

Using a novel β-lactamase based assay to measure protein surface expression, we found that ibogaine and bupropion can increase surface expression of DAT in HEK293 cells after overnight treatment. Subsequently we used radio-labeled dopamine uptake assays to demonstrate that transporter function was also increased. Immunoblotting showed increased mature DAT protein, indicating that the effect was due stabilization of DAT protein. We then looked at the ability of ibogaine and bupropion to rescue well-characterized ER-retained DAT mutants as well as DAT mutations that cause DDS. Ibogaine and bupropion significantly increased mature protein and uptake of both the ER-retained mutants and some of the DDS mutants, showing that these compounds are effective pharmacological chaperones of DAT. Because not all DDS mutants could be rescued, we investigated whether the mutants were stalled at different points in the maturation process, which is controlled by a heat shock protein (HSP) relay. HSP90 inhibition increased rescue in mutants responding to pharmacological chaperones while having no effect in non-responding mutants, suggesting these mutants may be stalled too early in the folding/maturation process for rescue to occur.

Pharmacological chaperoning of DAT may be a viable treatment for DDS, allowing maturation and functional expression of mutants that would otherwise be retained in the ER and be degraded.

WTH13-05

A novel and personalised approach to treating multiple sclerosis using patient-specific induced pluripotent stem cells

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Current multiple sclerosis (MS) therapeutics are not effective for all patients, often associated with severe side effects and do not promote regeneration. Furthermore, questions regarding pathogenesis, disease subtypes and response to therapy still need to be addressed. The production of cell lines from individuals with MS could therefore provide exciting new opportunities for MS researchers and clinicians. In the present study we utilized reprogramming technology to establish induced pluripotent stem cell (iPSC) lines derived from the dermal fibroblasts of three sets of MS patients and their unaffected siblings, including a set of monozygotic twins discordant for MS. We generated three iPSC lines from each individual patient, and employed two different reprogramming methodologies for generating iPSCs from monozygotic twins-retroviral transduction of Oct4, Sox2, Klf4, and c-Myc, and lentiviral transduction using a single excisable stem cell cassette encoding the same four factors. The pluripotency of each iPSC line was confirmed by teratoma formation. No significant differences were observed between MS-iPSCs and relevant control iPSCs in terms of their ability to differentiate into NSCs or mature neural cell types. Likewise, transcriptome analysis by RNA-seq revealed no obvious differences between fibroblasts, iPSCs, neural stem cells (NSCs) or FACS-purified neurons of MS patients versus healthy controls. The therapeutic efficacy of NSCs derived from monozygotic twins discordant for MS was assessed by transplantation into mice with experimental autoimmune encephalomyelitis. While intravenous injection had no effect, intrathecal injection significantly attenuated clinical and pathological signs of disease. Importantly, there was no difference in the beneficial effect of NSCs derived from MS and control. The successful generation of MS patient-specific NSCs with potential clinical applications represents an important step towards novel approaches for personalised regenerative therapies in MS.

WTH13-06

Atorvastatin prevents cell death and depressive-like behaviour induced by Aβ1-40 peptide via BDNF cleavage

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Infusion of Aβ1-40 peptide to mice has been shown to cause neurotoxicty and depressive-like symptons and it can be used to evaluate antidepressant-like and neuroprotective effects of drugs. Atorvastatin is a largely used statin that we demonstrated an antidepressant-like effect in predictable animal models and neuroprotective effects against Aβ1-40 peptide infusion.

The purpose of this study was to determine the effect of mice atorvastatin treatment against Aβ1- 40-induced changes in mood-related behavioral and biochemical parameters in hippocampal slices.

Atorvastatin (10 mg/kg, p.o.) treatment once a day during 7 consecutive days presented a prevention of depressive-like and anhedonic-like behavior induced by $A\beta_{1\text{-}40}$ peptide infusion (400 pmol/site, i.c.v.). Aβ1–40–induced hippocampal cell damage was prevented by atorvastatin treatment. Aβ1–40 infusion decreased glutamate uptake into hippocampal slices and atorvastatin did not altered it. Glutamine synthetase activity was not altered by any treatment. Atorvastatin also increased hippocampal mature BDNF (mBDNF) to pro-BDNF ratio, suggesting an increase of pro-BDNF to mBDNF cleavage. Therefore atorvastatin displays antidepressant-like and neuroprotective effects against Aβ1–40–induced toxicity and these effects may depend on the BDNF levels, pointing to a putative association between the neuroprotective and antidepressant-like effects of this statin.

WTH13-07

Axon stretch growth of adult primary motor neurons

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Growth of embryonic dorsal root ganglion (DRG) axons has been markedly enhanced by controlled *in vitro* stretching without the involvement of the growth cone. However limited work has been done on enhancement of axonal growth on adult motor neurons. The spinal cord was harvested from adult Sprague–Dawley rats (8–12 weeks) by bilateral neural arch dissection, demyelinated and separated neurons from the homogenized spinal cord using Papain (36 U/mL in 6 mLs processing media) digestion and trituration. Motor neurons were isolated from the homogenate using an established 4 step Optiprep density gradient centrifugation and plated onto poly-D-lysine (100 mg/mL) coated glass and aclar substrate (at a cell density of 320–500 cells/mm^3) within the axon stretch bioreactor. Cells were grown in the bioreactor using an optimized neuron culture media.

The bioreactor consisted of three sections, which were assembled, autoclaved, plated with neurons and sealed. Nerve cells were grown under controlled temperature (37°C) and 5% CO_2 atmosphere for 8 days prior to commencement of stretching of the axons. The axons were stretched using a motor driven device controlled by PIMikro-Move software, where the axons were subjected to stretching, resting and stretching in sequence at an incremental rate commencing at 0.5 mm per day in 2 μm increments with 500 ms resting between stretching. Stretching of axons was continued for up to 13 days. The amount of stretch was monitored using a micro-camera mounted onto the objective of an inverted microscope. Cultured neurons typically developed axons and dendrites by 4 days and remained viable in excess of 21 days. Cells were identified as motor neurons using HB9, Islet-1 and Neurofilament-M antibodies. Early results indicate feasibility in using this tailored adult primary motor neuron protocol for axon stretch growth *in vitro experiments.*

WTH13-08

DRP1 plays critical roles in the neurodegeneration of motoneurons in cellular and animal models of ALS

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The shape of mitochondria is dynamically changing through continuously fission and fusion, which is implicated in the quality control of mitochondria. The fission of mitochondria is known to be enhanced in many neurodegenerative diseases including Amyotrophic lateral sclerosis (ALS). In the pre-symptom period of ALS, for example, mitochondria are excessively fragmented, but functional significance and the underlying molecular mechanisms causing mitochondrial fragmentation are poorly understood. In the present study, we found that mitochondrial fission-promoting GTPase, Drp1 is de-phosphorylated at their S616 site in the motoneurons of G93A ALS model mice. Since de-phosphorylation of this site activates Drp1 function via enhancing oligomerization and mitochondrial targeting, we reasoned that this may cause mitochondrial fragmentation. In addition to the changes in the Drp1 phosphorylation, we also found the marked induction of Drp1 receptor, Fis1 expression. Inactivation of Drp1 by expression of shDrp1, shFis1 or treatment of specific small compound inhibitor, Mdivi-1, all consistently suppressed the G93A-induced neuronal death, suggesting that mitochondrial fragmentation is important early step for ALS-related neurodegeneration. Furthermore, treatment of Mdivi-1 efficiently mitigated the axonal degeneration in the zebrafish model of ALS *in vivo*. These data suggest that Drp1 is important therapeutic target for the ALS-related neurodegeneration.

WTH13-09

The microtubule stabilising agent epothilone D modifies disease progression in a mouse model of amyotrophic lateral sclerosis

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ALS is characterised by the degeneration of motor neurons, their axonal processes and neuromuscular junctions (NMJs). Therefore, stabilisation of the axon has been proposed as an attractive target for neurodegenerative disease therapeutics. The microtubule stabiliser EpothiloneD (EpoD) has showed promise in ameliorating the pathophysiology of disease models emulating Parkinson's, Tauopathies and Schizophrenia, however its effect on ALS pathology is yet to be elucidated. The well characterised ALS model, the mSOD1^{G93A} mouse, was treated with EpoD and subsequent motor behaviour, survival and histopathological at early and late disease stages were evaluated. This was accomplished by treating mSOD1^{G93A} mice and wild-type controls with 2 mg/kg EpoD or DMSO vehicle control every 5 days from 50 days to ethical endpoint. Rotarod, grip strength, weight and neurological scoring were investigated. Immunohistochemical evaluation of pathological changes were also established at 10 weeks, 20 weeks and endpoint. Disease onset, as measured by hindlimb grip strength, was delayed in EpoD treated mSOD1^{G93A} mice by 7 days ($p < 0.05$). Similarly, distal axon pathology was decreased in EpoD mice at 10 weeks of age ($p < 0.05$). However, axonal pathology normalized to levels similar to that of vehicle control mice by 20 weeks of age. At end stage EpoD treated mice had significantly ($p < 0.05$) reduced

rotarod performance and mean number of spinal motor neurons, in comparison to their respective controls. Indeed, EpoD administration significantly $p < 0.05$) decreased survival compared to control mice. We posit that EpoD is a disease-modifying agent; however, it has both positive and negative effects on specific aspects of motor function, but a negative influence on survival. EpoD administration was beneficial at an earlier time point when mice were beginning to show signs of disease onset. However, EpoD treatment was not beneficial during mid to late disease stages. These results highlight the heterogeneity of ALS, with a combination and varied doses of therapeutics most likely being needed to positivity modify or alleviate the disease phenotype.

WTH13-10

Learning and neurogenesis are improved with exercise after an endothelin-1-induced hippocampal stroke in adult mouse

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In the adult mammalian brain, the hippocampus continues to produce new neurons throughout life. There is substantial evidence that this neurogenesis is critical to the hippocampus' role in learning and memory and that changes in the rate of neuronal production affects hippocampal-dependent cognitive functions. Exercise has been established as one of the treatments that stimulates neurogenesis and enhances learning in aged animals. In stroke survivors, the hippocampus is reportedly reduced in size regardless of the lesion site, and this is associated with impaired cognitive abilities. We therefore hypothesised that stimulating neurogenesis with voluntary exercise following stroke would improve recovery of learning functions. We have found that exercise rescues a stroke-induced learning deficit and that this rescue is dependent on hippocampal neurogenesis. We observed that mice intrahippocampally injected with the vasoconstrictor endothelin-1, causing a lesion in the dentate gyrus, displayed deficits in learning and memory when tested in the active place-avoidance task, a hippocampal-dependent spatial learning task. Furthermore, we found that endothelin-1-injected animals with access to a running wheel displayed elevated neurogenesis and showed improved performance in the spatial learning task, when compared to endothelin-1-injected animals that did not exercise. Therefore, stimulating hippocampal neurogenesis after stroke may potentially lead to improved outcomes for stroke survivors.

WTH13-11

Immune suppression leads to arginine deprivation in a mouse model of Alzheimer's disease

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The pathogenesis of Alzheimer's disease (AD) is a critical unsolved question, and while recent studies have demonstrated a strong association between altered brain immune responses and disease progression, the mechanistic cause of neuronal dysfunction and death is unknown. We have previously described the unique CVN-AD mouse model of AD (hMaAPPSwDI/mNos2−/−), in which immune-mediated nitric oxide is lowered to mimic human levels, resulting in a mouse model that demonstrates the cardinal features of AD, including amyloid deposition, hyperphosphorylated and aggregated tau, behavioral changes and age-dependent hippocampal neuronal loss. Using this mouse model, we studied longitudinal changes in brain immunity. Contrary to the predominant view that AD pathology is driven by pro-inflammatory factors, we find that the pathology in CVN-AD mice is driven by local immune suppression. Our studies show that $CD11c^+$ microglia accumulate at sites of Abeta deposition, that these microglia show an immunosuppressive phenotype, that extracellular arginase accumulates in these same regions and that global arginine bioavailability significantly decreases. Importantly, $CD11c^+$ microglial and arginase accumulation in the hippocampus and subiculum in the CVN-AD mice corresponds to the brain areas associated with neuronal damage. Pharmacologic disruption of the arginine utilization pathway by an inhibitor of arginase and ornithine decarboxylase protected the mice from AD-like pathology and significantly decreased CD11c expression. Our findings indicate that immune suppression and arginine catabolism lead to a loss of arginine, a critical semi-essential amino acid, and this nutrient deprivation is followed by cell death. This is a novel and potentially critical mechanism that may explain the temporal and spatial induction of the slow and persistent loss of neurons in humans with AD.

WTH13-12

Interneuron loss and dysfunction in amyotrophic lateral sclerosis

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Interneuropathies are associated with disturbances in circuitry regulation, altering connectivity in key disease-associated regions. In amyotrophic lateral sclerosis (ALS), hyperexcitability of cortical motor circuitry is a prominent event, often preceding motor neuron degeneration; and whilst many factors may be attributed to this pathophysiology, a possible candidate, in the interneuron, has largely been overlooked.

Here, in a systematic study of interneuron subsets we demonstrate unique regional- and lamina-specific alterations to specific populations in human end-stage ALS and in $SOD1^{G93A}$ transgenic mice. Investigations are performed in motor and control somatosensory cortex from presymptomatic stages through to end-stage in a lamina-specific manner.

In SOD1 mice we find that in late symptomatic stages, specific interneuron subsets of the motor cortex display contrasting (and potentially complimentary) pathology; the density of calretinin (CR) populations is significantly reduced by 37% in supragranular lamina (WT, $n = 4$, 55.3 ± 6.9 mm^2; SOD1, $n = 4$, 35.3 ± 6.0 mm^2), whilst neuropeptide Y (NPY) populations are increased by 40% in the infragranular lamina (WT, $n = 4$, 18.6 ± 2.4 mm^2; SOD1, $n = 4$, 31.0 ± 4.2 mm^2) ($p < 0.05$, two-way ANOVA with Bonferroni *post hoc* test). Moreover, using morphometric approaches, we show that remaining CR populations have undergone early, and continuing alteration to neurite labelling patterns – with progressive

reductions in neurite complexity from presymptomatic- to late-symptomatic stages.

These findings indicate that inhibitory regulation of cortical circuitry may be impaired in a motor- and lamina-specific manner, prior to motor neuron loss, in ALS. Differential involvement of CR- and NPY-positive interneurons suggests interplay of these specific populations may drive altered regulation, as the majority of remaining cortical interneuron populations are not affected. Furthermore, analysis of human ALS post-mortem brain tissue revealed a cluster of ALS cases with reduced CR density in lamina II/III compared with controls. This may be suggestive of unique motor system vulnerabilities involving the early susceptibility of interneurons in the pathogenesis of ALS.

WTH13-13

Effect of curcumin against synaptic plasticity impairment in hippocampus induced by HIV-1GP120 V3 loop in rats

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Human immunodeficiency virus-1 (HIV-1)-associated neurocognitive disorders (HAND) is a significant consequence of HIV infection. Although highly active antiretroviral therapy (HAART) has dramatically decreased HIV-1 load in acquired immune deficiency syndrome (AIDS) patients, HAART does not completely protect against the development of HAND, therefore novel strategies for the prevention and treatment are urgently needed. Our previous studies have shown that the infection with the gp120 V3 loop can cause HAND. Curcumin has been shown to improve these effects to some degree, but the electrophysiological mechanism remains unkown. In this study, we observed that the long-term potentiation in the CA1 region of rat hippocampal was inhibited when incubated with gp120 V3 loop. And curcumin can improve this effect. We observed that the Ca^{2+} concentration in synaptosomes was elevated when incubated with gp120 V3 loop, which could be significantly reduced by curcumin. The effect of curcumin was similar to nimodipine, a clinical drug to block Ca^{2+} channels. Our results suggested that curcumin has a neuroprotective effect against gp120 V3 loop-induced neuronal damage.

WTH13-14

Metabolomics of neurodegeneration and aging in the ts65dn mouse model of premature aging and down syndrome

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Aging is often associated with impaired cognition and a progressive loss of organ function over time accompanied by an increased susceptibility for many disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), heart disease, type II diabetes, and many forms of cancer. With a rapidly aging population understanding the underlying mechanisms of both healthy aging and aberrant aging is of critical importance. In particular, finding interventions that may increase the healthspan of individuals by modulating these mechanisms may prove helpful at mitigating the negative impacts of aging and age-related disorders. The most successful interventions to date, dietary restriction and mTOR inhibition, are directly related to metabolic pathways. We have proposed to try and elucidate some of these age-related changes using the Ts65Dn mouse model of premature aging and Down syndrome using metabolomics. Metabolomics is the study of small molecules and intermediates that are the end products of reactions within a cell. They are altered depending on the physiological or pathological state of the cell, tissue, or organ, providing an opportunity to discover biomarkers of aging and age-related disorders. Changes in the metabolome are apparent in normal aging, but may increase in magnitude with accompanying disease states or with accelerated aging. Thus, studying aging in a disease state, or in a disorder characterized by accelerated aging, will facilitate identification of these changes. Down syndrome (DS) is an intellectual disability characterized by premature aging. We hypothesize that trisomy of chromosome 21 (HSA21) causes disruption of the metabolome leading to the accelerated aging phenotype. An initiative by the National Institute on Aging (NIA) Interventions Testing Program tested the efficacy of rapamycin treatment and demonstrated that mice treated with rapamycin show a significant increase in lifespan and healthspan. Therefore, we hypothesize that treatment of the Ts65Dn mouse model of premature aging and DS with rapamycin will ameliorate the accelerated aging phenotype.

WTH13-15

Modulation of astrogliosis in combination of stem cell transplantation as a new strategy for the treatment of spinal cord injury

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Profound biochemical and cellular changes occur in SCI affecting neurons, oligodendrocytes, and astrocytes. Reactive astrogliosis is characterized by the proliferation and hypertrophy of astrocytes which undergo phenotypic and morphologic changes expressing elevated levels of glial fibrillary acidic protein (GFAP), and release inhibitory extracellular matrix molecules, such as chondroitin sulfate proteoglycans (CSPGs) contributing to the formation of glial scar.

Growing evidence indicates that reactive astrocytes have both active and passive roles in regenerative processes after SCI. Reactive astrocytes per se cannot repair damaged SCI but can contribute to healing the area, protecting the lesion site from further extension of the damage. It seems that only in the proper environment can these cells jump to a higher regenerative level, modifying their own properties and creating a permissive niche for other endogenous processes and exogenous interventions to regenerate the damaged tissue.

We reported a novel function of transplanted human embryonic stem cell derivatives on modifying properties of reactive astrogliosis, classically considered as detrimental to axonal regeneration, showing their contribution in making a favourable environment for neuronal differentiation of transplanted cells.

Overall, a future repair strategy that will include a combinational cell/pharmacological therapy with pluripotent stem cell-derived neural cells, focused on minimizing the inhibitory properties of astrocytes and simultaneously maximizing their growth-promoting

properties, would be extremely attractive for the treatment of SCI. It will be challenging to navigate this "fine tuning" modification of reactive astrocyte function via selective blockade of molecules that inhibit axonal outgrowth yet otherwise permit reactive astrocytes to form a physical barrier to protect the intact tissue.

WTH13-16

Spread of pathology in motor neuron disease: assessment of PTDP-43 along axonal pathways

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Background: The progression of motor neuron disease (MND) through the brain has recently been staged using separate neuropathological and neuroimaging modalities. The two schemes tie into a concept of pathological spread through corticofugal axonal transmission, stemming from observations of oligodendrocyte pTDP-43 pathology that accompanies the neuronal inclusions.

Objectives: To assess evidence of transmission along axonal pathways by looking for pTDP-43 oligodendrocyte pathology in involved white matter tracts, and to present a first validation of the neuropathological staging scheme.

Methods: pTDP-43 immunohistochemistry was performed on 10 μm sections from MND autopsy cases ($n = 31$) in selected white matter tracts and neuropathologically staged grey matter regions. Double-labelling immunofluorescence was performed to confirm localisation of pTDP-43 immunoreactivity to oligodendrocytes.

Results: All cases assessed fit criteria for one of four neuropathological stages, although Stage 4 cases differed significantly in their age of onset (8–11 years older) and disease duration (shorter duration than Stage 3 and similar to Stage 2). Assessment of the white matter in the posterior limb of the internal capsule, corpus callosum and cingulum revealed no pTDP-43 pathology, whereas pTDP-43 immunoreactive oligodendrocytes were observed in the white matter under the motor cortex.

Conclusions: Regional assessment of pTDP-43 pathology can be used to distinguish four neuropathologically distinct stages. In our staged cohort, Stages 2 and 3 appear to lie on a continuum (same age at onset with increasing disease duration with stage) while Stage 4 had an alternative disease dynamic (older at onset and shorter duration). Assessment of white matter tracts implicated in neuroimaging studies of disease progression revealed no oligodendroglial (or other cellular) pTDP-43 pathology in MND questioning the spread via these hypothesised cellular mechanisms.

WTH13-17

D-serine content is elevated in TLS/FUS knockout mice

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Mutations in *Translocated in liposarcoma* (TLS)/*Fused in sarcoma* (FUS) gene are responsible for type6 familial amyotrophic lateral sclerosis (ALS6). ALS is an incurable disorder characterized by selective degeneration of upper and lower motor neurons. Although a specific cause of ALS6 has not been fully understood, motor neurons of ALS6 patients display cytoplasmic aggregates containing mutant TLS/FUS proteins, most of which carry point mutations in its C-terminus. Recently, we have found that an expression of C2' type NR1, a *N*-methyl-D-aspartate glutamate receptor (NMDAR) subunit with high Ca^{2+}-permeability, is significantly increased in TLS/FUS-deficient mouse neurons. As D-amino acid oxidase (DAO) is associated with SOD1-related familial ALS, aberrant accumulation of D-serine, an endogenous coagonist of NMDARs, may exert glutamate excitotoxicity to motor neurons. To determine a critical stage of ALS6 development, we investigated D-serine production in TLS/FUS-deficient mouse. Because TLS/FUS knockout mice (TLS−/−) die within 16 h after birth, the littermates (TLS−/−, TLS−/+, TLS+/+) obtained by crossbreeding of the heterozygotes were removed at 19.5dpc and used for the experiments or otherwise 4–8 week old heterozygotes were used. The whole brains were homogenized in MeOH followed by a centrifugation to obtain the supernatants containing amino acids. D-/L-serine content per each brain tissue amount was quantitated by 2D-HPLC system established for sensitive and selective determination of D-serine and L-serine (Miyoshi et al.; 2009, 2012). The analysis showed that D-serine content was significantly higher in TLS−/− mouse brains than TLS+/+ ones. Serine racemase, which converts L-serine to D-serine, also increased at both mRNA and protein expression levels in the TLS−/− fetuses and 4–8 week TLS−/+ mice while DAO expression was not detected in TLS−/− mouse brain mRNAs, indicating that the elevated D-serine content is due to increased activity of serine racemase in TLS-deficient mice. Our results suggest that motor neurons in ALS6 patients could be more vulnerable to glutamate excitotoxicity via NMDAR because of a chronic deficiency of functional TLS, although the aberrant metabolism of D-serine may only play a minor role in ALS6.

WTH13-18

Low-dose, continual enzyme delivery ameliorates aspects of brain disease in a mouse model of a childhood neurodegenerative disease

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Aim: to determine the capacity of continual low-dose lysosomal enzyme infusion into the cerebrospinal fluid of mucopolysaccharidosis type IIIA (MPS IIIA) mice to reverse established neurodegenerative disease. The rationale behind the study is that there is only limited animal model-derived evidence supporting treatment of symptomatic patients, principally because few studies have been designed to examine disease reversibility.

Methods: Twelve-week old MPS IIIA mice were implanted with indwelling unilateral intra-ventricular cannulae. These were connected to subcutaneous mini-osmotic pumps infusing recombinant human sulphamidase. Pump replacement was carried out in some mice at 16-weeks of age, enabling treatment to continue for a further month. Control affected/unaffected mice received vehicle via the same method. Behavioural, neuropathological and biochemical parameters of disease were assessed.

Results: Improvement in some, but not all, behavioural parameters occurred. Sulphamidase infusion mediated a statistically significant reduction in primary (heparan sulphate) and secondary (gangliosides G_{M2}, G_{M3}) substrate accumulation in the brain, with small reductions in micro- but not astrogliosis. There was no change in axonal spheroid number. All mice developed a humoral response, however no adverse clinical effects were observed.

Conclusions: Continual infusion of replacement enzyme partially ameliorates clinical, histological and biochemical aspects of MPS IIIA mice, when treatment begins at an early symptomatic stage.

WTH13-19

Old-age hippocampal sclerosis associates with grn but not with ABCC9 gene variation in the population

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Objective: To evaluate genetic risk factors of old-age hippocampal sclerosis (HScl) in population- based cohorts.

Background: HScl is a dementing disorder characterised by severe neuron loss in CA1 and presence of TAR-DNA-binding protein-43 (TDP-43)-positive intraneuronal inclusions. Polymorphisms in the progranulin (GRN) gene (rs5848) and the sulfonylurea receptor 2- encoding gene *ABCC9* (rs704178) have been proposed as genetic risk factors for HScl in selected US brain bank collections. GRN has direct neurotrophic and inflammatory response-modulating functions. *GRN* rs5848 T/T genotype is linked with decreased GRN levels.

Materials and Methods: 469 haematoxylin-eosin- stained 7–9 μm hippocampal sections from brains donated for the population-based clinicopathological Cambridge City over 75 Cohort (CC75C) or the Cognitive Function and Ageing Study (CFAS) were evaluated for HScl. HScl was defined as no more than five identifiable pyramidal neurons in over half of CA1 fields of view at 200-fold magnification. 25 cases met HScl criteria. Single nucleotide polymorphism (SNP) genotypes in *GRN* and *ABCC9* were determined using TaqMan 7500 assays.

Results: HScl was significantly associated with the T-allele of rs5848 in *GRN*. 46% of HScl cases were carriers of the T-allele, compared to 29% in none-HScl cases (χ^2= 6.17, p = 0.013). No differences in genotype and allele frequencies of ABCC9 rs704178 were seen between HScl and non-HScl cases.

Conclusion: GRN rs5848 T/T genotype is associated with loss of CA1 neurons in the ageing brain, which might be linked through lower progranulin levels. ABCC9 rs704178 was not confirmed as a genetic risk factor of HScl in these cohorts.

WTH13-20

Nicotinamide-A PARP inhibitor attenuates streptozotocin-induced experimental dementia in Wistar rats

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PARP over-activity has been suggested play important in neuronal demise associated with various neurodegenerative disorders, thus PARP inhibition is considered to be a viable strategy to combat neurodegenerative processes. In the present study we have investigated the therapeutic potential of nicotinamide – a endogenous inhibitor of PARP against streptozotocin (STZ) induced neurocognitive deficit and biochemical abnormalities in rats. Streptozotocin was administered intracerebroventrically (ICV 3 mg/kg) bilaterally on day 1 and 3 in Wistar rats. One week following 1st STZ infusion nicotinamide was administered (100 and 200 mg/kg/day p.o.) upto 21st day. Morris water maze and object recognition tasks were used to assess learning and memory. Terminally the level of oxidative stress, pro-inflammatory cytokines and acetylcholinesterase (AChE) activity was measured in rat's cortical and hippocampal brain homogenate. Bilateral infusion of STZ produced significant learning and memory impairment in rats and elevation in AChE activity. Cognitive deficit observed following ICV-STZ infusion in rat's was also associated with the increase in the level of neuroinflammatory cytokines and markers of oxidative-nitrosative stress. Nicotinamide dose dependently attenuated STZ induces cognitive decline and other biochemical abnormalities observed following STZ infusion in rats. The observed neuroprotective effect of nicotinamide may be due to its antioxidant and anti-inflammatory activities and restoration of cholinergic functions. The findings from the current study clearly suggest PARP inhibition could be a novel strategy to halt cognitive decline associated with sporadic Alzheimer's disease.

WTH13-21

ALS-linked mutant SOD1 affects the biochemical properties of TDP-43

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Amyotrophic lateral sclerosis (ALS) is a fatal, adult-onset, and progressive neurodegenerative disorder with no cure. Cu/Zn-superoxide dismutase (SOD1) was the first identified protein associated with familial ALS; and aggresome formation of misfoled SOD1 is closely associated with ALS pathogenesis. Recently, transactive response DNA-binding protein 43 (TDP-43) is a principal component of insoluble ubiquitinated inclusions in ALS. However, it remains unclear whether these ALS-linked proteins partly have a shared pathogenesis. To determine the association between mutant SOD1 and TDP-43 in an ALS model, we characterized TDP-43 expression in SOD1 G93A transgenic mice model and ALS *in vitro* cell model. In the present study, we observe that mutant SOD1 interacts with TDP-43 by co-immunoprecipitation assays using

cultured cells. Furthermore, we also observe C-terminal TDP-43 fragments in these end stage mSOD mice, but this is seen only in mice having advanced disease. Mutant SOD1 overexpression led to an increased amount of mutant SOD1 and its interacting proteins including TDP-43 in the detergent-insoluble fraction. These findings suggest that mutant SOD1 could affect the solubility/insolubility of its interacting TDP-43 through physical interactions and modified TDP-43 may be involved in motor neuron death in the spinal cord of SOD1 G93A transgenic mice model.

WTH13-22

A neurogenic perspective on sarcopenia and ageing

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Ageing results in a progressive decline in skeletal muscle mass and performance, known as sarcopenia that leads to frailty and loss of independence. Sarcopenia is associated with modification and loss of neuromuscular junctions on the surface of myofibres and our molecular analysis of aged muscles in mice has revealed striking changes in gene expression related to denervation. We are interested to discover the extent to which these age related changes are reflected in changes in the central and peripheral nervous systems, hence the present study examines sarcopenia from a neurogenic perspective.

The sciatic nerves innervating the lower limb muscles were collected from male C57BL/6J mice aged 4, 15, 18, 22 and 24 months and analysed using western blotting, immunohistochemistry and transmission electron microscopy (EM). Nerves appeared healthy until 15 months but by 18–22 months western blot analysis revealed increasing levels of various cytoskletal proteins (Tau, vimentin, SMI-32) and, the enzyme cholineacetyltransferase (ChAT). We also found, significantly increased expression of ubiquitin autophagy marker p62 in the aged nerves. Increases were seen despite the fact that axon counts were generally lower in immunostained longitudinal sections of nerves from 27 versus 3 month old male mice. Ultrastructural (EM) examination of transverse and longitudinal sections of nerves revealed distinct protein aggregates in 27 month old male mice. Additional studies in female C57BL/6J mice compared young adult (3 months) and very old (26 months) nerves to examine the influence of gender. Increased levels of cytoskeletal proteins Tau, ChAT, and autophagy markers were also seen in these female mice at 26 months. This comprehensive time-course study of sciatic nerves from ageing male mice combined with a comparison of nerves from young and old female mice, revealed many changes by 18 months that progressively increased by 22–27 months. Increased protein levels in aged nerves may be due to impairment degradation mechanism in these nerves. Further studies are needed to elucidate the exact temporal sequence of changes in ageing nerves versus muscles, which will help in clarifying the exact mechanistic events that result in sarcopenia.

WTH13-23

A transgenic zebrafish model of spinocerebellar ataxia-3 to test potential disease treatments

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Spinocerebellar ataxia-3 (SCA-3), also known as Machado Joseph disease (MJD), is a hereditary neurodegenerative disease that affects motor function. SCA-3 is caused by expansion of a trinucleotide (CAG) repeat region within the *ATXN3* gene, encoding a polyglutamine (polyQ) region within the ataxin-3 protein. Whilst the wild-type ataxin-3 protein contains 12-44 glutamine residues, as many as ninety glutamines are found in the ataxin-3 protein of SCA-3 patients. We have successfully established the first transgenic zebrafish model of SCA-3. These zebrafish express human ataxin-3 containing either 19Q (wild-type) or 84Q (SCA-3). Immunoblot analysis of protein lysates extracted from our transgenic SCA-3 zebrafish revealed the presence of ataxin-3 cleavage products similar to those found in SCA-3 patient samples. These fragments were present at all ages examined from 3dpf through to 12 months old. Consistent with SCA-3 being a progressive disease we identified a marked motor phenotype developed in ataxin-3Q84 zebrafish from 4 months old, with ataxin-3Q84 zebrafish swimming slower than ataxin-3Q19 fish. A more sensitive behavioural test (escape response during darkness) detected slower swimming speeds in ataxin-Q84 zebrafish than ataxin-Q19 as early as 6dpf. This motor phenotype provides a useful readout for drug screening assays because it is easily quantified and occurs at an age that zebrafish larva can be treated in small multi-well plates. Our results indicate that our transgenic zebrafish model of SCA-3 is relevant to the human disease and will be a valuable tool for testing potential disease treatments.

WTH13-24

A mechanism for neurodegenerative disease in the enteric nervous system

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Neurodegenerative diseases affect the central nervous system (CNS) causing cognitive or motor symptoms depending on the location of the degenerating neurons. In Parkinson's disease and the prion diseases there is also evidence that disease can manifest as a

loss of function in the gastrointestinal tract. We report the results of neurodegeneration in the enteric nervous system (ENS) in two well-characterised models of neurodegenerative disease, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) intoxication inducing Parkinson's disease and intracerebral inoculation of prions resulting in prion disease. Despite different modes of disease induction and clinical signs of CNS degeneration both models reveal remarkably similar pathogenic processes in the ENS. Reduced stool frequency was accompanied by a significant reduction of neurofilament medium immunoreactivity (a marker of intrinsic primary afferent neurons) within the myenteric plexus of the ileum. Further investigation suggested that this might be secondary to inflammation in response to oxidative stress as indicated by temporal changes in neuronal nitric oxide synthase and glial fibrillary acidic protein immunoreactivity and increased expression of pro-inflammatory molecule mRNA. These results provide a mechanism for gastrointestinal dysfunction reported in both prion and Parkinson's disease patients and suggest a common mechanism of neurodegeneration in the ENS.

WTH13-25

Protein tyrosine phosphatase, receptor type, D as a candidate locus that may impact on exceptional longevity via methylation

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DNA methylation is a major epigenetic mark and has recently been associated with human ageing and longevity. Mutations in the protein tyrosine phosphatase, receptor type, D gene (*PTPRD*) have been implicated in human diseases including such as cancer. These disease processes may be associated with DNA methylation changes that occur with age. PTPRD is a signalling molecule predominantly expressed in the brain and is involved in a variety of cellular process including promotion of neurite growth and regulation of axon guidance.

The current project seeks to determine if *PTPRD* is involved in the epigenetic modulation of human longevity via DNA methylation.

Top-associated SNPs from five genome-wide association studies (GWAS) were mapped to known Refseq genes based on their physical location (within a 50 kb window). The five GWAS datasets were used to conduct a gene overlap analyses using the web-based platform, *Galaxy*. The datasets included data for: exceptional longevity, trans-acting loci that regulate brain methylation, and late-onset Alzheimer's Disease. The final two datasets were obtained through work conducted in our lab. These were methylation GWAS datasets for the loci that regulate methylation of the apolipoprotein-A1 gene (*APOA1*) and the microtubule-associated protein tau (*MAPT*).

Pair-wise analyses showed significant overlap between all datasets examined (significance values ranging from $p = 2.89 \times 10^{-03}$ to $p = 6.28 \times 10^{-48}$). The overlap analysis identified two genes common to all datasets, *PTPRD* and the formin homology 2 domain containing 3 gene (*FODH3*). *PTPRD* was selected as the candidate locus in this project due to its recently described associations with age-related human disease.

PTPRD may be involved in the epigenetic modulation of human longevity, possibly via DNA methylation. Current work will involve over-expressing the candidate locus in immortalised human cells and using pyrosequencing to measure methylation levels in *APOA1* and *MAPT*. This is to determine if *PTPRD* has any influence on methylation in these genes.

WTH13-26

The Abl-interactor Abi regulates synaptic development and neuronal survival via inhibition of BMP signaling

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Abl is a nonreceptor tyrosine kinase that regulates neuronal morphogenesis via regulation of actin dynamics. Although Abi (Abl interactor) is known to be phosphorylated and, in turn, regulate Abl, its role in the nervous system is still poorly defined. In this study, we show that *Drosophila* Abi acts downstream of Abl to regulate synaptic development at the neuromuscular junction (NMJ). Loss of Abi causes an increase in synaptic growth and the synaptic level of phosphorylated Mad, a molecular read-out of bone morphogenetic protein (BMP) signaling that is known to control synaptic growth at the developing NMJ and neuronal survival in the adult brain. These synaptic phenotypes are also induced by loss of Abl, suggesting a functional link between Abi and Abl during synaptic development. Genetic interactions suggest that Abi works downstream of Abl to regulate BMP-dependent synaptic growth. Finally, depletion of neuronal Abi or Abl causes brain neurodegeneration in the adult fly, and this phenotype is suppressed by downregulation of neuronal BMP signaling. Taken together, our results support a model in which Abl/Abi inhibits synaptic growth and neuronal cell death by negatively regulating BMP signaling. We are currently investigating the mechanism underlying the Abl/Abi-mediated inhibition of BMP signaling.

WTH13-27

Noninvasive assessment of presymptomatic and symptomatic metabolic changes in the brain and brainstem of an ALS mouse model

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Amyotrophic lateral sclerosis (ALS) is a debilitating and fatal neurodegenerative disease of unknown etiology that affects motoneurons in the brain, brainstem and spinal cord. The aim of study was to longintutindally ^{1}H MRS studying metabolic evolutions in the G93A SOD1 mouse brainstem, motor cortex and striatum. The life spans of such animals were about P125 based on our preliminary physiological studies. Three phases were studied, P60, P100 and P115, as pre-symptomatic, minor post-symptomatic phases and the age of the treatment period as in ASL patients, respectively.

Regional metabolic profiles were distinguishable from both MR spectra and quantitatively ($p < 0.0001$). With such data, metabolic

changes were observed in motor cortex, striatum and brainstem of SOD1 mice as early as at pre-symptomatic phase, glutamate, GABA, lactate and ascorbate. At P100, increased number but different changes were observed in these three regions. We observed significantly decreased brain lactate contents at P100 through these regions. The brain lactate was further concluded not due to nearly identical plasma lactate levels when comparing to those WT mice. At P115, metabolic changes evolved further. Among these changes, only lactate, alanine and GABA had been consistently becoming lowered in all three brain regions while the disease evolved. Glutamine was consistently higher in SOD1 mouse in all three regions. Other changes, such as myo-inisotol, glutamate, glycine, aspartate, total creatine, *N*-acetyl-aspartate, taurine, total choline were more specific to region. Strikingly, motor cortex containing upper neurons presented metabolic changes through the entire study. All changes indicated that the progression of over-expressing superoxide dismutase in mice were variable. This could be explained by the possible multiple pathogenic mechanisms of the familial ALS diseases, such as inflammation and excitotoxicity at different phases.

WTH13-28

Tetrahydroxystilbene glucoside protects synapses and inhibits α-synuclein aggregation

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Neurodegenerative diseases have a high incidence with ageing. Alzheimer's disease (AD), Parkinson's disease dementia (PDD) and dementia of Lewy body (DLB) share some common pathogenesis including synapse dysfunction and α-synuclein aggregation.

Purpose: The present study was to investigate the effects of 2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside (TSG), a main component extracted from *Polygonum multiflorum*, on the memory and movement functions and its mechanisms related to synapses and a-synuclein in aged mice.

Methods: The memory ability of mice was detected by step-through passive avoidance task. The movement function was measured by the pole test and rotarod test. Transmission electron microscopy was used to observe the synaptic ultrastructure. Western blotting was applied to measure the expression of synapse-related proteins and a-synuclein.

Results: Intragastrical administration of TSG for 3 months significantly improved the memory and movement functions in aged mice. TSG treatment obviously protected the synaptic ultrastructure and increased the number of synaptic connections in the hippocampal CA1 region and striatum; enhanced the expression of synaptophysin, phosphorylated synapsin I and postsynaptic density protein 95 (PSD-95), elevated phosphorylated calcium/calmodulin-dependent protein kinase II (p-CaMK II) expression, and inhibited the overexpression and aggregation of a-synuclein in the hippocampus, striatum and cerebral cortex of aged mice.

Conclusion: TSG improved the memory and movement functions in aged mice via protecting synapses and inhibiting a-synuclein aggregation in multiple brain regions. The results suggest that TSG may be beneficial to the treatment of AD, PDD and DLB.

WTH13-29

Central nervous system pathology in the phytosphingolipid-deficient mouse

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Phytosphingolipids are characteristic sphingolipids with an additional hydroxyl group at sphinganine C-4. They are abundant in mammalian small intestine and kidney, but rarely detectable in the central nervous system (CNS). The physiological role of phytosphingolipids, *in vivo*, however is still enigmatic. In the present study, we characterized a mouse model with targeted disruption of dihydroceramide: sphinganine C-4-hydroxylase (DES2) gene that results in the complete lack of phytosphingolipids. About 25% of *Des2* knockout ($Des2^{-/-}$) mice showed growth retardation and body tremor at around 10 days after birth, and subsequently died before 2 weeks of age. The intestinal epithelial cells and renal tubular epithelial cells exhibited structural defects with impaired distribution of membrane proteins and lipids. Investigating the CNS of 12 day-old $Des2^{-/-}$ mice, we observed marked neuronal cell death in the granule neurons in the hippocampal dentate gyrus, and in the pyramidal neurons in the cerebral cortex layer II. Many of them were cleaved caspase-3 positive. Paired box 6 (PAX6) positive new neurons and Ki67 positive proliferating cells were decreased. In addition, vacuole formation was observed in the white matter of cerebellum. Electron microscopic observation revealed that the vacuoles were well delineated and circular. Some vacuoles contained membranous structures and some appeared to be empty and were surrounded by myelin sheath. Immuno-blot analysis revealed that the protein level of myelin basic protein (MBP) was drastically decreased in the cerebellum of $Des2^{-/-}$ mice. By the immuno-histochemical analysis, the expression of DES2 was weak but detected in the hippocampus and cerebellum at 10 days of age. These findings indicate that phytosphingolipids play a critical role *in vivo*, not only in the epithelial cells of small intestine and kidney, but also in the neuronal cells in the developing brain. Common molecular mechanisms that may underlie these phenotypes are discussed.

WTH13-30

Short term and long term behavioural and pathological changes in a novel rodent model of repetitive mild traumatic brain injury

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Repetitive mild traumatic brain injury (mTBI) is associated with chronic traumatic encephalopathy (CTE), a neurodegenerative disease characterised by both the abnormal accumulation of hyperphosphorylated tau within the brain and long-term deficits in learning and memory. Understanding the disease mechanisms is critical to develop treatments for this condition. However, current

in vivo models of repetitive mTBI do not sufficiently replicate the short and long term effects observed in humans. Therefore, we sought to characterise a novel model of repetitive mTBI that accurately portrays the short and long-term clinical and histopathological features of CTE. Male Sprague–Dawley rats (n = 41) recieved either sham surgery, 1 mTBI or 3 mTBIs, spaced 5 days apart, induced using a modified Marmarou impact acceleration diffuse TBI model. Motor and cognitive outcomes were assessed for 12 weeks post-injury and histopathology assessed at 24 h and 12 weeks post-injury. Repetitive mTBI animals showed mild learning deficits, as reflected by increased escape latency on the Barnes Maze, deficits in spatial memory as shown with a progressive decline in Y Maze performance and decreased exploratory behaviour on the Open Field, reflecting anxiety-like behaviours. Notably, no motor deficits were observed on the rotarod at any time-point following injury, comparable to clinical CTE. At 24 h post-injury, repetitive mTBI animals showed increased tau phosphorylation, astrocyte reactivity and microglial activation within the cortex, in the absence of overt tissue loss. Tau phosphorylation and microglial activation persisted at 12 weeks post-injury, in addition to increased APP immunoreactivity, suggesting changes in axonal integrity. The presence of persistent cognitive deficits associated with tau phosphorylation, in the absence of tissue loss or motor deficits, is consistent with descriptions of clinical CTE. Therefore this model represents a clinically relevant platform to further explore the underlying pathogenesis of CTE and screen novel therapeutics.

WTH13-31

Turning the head red: near-infrared light is neuroprotective in a non-human primate model of Parkinson's disease

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This study shows, for the first time, that near-infrared light (NIr) treatment reduces clinical signs and offers neuroprotection in a subacute MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) monkey model of Parkinson's disease. We implanted a laser optical fibre device that delivered NIr (670 nm; total dose = 25–125 J) to the midline midbrain of macaque monkeys, close to the substantia nigra of both sides. MPTP injections (1.5–2.1 kg) were made over a 5 to 7 day period, during which time the NIr device was turned on. This was then followed by a 3 week survival period. Monkeys were evaluated clinically and their brains were processed for immunohistochemistry and stereology. Our clinical evaluations revealed three groups of MPTP-NIr-treated monkeys, all of which had less clinical impairment than the MPTP-treated ones. While some MPTP-NIr-treated monkeys developed rather severe (n = 3) or more moderate (n = 6) clinical signs, others - quite remarkably - developed few signs (n = 5). No such groupings were evident among the MPTP-treated monkeys, where all animals had severe clinical impairment (n = 11). The NIr was not toxic to brain tissue and neuroprotected many dopaminergic cells and their terminations against MPTP insult, particularly in animals that had minimal clinical impairment. Overall, the lower NIr dosages resulted in fewer clinical signs and more extensive neuroprotection than the high dosage. In summary, our findings indicated NIr as an effective therapeutic agent in a primate model of the disease and lay the template for translation into clinical trial.

WTH13-32

Glutathione monoethyl ester prevents TDP-43 pathology in NSC-34 cells expressing mutant TDP-43

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Oxidative stress is recognised as central in a range of neurological diseases including Amyotrophic lateral sclerosis (ALS), a disease characterised by fast progressing death of motor neurons in the brain and spinal cord. Cellular pathology of ALS includes cytosolic protein aggregates in motor neurons and glia of which hyper phosphorylated fragments of the RNA-binding protein TDP-43 constitute a major component. This is associated with a loss of nuclear TDP-43 expression. Furthermore, mutations in TDP-43 cause familial ALS. In this study, we investigated the role of glutathione (GSH) in modulating oxidative stress responses in TDP-43 pathology in motor neuronal NSC-34 cells. Our results demonstrate that depletion of cellular GSH produces pathology similar to that of mutant TDP-43 including the occurrence of cytosolic aggregates, hyperphosphorylation and nuclear clearing of endogenous TDP-43. We also demonstrate that the introduction of the mutant TDP-43^{A315T}, or the silencing of endogenous TDP-43, result in similar pathology, including depletion of intracellular GSH that most likely results from a decreased expression of a regulatory subunit of ɣ-Glutamylcysteine synthetase (GCLM), the rate limiting enzyme in GSH synthesis. More importantly, by treating these cells with GSH monoethyl ester, an approach that directly increases intracellular GSH and bypasses the need for GSH synthesis, we were able to prevent mutant-induced TDP-43 pathology, including aggregate formation, nuclear clearance, ROS production and cell death. Our data suggest that oxidative stress is central in TDP-43 pathology and may result from a loss of function affecting GSH synthesis and that treatments directly aimed at restoring cellular GSH content may be beneficial in preventing cell death in TDP-43-mediated ALS.

WTH13-33

Neuroprotective effects of coumarin-based iron chelators in the MPP$^+$ model of Parkinson's disease

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A large body of evidence shows that disturbed iron homeostasis, often coupled to mitochondrial dysfunction, plays an important role in the development of common neurodegenerative diseases such as Parkinson's disease (PD) and Huntington's disease, together with a less typified group of disorders known as neurodegeneration with brain iron accumulation (NBIA). Prior work from our laboratory

demonstrated that intracellular iron mediated the damage to mesencephalic dopaminergic neurons (MDNs) triggered by the Parkinsonian neurotoxin MPP^+. Considering that natural coumarins have iron chelation capacities, here we evaluated the effect of three newly synthesized 7-hydroxycoumarin (7-HC)-based iron chelators on protection against dendritic tree collapse induced by sub-lethal concentrations of MPP^+. The synthesis of chelating agents 7-HC1, 7-HC5 and 7,8-HC1, was performed adapting standard protocols. SH-SY5Y cells and primary cultures of MDNs obtained from day-14 Sprague–Dawley fetuses. A dendritic tree protection assay was established co-incubating MDNs with MPP^+ and 7-HC derivatives. Length of neuritic processes was determined using the HCA-Vision program after tyrosine hydroxylase (TH) immunostaining. We found that nanomolar concentrations of 7-HC1 and of 7-HC5 exerted a protective effect against MPP^+-induced neurotoxicity in primary dopaminergic neurons. Against expectations, 7-HCs did not present mitochondrial localization, but were found concentrated in lysosome-like organelles. Protection of dendritic tree collapse by natural coumarins, carries expectations for the elaboration of preventive therapeutic strategies for PD and other NBIA disorders using coumarin-rich diets. Knowledge of the exact mechanism of action underlying the observed neuroprotective effects requires further investigations. Support: FONDECYT 1130068, PIA-ACT1114 from CONICYT, Chile and CONICYT PhD Fellowship grant 21130445 (to YM).

WTH13-34

Role of prosaposin in retinal degeneration

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Prosaposin (PSAP) is a precursor protein of four saposins (SAPs) A, B, C, and D, which are required for the intra lysosomal degradation of sphingolipids, and is also secreted into extracellular space as a trophic factor. Recently, deficiency of semaphorin 4A (Sema4A), a member of axonal guidance molecule semaphorin, were reported to cause photoreceptor cell degeneration due to the impaired secretion of PSAP from the retinal pigment epithelial cells (RPE). In the present study, we characterized the retinal phenotypes of *Psap* knockout (*Psap*-KO) mice and *PSAP* transgenic (*PSAP*-Tg) mice which have strong, stable, and ubiquitous expression of PSAP. Retinas of *Psap*-KO mice did not show apparent photoreceptor cell degeneration even at their terminal 4 weeks of age. In contrast, retinas of *PSAP*-Tg mice showed progressive photoreceptor cell degeneration at around 3 weeks of age and almost all the photoreceptor cells were disappeared by 6 weeks of age. This retinal phenotype is similar to those reported in cathepsin D-deficient mice and Sema4A-deficient mice. Electron microscopic analyses revealed that *Psap*-KO mice exhibited numerous membranous inclusion bodies in RPEs and ganglion cells, however *PSAP*-Tg mice exhibited highly disorganized outer segments of photoreceptor cells with numerous membranous inclusion bodies in inner segments. No apparent structural changes were observed in RPEs and ganglion cells in *PSAP*-Tg mice. By the immunohistochemical analyses using anti-SAP-B antibody and anti-PSAP specific antibody, in contrast to the dominant expression of SAPs in RPEs and ganglion cells, outer nuclear layer which contain the cell body of photoreceptor cells expressed PSAP. These findings indicate that PSAP and SAPs play a critical role in retina, and intra-cellular accumulation of PSAP and SAPs cause retinal photoreceptor cell degeneration.

WTH13-35

Deficits in the ubiquitin proteasome system and increased pro-inflammatory signalling in an iPSC model of ALS

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Amyotrophic lateral sclerosis (ALS) is caused by the degeneration of upper and lower motor neurons, often leading to paralysis and death within only 2–3 years from diagnosis. Genetic causes of ALS have identified proteostasis mechanisms, such as the ubiquitin proteasome system, as causative of some of the familial forms of the disease. The overall aim of this research was to test the contribution of the ubiquitin proteasome system to cellular changes and motor neuron dysfunction in ALS. We reprogrammed fibroblasts from five ALS patients and three healthy donors into induced pluripotent stem cells. The iPSCs were differentiated into motor neurons *in vitro* and used to investigate the role of protein degradation in motor neuron degeneration.

Many cases of ALS are characterized by cytoplasmic inclusions containing TDP43 within motor neurons, while phosphorylation of TDP43 is correlated with cytoplasmic rather than nuclear localization. To assess the contribution of the ubiquitin proteasome system to the ALS cellular phenotype, the expression and localization of phosphorylated TDP43 was used as a biochemical marker. Western blot analysis showed that phosphorylated TDP43 expression increased in cells from ALS patients compared to controls and demonstrated an increased translocation from the nucleus to the cytosol. To assess the effect of inhibiting the proteasome on ALS-relevant biomarkers, motor neurons were incubated with the proteasomal stressor MG132, which led to a larger increase in phosphorylated TDP-43 in ALS patient cells than controls.

To further model the environment of the central nervous system in motor neuron degeneration we co-cultured astrocytes and inflammatory cells differentiated from the iPSCs along with the motor neurons. We identified a pro-inflammatory phenotype in cells from ALS patients compared to controls. Together our work identifies deficits in the ubiquitin proteasome system and increased pro-inflammatory signalling in ALS patient cells.

WTH13-36

Cu^+ is a cofactor for ubiquitin conjugation and cellular protein degradation

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The initial steps of ubiquitination require ATP and inorganic cofactors, including Mg^{2+} and an unknown metal ion. Here we reveal this as Cu^+. Low micromolar Cu concentrations selectively and rapidly (3 h) induce the formation of ubiquitinated proteins in cell lines and primary neuronal cultures in the absence of cell damage, but do not inhibit the purified 20S proteasome. Cells overexpressing the CTR1 Cu-uptake transporter have greater copper uptake and higher ubiquitinated protein levels. By contrast, depletion of intracellular copper levels by diamsar, a high affinity Cu chelator, decreases both the endogenous levels of ubiquitinated proteins and the accumulation of ubiquitinated proteins induced by proteasome inhibition. Cu-induced ubiquitin protein were confirmed to be of a chemical nature similar to orthodox ubiquitin adducts, but are enriched with bound Cu. Pulse-chase experiments demonstrated that copper accelerates protein degradation in primary neuronal cell cultures. In addition, we found that Cu(I) is important for the degradation of a wide range of proteins in different cell lines and in primary neuronal cultures. In cell-free system, which mimics a reductive intracellular environment, Cu as Cu^+ induces ubiquitination catalyzed by E1/E2/E3 enzymes. In tissue culture, copper promoted degradation of p53, a substrate of UbcH5b E2 conjugating enzyme. We found that the cell-free activity of UbcH5b is regulated by Cu^+. These data indicate that Cu^+ is the previously unidentified metal ion cofactor for enzymatic ubiquitination, and intracellular protein degradation. These findings have implications for proteinopathies with altered Cu homeostasis, such as Alzheimer's disease and Lewy Body Disease.

WTH13-37

Effect of aging on monoamine and purine levels in the basal ganglia of mice carrying A53T and A30P alpha-synuclein mutations

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The risk of developing neurodegenerative diseases increases with age. Although Parkinson's disease causation is multifactorial, a number of genes have been implicated in its genesis. Mutations in the alpha-synuclein gene, A53T or A30P, lead to autosomal dominant parkinsonism. Although there are many documented effects of these mutations, their influence on monoamine and purine levels in basal ganglia during aging have not been documented. Understanding the alteration of these purines is important since adenosine is known to be involved in modulation of ATP. Adenosine also acts as an inhibitory neurotransmitter and neuromodulator and has been shown to alter catecholamine concentrations and turnover, which are critical factors that underlie motor symptoms in Parkinson's. Within the brain, adenosine levels are a combination of the additive actions of adenosine synthesis and breakdown and thus are only a snapshot of total adenosine content. Adenosine formation is controlled by the enzyme AMP-selective 5′ nucleotidases that catalyzes the breakdown of ADP and AMP. To determine if aging alters, we evaluated adenosine, ATP, ADP and AMP levels in the basal ganglia of wild-type and mice carrying mutations in the alpha-synuclein gene (A53T, A30P, and SNCA−/−) at 4, 12, 18 and %5C or 24 months. Chromatographic detection of striatal adenosine content show significant changes between wild-type and alpha-synuclein mice at each age examined. Alterations in ATP and ADP levels are seen at 4 and 24 months, respectively. No significant differences were observed in striatal AMP at any age. In the SNpc, adenosine levels shows significant changes between wild-type and alpha-synuclein at 12 and 24 months, ATP changes are seen at 12 months, ADP changes are seen mice at 4, 12 and 24 months and AMP changes are seen at 12 and 24 months. The age-related alterations in purinergic concentrations will be compared with synthesis and release of monoamines in the basal ganglia.

WTH13-38

Cholinergic dysfunction of mesopontine tegmentum is involved in the development of Alzheimer's disease

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Neuronal death, leading to overall brain atrophy, is one of the fundamental characteristics of Alzheimer's disease. Cholinergic neurons of the basal forebrain are particularly vulnerable in Alzheimer's disease, and the consequent cholinergic neurotransmitter decline affects other neurotransmitter systems. Epidemiological studies have shown that sleep apnoea - stopping breathing during sleep - is a risk factor for Alzheimer's disease. The neurons of another major cholinergic nucleus in the brain, the mesopontine tegmentum (MPT), project to upper motor neurons to control upper airway muscle tone during sleep, and are also implicated in initiating and maintaining rapid eye movement sleep, which is considered fundamental for learning consolidation and retention of memory. MPT neurons also project to the basal forebrain and produce nerve growth factor and thus may support basal forebrain neuronal survival and function throughout life. We found that lesions of MPT cholinergic neurons by stereotaxic injection of saporin toxin conjugated to the specific urotensin II receptor peptide ligand result in altered breathing. Furthermore, the lesions produce a subsequent and selective degeneration of basal forebrain cholinergic neurons and a resultant decline in spatial memory. Loss of MPT-derived nerve growth factor due to MPT neuronal dysfunction may in turn cause dysfunction of basal forebrain neurons with negative flow-on effects on cognitive function and the development of AD pathology.

WTH13-39

Amyloid-beta remains a membrane peptide after cleavage. implications for secretion and kinetics of abeta turnover in human brain

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The accumulation of the neurotoxic peptide amyloid-beta (Abeta) in the extracellular space is widely considered to play a causal role in the neurodegeneration observed in Alzheimer's disease(AD). However, the mechanism of Abeta toxicity remains unclear. Initially, the Abeta peptide functions as a transmembrane portion of the larger amyloid precursor protein. After cleavage Abeta is excreted to the extracellular space where it accumulates and eventually forms toxic soluble oligomers. How Abeta transitions after cleavage from a transmembrane peptide to a soluble peptide remains a mystery. We use a biochemical fractionation protocol to demonstrate that less than 1% of all Abeta is soluble with 40% associated with the membrane and the remaining 60% the plaque material. The significant association of Abeta with the membrane suggests the release into the soluble space is not a major mechanism in human brain as previously described. The fractionation allows us to estimate the total amount of Abeta in an AD brain, which has implications for the clearance rates of Abeta. Further, we show that the retention of amyloid imaging agents used for positron emission tomography correlate significantly with the level of membrane and plaque associated Abeta ($R^2 > 0.8$). Amyloid acumulation in the brain occurs for 15–20 years before symptom onset. Comparing the differnce in the total amount of Abeta between control and AD brain we find that over a 20 year period the difference in clearance rates between an individual that accumulates Abeta versus one who does not is only about 10% or 50 ng/h. This subtle difference is encouraging for the future development of therapies that may enhance the accumulation and thereby delay or prevent the onset of AD.

WTH13-40

P75NTR expression profile in motor neurons of mice that model motor neuron disease and as marker for disease progression

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The common neurotrophin receptor p75 (p75NTR) is expressed in embryonic and neonatal motor neurons, then down-regulated, and re-expressed in adult motor neurons during damage or disease, including motor neuron disease (MND). We found p75NTR signaling contributes to neuronal damage [1] and in this process the p75NTR extracellular domain (p75NTRECD) is cleaved and appears in urine of people and mice with MND [2]. We now aim to define p75NTR re-expression in $SOD1^{G93A}$G1H mice that model MND across development, and determine if urinary p75NTRECD levels follow progression.

Methods: p75NTR expression in ventral spinal cord motor neurons in newborn C57Bl/6J mice (PND 1–7 and d14; $n = 3$/age), and $SOD1^{G93A}$G1H mice and C57Bl/6J controls from 40 days to end-stage/140 days ($n = 4$/age) were examined. Motor function was measured $SOD1^{G93A}$G1H mice and C57Bl/6J controls across disease progression ($n = 24$). Motor neurons were detected by ChAT and apoptosis by cleaved caspase-3. Urinary p75NTRECD was measured by ELISA in $SOD1^{G93A}$G1H mice and controls from 40 days to end-stage/140 days ($n = 11$–21) with dilution corrected with creatinine.

Results: p75NTR was found in 92.6% of motor neurons in newborn C57Bl/6J mice, decreased to 44.5% at 7 days and by 14 days was 1.5%. $SOD1^{G93A}$G1H mice showed overt motor dysfunction at 120 days. Motor neurons re-expressing p75NTR in $SOD1^{G93A}$G1H mice, the majority of which were apoptotic, were first observed at 80 days, continuing to 140 days, peaking at 100–120 days to ~ 5%. Significantly higher levels of p75NTRECD were first detected in $SOD1^{G93A}$G1H mouse urine at 60 days, and increased to end-stage.

Conclusion: Significant levels of urinary p75NTRECD occur as early as 60 days in $SOD1^{G93A}$G1H mice, indicating neuronal damage caused by p75NTR occurs before motor function impairment at 120 days. Since p75NTR expression was observed in apoptotic motor neurons, we conclude that motor neurons rapidly die in $SOD1^{G93A}$G1H mice and shed p75NTRECD. Additionally, p75NTRECD may be a marker of disease progression.

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WTH13-41

Increased glutamate release in TRPML1 knock-out mice

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Deletion or loss-of-functional mutations in TRPML1 cause mucolipidosis type IV(MLIV) that is characterized by a psychomotor retardation, neurodegeneration, corneal opacity, retinal degeneration, and achlorhydria. Our previous work with the $Trpml1^{-/-}$ mice showed that $Trpml1^{-/-}$ mice recapitulate many features of the human disease, including neuronal degeneration and achlorhydria. Achlorhydria was associated with a permanent stimulated morphology of the parietal cells, suggesting that TRPML1 may have role in regulated exocytosis, perhaps in membrane retrieval. To further explore the role of TRPML1 in regulated exocytosis we are studying salivary gland and pancreatic exocytosis *in vivo* and in isolated acini. In the present study, there are no apparent effect on fluid secretion in both of 10 mg/kg pilocarpine and mixture of 1 mg/kg pilocarpine and 0.6 mg/kg isoproterenol in the saliva secretion. However, amylase secretion was increased in parotid gland *in vivo* and in isolated acini also. Further, the agonist-related amylase secretion was increased in time-dependent and dose-dependent manner in the isolated acini. Interestingly, TEM images both of

pancreas and parotid gland show enlarged vesicles. Some of the enlarged vesicle shows fusion-like structure between lysosome and secretory vesicles. Moreover, we found an increased synaptic vesicle in the synaptic terminal of the TRPML1 knock-out (KO) mice brain cortex. Similar with the exocrine glands, we found an increased glutamate release in the cultured neuron. The glutamate release was increased in TRPML1 KO both of the resting state and KCl-stimulated state. This may be an explanation of a neuronal toxicity, which is the major symptom of the MLIV patient. Then, we found an increased activity of an acid phosphatase, the lysosomal enzyme located in the lumen of lysosome, in the saliva and pancreatic fluids. However, a mouse model of the niemann-pick disease type C did not represent the increased exocytosis and synaptic vesicle size was within the normal range. From these results, we suggest that Trpml1$^{-/-}$ deletion may relate with exocytosis of the secreting vesicles or related with fusion of the vesicles with the lysosome. Therefore, it would be useful to show new function of Trpml1$^{-/-}$ in exocytosis machinery of the cells, and suggest that novel mechanism that affects the neuronal disease in the MLIV.

WTH13-42

Triheptanoin delays symptom onset in the superoxide dismutase 1 (SOD1G93A) mouse model of amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder characterized by degeneration of upper motor neurons in the motor cortex and lower motor neurons in the spinal cord leading to muscle weakness, paralysis and death due to respiratory failure. The exact causes of the disease are unknown although glutamate toxicity, neuroinflammation, aberrant axonal transport and mitochondrial dysfunction are implicated. Impaired energy metabolism has been shown in patients with ALS and in animal models of ALS. There is an increased energy requirement as a result of hypermetabolism (higher energy expenditure), reduced food intake and weight loss. Alternative metabolic substrates such as triheptanoin, a triglyceride of heptanoate can be used to meet the high energy demand. Also, heptanoate can refill the tricarboxylic acid (TCA) cycle intermediates, which allows increased cycling of the TCA cycle and subsequently improved ATP production. We found metabolic abnormalities in gastrocnemius muscle of hSOD1G93A overexpressing mice at different disease stages that signify impaired glycolysis and TCA cycling. When triheptanoin (35% caloric content) was initiated in presymptomatic hSOD1G93A mice at P35, it delayed the onset of loss of grip strength, onset of body weight loss and seemed to attenuate some skeletal muscle abnormalities. However, triheptanoin appeared to have no benefit in improving motor symptoms and survival when it was administered after symptom onset at 70 days of age. Further studies are ongoing to reveal the effects and mechanisms of triheptanoin in ALS disease progression.

WTH13-43

The neuroprotective effect of puerarin against glutamate-induced cell injury in neuronally differentiated Y-79 retinoblastoma cell

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Puerarin is a major isoflavonoid derived from the Chinese herb *Radix puerariae* (kudzu root), which has been shown to be a potential treatment of various retinal disorders. However, to date, the molecular mechanisms underpinning its therapeutic effects on retinal diseases are not fully understood. Neuronal degeneration is a critical event in the occurrence and development of retinal diseases. Since the neuro-protective activity of Puerarin was reported before, the current study focused on the effect of Puerarin against neuronal degeneration induced by glutamate insults in neuronally differentiated Y-79 retinoblastoma cells. Human Y-79 cells were cultured in a synthetic medium and differentiated to be photoreceptor-like neurons. With the treatment of indicated drugs, the protective effect of Puerarin against glutamate-induced cell injury in neuronally differentiated Y-79 retinoblastoma cells was determined by MTT and Annexin V-FITC and PI double staining assays. Furthermore, the molecular events of related signaling pathways involved in this cellular process were also evaluated. Our data showed that cell injury was significantly increased with the treatment of glutamate (5 mM) in neuronally differentiated Y-79 cells, however, the treatment of Puerarin at 0.5, 5 and 50 μM attenuated the glutamate-induced cytotoxicity and cell apoptosis at a dose dependent manner. Furthermore, our study revealed that the neuroprotective effect of Puerarin is possibly mediated through inhibiting the death receptor-initiated extrinsic apoptosis pathway and the mitochondria-initiated intrinsic apoptosis pathway activated by glutamate. In summary, this study demonstrated the neuroprotective property of Puerarin against glutamate-induced cell injury in neuronally differentiated Y-79 retinoblastoma cells and related molecular mechanisms. This information may significantly contribute to better understanding the therapeutic effect of Puerarin and form the basis of the clinical applications of Puerarin in treating human retinal neurodegenerative diseases.

WTH13-44

M1-dominant microglial response precedes Prukinje cell loss in the cerebellum of SCA6-knockin mouse models

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Spinocerebellar ataxia type 6 (SCA6) is a dominantly inherited neurodegenerative disease, caused by an expansion of a CAG repeat

encoding a polyglutamine (PolyQ) tract in the $Ca_v2.1$ calcium channel. Pathologically, it is characterized by selective degeneration of the cerebellar Purkinje cells (PCs), which are a common target for PolyQ-induced toxicity in various SCAs. Molecular pathogenesis of SCA6 is largely unknown and it remains elusive whether SCA6 shares common pathogenic pathways with other SCAs. Here we studied the cerebellar gene expression patterns of young *Sca6* $MPI^{118Q/118Q}$ knockin (KI) mice, which express mutant $Ca_v2.1$ from an endogenous locus and recapitulate many features of human SCA6. Concordance analysis revealed that transcriptional profiles in the $MPI^{118Q/118Q}$ mice were distinct from those in the $Sca1^{154Q/2Q}$ mice, a faithful KI mouse model of SCA1. Gene ontology analysis indicated that genes involved in chemokine activity and chemokine receptor binding were significantly enriched in the $MPI^{118Q/118Q}$ cerebellum, suggesting that the early inflammatory response might precede the onset of PC loss. qPCR analysis showed that the upregulation of genes associated with microglial activation was initiated before PC degeneration was apparent and was augmented as the disease progressed. Histological analysis of the $MPI^{118Q/118Q}$ cerebellum confirmed the presence of Iba1-positive activated microglia. These microglia were predominantly M1-like pro-inflammatory microglia, and this was concomitant with an increased expression of pro-inflammatory cytokines and Toll-like receptor (TLR) -2 and-7. These results suggest that the unique transcriptional response, which highlights the upregulation of neuroinflammatory genes, may play a pivotal role in the pathogenesis of SCA6 and modulation of the innate immune system could pave the way for slowing the disease progression.

WTH13-45

Assessing the neuroprotective and therapeutic properties of a strawberry anthocyanin extract *in vitro* and in a mouse model of ALS

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Evidence implicates oxidative stress and the loss of principal antioxidant defenses in specific regions of the brain and spinal cord as underlying causes of the neuronal cell death observed in diverse neurodegenerative diseases. Thus, supplementation of antioxidant defenses may be an effective therapeutic strategy for diseases such as amyotrophic lateral sclerosis (ALS), Parkinson's disease, and Alzheimer's disease. In this context, nutraceutical antioxidants have provided promising insight into prospective treatment strategies. Anthocyanins, a class of flavonoid compounds responsible for the brilliant red, blue, and purple coloration of many fruits and vegetables, are of particular interest as these compounds have been previously shown to cross the blood brain barrier and exert neuroprotective and anti-neuroinflammatory effects in numerous models of neurodegeneration and aging. However, the use of anthocyanins for the treatment of ALS has never been examined. We explored the capacity of an anthocyanin-enriched extract from strawberries (SAE) and its principle anthocyanin constituent, callistephin (pelargonidin-O-3-glucoside) to protect cerebellar granule neurons (CGNs) *in vitro* from damage induced by oxidative stress. The neuroprotective and anti-inflammatory properties of SAE were then examined in the G93A mutant Cu, Zn-superoxide dismutase (SOD1) mouse model of ALS. Our work demonstrates that SAE and callistephin are potent neuroprotective agents *in vitro* against glutamate excitotoxicity, which features prominently in the pathogenesis of ALS. Moreover, transgenic mice harboring the G93A mutation of SOD1 displayed a significant delay in disease onset and extension in survival when orally administered SAE pre-symptomatically. This was accompanied by a corresponding preservation of grip strength in the hind limbs of treated animals, enhanced motor neuron survival, and reductions in neuroinflammatory markers within lumbar spinal cord such as reactive astrogliosis. Collectively, our data suggest that anthocyanins may be promising therapeutic agents for the treatment of ALS.

WTH13-46

Involvement of 17β-hydroxysteroid dehydrogenase type 10 (17β-HSD10) in neurodegenerative disorders

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17β-HSD10 is a mitochondrial multifunctional enzyme encoded by the *HSD17B10* gene mapping at Xp.11.2. It is a homotetrameric protein with a molecular weight of 108 kDa, having a crucial role to play in isoleucine and neurosteroid metabolism. For example, the catalysis of 17β-HSD10 is essential for both the inactivation of 17β-estradiol and allopregnanolone (a positive allosteric modulator of gamma-aminobutyric acid type A ($GABA_A$) receptors). Therefore, the maintained homeostasis of 17β-HSD10 in brain cells is important to avoid imbalances in neurosteroid metabolism. Extraordinarily high levels of 17β-HSD10 in hyppocampi have been associated with Alzheimer's disease (AD), while decreased 17β-HSD10 levels were found in the ventral midbrain of patients with Parkinson disease (PD). 17β-HSD10 mutations result in HSD10 deficiency, an infantile neurodegenerative disorder with intellectual and developmental disabilities. Abnormal quantity, quality and dynamics of mitochondria are revealed in the neurons of such patients because 17β-HSD10 is a member of the Parkin/PINK1 mitochondrion-quality control pathway. Moreover, a normal level of 17β-HSD10 appears to be vital to the electron transfer chain (ETC.) and to ATPase efficiency. Neurons, especially their synapses, rely heavily on oxidative phosphorylation for ATP production. Altered 17β-HSD10 levels can lead to bioenergetic crises, which underlies various forms of neurodegeneration.

WTH13-47

Morroniside, a potential protein phosphatase 2A activator, antagonizes tau hyperphosphorylation in a neurodegeneration model

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Background: Protein phosphatase 2A (PP2A), a major protein phosphatase, have been reported to be involved in the microtubule-associated protein tau hyperphosphorylation and aggregation in Alzheime disease (AD). Morroniside (MOR) is the isolated component of Corni Fructus. The present study is to investigate

the inhibitory effect of MOR on tau hyperphosphorylation and the underlying mechanisms.

Methods: SK-N-SH cells were pretreated with MOR 50–200 μmol/L for 24 h and then treated with okadaic acid (OA) (20 nM) for 6 h to induce tau hyperphosphorylation by inhibiting PP2A activity. To observe the inhibitory effect of MOR on tau hyperphosphorylation is dependent of PP2A directly or not, we transfected PP2Ac siRNA into HEK293 cells. Cell morphology was visualized under contrast microscope. Western blotting was used to measure the expressions of phosphorylated tau, total tau, Protein phosphatase-2A (PP2A), phosphorylated PP2A at Tyr307 (P-PP2A), demethylated PP2A at Leu309 (DM-PP2A), protein phosphatase methylesterase 1 (PME-1), Leucine carboxyl methyltransferase 1 (LCMT-1), phosphorylated Src at Tyr416 and Tyr529, total Src, Glycogen synthase kinase-3β (GSK-3β) and phospho-GSK3β (Ser9). The activity of PP2A was measured by a protein phosphatases activity assay kit.

Results: Compared with the control, the OA-treated cells became retracted and rounded up and their tau phosphorylation levels at pSer199/202, pT205, pT212, pS214, pT217 markedly increased. Pretreatment with MOR improved the morphology of cells and reduced OA-induced tau hyperphosphorylation. In addition, MOR treatment increased PP2A activity accompanied by a decrease of DM-PP2A and P-PP2A expression. MOR decreased PME-1 expression and the ratio of PME/LCMT-1. Furthermore, MOR treatment altered the level of Src phosphorylated at Tyr416, which can regulate phosphorylation of PP2A. PP2Ac siRNA could inhibit PP2Ac expression and induce tau hyperphosphorylation. MOR had no effect on PP2Ac expression, correspondingly, didn't affect tau hyperphosphorylation in PP2Ac siRNA transfected HEK293 cells.

Conclusions: Morroniside attenuates OA-induced tau hyperphosphorylation through regulating PP2Ac posttranslational modification. MOR could be a potential protein phosphatase 2A activator to be used as a therapeutic drug for AD and other tau pathology-related degenerative diseases.

WTH13-48

Characterising a novel isoform of the EphA4 gene

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Alternative splicing is a common and essential phenomenon in gene regulation, and disruption of this process has been associated with human genetic diseases. Amyotrophic lateral sclerosis (ALS) has been associated with mutations in more 20 genes, and one quarter of these genes are known to play a role in RNA metabolism, including TDP43. *EPHA4*, a target gene of TDP43, has been implicated in ALS with high levels being found in ALS patient's blood [1]; however, its exact role is yet to be elucidated. In the present study, alternative isoforms of *EPHA4* were investigated. Expressed sequence tags from the UniGene clusters of both mouse and human *EPHA4* were searched, and possible novel isoforms identified. Two of these novel isoforms were found in both human and mouse brain and spinal cord. To determine whether these mRNA isoforms encode proteins, we performed Western blot hybridisation and identified several protein bands, one of which was consistent with the predicted protein size of the novel mRNA isoform. The novel isoform, EphA4-N, contained the extracellular component of full-length EphA4, including the transmembrane domain. Additionally, we found EphA4-N and full-length EphA4 RNA expression profiles were different in $SOD1^{G93A}$ and $TDP43^{A315T}$ transgenic mouse models, and also differed as the disease progressed. This supports our hypothesis that EphA4-N may be regulated by TDP43. In addition, we stably overexpressed EphA4-N and full-length EphA4 in CHO cells, and found EphA4-N to exert an inhibitory effect on the phosphorylation level of the full-length EphA4, following stimulation with either ephrinA4 or ephrinA5 ligands. Because it repressed the function of full-length EphA4 *in vitro*, EphA4-N may be an endogenous inhibitor of full-length EphA4 and, thus, may be important in regulating the damaging effect of full-length EphA4 in ALS patients.

[1] Van Hoecke, A., et al., EPHA4 is a disease modifier of amyotrophic lateral sclerosis in animal models and in humans. Nat Med, 2012.

WTH13-49

Angiotensin type 1A receptor deficiency decreases amyloid β-protein production and ameliorates brain amyloid pathology

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Alzheimer's disease is characterized by neuronal loss and cerebral accumulation of neurotoxic amyloid-β protein (Aβ) and lowering the production of Aβ before the disease onset is a pivotal approach in the strategy of Alzheimer's disease treatment. Midlife hypertension is one of the major risk factors for the future onset of sporadic Alzheimer's disease and the use of some antihypertensive drugs may decrease the incidence of Alzheimer's disease. However, it is largely unknown how the blood pressure regulation system is associated with the pathogenesis of Alzheimer's disease. Activation of angiotensin type 1a receptor (AT1a) plays the key role in the renin-angiotensin system to elevate blood pressure. Here we found that AT1a deficiency significantly decreased Aβ production and amyloid plaque formation in a mouse model of Alzheimer's disease. The lack of AT1a led to the decreased endocleavage of presenilin-1, which is essential for γ-secretase complex formation and Aβ production. Notably, the ligand of AT1a, angiotensin II, enhanced Aβ production, presenilin-1 endocleavage and γ-secretase complex formation. Our results suggest that AT1a activation is closely associated with Aβ production and brain amyloid accumulation by regulating γ-secretase complex formation. Thus, removal of life style factors or environmental stresses that stimulate AT1a to elevate blood pressure may decrease Aβ production and brain amyloid accumulation, thereby preventing the pathogenesis of Alzheimer's disease.

WTH14 Psychiatric Disorders and Drug Abuse (Part 2)

WTH14-02

Increased expression of prohibitin and DISC1 in oligodendroglial cells in Schizophrenia

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Prohibitin and DISC1 have previously been implicated in the neuronal pathology of schizophrenia. The recently discovered abundant expression of both proteins in human brain oligodendrocytes raises the issue, whether these proteins might also be part of the well-known white matter abnormalities in schizophrenia. Using a direct, 3D-counting technique we morphometrically analyzed the number and density of prohibitin-immunoreactive oligodendrocytes in the left and right dorsolateral, anterior cingulate, and orbitofrontal cortex white matter as well as of DISC-immunopositive oligodendroglial cells in temporal cortex white matter of individuals with schizophrenia and controls. We could confirm the strong expression of prohibitin and DISC1 in oligodendrocytes. In schizophrenia, the numerical density of prohibitin-expressing oligodendrocytes was significantly increased in the right dorsolateral white matter area. The density of DISC1 immunoreactive oligodendrocytes was also elevated in schizophrenia. Taking into consideration the dual intracellular localization of prohibitin in oligodendrocyte mitochondria and cell nuclei, one may suggest an involvement of the protein in mitochondrial dysfunction and/or cycle abnormalities in schizophrenia. The increased expression of DISC1 might be of significance for the abnormal proliferation behaviour of oligodendrocytes and the impaired myelin formation in schizophrenia.

WTH14-03

Increased serotonin release in the brain underlies the effect of 4-MTA on olfactory responses in drosophila

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4-MTA (4-Methylthioamphetamine) is a "designer drug" which induces prolonged stimulation and euphoria, and has been associated with neurotoxicity and even death. It was designed to specifically block the serotonergic plasma membrane transporter (SerT), leading to an increase in the extracellular content of this amine. However, it has been also shown to act on other two molecular targets: the dopamine transporter and MAO-A. Thus, the behavioral consequences of 4-MTA exposure in an animal depend on the effects induced by this drug on all these targets. Invertebrates exposed to drugs of abuse display a set of behaviors that depend on the activation of aminergic systems, which are highly conserved when compared to vertebrate counterparts. In our lab, we are using some of the behavioral, physiological and genetic tools available in the fly *Drosophila melanogaster* to dissect out the contribution of different amine systems to the behavioral effects induced by 4-MTA.

Our data show that 4-MTA at different concentrations induce differential effects on fly olfaction and motor responses. Chronoamperometry studies indicate that 4-MTA induces the release of endogenous BAs in the fly brain, with a slow kinetics as compared to the effects observed for nicotine. Experiments in mutant flies suggest that the amine whose release is being modified by 4-MTA is serotonin. We further show that the effects on olfaction are not observed in animals expressing a mutation for SerT. Altogether, this data supports the proposition that 4-MTA induces the release of serotonin to modulate olfactory responses in *Drosophila*.

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WTH14-04

Selenium as a potential biomarker for Alzheimer's disease

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Prevalence of Alzheimer's disease (AD) is growing, and it is estimated that the number of affected people will exceed 81 million by the year 2040. Molecular pathology of AD may begin 20 or more years before symptoms appear, and in general the diagnosis is made when the disease is too advanced, and treatment is less effective. Therefore, the search for biomarkers of AD in its earliest stages is one of the most important research fields, supporting strategies to avoid the irreversible brain damage or mental decline. Selenium, recognised as essential component of selenoproteins that provide protection from oxidative cell damage, have an interesting association with AD risk and progression. We previously showed that deficiency of selenium contributes to cognitive decline among ageing population, confirming other studies that report that the deficiency of selenium, even subclinical, could be a risk factor for AD. Current approaches to studying selenium in AD tend to focus on total selenium levels, whereas all selenium species, including different selenoproteins are essential for brain function, and selenium deficiency and its role in disease may be confined to a single protein species. We propose to analyse selenium species in serum of participants from the Australian Imaging, Biomarker & Lifestyle Flagship Study of Ageing (AIBL) study, which aims to discover which biomarkers, cognitive characteristics, and health and lifestyle factors that determine the development of AD. Highly accurate and sensitive technique using high-performance liquid chromatography coupled to inductively coupled plasma mass

spectrometry (HPLC-ICP-MS) were developed to evaluate selenium status and the selenoproteome, which allows us to better understand how selenoproteins protect brain cells. The serum selenoproteome is a promising biomarker of early dementia, since its assessment is minimally invasive and has a clear association with cognition status.

WTH14-05

Transcriptional and epigenetic factors underlying the extinction of nicotine-seeking behaviour in the rat

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Relapse to cigarette smoking is a principle characteristic of tobacco addiction. This may be due to the persistence of drug-associated memories that prompt drug use across abstinence. This clinical finding can be modelled in rodents, as rats previously trained to self-administer nicotine are susceptible to reinstatement of drug-seeking following exposure to priming doses of nicotine, or cues previously associated with its delivery. Like other forms of memory, drug-associated memories appear to depend on changes in gene expression that are coordinated, in part, by epigenetic mechanisms. Research in this field suggests that the formation and extinction of contextual drug memories are regulated by dynamic modifications to chromatin. Previous work in our laboratory has demonstrated that administration of the histone deacetylase (HDAC) inhibitor, sodium butyrate (NaB), facilitates the extinction of nicotine-seeking in a manner that provides resistance to reinstatement. The present study aimed to investigate the molecular mechanisms involved in this potentiated extinction learning, examining the effect of both nicotine exposure and NaB treatment on mRNA expression and histone acetylation in the rat medial prefrontal cortex. Sodium butyrate induced a significant increase in gene expression of the neuronal protein kinase, Cdk5, in both saline and nicotine treated rats. In contrast, NaB increased BDNF mRNA only in saline control rats. Furthermore, nicotine, but not saline, exposure induced a decrease in histone H3K14 acetylation at the BDNF Exon IV promoter that was normalised by NaB treatment. These findings indicate that NaB and nicotine may act individually and in concert to regulate the genetic profile of the brain, though the behavioural consequences of their interactions are yet to be fully elucidated.

WTH14-06

Paraventricular thalamic pathways and fear memory retrieval

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The paraventricular thalamus (PvT) links prefrontal and amygdala circuits for emotion and learning. Here, we examined the role of PvT in retrieval of fear memories after extinction training and the circuit level mechanisms that enable this role. First, we used a double retrograde tracing approach to identify the PvT neurons at the origin of PvT – prelimbic (PL), PvT – infralimbic (IL), PvT-basolateral amygdala (BLA), and PvT-central amygdala pathways. Next, we combined retrograde tracing with detection of the activity marker (c-Fos) to identify the PvT pathway recruited during fear expression. Specifically, we trained rats to fear an auditory CS via pairings with shock in a distinctive context (A) and then extinguished that fear in a second, distinctive context (B). Rats were tested for fear in context A (ABA), context B (ABB), or were not tested (AB0). ABA renewal of fear was associated with activation of PvT projections to IL. Then, we asked whether PvT has a causal role in mediating this fear expression. To do so, we used adeno-associated viral vectors to express the inhibitory hM4Di DREADD in PvT. We injected CNO prior to ABB and ABA tests. Relative to rats expressing eYFP, the hM4Di rats showed reduced expression of fear on ABA test. These findings implicate a PvT – IL pathway in the expression of fear. Further experiments will examine the causal role of this pathway using optogenetic inhibition of PvT terminals in IL. Taken together, these results implicate PVT in retrieval of fear memories after extinction and suggest that this depends on interactions with IL prefrontal cortex.

WTH14-07

Stress-induced synaptic changes in the lateral habenula

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A number of brain area has been suggested to undergo synaptic changes in animal models of depression. One such region is the lateral habenula (LHb), a part of the epithalamus that mediates communication between forebrain and midbrain structures. Several lines of recent evidences suggest that the LHb is likely to play a critical role in depressive disorders. Previously, we have shown that excitatory synaptic inputs onto the LHb are abnormally potentiated, which is likely to mediate helplessness in animal models of depression. Given that the activity of the LHb is correlated with negative affective states of animals, we hypothesized that synaptic efficacy in the LHb could undergo alterations depending on emotional valence of external cues or experiences. Here, we investigated how exposure to stressful experience may modulate synaptic efficacy in this particular brain area.

WTH14-08

The effect of the partial receptor agonist varenicline on responding maintained by nicotine-associated cues

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It is widely recognized that cues associated with drug use (e.g., people, places, objects and paraphernalia) play an important role in maintaining drug addiction. However, the behavioural and neurobiological mechanisms by which they influence drug seeking are largely unknown. The present study used a laboratory model,

nicotine intravenous self-administration in rats, to track the evolving contribution of response-contingent cues in maintaining drug-seeking across extended training. In Experiment 1, the pattern of nicotine self-administration changed across extended training as rats shifted from responding in distinct clusters, to more regular nose-poking across the session. This change in how the drug was administered was accompanied by a shift in the contribution of cues to drug-seeking. Following brief training response-contingent cues were ineffective in maintaining responding in the absence of nicotine. In contrast, after extended training previously neutral cues maintained a high level of drug-seeking, even in the absence of nicotine. In Experiment 2, the impact of the partial nicotine agonist varenicline (VAR) on cue- or nicotine-maintained drug-seeking was assessed. Following brief training, VAR had little impact on responding for nicotine alone, yet significantly enhanced responding for previously nicotine-associated cues. After extended training, VAR significantly blocked responding for nicotine, but was ineffective in reducing cue-maintained responding. Together, these results highlight an increasing contribution of nicotine-associated cues in maintaining drug-seeking across an extensive drug-taking history. Furthermore, the popular smoking-cessation agent varenicline appears to be largely ineffective in disrupting responding maintained by nicotine-associated cues.

WTH14-09

A framework for holistic management of Schizophrenia

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Background: Schizophrenia is a brain disorder that affects the way a person acts, thinks, and sees the world. People with schizophrenia have an altered perception of reality, often a significant *loss* of contact with reality. They may see or hear things that don't exist, speak in strange or confusing ways, believe that others are trying to harm them, or feel like they're being constantly watched. With such a blurred line between the real and the imaginary, schizophrenia makes it difficult—even frightening—to negotiate the activities of daily life. In response, people with schizophrenia may withdraw from the outside world or act out in confusion and fear.

Method: The definition of holistic is relating to the idea that things should be studied as a whole and not just as a sum of their parts. In Schizophrenia a holistic framework is of paramount importance. This framework includes:

Clinical intervention – medical treatment, Suicide prevention, Counselling, Finance, Insurance needs, Public trustees and guardianship, Accommodation – Community living, Independent living skill, Relationship, Friendship, Entertainment, Stigma, Job, Education, AOD – Alcohol and Other Drug issues, Domestic Violence, Regular exercise for overweight due to medication, Any other health issues, Yoga Therapy – This is an emerging paradigm in India.

This research study aims to investigate and provides potential solutions for all of the above elements.

Expected Results: The framework, implemented in full form, will greatly improve the life style of schizophrenia sufferers. The effectiveness of all the elements in the proposed framework will be evaluated thru a web-based survey and the responses statistically analysed by back end computing processes.

Discussion: Though universal in nature, framework is based on resources available in Sydney, Australia. This framework needs customisation depending on the geographical location and available resources. Again with implementation of this framework and advancement in more meaningful clinical intervention for Schizophrenia, we firmly believe that with time Schizophrenia suffers will have more and more meaningful and productive life.

WTH14-10

Optogenetic evidence for opposing roles of the striatopallidal and striatohypothalamic pathways in relapse to alcohol seeking

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The nucleus accumbens (Acb) is a ventral striatal structure implicated in both relapse to, and abstinence from, drug seeking. Here we used optogenetic approaches to map the critical ventral striatal pathways mediating these processes. Of interest was the differential projection pattern between the two main nuclei of the Acb: the accumbens shell (AcbSh) and the accumbens core (AcbC). We applied AAV encoding eYFP, ChR2(H134R), or eNpHR3.0 to the accumbens shell (AcbSh) or accumbens core (AcbC) and implanted bilateral fibre optic cannulae into the lateral hypothalamus (LH) or ventral pallidum (VP) to allow for bidirectional optogenetic manipulation of the AcbShàLH or AcbCàVP pathways. Rats were trained to self-administer alcoholic beer in a distinctive context (A) prior to extinction in a second distinctive context (B). They were tested for ABA renewal of alcohol-seeking and then for reacquisition of alcohol-seeking in context A. Photoinhibition of the AcbCàVP projection impaired reacquisition of alcohol seeking as measured either by number of alcohol seeking responses or amount of alcohol consumed. In contrast, photoactivation of the AcbShàLH pathway attenuated reacquisition of alcohol seeking as measured either by number of alcohol seeking responses or amount of alcohol consumed. These findings demonstrate the key role of Acb circuits in relapse to alcohol seeking. Importantly, they highlight the opposing functions of the AcbShàLH or AcbCàVP pathways in this relapse.

WTH14-11

Inflammatory cytokines and glutaminergic excitotoxicity in patients with obsessive compulsive disorder

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In-vitro studies have demonstrated possible neuro protective effects of the following pro-inflammatory cytokines: IL-6, IL-1β and TNF-α against glutaminergic excitotoxicity in brain through different pathways. In the present study, we investigated the correlation among the level of the above pro-inflammatory cytokines in serum with Glx (glutamate+glutamine) levels in head of caudate nucleus (in brain) measured using *in vivo* proton Magnetic Resonance Spectroscopy (^{1}H-MRS) in patients with obsessive compulsive disorder (OCD), a neuropsychiatric illness with a possible multifactorial aetiology including immunological and excitotoxic factors. Thirty psychotropic-naïve patients with OCD (not on medication) and an equal number of gender and age matched normal controls

were recruited in the study. A detailed psychiatric assessment was carried out including sociodemographic and clinical variables (e.g. duration of illness and disease severity). ^{1}H MRS was performed using a 3T human MR scanner (Achieva, Philips) of the caudate nucleus (part of basal ganglia, implicated in OCD). Further, absolute quantification of Glx level was obtained using LC model. Further, 5 mL of blood sample was collected and assayed for the above pro-inflammatory cytokines (Siemens, Immulite™). The concentration of Glx as measured by ^{1}H-MRS was correlated with the cytokine levels in patients with OCD. The level of Glx was significantly higher in patients with OCD as compared to controls ($p < 0.05$). The Glx level negatively correlated with two of the three pro-inflammatory cytokines: IL-6 and TNF-α ($r = 0.807$, $p < 0.05$; $r = 0.838$; $p < 0.05$ respectively). The cytokine or Glx levels did not vary with age, gender, duration or severity of illness. The findings provide preliminary evidence regarding the possible neuroprotective effects of some pro-inflammatory cytokines against glutaminergic excitotoxicity in patients with OCD. Further studies on a larger sample size are required for confirmation of the above findings. The study was carried out with financial support from DBT (Department of biotechnology), Government of India under the INCRE (Initiative in Neuroclinical Research Education) initiative.

WTH14-12

Optimisation and utilisation of a novel whole-brain imaging technique (clarity) to analyse addiction-like behaviours

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Addiction, like many complex behaviours, requires communication and integration from multiple brain regions to produce the desired response. While much research has focused on individual cells or neuroanatomical areas, analysing these behaviours from a systems perspective often provides greater insight. However, current forms of molecular analysis such as immunofluorescence cannot provide systems level resolution. Immunofluorescence continues to be hampered by the inability of photons (light) and antibodies, to pass through complex, dense, and opaque brain tissue. Consequently, researchers have relied upon serial sectioning, and subsequent reconstruction via image analysis software, to generate a systems perspective. Research over the past 2 years however, has seen an influx of methods designed to overcome these problems. CLARITY, the most well known method, fixes nucleic acids, proteins and other macromolecules in place using a hydrogel superstructure, while allowing the removal of lipids via a detergent. After several weeks of washing, an antibody and photon permeable tissue remains. Subsequent methodological variations have recently been published, such as PARS and iDISCO, which respectively utilise higher concentrations of detergent or alternate detergent compounds to speed up the process. Although these techniques have focused on CNS tissue (predominately the brain), they have the potential to be used for most organs and tissues. These techniques however, are still in their infancy and require substantial trouble-shooting, and a significant investment of time and resources. We present, an optimised easy to follow, CLARITY protocol and provide examples of how it can be used to aid in the analysis of complex behaviours such as alcohol addiction.

WTH14-13

Genetic variation in glutamate and glycine neurotransmission pathways associated with the length of sobriety in alcoholics treated

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Objectives: To identify genetic variations associated with sobriety length in alcoholics treated with acamprosate.

Methods: Association analyses were conducted in the discovery sample of 225 alcoholics treated with acamprosate for 3 months in the community-based treatment programs affiliated with Mayo Clinic in Rochester Minnesota. Data from 110 male alcoholics treated with acamprosate in the study PREDICT were used for replication of the top findings. Statistical models were adjusted for relevant covariates, including recruitment site and clinical variables associated with response. Gene-level tests were performed using principal components analysis. Gene-set analyses were performed using the PC-Gamma approach with varying soft truncation threshold (STT) for the Gamma method for combining gene-level *p*-values.

Results: In the discovery sample, shorter abstinence was associated with increased intensity of alcohol craving and lower number of days between last drink and initiation of acamprosate treatment. After adjustment for these covariates, sobriety length was associated with the *GRIN2B* rs2058878 ($p = 4.6 \times 10^{-5}$). In the replication sample, shorter abstinence was associated with increased craving, increased depressive mood score, and higher alcohol consumption. After adjustment for these covariates, association of abstinence length with *GRIN2B* rs2058878 was marginally significant ($p = 0.0675$); as in the discovery sample, the minor A allele was associated with longer abstinence. Furthermore, rs2300272, which is in strong linkage disequilibrium with rs2058878, was also associated with abstinence length ($p = 0.049$). At gene level nominally significant association of abstinence length was observed with variation in the *AMT* ($p = 0.024$), GRIN3A ($p = 0.016$) and SHMT2 ($p = 0.039$) genes, and marginally significant evidence for association with the GRIN2B ($p = 0.067$) and GLRB ($p = 0.060$) genes. At the gene-set level, association of abstinence length with variation in the glycine pathway was nominally significant ($p = 0.042$, STT = 0.37). Marginal evidence of association with abstinence length was also observed for variation in the NMDA-receptor subunits ($p < 0.1$, STT < 0.15).

Conclusions: The length of abstinence in acamprosate-treated alcoholics is associated with genetic variation in the glutamate and glycine neurotransmission pathways. Search for the underlying mechanisms of this association and its utility for individualized treatment selection should follow.

WTH14-14

Novel methylation markers of the dysexecutive-psychiatric phenotype in FMR1 premutation women

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Objective: To examine the epigenetic basis of psychiatric symptoms and dysexecutive impairments in *FMR1* premutation women (PM: 55 to 199 CGG repeats).

Methods: A total of 35 *FMR1* PM women aged between 22 and 55 years and 35 age- and IQ-matched control women (< 45 CGG repeats) participated in this study. All participants completed a range of executive function tests and self-reported symptoms of psychiatric disorders. The molecular measures included DNA methylation of the *FMR1* CpG island in blood, presented as *FMR1* activation ratio (AR), and 9 CpG sites located at the *FMR1* exon1/intron 1 boundary, CGG size, and *FMR1* mRNA levels.

Results: We show that *FMR1* intron 1 methylation levels could be used to dichotomize PM females into greater and lower risk categories ($p = 0.006$ to 0.037; odds ratio = 14–24.8), with only *FMR1* intron 1 methylation, and to a lesser extent AR, being significantly correlated with the likelihood of probable dysexecutive or psychiatric symptoms ($p < 0.05$). Furthermore, the significant relationships between methylation and social anxiety were found to be mediated by executive function performance, but only in PM women. *FMR1* exon 1 methylation, CGG size, and *FMR1* mRNA could not predict probable dysexecutive/psychiatric disorders in PM women.

Conclusions: This is the first study supporting presence of specific epigenetic etiology associated with increased risk of developing comorbid dysexecutive and social anxiety symptoms in PM women. These findings could have implications for early intervention and risk estimate recommendations aimed at improving the outcomes for PM women and their families.

WTH14-15

Effect of melatonin on NMDA receptor subunit 2A and 2B expression in hippocampus of methamphetamine-treated postnatal rats

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Methamphetamine (METH), an addictive psychostimulant drug, effect to the nervous system. Chronic administration induced damage both of anatomical and functional of brain. Hippocampus, brain area involving in learning and memory, expresses of NMDA receptors especially subunits 2A and 2B. Additionally, METH induces glutamate release, NMDA receptor over-activation, excitotoxicity and leading to damage glutamatergic neuron. Melatonin, a circadian regulatory hormone from the pineal gland, is a powerful antioxidant agent in the nervous system. It can protect the degeneration of neurons by METH-induced in various areas. Therefore, the aim of this study is to examine the effect of METH on NMDA receptor subunit 2A and 2B alteration in hippocampus and the protective effect of melatonin. Four-day postnatal rat pups were divided into 4 group consists of control, METH-treated, melatonin and METH-treated, and melatonin-treated groups. Each group was subcutaneously injected for 7 days with normal saline, 5–10 μg/kg of METH, 30 min pretreatment of 10 μg/kg melatonin following METH, or 10 μg/kg of melatonin respectively. The results from the western blot and immunohistochemical studies showed that METH induced significantly decrease of NR2A/B level in hippocampus and NR2A/B-immunoreactivity in CA1, CA3, and dentate gyrus. In addition, melatonin can attenuate the effect of METH by enhancing NR2A/B expression in hippocampus. In conclusion, melatonin may protect hippocampus by its antioxidant effect against METH.

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WTH14-16

Conditioned stimulus extinction attenuates incubation of cocaine craving in adolescent and adult rats

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Relapse to drug use can occur in addicted individuals despite long periods of abstinence, and this is often precipitated by exposure to drug associated cues that evoke craving. In both animals and humans it has been observed that cue-induced drug craving increases over the first few weeks of abstinence and remains high over extended periods, a phenomenon termed "incubation of craving". As adolescence represents a unique period of vulnerability to developing drug addiction, potentially due to increased reactivity to drug associated cues, the first aim of the present study was to compare incubation of cocaine craving in adolescent and adult rats. P35 (adolescent) and P70 (adult) rats were trained to lever press to obtain intravenous cocaine at a dose of 0.3 mg/kg/infusion in daily, 6 h sessions. A stimulus light located above the active lever illuminated to coincide with each drug delivery, and this served as the conditioned stimulus (CS). Following acquisition of stable responding, rats were tested for cue-induced cocaine-seeking after either 1 or 30 days of withdrawal. A third group rats were tested for cue-induced cocaine-seeking after 30 days of withdrawal, however during this period they received 4 weekly sessions of CS extinction training. During CS extinction training, rats were placed in the self-administration chambers and exposed to 120 non-contingent CS presentations. Adolescent and adult rats acquired and maintained a similar level of cocaine self-administration, and rats of both ages exhibited a higher level of cue-induced cocaine-seeking if they were tested after 30 days of abstinence compared to 1 day. Incubation of cocaine craving was significantly reduced in both adults and adolescents that received CS extinction training. These results indicate that CS extinction training during abstinence may effectively reduce cue-induced relapse at a time when cue-induced drug craving is usually high.

WTH14-17

Synaptic mirnas coordinately regulate synaptic mrnas: perturbation by chronic alcohol consumption and manipulation *in-vivo*

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Local translation of mRNA into protein in synapses is a well characterized mechanism by which cells rapidly react to stimuli. Chronic alcohol consumption changes mRNA expression, likely causing persistent remodeling of synaptic structures via altered translation of mRNAs within synaptic compartments of the cell. MicroRNAs are short non-coding RNAs that have the ability to regulate mRNA translation. We previously showed that several microRNAs and mRNAs respond to alcohol consumption specifically in mouse synapses, including up-regulating GABA-related mRNAs and down-regulating glutamate-related mRNAs (Most et al. 2014). Furthermore, synaptic gene co-expression analysis revealed a highly connected network, demonstrating coordinated patterns of gene expression and highlighting the potential role of microRNAs as regulators. We identify alcohol-altered microRNA-mRNA synaptic interactions by using a combination of unbiased bioinformatics methods such as differential expression, correlation, co-expression, microRNA-mRNA target prediction, co-targeting and cell type specific analyses. Results show several microRNAs and mRNAs with overlapping patterns of expression that are correlated with alcohol consumption. Cell-type specific analysis revealed that a significant number of alcohol-responsive microRNAs and mRNAs were unique to glutamate neurons and were predicted to target each other. Here we study whether the manipulation of microRNAs *in-vivo* affects alcohol consumption and preference and if this process is reversible. We treat mice with microRNA mimics and antagomiRs and use a non-biased high-throughput microarray approach to measure the molecular effects on microRNAs and mRNAs. Finally we confirm changes in the expression of the predicted GABA and glutamate mRNAs using PCR, in-situ hybridization and immunohistochemistry. Results indicate that chronic alcohol consumption perturbs the coordinated microRNA regulation of mRNAs in synapses, a mechanism that may explain the aberrations in synaptic plasticity affecting the alcoholic brain.

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WTH14-18

Schizophrenia-like phenotypes of a novel transgenic mouse model for neuregulin-1 type III

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Neuregulin-1 (NRG1) is a neurotrophin that mediates neuronal migration, survival and synaptic plasticity. Neuregulin-1 Type III (NRG1-III) is a structurally unique isoform of NRG1 and is abundantly expressed. Individuals carrying a risk haplotype for the *NRG1* gene have an elevated mRNA expression of NRG1-III in their prefrontal cortex and are at risk of developing schizophrenia. Currently, it is unknown which symptoms of schizophrenia are mediated by an overexpression of NRG1-III or how this overexpression contributes to the neuropathology of schizophrenia. To address this, we have recapitulated an overexpression of NRG1-III *in vivo* in order to understand which schizophrenia-relevant behaviours are mediated by the overexpression and their underlying molecular mechanisms. A transgenic mouse overexpressing Nrg1-III specifically in forebrain regions was generated (*Nrg1-III tg* mice). Overexpression of Nrg1-III mRNA was confirmed in forebrain regions via qPCR. Mice underwent testing for cognitive, sensorimotor gating and social behaviour in order to detect schizophrenia-like behavioural deficits. qPCR revealed that male *Nrg1-III tg* mice have a ~ 2.5 fold increase in Nrg1-III mRNA in the olfactory bulb ($p = 0.0264$, $n = 4–5$) and prefrontal cortex ($p < 0.0001$, $n = 13–15$). Transgenic mice displayed impaired learning of a fear-eliciting context ($p = 0.024$, $n = 7–10$), a deficiency in prepulse inhibition ($p = 0.0331$, $n = 10$), and spent less time interacting with a novel social conspecific in a test for social preference ($p = 0.0029$, $n = 9$). Results indicate that *Nrg1-III* transgenic mice faithfully recapitulate an overexpression of Nrg1-III in forebrain regions, similar to that found in the disease state of a subset of schizophrenia patients (i.e. construct validity) and that these mice exhibit several schizophrenia-related behaviours (i.e. face validity) making them a prime model for research into the molecular pathologies caused by an overexpression of NRG1-III in schizophrenia patients.

WTH14-19

Pindolol, an FDA approved antihypertensive drug, decreases ethanol consumption in mice following long but not short-term exposure

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Alcohol dependence is a debilitating disorder with current therapies displaying limited efficacy and/or compliance. Previous studies have shown that stress contributes to the development of alcohol dependence. Long term alcohol consumption simulates chronic stress-like conditions that cause sensitization to negative emotional states of alcohol withdrawal. Stress induced changes in brain noradrenaline signaling has been shown to contribute to alcohol consumption and studies have shown that β-blockers including propranolol, which inhibit the activity of norepinephrine at beta-adrenoceptors, reduce ethanol consumption in animal models. We have extended these studies using a series of β-blockers to determine their effect on short and long term ethanol consumption using the drinking in the dark (DID) protocol in mice. Briefly, C57BL/6J mice were housed individually in a reverse light-dark cycle room and given access to 1 bottle of 20% ethanol (v/v) and 1 bottle of filtered water for a 2 h period, 5 days a week, 3 h into the dark cycle. Bottles were weighed 30 min and 2 h after presentation to determine daily ethanol consumption. Following 4 or 12 weeks of ethanol exposure, the mice received drugs or vehicle via sub-cutaneous or intra peritoneal injection. Our results show that pindolol, an FDA approved drug for the treatment of hypertension, with dual pharmacological activity at both noradrenergic and serotonergic receptors, significantly decreases ethanol consumption in mice following long-term exposure. These results implicate changes in noradrenergic and serotonergic signaling following prolonged alcohol use. Since pindolol is FDA approved, we have a great opportunity to advance this drug to a small scale clinical trial in humans as a potential pharmacotherapeutic treatment option for alcohol dependence.

WTH14-20

mGlu5 receptors in the extinction of cocaine-associated cues

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Drug-associated cues motivate drug-seeking, and cue extinction forms the basis of behavioural therapies targeting substance abuse. Here we examined whether Pavlovian extinction of a drug-associated stimulus (CS) would decrease the ability of that CS to reinstate an instrumental drug-seeking response, and the role of the mGlu5 receptor in CS extinction. In Experiment 1, all rats were trained to lever press for intravenous administration of cocaine (0.3 mg/kg/infusion), paired with a light CS. The lever pressing was extinguished in the absence of the CS. CS extinction occurred on the day following last day of lever extinction. Half of the rats were placed in the operant chambers and given 120 non-reinforced presentations of the CS, but with the levers retracted. The remaining rats were handled but received no further training. All rats received an intraperitoneal injection of either an mGlu5 negative allosteric modulator (MTEP) (2 mg/kg) or vehicle 20 min prior to this session. Cue-induced reinstatement was tested the following day by re-pairing the lever with the CS in the absence of any further primary reinforcement. Rats gave fewer drug-seeking responses following CS extinction than following handling alone ($p < 0.05$). This effect was attenuated by MTEP ($p < 0.05$). Experiment 2 followed the same protocol as Experiment 1, except that a positive allosteric modulator CDPPB (60 mg/kg) or vehicle was administered i.p. 20 min prior to CS extinction. At reinstatement (drug free), cue-elicited cocaine seeking was lower for the animals that had previously been administered CDPPB, regardless of extinction condition ($p < 0.05$). This study shows that Pavlovian CS-drug associations are important for driving instrumental drug-seeking behaviour during reinstatement; and that mGlu 5 signalling is necessary and sufficient for extinction of CS-drug associations.

WTH14-21

The GABA, glutamate and serotonin interplay in animal models of psychosis, parmacological and neurochemical studies

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The mechanism of action of presently used atypical neuroleptics is based not only on the blockade of D_2 receptors, but also on their affinity to other types of receptors, such as serotonergic, muscarinic, histaminergic or adrenergic. In majority of patients, those drugs are more effective especially in the context of negative and cognitive symptoms, in contrast to typical neuroleptics. Moreover, several clinical studies indicate, that the supplementation of the treatment with glutamatergic agents (e.g. modulators of NMDA receptor) is even more effective. Therefore it seems that the pharmacotherapy based on the activation of several kinds of receptors may bring an optimal therapeutic effect. In several studies we proposed the novel mechanism of action of antipsychotics, based on the stimulation of metabotropic glutamatergic receptors, with concomitant stimulation of serotonergic and/or $GABA_B$ receptors. We showed, that the concomitant stimulation of $mGlu_4$/5-HT_{1A} tandem, or $mGlu_5$/$GABA_B$, exerts clear antipsychotic-like activity.

Here we focused on the investigations of a novel mechanism that may underlie the above-mentioned interactions. We examined DHPG (group I agonist)-stimulated PI hydrolysis in the presence of a $GABA_B$ receptor agonist (SKF97541) and SKF97541-induced inhibition of cAMP formation in the presence of DHPG; we also checked if WAY100635 (5-HT1A agonists) and LSP4-2022 (mGlu4 agonists) exert mutual action on cAMP accumulation. We also investigated, if WAY100635 antagonizes LSP4-2022-induced inhibition of glutamate release in MK-801 treated rats. Moreover, the same schedule of experiments was performed in electrophysiological studies. Our results show, that there is no mutual interaction between $mGlu_5$/$GABA_B$ receptors or between $mGlu_4$/5-HT_{1A} receptors on the level of second messenger system. Similarly, such an interaction was not observed in electrophysiological studies, as WAY100635 did not blocked the action of LSP4-2022 in the patch-clamp experiments. Our results indicate, that the mutual interactions between above-mentioned tandems of receptors are dependent on more complex mechanisms that regulate the activity of neuronal network through independent neuronal pathways.

WTH14-22

Gene expression of androgen receptor and tyrosine hydroxylase are positively related in the substantia nigra in Schizophrenia

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Men are most often diagnosed with schizophrenia in their late teens to early adulthood, a time coinciding with increased circulating testosterone. In woman, psychosis is ameliorated when estrogen levels are high. This suggests that sex steroids modulate psychotic symptoms. Studies show increased presynaptic dopamine synthesis capacity in schizophrenia. It is unknown how sex steroid receptor gene expression is changed in the substantia nigra (SN) in schizophrenia or how sex steroid receptor gene expression is related to gene expression of tyrosine hydroxylase (TH), the rate limiting enzyme in dopamine synthesis. We tested for diagnostic differences in mRNA qPCR of estrogen receptors (ERa, ERb), androgen receptor (AR) and TH in the SN of control ($n = 29$) and schizophrenia post mortem brains ($n = 29$) provided by the New South Wales Tissue Resource Centre, (covaried for RNA integrity and brain pH). We found no change in overall TH ($F = 0.18$, df = 54, $p = 0.67$), ERa or ERb (both $F < 0.85$ and both $p = 0.40$) mRNA in SN from schizophrenia brains compared to control brains. There was a trend for AR mRNA to be decreased in schizophrenia ($F = 2.85$, df = 54, $p = 0.097$). Pearson's partial correlations revealed no significant correlations between ERs and TH mRNA. Overall, we found that the levels of TH and AR mRNA were positively correlated ($r = 4.75$, df = 49, $p < 0.001$) and in particular, there was also a strong positive correlation between AR and TH mRNA in SN from schizophrenia brains ($r = 0.55$, df = 22, $p = 0.005$). These data indicate that although gene expression of sex steroid receptors and TH may not be dramatically different in the SN with diagnosis, AR mRNA is strongly positively correlated with TH mRNA in schizophrenia. This provides evidence that AR activation may contribute to increased dopamine synthesis in the SN of schizophrenia patients and contribute to dysregulated dopaminergic activity in schizophrenia.

WTH14-23

Behavioural sensitisation to methamphetamine alters protein expression in the ventral hippocampus: implications for psychosis

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Purpose: Methamphetamine ("ice") is a widely abused illicit drug that can induce psychosis that is indistinguishable from schizophrenia. In rats, repeated administration of methamphetamine results in sensitised locomotor activity that is believed to model the neurochemical changes that underlie psychosis. Evidence for dysfunctional neurotransmission in the ventral hippocampus (VHipp) in schizophrenia and psychoses suggests that this region may be involved in the development of positive symptoms of psychosis. However, the underlying biological mechanisms that cause this dysfunction in the VHipp are largely unknown. In this study, we investigated alterations in protein expression in the VHipp following behavioural sensitisation to methamphetamine.

Method: Using a 2 × 2 experimental design, male Sprague–Dawley rats ($n = 48$) were administered methamphetamine (1 mg/kg i.p. on days 1 & 7; 5 mg/kg on days 2–6) or saline (1 mg/kg i.p.) for 7 days, followed 14 days later with an acute methamphetamine (1 mg/kg i.p.) or saline (1 mg/kg i.p.) challenge. Locomotor activity was then measured for 60 min, rats were euthanased and the VHipp was dissected out for label-free quantitative shotgun proteomic analysis ($n = 12$).

Results: Methamphetamine challenge resulted in a significant sensitised locomotor response in rats pre-treated with methamphetamine when compared to all other groups ($p < 0.05$). Proteomic triplicate analysis of the VHipp resulted in a total of 596 differentially expressed proteins over the four groups, with 370 proteins uniquely changed in methamphetamine sensitised rats. Changes to protein expression in the VHipp of methamphetamine sensitised rats were associated with dysfunctional energy metabolism, glutamatergic overactivity, reduced GABAergic activity, and neurotoxicity.

Conclusion: Proteins found to be differentially expressed in the VHipp following behavioural sensitisation to methamphetamine are consistent with studies of molecular changes in schizophrenia and suggest that the VHipp may be involved in psychoses. Our findings may facilitate discovery of novel pharmacological treatments for positive pathological features of methamphetamine-induced psychosis and psychotic disorders.

WTH14-24

Impairment of molecular transport in a mouse model of Schizophrenia

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Schizophrenia is a devastating psychiatric disorder. Genetic studies of patients with the disease have identified several susceptibility genes, including DISC1, 14-3-3 and NDEL1/NDE1. DISC1 is implicated in several cellular processes during neurodevelopment via its interaction with NDEL/NDE1. We previously reported that the mutations in *NDE1, ARHGAPs* and *YWHAE*/14-3-3e contributed to schizophrenia susceptibility and that DISC1 functions as a adaptor molecule linking the 14-3-3/NDE complex to Kinesin-1 for axonal outgrowth. In this study, we identified several RNA-binding proteins, including HZF, as novel DISC1-interactors. HZF participates in the mRNA localization of inositol 1,4,5-trisphosphate receptor type 1 (IP3R1), which plays a key role in synaptic plasticity. DISC1 co-localized with HZF protein and *IP3R1* mRNA in hippocampal dendrites and directly associated with neuronal mRNAs including *IP3R1* mRNA. Studies of *Disc1* knockout mice revealed that DISC1 regulates the dendritic transport of *IP3R1* mRNA by directly interacting with its mRNA. The DISC1-mediated mRNA regulation was involved in synaptic plasticity. We will discuss the physiological meaning of the transport of the NDE1 complex, ARHGAPs and the neuronal mRNAs mediated by DISC1.

WTH14-25

Regulation of a neuronal glutamate transporter by methamphetamine

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Amphetamine (AMPH) and methamphetamine (METH) are psychostimulants that affect behavior primarily through modulation of dopaminergic and glutamatergic neurotransmission. AMPH and METH differ by only one methyl group, however their behavioral actions, therapeutic utility and potential for addiction differ. In addition to their effects on dopamine reuptake and efflux, AMPH and METH also regulate extracellular dopamine concentrations through their ability to cause internalization of the dopamine transporter, DAT. Our recent work has shown that AMPH also modulates excitatory signaling through endocytosis of the glutamate transporter, EAAT3 which results in the potentiation of excitatory neurotransmission. AMPH enters the cytoplasm through the DAT and once inside it initiates a biochemical cascade that activates endocytosis of the DAT and EAAT3 through a RhoGTPase- and dynamin-dependent mechanism. Whether METH activates transporter internalization through a similar mechanism within neurons has not yet been established. To address this question we used transfected HEK293 cells and primary midbrain dopamine neuron cultures to examine the effects of METH on DAT and EAAT3 trafficking and surface expression. Pretreatment of cells with either METH or AMPH resulted in a dramatic loss in both EAAT3 and DAT surface expression. Inhibition of Rho or dynamin activity blocked AMPH- and METH-mediated loss of DAT and EAAT3 activity. However, only AMPH-mediated trafficking of the carriers was blocked by pre-incubation with the DAT inhibitor, cocaine. Furthermore, METH induced a loss of EAAT3 activity in HEK293 cells without DAT co-expression. In neuronal cultures, METH treatment led to a loss of cell-surface EAAT3 in multiple neuronal subtypes, whereas AMPH led to a loss of EAAT3 only in DAT-expressing neurons. Finally, in acute brain slices from regions devoid of DAT, we observed internalization of EAAT3 following METH, but not AMPH treatment.

These findings indicate that unlike AMPH, METH has the capacity to alter glutamate transporter surface expression in a manner that does not depend on DAT expression. These data imply actions of METH on both glutamatergic and dopaminergic signaling, which could contribute to the different behavioral and neurochemical profiles of METH and AMPH.

WTH14-26

Modelling methamphetamine psychosis in mice: role of brain bdnf or reelin

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The abuse of methamphetamine (METH a.k.a. ice or crystal meth) has reached near-epidemic proportions in Australia and worldwide. Abuse is particularly prevalent among young adults. METH abuse is associated with a heavy burden on the health care and criminal justice system. Among regular METH users, the prevalence of psychosis is 11 times higher than in the general population. This psychosis closely resembles paranoid schizophrenia with persecution delusions, auditory and visual hallucinations, and social withdrawal. Late adolescence may be a period of particular developmental vulnerability. However, the brain mechanisms involved in vulnerability to methamphetamine psychosis and their overlap with those involved in schizophrenia, remain unresolved.

We developed an animal model of methamphetamine abuse and psychosis where mice are treated chronically during late adolescence/young adulthood. The animals receive escalating doses of methamphetamine when they are 6, 7 and 8 weeks of age and the behavioural effects of this chronic "binge"-like protocol are assessed in adulthood in a range of tests relevant for psychosis-like behaviour, sensorimotor gating, learning and memory, and social behaviour. Because several studies have shown that levels of brain-derived neurotrophic factor (BDNF) and reelin are reduced by about 50% in post-mortem brain samples from patients with schizophrenia, we used BDNF- and reelin heterozygous mice.

BDNF heterozygous mice showed significantly greater sensitization to METH than wildtype control mice and showed selected deficits in social behaviour and spatial memory (Manning & van den Buuse, in preparation). In contrast, after a METH challenge dose, METH-pretreated reelin heterozygous mice tended to show less hyperactivity than similarly pretreated wildtype controls, suggesting a protective effect of partial reelin depletion although male METH-treated reelin heterozygous mice displayed reduced short-term spatial memory. Anxiety was increased in both METH-treated mice

controls and reelin heterozygous mice but no differences were found for prepulse inhibition, a measure of sensorimotor gating.

These preliminary results reveal involvement of BDNF and reelin in selected behavioural changes in a mouse model of METH abuse and psychosis.

WTH14-27

Using a transgenic zebrafish model of Machado-Joseph disease to study the role of calpains, caspases and cathepsins

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Machado Joseph Disease (MJD), also known as spinocerebellar ataxin-3, is a fatal hereditary neurodegenerative disease affecting neurons of the brain and spinal cord disrupting the ability to control movement of muscles. MJD is caused by a long trinucleotide (CAG) repeat region within the gene ATXN3. This CAG repeat region encodes for a polyglutamine (Q) tract, greater than Q40, compared to healthy subjects with lengths ranging Q1-Q40. Here we have investigated ataxin-3 proteolytic cleavage, using transgenic MJD zebrafish. Western blot of protein lysates extracted from our transgenic zebrafish revealed the presence of faint full-length ataxin-3 bands and ataxin-3 positive cleavage fragments. Similar sized cleavage fragments (50 kDa and 30 kDa) have been identified in human MJD patient brains and animal/cell models. We explored whether ataxin-3 cleavage is caused by proteases such as calpains, caspases or cathepsins by examining whether inhibiting calpain/caspase/cathepsin activity prevents formation of the fragments. Treatment with calpain inhibitors (ALLN, calpeptin and MDL28170) lead to preservation of full-length ataxin-3 protein and decreased cleavage fragments. On the other hand, caspase and cathepsin inhibitors was not successful at conserving full-length ataxin-3. From these results, we conclude that calpain activity, and not caspase activity, is responsible for the cleavage of ataxin-3 protein. Therefore calpain inhibitor drugs warrant further investigation for MJD treatment.

WTH15 Mechanism of Neuroprotection (Part 2)

WTH15-01

Anti-amyloidogenic, anti-inflammatory and antioxidant potential of Caesalpinia crista

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Amyloidosis, oxidative stress and inflammation have been strongly implicated in neurodegenerative disorders like Alzheimer's disease. Traditionally, Caesalpinia crista leaf extracts are used to treat brain related diseases in India. C. crista is used as a mental relaxant drink as well as to treat inflammatory diseases and is reported to be used to enhance memory and to treat dementia. Amyloid beta is the major etiological factor implicated in Alzheimer's disease (AD). Amyloidbeta(42) selfassembles to form oligomers and fibrils via multiple aggregation process. The recent studies aimed to decrease Amyloidbeta levels or prevention of Amyloidbeta aggregation which are the major targets for therapeutic intervention. Natural products as alternatives for AD drug discovery are a current trend. The studies on pharmacological properties of C. crista are very limited. We evidenced that Caesalpinia crista leaf aqueous extract has anti-amyloidogenic potential. The aggregation kinetics was monitored using thioflavin-T assay and transmission electron microscopy (TEM). The results showed that C. crista aqueous extract could able to inhibit the Amyloid beta(42) aggregation from monomers and oligomers and also able to disaggregate the pre-formed fibrils. Further,leaf extract of C. crista exhibited antioxidant properties and inhibited 5-lipoxygenase (anti-inflammatory) in a dose dependent manner. The better activity of C. crista is attributed to high gallic acid and ferulic acid present in the leaf extract: Thus, the leaf extract of C. crista can be a potential therapeutic role for Alzheimer's disease. The study provides an insight on finding new natural products for AD therapeutics. Our lab is currently working on characterizing the active molecules responsible protective activity of leaf extract of C. Crista.

WTH15-02

Genetic deletion of D3 receptors abolishes the influence of prolonged exposure to pramipexole upon the dopamine transporter

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The dopamine transporter (DAT) is a transmembrane glycoprotein essential for the maintenance of normal dopamine (DA) homeostasis in the brain. Several presynaptic proteins like protein kinases, α-synuclein, D_2 and D_3 autoreceptors tightly control DAT, whose dysfunction is involved in neurological and psychiatric conditions such as Parkinson's disease (PD), depression and attention-deficit hyperactivity disorder. In this study we show that prolonged pramipexole administration (0.1 mg/kg/day, 6–21 days), a D_3-preferrent agonist widely used in PD treatment, leads to a decrease in DA uptake in mouse striatum with a reduction in DAT affinity for DA and no changes in DAT density or subcellular distribution. The effect of pramipexole was absent in mice with genetically-deleted D_3R ($D_3R^{-/-}$), yet unaffected in mice genetically deprived of D_2R ($D_2R^{-/-}$). Moreover, Pramipexole promotes a physical interaction between D_3R and DAT. Treatment also modified DAT interactome in a D_3-dependent manner, promoting the formation of DAT dimers and DAT interaction with both D_2R and α-synuclein, effects that were abolished in $D_3R^{-/-}$ mice, yet unaffected in $D_2R^{-/-}$ mice. Our findings contribute to a novel understanding into the D_3R agonist long-term effects, with relevance in its use as antiparkinsonian and antidepressant.

WTH15-03

Neuroprotective effect of salidroside on AD transgenic drosophila and beta-amyloid-induced toxicity in PC12 cells via the PI3K/A

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Objective: Alzheimer's disease (AD) is an age-related and progressive neurodegenerative disease that causes substantial public health care burdens. Intensive efforts have been made to find effective and safe treatment against AD. Salidroside (SDS) is the

main effective component of Rhodiola rosea L, which has several biological properties.The objective of this study was to investigate the efficacy of SDS in the treatment of AD transgenic Drosophila and beta-amyloid-induced toxicity in PC12 cells as well as explore the possible mechanisms.

Methods: we used five different AD transgenic Drosophila lines (Aβ1–42, Aβ1–42; Aβ1–42, Aβ1–42E22G, APP;BACE transgenic Drosophila lines and Tau expressing Drosophila line). All expressed in the Drosophila central nervous system and eyes by the Gal4/UAS system.Canton S (CS) flies were used as WT controls. SDS and Aricept were diluted into Nestle Infant Cereal to a final concentration of 2 μM, 6 μM, 20 μM and 3 μM, 10 μM, 30 μM respectively. The number of dead flies was recorded every day. The fly behavior was recorded with a video camera. The accumulation of human Aβ42 in Fly head extracts was quantified using ELISA kits. Micrographs of Drosophila brains showed fluorescence from Aβ-antibody 6E10 staining. PC12 cells were pretreated with SDS (50 μM, 100 μM, 200 μM) for 2 h before treatment with Aβ25–35 for 24 h. After exposure to Aβ25–35, the levels of AKT/P-AKT,P-GSK3β,P-tau396,t-tau,Bax and Bcl-2 were evaluated by western blot analysis. The levels of TNF-α, IL-1β and IL-6 were determined by ELISA kits. The antiapoptotic and antioxidative mechanisms of SDS were also studied in Aβ-treated PC12 cells.

Results: *In vivo* studies, the longevity as well as the locomotor activity of five different AD model genotypes were improved remarkably in the SDS treated group. We also observed less β-amyloid deposition following the drug treatment. *In vitro* studies illustrated that SDS increased the cell viability of Aβ-treated PC12 cells in dose dependent manner, probably through attenuation of Aβ-induced oxidative and apoptotic stress. SDS also significantly upregulated the levels of P-AKT, P-GSK3β, Bcl-2 and downregulated the expression of P-tau396,Bax,TNF-α, IL-1β and IL-6.

Conclusion: In conclusion, our data presented the evidence that SDS was capable of reducing the neurodegeneration in AD transgenic Drosophila, inhibiting apoptosis, reducing oxidative stress, possibly through the PI3K/Akt pathway. Salidroside could be developed as a promising herbal agent for neuroprotection and novel adjuvant therapies for Alzheimer's disease.

WTH15-04

Synergistic action of camp and omega-3 in protecting central neurons after injury

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Injury to central nervous system causes progressive degeneration of injured axons and the neuronal bodies. Neuronal survival after injury is a prerequisite for successful regeneration of axons and restoration of lost functions. Using rodent visual system as a CNS model, we investigated the effect of cAMP elevation and over-expression of Omega-3 on retinal ganglion cell survival after optic nerve injury. We found that either sustained elevation of cAMP by intravitreal injections of cAMP analogue cpt-cAMP or over-expression of Omega-3 in transgenic mice enhanced retinal ganglion cell survival by 2-folds after optic nerve injury. In addition, further enhancement (3-fold) of retinal ganglion cell survival was seen in Omega-3 over-expression mice with intravitreal injections of cpt-cAMP. These results demonstrate that cAMP elevation and over-expression of Omega-3 protect retinal ganglion cells after injury, and there is a synergistic effect of cAMP elevation and Omega-3 over-expression in protecting central neurons. This study thus provides important information for possible therapeutic intervention for neural repair in the future. The underlying mechanisms for the observed neuroprotections are currently under investigation.

WTH15-05

Early decompression following traumatic cervical spinal cord injury in Australia: access to care from the accident site to surgery

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Early decompression surgery may improve neurological outcome after traumatic spinal cord injury (SCI) but can be difficult to achieve because of times for transportation, investigation and organisation of surgery. We examined cervical SCI cases in Victoria, Western Australia and South Australia to determine average times to decompression and identify delays. Data were extracted from medical records from 2010 to 2013 including patients (n = 47, VIC; n = 27, WA; n = 10, SA) aged between 15 and 70 with C3-T1 traumatic SCI and excluding multi-trauma cases and those not undergoing decompression. Non-parametric data were compared using Mann–Whitney U t-test (significance $p < 0.05$). Median times from accident to decompression were similar in VIC and WA (21 h and 22 h, respectively) and 14 h in SA. Between 2010 and 2013, there was a substantial improvement in the median time to decompression in all states. Delays resulted from pre-surgical hospital admission with median times to decompression being 26 h in VIC, 35 h in WA and 22 h in SA; cases taken straight to a surgical hospital were 10 h in VIC and SA and 19 h in WA. Across the three states, cases taken straight to the surgical hospital had a significantly lower time to decompression compared to those taken to a pre-surgical hospital (11 ± 65 and 26 ± 73, respectively, $p < 0.01$). Other delays resulted from times taken to complete investigations and organise theatre at the surgical hospital. We conclude that the median time to decompression surgery in cases of cervical SCI in the included states improved between 2010 and 2013. Early identification of SCI in the field, direct admission to a surgical centre and improved post-admission management could further reduce these times. Discussions with paramedics and clinicians are underway to improve patient access to care.

WTH15-06

A rare P2X7 variant ARG307GLN with absent pore formation function protects against neuroinflammation in multiple sclerosis

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Multiple sclerosis is a chronic relapsing-remitting inflammatory disease of the central nervous system characterized by oligodendrocyte damage, demyelination and neuronal death. Genetic association studies have shown a two-fold or greater prevalence of the HLA DRB1*1501 allele in the MS population compared with normal Caucasians. In three large cohorts of patients with multiple sclerosis (total 2941 patients, 3008 controls) we examined the associations of twelve functional polymorphisms of P2X7, a microglial/macrophage receptor with proinflammatory effects when activated by extracellular ATP. In initial and replication cohorts the SNP, rs 28360457, coding for Arg307Gln was associated with MS and combined analysis showed a two-fold lower minor allele frequency compared with controls (1.11% for MS and 2.15% for controls, $p = 0.000007$). Meta-analysis of Australasian and three European case-control cohorts confirmed this association ($p = 0.00006$, OR 0.49). In subgroup analysis, Arg307Gln showed a relatively greater association with the relapsing-remitting subtype of MS than other subtypes (odds ratio 0.30, $p = 0.006$) and with HLA DR15 negative compared to DR15 positive patients. Fresh human monocytes heterozygous for Arg307Gln have > 90% loss of "pore" function of the P2X7 receptor measured by ATP-induced ethidium uptake. In transfection experiments, the Arg307Gln variant of P2X7 supported robust uptake of 1.0 μm beads equal to wildtype P2X7. Modelling based on the homologous zP2X4 receptor showed Arg307 was located in a region rich in basic residues located 12 Å from the ligand binding site. Our data show the protective effect against MS of a rare genetic variant of P2RX7 with heterozygotes showing near absent "pore" (proinflammatory) function but intact scavenger function.

WTH15-07

Mechanisms of chemobrain and chemopain

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Progress made in the treatment of cancer has led to a sharp increase in the number of survivors. However, cancer treatment poses severe side effects like pain, fatigue and cognitive deficits which persist long into survivorship. Currently, there are no drugs to manage chemotherapy-induced neuropathy (CIPN) and cognitive dysfunction. We have shown before in a model of hypoxic-ischemic brain damage that the mitochondrial protectant pifithrin-m (PFT-m) acts by inhibiting translocation of p53 to mitochondria thereby preventing neuronal damage. Cisplatin is a platinum-based drug and is widely used for many cancer types. Apart from killing tumor cells, it also affects healthy tissues leading to toxic side effects like damage to the peripheral and central nervous system.

In this study C57/Bl6 mice were treated with 3 cycles of 2.3 mg/kg of cisplatin (5 gifts per week for 3 weeks). CIPN was measured as mechanical hyperalgesia using von Frey hairs and peripheral numbness was quantified by the adhesive removal test. Cognition was determined by the novel object and place recognition task (NOPRT). Neuronal arborization was measured by Golgi and MBP staining. Mitochondrial function was analyzed by Seahorse technology. PFT-m was administered in a dose of 8 mg/kg.

Cisplatin treatment induced persistent mechanical hyperalgesia long after cessation of treatment. Cisplatin also induced numbness in the hind paw as a characteristic of CIPN. Moreover, cisplatin induced a decrease in cognitive function when using the NOPRT. Cisplatin also induced a neuronal mitochondrial dysfunction such as decrease in mitochondrial oxygen consumption rate and spare respiratory capacity as measured with Seahorse technology. Cisplatin treatment was also associated with a decrease in neurogenesis as shown by the number of DCX^+ precursors in the SVZ as well as a decrease in arborization of white matter.

When animals were treated with PFT-m during cisplatin treatment, we observed that chemopain as well as chemobrain were prevented in association with a restoration of the mitochondrial oxygen consumption rate.

WTH15-08

coffee induces vascular endothelial growth factor expression in human neuroblastoma SH-SY5Y cells

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Objectives: Coffee is one of the most world-widely consumed beverage on a daily bases. Recent epidemiological studies have reported that amyotrophic lateral sclerosis (ALS) patients are less frequent and prolonged to coffee intake than other neurodegenerative diseases patients or healthy persons. However, the precise molecular mechanisms of the effects of coffee are yet uncertain. Vascular endothelial growth factor (VEGF) is known to have protective effects on ALS in development of symptoms and prolongation of life. Therefore, we investigated the effects of coffee on VEGF expression in human neuroblastoma SH-SY5Y cells.

Methods: SH-SY5Y cells were cultured in Ham's F-12/DMEM (1 : 1) medium supplemented with 15% fetal bovine serum. The cells were exposed to coffee or coffee extracts up to at 2.0% (v/v). After 4 hours, the whole cell lysates were isolated and subjected to immunoblotting for HIF-1α. VEGF gene expression was monitored by qPCR using RNAs isolated from the cells treated with coffee for 8 hours. After 12 hours, the amount of VEGF in the culture medium was measured with an ELISA kit (eBioscience). Deferoxamine (100 μM) was used as a positive control to induce HIF-1α.

Results: Coffee induced VEGF expression in time- and dose-dependent manner whereas decaffeinated coffee or caffeine (100 μM) showed no effects. The induction profile of VEGF was corresponding to that of an activation of HIF-1α by coffee. The active constituents of coffee were produced by roasting process of coffee beans and was extractable with *n*-butanol.

Conclusion: Coffee induced VEGF expression via the HIF-1α activation in the human neuroblastoma cells. This activity may contribute to the preventive effects of coffee on ALS. Further study to identify active components and to elucidate the mechanism of the effects is needed to clarify the molecular basis of neuroprotective effect associated with daily coffee consumption.

WTH15-09

A novel neuroprotective activity of Otx2 in mouse retinal neuron

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OTX2 (*orthodentricle homeobox 2*) plays critical roles in the development of the structures derived from the anterior neural tube, including forebrain, midbrain, and eyes, in gene dose and activity dependent manners. Thus, *OTX2* haplodeficiency in human resulted in wide range eye defects from anophthalmia to retinal distrophy. In $Otx2^{+/-}$ mouse retina, we found a progressive loss of OFF-cone bipolar cells and cone photoreceptors that lead to visual impairment of the mice. We discovered a neuroprotective activity of Otx2, which was obtained from external sources without autonomous *Otx2* gene expression in OFF cone bipolar cell subset. Moreover, the exogenously-derived Otx2 mobilized to the mitochondria and facilitate ATP synthesis. Together with successful protection of retinal neurons in $Otx2^{+/-}$ mice by intraocular Otx2 injection, our study suggests that Otx2 can be applicapable to prevent retinal dystrophy caused by *Otx2* haplodeficiency in mice and potentially in human.

WTH15-10

Enbrel treatment promotes transplanted donor human mesenchymal precursor cell survival following spinal cord injury

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Immediately following spinal cord injury (SCI), pro-inflammatory cytokines are released from damaged cells and other resident cells within the spinal cord. This cytokine release is involved in the inflammatory response to injury and the extensive and persistent spread of secondary damage following SCI. Activation and infiltration of immune cells into the spinal cord contributes to ongoing secondary damage with increased death of neurons and glia, demyelination of intact axons and cyst formation at the injury site. We have previously shown a marked improvement in functional and morphological outcomes in host tissue following transplantation of adult human mesenchymal precursor cells (hMPCs) into the contused spinal cord in Nude rats. However, donor hMPCs do not survive to 4 weeks post-transplantation due to a host immune response. Modulation of pro-inflammatory cytokine levels and immune cell activity immediately following injury may lead to decreased secondary degeneration and increased donor hMPC survival, potentially leading to greater improvement in functional and morphological outcomes. Enbrel is a TNFα antagonist that can have neuroprotective effects following SCI by reducing apoptosis, tissue damage, and immune cell activation. Rats were given a moderate contusive SCI and treated with hMPCs alone or in combination with Enbrel. Enbrel treatment immediately after SCI improved donor cell survival in all Enbrel+hMPC treated animals, with at least some hMPCs still present in the spinal cord 4–6 weeks post-transplantation. Although transplanted hMPC survival was increased, there was no significant improvement in functional recovery at 5 weeks post-injury for any treatment group. Also, combined Enbrel+hMPC treatment did not further reduce cyst size compared to hMPC transplantation alone. Enbrel may have a moderate impact on SCI repair alone by reducing secondary damage, and may be useful in combinatorial clinical applications to enhance donor cell survival in transplantation based therapies.

WTH15-11

Systemic delivery of a mimetic peptide against CONNEXIN43 GAP junction protein in rats following spinal cord injury

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Injuries of peripheral nerves can cause chronic inflammation along the pain pathway involving diverse immune cell types and immune-like glial cells (e.g., astrocytes and microglia). Numerous studies show significant glial activation and infiltration of leukocytes, such as macrophages and T cells, at the site of the initial nerve lesion, at the ipsilateral dorsal root ganglia and at the dorsal horn of the spinal cord following peripheral nerve injury. Activated immune and glial cells produce and secrete cytokines and chemokines, which can be pro-inflammatory or anti-inflammatory. Dysregulation of cytokines has been implicated in a variety of painful neurological diseases and in animal models of neuropathic pain. We have previously demonstrated the contribution of pro-inflammatory cell types and their mediators to neuropathic pain and the beneficial effects of immunosuppressive T cells in controlling chronic inflammation and attenuating pain hypersensitivity. This presentation will focus on changes in cytokine profile and pain behaviours following modulation of the immune response in animal models of neuropathic pain due to peripheral nerve injury and in patients with painful peripheral neuropathy. Targeting of neuroinflammation in neuropathic pain may provide potential therapeutic opportunities.

WTH15-12

Dynamin-related protein-1 regulates neurodegeneration in experimental Huntington's and Parkinson's diseases

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Mitochondria are central to the proper functioning of cells, and their involvement in the pathogenesis of neurodegenerative diseases (NDD) is well documented. Mitochondrial electron transport chain (ETC.) dysfunctions, generation of reactive oxygen species, oxidative damage and programmed apoptotic or necrotic or autophagic cell death are factors that have been linked causally to NDD processes. The present study examined mitochondria dynamics, its fission-fusion status in relation to neuropathology of two NDD, Parkinson's disease (PD) and Huntington's disease (HD). We used 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mediated parkinsonism in mice or MPP^+-induced SH-SY5Y neuronal death model of PD for examining mitochondrial dynamics. For investigating HD, we used 3-nitropropionic acid-induced rat model or Neuro2a cell line that expresses the tNhtt with either Htt 16 or 150 CAG (Q) under an inducible promoter, tagged with e-GFP. Both these models showed decreased mitochondrial ETC. complex activities and loss in respiratory rate. While MPTP animal model exhibited loss, HD model showed a substantial increase in striatal dopamine levels. Melatonin and a novel isoquinoline derivative attenuated specific alterations in the fission protein DRP1 and the fusion proteins Mfn1 and 2 respectively, in PD and HD models. These molecules also corrected all the mitochondrial dysfunctions observed in these disease models. It is possible that phosphorylation of AMPK might be a target by which these molecules influence the expression levels and translocation of mitochondrial dynamics regulating proteins to regulate their dynamics and bioenergetics which is important to protect neurons from degeneration. This information suggests that molecules that act as mitochondrial stimulants may be used in human disease conditions to control NDD processes to contain the disease.

WTH15-13

Pharmacokinetics of intranasal guanosine administration

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Purines have been largely studied as neuroprotective molecules; however, few groups studied the neuroprotective potential of guanine-based purines (GBP). Our and other groups investigate neuroprotective potential of the nucleoside guanosine (GUO) in several models of experimental brain injuries; however, few studies have shown pharmacokinetics of GUO administration aiming to understand mechanisms of GUO neuroprotective effects, specially in a new pathway of administration. An approach to improve GUO potential is to search intranasal (i.n) pathway, since several neuroprotective drugs enhanced its effects through this way when compared to usual pathway of administration, intraperitonial (i.p). Thus, in this work we evaluated pharmacokinetics parameters of i.n Guo administration.

Methodology: Male adults Wistar rats were used. Animals were divided in control and i.n [H^3]Guo groups; the animals were anesthetized with xilazine/ketamine before GUO or saline solution i.n administration. The time and concentration curve was performed by scintillation analysis and purine analysis was performed with defined concentration, time and volume based on our previous pharmacokinetics study.

Results: The maximum of [H^3]GUO levels were detected as earlier as 5 min (first time evaluated) after its administration, in all brain structures, blood plasma in all doses evaluated. I.n [H^3]GUO have shown a dose response until 30 mg/mL concentration, revealing a plateau in GUO amount in brain structures; however, in blood there was no plateau. In brain structures and blood plasma, the bulk of purines were not affected by i.n GUO administration (except Adenosine levels in blood plasma after 15 min of i.n GUO administration). The most relevant results in this work relay on the comparison i.n vs. i.p, since radioactivity in CSF is similar in both pathways, but only i.n [H^3]GUO have shown an altered purine profile. It is important to note that radioactivity levels were higher through i.p than i.n administration in blood plasma and brain structures.

WTH15-14

Re-examining the chaperone efficacy of heat shock proteins in neuro-2A using novel bicistronic expression constructs

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Cell-based studies of the heat shock proteins (Hsps) have demonstrated their remarkable ability to prevent the aggregation of neurodegenerative disease-associated proteins and enhance cytoprotection. One potential limitation with many of these studies is that they have examined Hsp chaperone function by linking fluorescent protein tags (such as GFP) to the N- or C-terminus of the protein. Indeed, a recent study investigating the functions of the sHsps, Hsp27 and αB-c, demonstrated that tagging them on the N- or C-termini with fluorescent GFP derivatives severely affected their oligomeric assembly and subsequent chaperone activity compared to non-tagged proteins. Therefore, chaperone functions of tagged-Hsps may differ considerably to non-tagged versions in cells. Another limitation is that previous studies have not taken into account differences in transfection efficiencies that occur when using different constructs to over-express proteins in the cell. Here, we describe the use of bicistronic pIRES2-mCherry constructs for the correlated expression of (non-tagged) Hsps and mCherry (fluorescent reporter) proteins. This strategy avoids the use of adding bulky fluorescent tags but enables differences in transfection efficiencies to be taken into account and as such provides a novel method to re-evaluate Hsp chaperone functions in cells. Neuro-2a cells were co-transfected with Hsp-encoding bicistronic constructs (Hsp40, Hsp70, Hsp90, Hsp27 or αB-c) and an aggregation-prone double mutant of firefly luciferase. The efficacy of the Hsps to inhibit the formation of firefly luciferase intracellular inclusions was assessed

by flow cytometry using pulse shape analysis. Hsp40 and Hsp70 were the most potent inhibitors of inclusion formation, reducing the proportion of cells with inclusions by < 40% compared to the chaperone-negative control. Such findings highlight the potential of these Hsp-encoding bicistronic constructs to be used in future cell studies to investigate the roles of Hsps in models that recapitulate neurodegenerative disease pathologies.

WTH15-15

Targeting the plasminogen activation system in traumatic brain injury

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Taumatic brain injury (TBI) is the leading cause of death and disability in young adults. TBI causes physical and biochemical breakdown of the blood brain barrier (BBB) leading to cerebral oedema and acute traumatic coagulopathy (ATC). ATC occurs in ~ 1/3 of TBI patients and is a powerful predictor of mortality. It occurs due to dramatic changes in the haemostatic system not only as a result of coagulation dysfunction, but also due to excessive fibrinolysis. Tranexamic acid (TXA) is a lysine analogue that blocks the activation of plasminogen to plasmin, a protease which degrades fibrin clots. Tissue plasminogen activator (t-PA) is the primary plasminogen activator. While activation of the fibrinolytic system promotes bleeding, we have shown that t-PA and plasmin also promotes blood-brain barrier (BBB) disruption following TBI. t-PA activity increases in the brain following injury. Paradoxically, we demonstrated that t-PA can also worsen BBB damage via formation of complexes with its natural inhibitor, plasminogen activator inhibitor-1 (PAI-1). These complexes in turn activate signalling events after binding to LDL receptors and blockade of this interaction reduces BBB permeability. We have also shownthat tPA:PAI-1 complexes are elevated in cerebrospinal fluid of patients with severe TBI. We have now confirmed this in a separate clinical study; levels of tPA:PAI1 complex are also elevated in the plasma of severe TBI patients. Activation of the fibrinolytic system following TBI therefore promotes both ATC due to plasmin-driven hyperfibrinolysis, and also BBB disruption via active t-PA/plasmin and via the formation of t-PA:PAI-1 complexes. This suggests that TXA could have added clinical benefit via inhibition of t-PA/plasmin-mediated BBB permeability. Indeed, our data has shown that TXA administration in mice subjected to TBI caused significant reduction in BBB permeability. Therefore administration of agents that block the downstream actions of t-PA:PAI-1 complexes on LDL receptors alone or in combination with TXA will further reduce neurovascular permeability and improve outcome following TBI.

WTH15-16

tPA promotes axonal regeneration into and through a dorsal column spinal cord injury

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Plasminogen activators tPA and uPA have been shown in our prior studies to promote cerebellar neuronal cell migration, PNS axonal growth *in vitro*, motor neuron synaptic remodeling following spinal cord injury, and PNS axonal regeneration across prohibitive myelin substrates. Studies on spinal cord axonal regeneration have been initiated by characterizing dorsal column injuries at C4. Longitudinal sections spanning the spinal cord injury have been observed by immunohistochemistry for axonal regeneration following cholera toxin B injections into the sciatic nerve. We compared mice receiving a sciatic nerve injury ("priming or conditioning" event) with uninjured "non-primed" wildtype mice as well as "primed" tPA−/− mice, and "non-primed" mice receiving exogenous tPA via saturated gelfoam placed over the lesion site. Mice were examined after 75d and extensive axonal re-growth is seen into the filled-in lesion site in "primed" mice, as compared to bulbus terminals with no axonal regrowth in "non-primed" mice or the "primed" tPA−/− mice, which fail to regenerate. Excitingly, "non-primed" mice receiving exogenous tPA soaked gelfoam, overlying the lesion site showed in axonal regeneration into the lesion site and for several hundred micrometers beyond the lesion site, suggesting that tPA can promote axonal regeneration in the SC dorsal column. (Supported in part by NIH-NS044129).

WTH15-17

Bioactive polyphenol interactions with β amyloid: a comparison of binding, fibril inhibition and neuroprotection

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We compared the effects of a diverse set of natural polyphenolics ligands on *in silico* interactive modelling, *in vitro* anti-aggregative properties and neuronal toxicity of β amyloid. The β amyloid-binding characteristics of optimised structural conformations of polyphenols with ascribed neuroprotective actions including punicalagin, myricetin, luteolin and honokiol were determined *in silico*. Thioflavin T and transmission electron microscopy were used to assess *in vitro* inhibitory effects of these polyphenols on $A\beta_{1\text{-}42}$ fibril and aggregation formation. Phaeochromocytoma (PC12) cells were exposed to $A\beta_{1\text{-}42,}$ alone and in combination with test concentrations of each polyphenol (100 μM) and viability measured using MTT assay. $A\beta_{1\text{-}42}$ evoked a concentration-dependent loss of cell viability in PC12 cells, in which all four polyphenols demonstrated significant inhibition of neurotoxicity. While all compounds variably altered the morphology of Aβ aggregation, the flavonoids luteolin and myricetin and the lignan honokiol all bound in a similar hydrophobic region of the amyloid pentamer and exerted a pronounced inhibition of $A\beta_{1\text{-}42}$ aggregation. However, each of the polyphenols demonstrated neuroprotective effects in PC12 cells exposed to $A\beta_{1\text{-}42}$. These findings highlight some structure-activity insights that can be gleaned into the anti-aggregatory properties of bioactive polyphenols based on modelling of

their binding to β-amyloid, but also serve to highlight the more general cellular neuroprotective nature of such compounds.

WTH15-18

Vascular endothelial growth factor reduces alterations associated to blood-brain barrier disruption after ischemic stroke

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Ischemic stroke is a leading cause of death and neurological disabilities worldwide for which no effective therapy exists. Brain injury in stroke appears either as infarct core, caused by blood flow blockage, or as penumbra, generated by cellular death at the core. In these regions blood-brain barrier (BBB) integrity is lost allowing the entry of toxic substances and activated cells from the peripheral immune system, which further contribute to impair tissue homeostasis leading to neuronal death and exacerbation of neurological decline. BBB dynamics are modulated by vascular endothelial growth factor (VEGF) that is known to induce vascular leakage and remodeling. Conversely, VEGF is also a potent neuroprotector in experimental models of stroke, but the mechanisms for protection are not entirely understood. We tested whether administration of VEGF during reperfusion would modulate BBB permeability affecting tissue viability. We used the transient middle cerebral artery occlusion (MCAO) model in the rat that allows focal blockage of brain blood flow followed by reperfusion. At 30 min of reperfusion, we administered via i.c.v. different concentrations of recombinant VEGF that resulted in preservation of brain viability as determined by tetrazolium-chloride staining 24 h after MCAO, a time-point of peak vascular leakage assessed by two-photon microcopy *in vivo*. We tested how VEGF would modulate BBB disruption by analyzing the extravasation of intravenously-administered Evans blue, which do not cross the intact BBB. We found that stroked animals showed intense blue staining at the infarct core that was significantly reduced by VEGF. Staining at the penumbra did not change. We also evaluated the maintenance of the BBB molecular structure by immunofluorescence of claudin 5, a tight junction protein essential for BBB integrity. Our findings indicate that despite VEGF is a known inducer of vascular permeability, at low concentrations it can modulate the dynamics and stability of BBB which could constitute a mechanism for protection that has not been described before.

WTH15-19

The neuroprotective effect of simvastatin in an induced Müller cell disruption mouse model

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Loss of vision in retinal diseases, the commonest causes of blindness, is a result of progressive degeneration, dysfunction and death of the photoreceptors. How this occurs is poorly understood. Retinal Müller cells are believed to be important for the health of photoreceptors. Müller cell dysfunction has been implicated in several retinal diseases, especially Macular Telangiectasis and Diabetic Retinopathy. We have developed a unique transgenic mouse model where patches of Müller cells are induced disruption while surviving Müller cells become active, or gliotic. Recently we have reported that significant up-regulation of TNFa has been found after induced Müller cell disruption, which leads to markedly reduced photoreceptor specific marker Interphotoreceptor Retinoid-Binding protein (IRBP) and then photoreceptor degenerations. In this study, we used simvastatin (Zocor) to pre-treat the mice before inducing Müller cell disruption. This marketed drug is a known inhibitor of TNFa. Simvastatin was administered to the mice in drink water 1 week before and continued until 1 week after induced Müller cell disruption. We found simvastatin treatment attenuated the up-regulation of TNFa and the activation of JAK/STAT signaling pathway in the retina usually occurred after induced Müller cell disruption. Additionally, simvastatin recovered 81% of IRBP expression 1 week after induced Müller cell disruption. More importantly, this pre-treatment resulted in significantly reduced photoreceptor degeneration induced by the stress of Müller cell dysfunction with recovering 70% of the loss of the staining photoreceptor outer segment by flat mount PNA staining. Our study shed a novel light on the molecular regulation of Simvastatin on IRBP under Müller cell dysfunction and its neuroprotective effect on photoreceptor degeneration, which novel pathogenic mechanism for retinal diseases might be open to therapeutic manipulation.

WTH15-20

Tetramethylpyrazine protects neuronally differentiated Y-79 retinoblastoma cells from oxidative stress-induced apoptosis

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Tetramethylpyrazine (TMP), one of the alkaloids contained in *Ligusticum wallichii Franch* (L. wallichii), has been widely used to treat many neurodegenerative diseases by Chinese herbalists due to its strong neuroprotective and antioxidant activities. TMP is also used in the treatment of retinal neurodegenerative disorders in China, however, the molecular basis of its application on these eye diseases are not well understood. Because it is proposed that oxidative stress-induced neuronal apoptosis is a major cause of retinal neurodegenerative diseases, this study was conducted to investigate the protective effect of TMP against oxidative stress-induced apoptosis in neuronally differentiated Y-79 retinoblastoma cells, which is an *in vitro* model with characteristics of photoreceptor. Human Y-79 cells were differentiated to be photoreceptor-like neurons and subjected to oxidative stress induced with hydrogen peroxide. Following that, the protective effect of TMP against hydrogen peroxide-induced cell injury in neuronally differentiated Y-79 retinoblastoma cells was evaluated by MTT assay and Annexin V-FITC and PI double staining assay. Furthermore, the expression profiles of apoptosis-related molecules involved in this cellular process were assessed by western blot analysis. Our data indicated that the pre-treatment of TMP at 5, 10 and 25 μM reduced hydrogen peroxide-induced cytotoxicity and cell apoptosis in neuronally differentiated Y-79 retinoblastoma cells in a dose dependent manner. The effect of TMP is possibly mediated through

attenuating the up-regulation of Bax and down-regulation of Bcl-2, preserving mitochondrial potential and preventing cytochrome c release from mitochondria. In conclusion, our study is the first to elucidate the detailed molecular mechanisms underpinning the neuroprotective effect of TMP against oxidative stress-induced cell injury in neuronally differentiated Y-79 retinoblastoma cells. Our novel findings significantly contribute to understanding the therapeutic effect of TMP in treating human retinal neurodegenerative diseases.

WTH16 Cell Death

WTH16-01

Increased active caspase 3 expression in the sudden infant death syndrome (SIDS) infant medulla

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Despite remaining a diagnosis of exclusion, Sudden Infant Death Syndrome (SIDS) is a leading cause of post-neonatal mortality worldwide. Increased expression of apoptotic markers has previously been identified in the brainstem of SIDS infants. Examining infant deaths between 2008 and 2012 this study quantitatively assessed neuronal expression of the specific apoptotic marker, active caspase 3, in the human infant medulla via immunohistochemistry. Seven nuclei from the open medulla were quantified, including; the Hypoglossal (XII), Dorsal Motor Nucleus of the Vagus (DMNV), the Nucleus of the Solitary Tract (NST), Vestibular (Vest), External Cuneate (ECun), Arcuate Nucleus (AN) and the Inferior Olivary Nucleus (ION). Comparing infants diagnosed with SIDS (SIDS, $n = 6$) and sex and age matched control infants (non-SIDS, $n = 5$), increased expression of active caspase 3 was observed in the SIDS DMNV ($p = 0.019$) and AN ($p = 0.047$). These findings support the major hypothesis that neuronal cell death is increased in SIDS compared to non-SIDS infants. This recent post-mortem study highlights the continual prevalence of apoptotic expression in the SIDS medulla.

WTH16-02

Retinal function and neuronal loss in a model of retinitis pigmentosa

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Purpose: Retinitis pigmentosa (RP) is a group of heterogeneous retinal diseases characterized by loss of the light detecting neurons, the photoreceptors. This work characterizes the progressive retinal dysfunction, altered retinal structure and photoreceptor loss in a transgenic rat model harboring a mutation in the photopigment, rhodopsin (Pro23His).

Methods: Age-related changes in retinal function and structure were assessed in Pro23His and Sprague–Dawley (SD) control rats at 1, 2 and 3 months. Retinal function was assessed using the electroretinogram (ERG), while outer retinal structure was assessed using optical coherence tomography (OCT) and retinal histology. Photoreceptor loss was quantified using TUNEL and markers of apoptosis.

Results: ERG data showed that the rod photoreceptor response was significantly reduced in Pro23His rats from 1 month of age compared to age-matched controls (-30%, 587 ± 15 μV vs. 838 ± 28 μV). The functional deficit was more evident after 2 ($-41 \pm 2\%$) and 3 ($-57 \pm 7\%$) months of age. Cone photoreceptors also exhibited a functional deficit, however, it was less severe than that observed for the rod photoreceptors (-26 ± 5; -17 ± 4; $-18 \pm 6\%$). *In vivo* OCT imaging showed neuronal loss in the outer retina of the Pro23His rats in which the outer nuclear layer (ONL) was significantly thinner than age-matched controls. TUNEL labeling was mostly confined to the outer retina and showed a temporal response reflecting the functional data, with the peak number of TUNEL positive photoreceptor cells occurring before 3 months of age.

Conclusion: These data indicate that the majority of the photoreceptor degeneration in the Pro23His rat occurs within the first 3 months of age and that it is principally characterized by a loss of rod photoreceptors in the outer retina. This model will provide insights into the mechanisms that are responsible for RP.

WTH16-03

AS,CD AND PB metal mixture up-regulates PPARγ inducing PPRE mediated PARP activation and apoptosis in the rat astrocytes

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Glial fibrillary acidic protein (GFAP) is essential for maintaining morphology and function of astrocytes. Modulation in GFAP expression is a prominent feature of various neuropathological conditions. We investigated the cellular and molecular mechanisms behind GFAP alteration during Arsenic, Cadmium and lead mixture (MM) exposure in developing rats. Peroxisome-proliferator-activated-receptor-γ (PPARγ) regulates astrocyte functioning; however, its role in the expression of GFAP and astrocyte apoptosis is still elusive. Here, we recognized a GFAP-trans-repressing role of p-PPARγ (ser112), in causing astrocytes apoptosis through the genomic activity of PPARγ. We first observed that a treatment with MM or PPARγ agonist, troglitazone, down-regulated GFAP levels, interlinked with an increase in the p-PPARγ which leads to enhancing astrocyte apoptosis in the rat brain and also in astrocytes primary culture. Moreover, through an in-silico prediction studies, we scanned several apoptotic genes and found that non-coding regions of PARP revealed two PPARγ response elements (PPREs); direct repeat 6 (DR6) and evert repeat 1 (ER1) sequences. Electrophoretic mobility shift assay and chromatin immunoprecipitation assays demonstrated enhancement in binding of PPARγ to the PARP-PPRE region. Further, luciferase reporter assay also revealed strong suppression of GFAP via PPREs, in response to MM. In addition, we examined the non-genomic signaling of metal mixture mediated upregulation of PPARγ via targeting the axis

where PI3K-AKT is in upstream and PARP is in down-stream module. Further, using PI3K-AKT inhibitor i.e. LY294002 leads with decrease in GFAP levels, which was further enhanced by siRNA mediated PARP1 knockdown in primary astrocytes culture. Taken together, these results are the first to demonstrate the direct transcriptional regulation of PARP by PPARγ, through its interaction with the PPREs, and bring forth the contribution of PPARγ in astrocyte apoptosis and damage.

WTH16-04

MALDI-TOF/TOF-IMS examination of peptide expression in the medulla in sudden infant death syndrome (SIDS)

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The brainstem contains both specific and interacting groups of neurons (nuclei) that have unique and critical roles in the central regulation of respiration, cardiovascular activity, metabolic/energy homeostasis, and the control of the transition between sleep and wakeful states. These nuclei are critical for basal regulation and adaption of these systems to internal and environmental factors. So far 23 neurochemical/receptor abnormalities have been identified in the rostral medulla of SIDS infants. This study aimed to (i) optimise MALDI-TOF/TOF-IMS for use on archived (1–6 years) FFPE human brainstem tissue to determine differences in peptide expression amongst the major nuclei. (ii) Examine the proteomic changes in the rostral medulla in SIDS using LC MALDI and IMS.

Comparisons between the nuclei in control infants demonstrated common peptides for neuronal and non-neuronal structures allowing for the identification of specific nuclei. The use specific m/z values allowed for computational modelling (using SCiLS lab) of particular nuclei of interest. These models could then be applied to other tissue sections to identify the same nucleus. Abnormal expression patterns of glial fibrillary acidic protein (GFAP), myelin basic protein (MBP) and tubulin, beta class I (TUBB) were strongly observed within the SIDS medulla compared to controls ($p \leq 0.05$). These abnormalities varied between nuclei, with the large variations occurring in the rostral ventral lateral medulla. These results demonstrate the effectiveness of MALDI IMS in identifying and localising protein expression changes in both control and in diseased tissue.

WTH16-05

Cypermethrin aberrantly up-regulates CA^{2+}, ROS, JNK, MMP-2 and reelin signaling proteins leading to astrocyte damage

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Introduction: Cypermethrin is a synthetic type II pyrethroid, known to cause neurotoxicity however, relatively less is known of its effect on astrocyte development and migration. Astrocytes are one of the component of blood brain barrier (BBB) and their damage along with BBB dysfunction impairs the tight junction (TJ) proteins. Here, we studied the mechanism of cypermethin mediated BBB disruption in rat brain, rat astrocytes damage and determined any change in expression of reelin signaling proteins associated with cell migration.

Methods: We treated 24-day old rats with cypermethrin (10 mg/Kg) for 3 weeks and astrocyte cells (100 μM) for 48 h. Through evans blue extravasation, western-blotting, immunocytochemistry and Ca^{2+}, ROS generation assay we detected BBB and astrocyte damage.

Result: Through MTT assay we found that cypermethrin reduced viability of cultured rat astrocytes. Immunolabelling with astrocyte marker, glial fibrillary acidic protein, revealed alteration in astrocyte morphology. The astrocytes demonstrated an enhanced release of intracellular Ca^{++} and ROS, and up-regulation in p-JNK levels in a time-dependent manner. Cypermethrin disrupted the BBB (*in vivo*) in developing rats and attenuated the expression of claudin-5 in cultured astrocytes. We further observed an augmentation in the levels of matrix metalloproteinase 2 (MMP2), known to modulate cellular migration and disrupt the ECM and BBB. We observed an increase in the levels of reelin, involved in cell migration, in cultured rat astrocytes. The reelin receptor, α3β1integrin, and a mammalian cytosolic protein Disabled1 (Dab1) were also up-regulated.

Discussion: Overall, our study demonstrates that cypermethrin induces astrocyte injury via modulation in Ca^{++}, ROS and JNK pathways, which may alter MMP expression and reelin dependent astrocyte migration during brain development.

WTH16-06

Inhibition of multidrug resistance-associated proteins exerts cytotoxicity to neuroblastoma without neurotoxicity

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The current chemotherapeutic drugs sometimes cause side effects including neurotoxicity, and they cannot sufficiently treat high-risk neuroblastoma. Therefore, the development of drugs, which possess selective cytotoxicity toward neuroblastoma via different mechanisms from the conventional ones, are desired. The aim of the present study was to clarify whether the inhibition of multidrug resistance-associated proteins (MRPs) selectively exerts cytotoxicity to neuroblastoma, but not neurons. Mouse primary cultured cortical neurons (MCN) and neuroblastoma Neuro2a cells (N2A) were used as neuronal and neuroblastoma model cells, respectively. Real-time PCR analysis revealed that expression of MRP3 mRNA in N2A was remarkably higher than that in MCN. In N2A, transfection with small interfering RNA (siRNA) for MRP3 largely decreased expression of MRP3 mRNA, but markedly increased the number of cells with high fluorescence intensity of MRP3 substrate Fluo-8, which is a fluorescent Ca^{2+} indicator. Knockdown of MRP3 significantly decreased MTT reduction activity with a concomitant increase in lactate dehydrogenase released into culture medium in N2A, suggesting that inhibition of MRP3 may induce cytotoxicity to neuroblastoma cells. In N2A, fluorescence intensity was minimally detected after incubation with Fluo-8, but exposure to MRP inhibitor probenecid increased the number of cells with high fluorescence intensity of Fluo-8 in a dose-dependent manner. On the other hand, many cells with high fluorescence intensity of Fluo-8 were observed in MCN regardless of the addition of probenecid, indicating that

functional expression of MRPs in N2A is much higher than that in MCN. The addition of probenecid over a concentration of 1 mM significantly decreased MTT reduction activity in N2A whereas minimal effect of probenecid was observed in MCN, suggesting that cytotoxicity of probenecid in N2A is more remarkable than that in MCN. These results suggest that inhibition of MRPs by traditional therapeutic drugs may exert selective cytotoxicity toward neuroblastoma with minimal neurotoxicity via different mechanisms from the conventional chemo drugs.

WTH16-07

Dehydroascorbic acid promotes cell death in neurons under oxidative stress: a protective role for astrocytes

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Ascorbic acid (AA), the reduced form of vitamin C, is incorporated within neurons through the sodium ascorbate co-transporter, SVCT2. However, this transporter is not expressed in astrocytes, which uptake the oxidized form of vitamin C, dehydroascorbic acid (DHA), through the facilitative hexose transporter, GLUT1. Therefore, neuron and astrocyte interactions are thought to mediate vitamin C recycling in the nervous system. Although astrocytes are essential for antioxidant defense of neurons under oxidative stress, conditions in which a large amount of ROS is generated may favor the extracellular oxidation of AA and subsequent DHA uptake by neurons through GLUT3, which may increase oxidative stress in neurons. In this study, the effect of *in vitro* neuronal cell death by oxidative stress and DHA uptake was analyzed. Different analyses revealed the presence of the DHA transporters, GLUT1 and GLUT3, in Neuro2a and HN33.11 cells as well as cortical neurons. Kinetic analysis confirmed the presence of functional GLUTs that uptake 2-deoxyglucose and DHA in all cells analyzed. In the present study, Neuro2a, HN33.11 cells and rat cortical neurons subjected to oxidative stress and DHA treatment had significantly increased cell death compared to cells not treated with DHA, suggesting that neuronal death occurs preferably in pathological conditions in which DHA levels significantly increase. Additionally, the presence of astrocytes and DHA recycling reverses the cell death of stressed neurons. Taken together, these data suggest that astrocytes promote the maintenance of optimal levels of AA, thereby attenuating neuronal death induced by oxidative stress such as glutamate cytotoxicity, oxidative stress, and acidosis during ischemia and reperfusion. However, in extreme pathophysiological conditions, we propose that the neuroprotective effects of astrocytes are significantly reduced, resulting in massive neuronal death.

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WTH16-08

Comparative microarray analysis identifies commonalities in neuronal apoptosis

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Purpose: To examine the transcriptomic profile of multiple neurodegenerative models to obtain a more in-depth understanding of the temporal recruitment of cellular signaling.

Method: Cultured mouse cortical neurons were treated with (i) 10 nM rotenone for 8 h, 15 h and 24 h, or (ii) 1 μM lactacystin for 7.5 h, 24 h and 48 h, or (iii) 200 μM NMDA for 5 h, 15 h and 24 h, respectively. RNA was harvested for Illumina Mouse Ref8 Ver.1.1 arrays (Rotenone or NMDA treatment) or Affymetrix Genechips (Lactacystin treatment). The absolute data was analyzed using GeneSpring GX. Genes with fold change ± 1.5-fold against controls in at least one of three time point conditions were annotated using DAVID and PubMed search. Statistical analysis was performed using One-way ANOVA ($p < 0.05$).

Results: We identified changes in three biological processes, response to oxidative stress, calcium homeostasis and autophagy lysosome pathway. Several genes involved in oxidative stress (*Atf4, Cebpb*, *Gsta4*, *Hmox1* and *Mt2*) and aberrant calcium homeostasis (*Anxa2* and *Anxa5*) were upregulated, which have also been demonstrated to induce autophagy. In addition, our data revealed that an antioxidant, sulfiredoxin (Srxn1), is upregulated by all the treatments.

Conclusion: Biological events occurring during mitochondrial inhibition, proteasome ubiquitin inhibition and NMDA receptor activation involve oxidative stress, aberrant calcium homeostasis, and the autophagy-lysosomal pathway. These three biological events are interconnected and the activation of autophagy is believed to be pro-survival. Therefore, further studies focusing on the genes that modulate autophagy could provide useful information for the treatment of neurodegenerative diseases.

WTH17 Motor Systems

WTH17-01

Deficient corticostriatal activity in ageing shortens patterns of goal-directed action

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Humans and other animals execute thousands of different actions, most of which are aimed at attaining the numerous goals arisen in everyday life. Individuals often learn to optimise the way they execute goal-directed actions in order to maximise the fulfilment of their needs, a process that is largely dependent on cortico-basal ganglia circuits. With training, separate unitary actions are gradually integrated into automatic action sequences, which improve task efficiency while reducing memory load during ongoing performance. Although the automatisation of behaviour during learning of novel motor skills is known to be severely affected in ageing, the precise alterations in action structure and the neuronal correlates leading to these deficits remain to be determined. Here, we analysed the effects of age on goal-directed performance by studying the detailed structure of behaviour in young and aged mice engaged in a fully-automated instrumental task. Our results showed a dramatic decrease in the latency of action in aged mice, as revealed by the premature termination of ongoing execution programs. Importantly, when forced to extend their latency of action through uncued penalising regimes, aged mice displayed compressed patterns of behaviour, which were characterised by shorter but ultra-fast sequences of action. Using large-scale functional profiling of neurons, we observed that this deficit was accompanied by deficient patterns of activity throughout the cortico-striatal network, which displayed higher levels of coherence in rostral as opposed to caudal regions of the brain, suggesting aberrant automatisation processes. Altogether, our findings reveal that profound functional deficits in cortico-basal ganglia networks of aged animals translate into shorter patterns of goal-directed action, a process that likely compromises the ability of older individuals to acquire new skills.

WTH17-02

Respiratory neuroplasticity within reticular nuclei following cervical spinal cord injury

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Cervical spinal cord injury (SCI) results in impaired breathing, which is primarily attributed to disruption of the phrenic circuitry mediating diaphragm function. Spontaneous improvement in breathing post-SCI has been reported experimentally and clinically, but the extent of respiratory recovery is minor and long-term deficits persist. Furthermore, while neuroplastic changes have been reported throughout the entire central and peripheral nervous systems, the focus of previous work has been on changes within the spinal cord. Little is known about anatomical reorganization and functional changes at supraspinal levels that may contribute to neuroplasticity. Understanding the mechanisms of recovery is an important step in developing new - or modifying existing – therapies to improve breathing following cervical SCI.

The present work explores the anatomical and functional changes that occur within medulla that are associated with respiration following high cervical (C2) lateral hemisection (Hx). This injury disrupts descending bulbospinal and ascending spinobulbar pathways between the medulla and spinal phrenic neurons, resulting in immediate hemidiaphragm paralysis. Specific focus is on the phrenic motor system, using the adult Sprague-Dawley rat. To first define the neuronal circuitry associated with phrenic function, a retrograde transynaptic tracer (pseudorabies virus; PRV) was applied to the hemi-diaphragm. PRV-positive neuronal labelling was seen throughout the spinal cord, brainstem and brain. Early brainstem labelling was seen within the raphe and reticular nuclei suggesting a close synaptic integration with spinal phrenic circuitry.

Bilateral phrenic neurograms were made and the caudal medulla was systematically mapped medulla for the presence of inspiratory and expiratory activity. The distribution of respiratory-related activity was mapped in uninjured animals and acutely following C2Hx. Partial recovery of ipsilateral phrenic activity was observed spontaneously 4–6 post- C2Hx. At that time electrophysiological mapping was conducted. An increase in respiratory activity was seen within the reticular nuclei post-C2Hx. This recruitment of activity may indicate reticular involvement with post-injury respiration and neuroplastic changes within respiratory network after cervical spinal cord injury.

WTH17-03

Visuospatial and somatomotor representations of the direction and extent of reaching movements in the parietal cortex of monkeys

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Many psychophysical studies suggest that the direction and extent of arm reaching movements are processed in functionally segregated visuomotor channels. Furthermore, several works have shown that visual information is important for the specification of the reach direction, whereas proprioceptive input is crucial for the control of movement extent. The medial posterior parietal cortex contains several areas that combine visual and somatosensory inputs and are involved in arm reaching, however the processing of reach extent information and its relationship with reach direction are poorly understood. In the medial parietal area V6A of monkeys we have recently demonstrated a widespread convergence of extent and direction information on the discharge of single neurons during several phases of arm movements in 3D space. Using the same, foveal reaching task, we studied direction and extent tuning in

macaque area PEc and compared the results with V6A. In PEc, the effect of direction was more prevalent than the effect of the reach extent before reaching execution, i.e. during target fixation and movement planning. Modulations of neural activity by reach extent and convergence of direction and extent signals on single cells became prominent after the start of arm movement. Comparing the two areas revealed that PEc cells processed mostly the information related to the extent of the arm movement, whereas many V6A neurons processed both the eye position information related to target distance and the information related to movement extent. These findings suggest the involvement of both areas in visuospatial and hand movement representations in 3D peripersonal space, with a caudo-rostral trend from a representation of both space and movement in V6A to movement prevalence in PEc.

WTH17-04

Do somatic neural adaptations occur in lower limb of elite football players?

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High repetition of motions, high muscular forces and extreme skillful foot positions in playing football may induce structural and functional changes in somatic neurons, the training effects yet not explored and clear in such rhythmic exercise. This study was aimed to compare nerve conduction in lower limb of football players with controls. Players (n = 27, age 22.74 ± 2.52 years.) with excellent cardio-respiratory fitness and with no signs of injuries, and sedentary controls (n = 29, age 23.41 ± 2.95 years.) were recruited for the study. Standard nerve conduction techniques were applied to evaluate tibial and sural nerves of each individual. The players had significantly lowered resting heart rate, systolic and diastolic blood pressure than controls. Tibial compound muscle action potential (CMAP) showed higher amplitude compared with controls; right tibial proximal CMAP amplitude [12.30(10–17.70) vs. 11.10(8.0–13.50) mV, p = 0.035], left tibial proximal [14.0(10.20–17.0) vs. 11.20(7.33–14.30) mV, p = 0.045] and distal [16.80(13.80–19.50) vs. 14.20(10.15–17.80) mV, p = 0.049] amplitude. Whereas right tibial proximal [7.50(7.0–8.80) vs. 9.0(7.30–10.75) ms, p = 0.005] and distal [7.20(6.70–8.10) vs. 8.30(7–10.25) ms, p = 0.046] CMAP duration were shortened in players, similar results were obtained for left tibial CMAP duration. Left sural nerve revealed shortened sensory nerve action potential duration [1.80(1.50–1.80) vs. 2.00(1.69–2.16) ms, p = 0.018]. Increased tibial CMAP amplitude and decreased CMAP duration in players suggest excitation of more number of motor units and higher synchronicity of muscle fibers' discharge than in controls respectively. Higher amplitude can also be attributed to increase in muscle fiber size and/or efficiency of neuromuscular transmission. Increased synchronicity indirectly reflects narrow range of conduction velocity among tibial neurons. Latency and conduction velocity that measure fastest conducting fibers were similar, thus simultaneous firing of motor units in players may be due to increased diameter of slower conducting axons. Similarly synchronous firing of sensory neurons is evident. The adaptive alterations in somatic nerves need more crucial research to exact identification of sites and the structures responsible.

WTH17-05

Transplantation of neural progenitor cells promotes respiratory recovery after cervical spinal cord injury

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Impaired breathing is a devastating consequence of cervical spinal cord injury (SCI) that increases morbidity and the risk of mortality. Injuries at high-to-mid cervical levels (C1-4) result in the most severe deficits as the phrenic motor circuitry – controlling the diaphragm – is directly compromised. While there is mounting evidence for spontaneous respiratory improvement, the extent of recovery – or functional plasticity – remains limited. Thus, there is a need to develop therapeutic strategies for enhancing repair and recovery of respiratory pathways.

Our ongoing research aims to elucidate spinal and supraspinal changes that may influence respiration post-SCI, and assess whether treatments can harness ongoing neuroplasticity to improve function post-injury. With a particular focus on the phrenic motor system, the goal of the present work is to assess whether transplantation of neural precursor and stem cells (NPCs/NSCs) can facilitate repair of the injured adult rat cervical spinal cord and promote functional recovery. We hypothesize that spinally derived NPCs will provide a source of neurons that facilitate a novel neuronal relay capable of restoring input to phrenic motoneurons.

Adult, female Sprague-Dawley rats (~250 g) received lateralized C3/4 contusions (200 kilodynes, Infinite Horizons Pneumatic Impactor). One week post-injury, NPCs derived from developing rat spinal cord (E13.5 Fisher rat, expressing green fluorescent protein) were injected directly into the injury cavity (~1 million cells). All transplant recipients were immunosuppressed (1 mg/kg cyclosporine i.p. daily). Transplanted animals are compared against injured, untreated animals. Four weeks later, a transynaptic, retrograde tracer (pseudorabies virus) was delivered to the ipsilateral hemidiaphragm or directly into the transplant. Tracing revealed synaptic integration between donor neurons and host phrenic circuitry, and host neurons with donor cells. Ventilatory function was assessed using whole-body plethysmography weekly pre-and post-injury. Phrenic function assessed terminal electrophysiology revealed enhanced phrenic and diaphragm recovery in those animals that received NPC transplants following cervical contusion injury. These ongoing studies are providing insight into the therapeutic potential for NPC therapy in the injured spinal cord.

WTH18 Sensory Systems (Part 2)

WTH18-01

Temporal dynamics of sensory adaptation in rat barrel cortex

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Exposure of sensory neurons to repeated stimulation results in changes in neuronal response properties – a phenomenon known as sensory adaptation. Although adaptation is a common feature of all modalities, there is a high level of diversity in the way individual neurons adapt to repeated stimulation. Here, we quantify the time course of adaptation in individual neurons recorded across layers of the rat somatosensory "barrel" cortex. In urethane-anaesthetised rats (n = 32), we juxta-cellularly recorded the activity of single cortical neurons (n = 95) with patch pipette filled with neurobiotin (1–2%) while applying a series of whisker deflections (100–200 μm in magnitude) at various temporal intervals (33–500 ms). The recorded neurons were then histologically reconstructed to obtain their topography and cortical layer. For over 90% of neurons, the net neuronal response did not increase with stimulation frequency. The depth of adaptation increased over time and was strongest at highest frequency (96 repetitions in 3s). However, in 10% of neurons, we observed strong response facilitation over time, where sustained stimulation significantly increased the response to subsequent stimulations. Across all neurons, response latency increased over the time course of stimulation, irrespective of changes in the response rate (facilitation vs. adaptation). Reconstruction of the recorded neurons (n = 18) revealed no systematic cell type difference between the adapted and facilitated neurons. We further formulated the population response dynamics as an exponential function of time and frequency with one parameter accounting for changes over time and frequency (r^2 = 0.96). Additionally, we applied an irregular train of deflections with a mean frequency of 8 Hz. For 70% of neurons, the response to irregular stimulation was greater compared to regular stimulation. This increase was significantly correlated (0.3451, $p < 0.002$) with the degree of adaptation. In conclusion, our findings demonstrate a high level of diversity among cortical neurons in their response to a train of stimulation, with a significant proportion of neurons showing facilitation at specific temporal intervals.

WTH18-02

Audiovisual integration in areas mt & mst of marmoset monkeys

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The traditional model of sensory processing states that each modality is processed independently and is only integrated in higher-level multisensory cortical areas. However, recent studies have shown anatomical and physiological evidence for multisensory integration in regions that have historically been attributed to one modality. The middle temporal area (MT) and medial superior temporal area (MST) of the primate cerebral cortex have well known roles in processing visual motion, but in marmosets they are known to have direct connections with auditory cortex (although more so for MST). We tested if neurons in these areas are responsive to auditory motion stimuli, and whether these neurons can integrate auditory and visual motion cues. We performed extracellular recordings (n = 29, 24 in MT, 5 in MST) in 3 anaesthetised marmosets and measured the neurometric thresholds of neurons for visual, auditory, and audiovisual motion stimuli. Visual stimuli were random dot kinematograms and auditory stimuli were interaural level difference ramps of 6–12 kHz bandpass noise which simulated motion. Auditory and visual stimuli were presented at the same spatial location, direction and speed for 1 s with matching noise levels to maximise the chance of multisensory integration. No neurons in either area were reliably responsive to auditory stimuli alone. While there were some circumstances where auditory stimuli appeared to modulate visual responses in MT, auditovisual stimuli did not reliably improve neurometric thresholds in any of our MT cells. Only one neuron from area MST showed a statistically significant change in threshold between the visual and audiovisual conditions. These data, while not ruling out minor modulatory auditory influences, suggest that MT does not integrate auditory cues for motion. It is more likely this occurs in downstream motion areas such as MST or beyond.

WTH18-03

Are rats capable of selective, spatial attention?

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Background: Selective attention is the process by which brain focuses on events that offer organisms survival advantages. Non-human primates are currently the primary animal model for selective attention. Here, we ask whether selective visual attention can be demonstrated in rats.

Methods: We trained Long-Evans rats (n = 2) in a visual detection task, where they were required to respond to a change in the luminance or the orientation of a visual stimulus. Rats initiated a trial by nose-poke into a small aperture, after which a visual change (reduction in luminance, or rotation in orientation) occurred in either the left or right visual field, at a random time (0.45–1.45 s, uniform distribution). Change on either left or right cued the animal to leave the aperture and seek sugar water reward from a small spout below it. To manipulate attentional load we varied the probability with which the signal was presented on left or right: either fully-predictable (100% on one side), random (50% on

each side), or in blocks where it was more likely (90%) to be presented on one side.

Results: Rats performed over 400 trials per session with high performance (above 80%) on both the luminance and the orientation change detection tasks. The reaction time (the time between signal onset and the animal's exit from the nose-poke) was systematically modulated by attention: it was fastest for the fully-predictable (204 ms) and slowest for the random signals (235 ms; $p < 0.01$). The reaction time was on average 6 ms faster on low-probability than high-probability trials ($p < 0.01$).

Conclusion: Reaction time difference between fully-predictable and random conditions suggests capacity for spatial attention engaged by top-down mechanisms that have access to the predictability of stimulus location. Slower reaction time for the high-probability than low-probability signals may be related to more feed-forward mechanisms, repetition suppression and surprise.

WTH18-04

Optogenetic dissection of a parvalbumin interneuron microcircuits within the superficial dorsal horn of the spinal cord

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The superficial dorsal horn (SDH) of the spinal cord is an important site for modality specific processing of sensory information related to nociception, touch, temperature and itch. Segregation of these modalities is essential for contextually relevant sensory experiences. Furthermore, when modality segregation fails aberrant sensory experiences such as allodynia may emerge. We have recently described a population of inhibitory parvalbumin-positive interneurons (PV^+INs) with functional properties and connectivity that would enable them to segregate tactile and nociceptive information (Hughes et al., 2012 J Physiol 16:3927). To better understand this microcircuit, our current experiments use an optogenetic approach, employing transgenic mice that express Channelrhodopsin-2 in PV^+INs. Adult mice (2–12 months old, both sexes) were deeply anaesthetized with ketamine (100 mg/kg, i.p.) and decapitated. Parasagittal spinal cord slices were prepared from the lumbar cord. Whole cell patch clamp recordings were made from both unidentified and targeted PV^+INs using a CsCl-based internal (holding potential −70 mV). Following 1 ms of photostimulation, inhibitory postsynaptic currents (IPSCs) were recorded in 37/38 PV^+INs and 31/42 unidentified interneurons. Photostimulation evoked IPSCs could be blocked by bath application of Bicuculline and Strychnine, suggesting co-release of the inhibitory neurotransmitters GABA and Glycine. In order to assess the nature (monosynaptic or polysynaptic) of PV^+IN connections we applied TTX to block action potentials in the slice. We then applied 4AP to facilitate photostimulation-induced release from PV^+IN synaptic terminals. Monosynaptic IPSCs were recorded in 9/9 of PV^+INs and 12/14 of unidentified interneurons. Together these data suggest that PV^+INs regulate a range of inhibitory SDH microcircuits likely to be important for normal sensory processing. Given this role, we predict that modulation of PV^+IN function by pathological conditions such as nerve injury will contribute to the development of aberrant sensory coding.

WTH18-05

Characteristics of dorsal horn neuron excitability and synaptic input in aged mice

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The incidence of pain rises in late middle age and continues to rise thereafter. The reasons for this are multifactorial. The fact that pain mechanisms differ in aged individuals is thought to be central. Our understanding of the mechanisms that mediate pain signalling in the aged is limited, in part because the classic electrophysiological recordings required to understand nervous system dysfunction are not thought to be possible in aged CNS preparations. Our study assessed the viability of patch clamp recordings in spinal cord slices from aged (28–30 months), and young (3 months) mice. Mice were deeply anesthetized (ketamine, 100 mg/kg) and parasagittal spinal cord slices were prepared. Patch clamp recordings were obtained in the superficial dorsal horn (SDH) of aged ($n = 21$) and young ($n = 20$) tissues. Stability and health of aged recordings was comparable to young recordings as evidenced by the similarity of, input resistance (424 ± 49 MOhm vs. 471 ± 55 MOhm, aged vs. young) and resting membrane potential (−51 ± 6 mV vs. −54 ± 2 mV, aged vs. young). In contrast, intrinsic excitability was significantly increased in old SDH neurons. Specifically, we identified three forms of action potential discharge during step current injections: tonic firing, initial bursting, and delayed firing. Tonic firing, the most excitable form of discharge, dominated in aged recordings (11/22 vs. 5/20, aged vs. young), whereas initial bursting was most common in the young sample (4/22 vs. 14/20, aged vs. young). Recordings of spontaneous excitatory currents (sEPSCs) were used to assess levels of excitatory drive. Both groups received a similar frequency of sEPSCs (4.0 ± 1.5 Hz vs. 3.4 ± 1.9 Hz, young vs. aged), however, sEPSC amplitude was smaller in aged recordings (15.3 ± 0.8 vs. 25.5 ± 3.5, aged vs. young). These data confirm that the cellular properties of aged pain circuits are different. These differences would be predicted to render aged pain pathways more vulnerable to central sensitization and neuropathic pain.

WTH18-06

Contribution of cortical layers 2/3 to sensory processing in mouse barrel cortex

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The mammalian neocortex is organised in a laminar structure based on the neuronal type, size and density. How does this elegant structure underpin the transformation of sensory information? Here, we combined optogenetics, *in vivo* juxta-cellular recording/labelling, information theoretic approach and network modelling to characterise how activation of layer 2 and 3 (L2/3) neurons affects sensory processing in deeper layers. We used the well-characterised mouse

whisker-barrel system. Channelrhodopsin-2 was expressed selectively in L2/3 by *in utero* electroporation at embryonic day 15.5. We recorded single cell activity (n = 266) from granular and infra-granular layers while applying mechanical stimuli of varying amplitudes (0–256 μm) to the whiskers of urethane-anaesthetised mice (n = 30). We then quantified neuronal response functions with and without activation of L2/3 neurons. Out of 96 whisker responsive neurons, the baseline activity of 56% of neurons increased by optogenetic stimulation, while that of 32% showed suppression. We then quantified how optogenetic activation affected the neuronal response range evoked by mechanical stimulation of whiskers. Across neurons, activation of L2/3 reduced the range of stimulus response function by 14.8% and thus decreased the encoding capacity of the population. There was no systematic correlation between changes in the baseline and changes in the neuronal response range. We applied information theory to characterise the information that individual neurons conveyed about the sensory stimulus (deflection amplitude): Optogenetic stimulation reduced the information content of 60% of neurons and increased that of the remaining 40%. This resulted in 26.5% decrease in the average information content. These findings are compatible with previous studies of the circuitry revealing that infra-granular layers receive mostly excitatory connections from L2/3. Optogenetic activation of L2/3 exerts a net sub-linear transformation on the sensory input. We discuss these findings in the context of a normalisation model.

WTH18-07

Dysregulation of metabotropic glutamate receptor 5 in periaqueductal gray perpetuate chronic neuropathic pain

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We measured glucose metabolism of the whole-brain using positron emission tomography from neuropathic pain model animals to see the altered state of the brain. Result showed that metabolic activities of periaqueductal gray (PAG) and rostral ventromedial medulla (RVM), the endogenous pain-modulation pathway, were lower in neuropathic pain group compared to control. Activating group I metabotropic glutamate receptor (mGluR) in ventrolateral PAG (VL-PAG) increased metabolic activities of PAG and RVM and completely cancelled out neuropathic allodynia in pain group. Interestingly, response to PAG-mGluR activation was different in control group in that weak or no metabolic increase was observed from VL-PAG and RVM.

One of the possible explanations is that the mGluR signaling within VL-PAG is spontaneously active in normal state to maintain normal sensory perception, and is lost following chronic neuropathic pain so that neuropathic symptoms are maintained. To address this issue, we deactivated mGluR5 and measured excitability of the VL-PAG neuron from brain slices of both groups before and after mGluR5 deactivation. Indeed, deactivation of mGluR5 resulted in decrease of neuronal excitability of VL-PAG neuron in control group, which indicates that mGluR5 signaling is persistently active in normal condition to maintain neuronal excitability. As we expected, baseline excitability of VL-PAG neuron was significantly lower in pain group and deactivation of mGluR5 failed to reduce excitability, suggesting that PAG-mGluR5 signaling is already occluded in pain condition.

Behavioral study further supported the results above. Deactivation of mGluR5 in bilateral VL-PAG of naïve animals reduced paw withdrawal threshold and induced mechanical allodynia, a representative symptom of neuropathic pain. Beyond our expectations, allodynia persisted even after a month after drug administration. Overall, these data suggest that persistently active mGluR5 signaling in VL-PAG plays a key role in normal sensory transmission, and a disturbance of it causes induction and maintenance of chronic neuropathic pain.

WTH18-08

Identification of the spinal afferent nerve endings that encode noxious & innocuous stimuli in the stomach and oesopahgus

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The nerve endings of spinal afferents innervating the oesophagus and stomach have not been identified. We have recently developed an anterograde tracing technique which facilitates selective labeling of only spinal afferent axons and their nerve endings in visceral organs. Mice were anesthetized, thoracic DRGs (T8-T12) surgically exposed, then injected with dextran-amine. Seven days post-surgery, the entire stomach and esophagus was removed, fresh fixed and stained for CGRP. The characteristics of 7 major types of spinal afferent nerve endings were identified throughout the corpus, antrum and fundus of stomach of 11 control C57BL/6 mice. The greatest proportion of nerve endings was in the myenteric ganglia (44%), and circular muscle (23%). These nerve endings consisted of fine intraganglionic varicose endings (IGVEs) that ramified through multiple myenteric ganglia, very similar to the IGVEs we recently identified in the colorectum. Greater than 90% of IGVEs and "simple" type varicose nerve endings in the CM layer were CGRP-positive (n = 10). No intraganglionic laminar endings (IGLEs) were observed that resembled those reported for vagal afferent endings. The other types of spinal afferent endings consisted of "simple" fine varicose nerve endings that innervated internodal strands, the submucosa and mucosa, blood vessels and longitudinal muscle (n = 10). Spinal afferent endings were rare in the esophagus, but in 2 animals consisted on "simple" type fine varicose endings that ramified within the skeletal muscle and/or myenteric ganglia. We present the first complete characterization of the different types of spinal afferent nerve endings that innervate the upper gastrointestinal (GI) tract of a mammal. The findings reveal a complex array of different types of primary afferent endings that innervate specific layers of the stomach. Some of the novel classes of nerve endings identified must underlie the transduction of noxious and/or innocuous stimuli from the upper GI-tract *in vivo*.

WTH18-09

A rodent model of sensory prioritisation: behavioural performance and neural correlates

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Introduction: In a natural environment, animals need to assess when to initiate actions based on uncertain sensory evidence. This is evident when dealing with weak sensory inputs, such as small changes in luminance or vibrations induced by predators. In such scenario, animals benefit from prioritising sensitivity in the modality that is more likely to provide the key information.

Methods: We trained rats in two detection tasks: a go/no-go detection ($n = 4$) and a two-alternative-forced-choice ($n = 4$) where they detected the stimulus position (left/right). In each trial, the stimulus was either a vibration (3–8 μm; 40 Hz) applied to whiskers or a visual flicker (4–12% luminance change). Both stimuli were adjusted independently in difficulty across rats to achieve 20% miss rate. We manipulated attention by controlling the likelihood with which the stimulus was presented in each modality. In a *whisker session*, 80% of trials were whisker vibration and the remaining 20% were visual flicker. Likelihoods were reversed in a *visual session*. As rats performed the task, we recorded single-cell activity from primary somatosensory cortex ($n = 10$) along with local field potential (LFP).

Results: Across rats, the earliest behavioural manifestations of detection (when *d'* deviated from chance) were remarkably fast (vibration: M = 47.5 ms; flicker: M = 56 ms). High-likelihood trials (e.g. vibration during *whisker session*) resulted in lower miss rates (vibration: 18% in high-likelihood vs. 34% in low-likelihood, $p < 0.01$; flicker: 19% vs. 32%, $p < 0.01$) and faster decisions (vibration $_{\text{low-high likelihood}}$: 187.5 ms, $p < 0.01$; flicker $_{\text{low-high likelihood}}$: 131.3 ms, $p < 0.01$) than on low-likelihood trials (e.g. flicker during *whisker session*). Reflecting changes in whisking behaviour, single-unit activity increased during entrance and exit from the stimulus presentation port. As rats nose-poked to initiate trials, LFP showed higher variability during *whisker sessions* compared to *visual sessions.* This variability was also predictive of rats' performance.

Conclusion: Our study provides both behavioural and neuronal evidence for sensory prioritisation in rodents.

WTH18-10

Transformation of orientation bias to sharp orientation selectivity within the primary visual cortex of the tree shrew

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Introduction: There is still no consensus on the origin of the orientation selectivity seen in the primary visual cortex (V1) of mammals. One theory is that the selectivity derives from biases to orientation already present in the thalamic input to V1. We tested this hypothesis in the tree shrew, a species where the input layer 4 of V1 contains neurons whose response characteristics are similar to subcortical neurons. They lack sharp orientation tuning to bars, but when using gratings they exhibit steadily increasing selectivity to orientation as the spatial frequency of the grating stimulus increases. We compared spatial frequency and orientation tuning of cells in layer 4 and in supragranular (2/3) layers in the same electrode penetration to see whether the simple loss of responses to low spatial frequencies due to non-specific inhibition, as the signal goes from layer 4 to layer 2/3, can completely explain the sharper orientation tuning of the 2/3 cells.

Methods: In tree shrews under isofluorane anaesthesia, we recorded in 7 penetrations the spatial frequency (SF) and orientation response functions of layer 2/3 and layer 4 neurons. To predict the preferred orientation and tuning width of layer 2/3 cells, the responses of layer 4 cells at different spatial frequencies and orientations were convolved with the spatial frequency tuning of layer 2/3 units.

Results: Orientation tuning of the responses of layer 4 neurons were significantly broader at low frequencies compared to high ($p < 0.01$; Student's *t*-test). Furthermore, the predicted width of orientation tuning of layer 2/3 cells showed moderate correlation with the measured width (Pearson's $r = 0.67$; $p = 0.09$).

Conclusions: Our data shows that sharp orientation tuning of layer 2/3 neurons can be derived from the broadly tuned responses of layer IV neurons as a direct result of non-specific inhibition. This inhibition acts by attenuation of the responses to low spatial frequencies.

WTH18-11

Water channel permeability in retinal degeneration. the role of aquaporins

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Aquaporins are small trans-membrane water channels important for water transport and maintaining ocular homeostasis. Different aquaporin isoforms have been detected in areas of photoreceptor degeneration in the retina, but how they relate to disease remains largely unknown. This study characterized the distribution of various aquaporins in the normal and degenerating retina. Retinal sections of normal and age-related macular degeneration (AMD) donor eyes were immunofluorescently stained for various aquaporin isoforms. Normal labeling was revealed in photoreceptors and the inner retina including the ON sublayer of the inner plexiform layer. In AMD retina, there was abnormal distribution of aquaporin markers in the inner and outer retina. These results suggest aquaporin may be reliable marker of retinal remodeling and part of the retinal degeneration process.

WTH18-12

Effect of spike rate on response characteristics of koniocellular cells in the lateral geniculate nucleus of common marmosets

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Purpose: We previously reported that some neurons in the intercalated (koniocellular, KC) layers of the lateral geniculate nucleus (LGN) show high variability in spike rate. The spike rates of K-cells are higher when low frequency power in the electroencephalogram (EEG) in primary visual cortex (V1) is lower [1]. Our purpose here is to find out if variability in maintained discharge rate has an influence on the responses of LGN cells to visual stimuli.

Methods: Extracellular spike activity of KC cells in the LGN (n = 48) was recorded in Sufentanil-anaesthetised marmosets (*Callithrix jacchus*). Visual stimuli were presented against a uniform grey field (50 Cd/m^2). Spike rate was considered high if firing rate exceeded 20 imp/s in the 350 ms before stimulus onset. Receiver Operator Characteristic (ROC) and regression analyses were performed on K-cell responses.

Results: Response profiles to uniform high contrast stimuli were additive (mean regression slope: 0.98 ± 0.24). ROC analysis showed no statistical difference in area under the curve for preferred stimuli when firing rate before the stimulus was low (0.94 ± 0.076) or high (0.91 ± 0.11). Analysis of patterned stimuli with increasing contrast revealed that for presentations with high firing rate before stimulus onset average firing rate (F0) did not change. However, when looking at the patterned response (F1) high firing rate before stimulus onset increased area under the curve at 14% contrast (high: 0.77, low: 0.64).

Conclusion: In the LGN, K cells show additive response properties with varying firing rate before stimulus onset. A high firing rate is not detrimental in detecting stimuli of varying contrast.

[1] Cheong S.K. et. al., (2011) *PNAS* 35, 14659–14663.

WTH18-13

Paradoxical effect of endocannabinoids on visually-evoked responses of mouse retinal ganglion cells

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The endocannabinoid (eCB) system and all of its components (receptors, synthesising and degrading enzymes and agonists) have been detected in the retina. We have previously found that the exogenous cannabinoid agonist WIN55212-2 decreased the strength of synaptic transmission whilst the antagonist AM251 enhanced synaptic transmission onto retinal ganglion cells (RGC). Whether or not eCBs are tonically released in the retina, however, is still unknown. In this study we aimed to establish whether or not eCBs are released in physiological conditions in the retina, and if so, how they affect retinal function. We examined how the eCB system modulates response strength of RGCs to visual stimulation and their receptive field organisation. In addition, we assessed the effects of modulating the eCB system on RGC excitability by monitoring the activity of sodium channels in RGCs. We recorded spontaneous activity, visual-evoked responses and voltage-gated sodium currents from large ON-RGCs using whole-cell patch-clamp recordings before and after bath application of either the endocannabinoid anandamide (15 μM) or the inhibitor of its degrading enzyme URB597 (1 μM). Anandamide and URB597 reduced the amplitude of visual-evoked postsynaptic potentials in RGC but paradoxically both drugs increased RGC spike responses; both drugs also modified the receptive field organisation of RGCs. In addition, we found that URB597 and anandamide increased the occurrence of sodium currents for any given voltage-step and that the current–voltage curve of the sodium current was shifted to the left. We conclude that eCBs are tonically released in the retina and they modulate retinal synaptic transmission, consistent with their function in other central nervous system areas. Their net effect on retinal output, however, will depend on the balance of their effects at the circuit level and directly on RGCs sodium channels.

WTH18-14

Spatial relationship between cones and midget bipolar cells in human retina

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The midget-parvocellular pathway is responsible for high acuity and red-green colour vision in primates including humans. The anatomical substrate for red-green colour vision is the one to one connectivity in the midget pathway. That is in central (foveal) retina midget bipolar cells contact a single cone and thus transfer the chromatic signal of this cone to midget ganglion cells. Here we addressed the question whether this one-to-one connectivity persists outside the fovea thus enabling colour specific input to midget ganglion cells outside the fovea. *Post mortem* human eyes were obtained following ethical approval and consent from the Lions NSW Eye Bank at Sydney Eye hospital. Pieces of defined eccentricity were embedded in Agarose and sectioned vertically at 100 μm thickness along the horizontal meridian using a Vibratome. Sections were processed for immunofluorescence using antibodies against cone arrestin to label cone photoreceptors, and antibodies against recoverin to label OFF midget bipolar cells. Subsequently, confocal image stacks were obtained and the specific cell types were counted. The peak density of 141,935 cones per mm^2 at the centre of the fovea drops to 50,000 cones per mm^2 at 0.3 mm and remains stable at ~ 1000 cones per mm^2 between 4 and 7 mm eccentricity. The density of recoverin positive OFF midget bipolar cells matches the density of cones from 2 mm to an eccentricity of at least 7 mm. Our results suggest that single cone contacting midget bipolar cells

are present in peripheral retina and thus could provide chromatic input to the parvocellular pathway.

WTH18-15

Sub-threshold stimulus-response function in mice layer 2/3 barrel cortex

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The mammalian neocortex is organised into distinct layers that process sensory information to generate a reliable representation of the world. Neurons located in the superficial layers (L2/3) of sensory cortex form a hub in intra-cortical processing: they reciprocally connect to other cortical areas but do not project to subcortical sites. This suggests that L2/3 neurons are involved in hierarchical sensory processing and integration. Here we use two-photon Ca^{2+} imaging and *in vivo* whole-cell recording in the mouse somatosensory "barrel" cortex to characterise the response properties of L2/3 neurons to whisker stimulation. Bolus injection of Ca^{2+} indicator Cal-520 allowed us to simultaneously image a population of up to 65 L2/3 neurons in urethane anaesthetised mice ($n = 5$). We performed two-photon imaging while a calibrated piezoelectric device stimulated the contralateral whisker pad with a wide range of brief semi-Gaussian deflections (9 ms pulses at 11 amplitudes: 0–129 μm, 25 trials each) and an additional "instantaneous" high-velocity deflection produced by a step command. Consistent with previous studies, sensory stimulation evoked significant calcium transients in a small fraction of L2/3 neurons (~10%). However, the intense deflection activated > 90% of L2/3 neurons. We further performed blind whole-cell recordings from L2/3 barrel cortex neurons. The 59 μm stimulus produced a significant synaptic response defined as the maximum membrane potential (V_m) within the 130 ms post-stimulus window (Wilcoxon rank-sum test, $p < 0.05$). V_m further increased at the next two amplitudes (72 and 85 μm, compared to 59 μm and each other, $p < 0.05$) and saturated at 100 μm (with no further increases at 114 and 129 μm). No AP was evoked in response to these 11 amplitudes. In two cells, we applied the "instantaneous" deflection. Despite a saturating synaptic response function, the sharp stimulus evoked action potentials (0.68 ± 0.09 and 0.48 ± 0.10 APs/trial) in these cells. Overall, we found prominent sub-threshold synaptic response in all L2/3 neurons, with a sparse code where APs were only generated in response to highly intense stimulation.

WTH18-16

Neuronal imaging of intrinsic sensory neurons during colonic migrating complexes

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Dogiel Type II neurons have been shown to be intrinsic sensory neurons in the gastrointestinal tract but it is not clear whether they behave only as sensory neurons. In this study, we used the latest high resolution, EMCCD camera (Evolve Delta; Photometrics) to visualize temporal activation properties of multiple Dogiel Type I and Type II neurons simultaneously in a number of neighbouring myenteric ganglia along the isolated whole intact mouse colon at 35°C. Intracellular electrophysiological recordings from the circular muscle confirmed the presence of CMMCs every 2–4 min that propagated along the colon. Each CMMC consisted of repetitive discharge of hexamethonium-sensitive cholinergic excitatory junction potentials (EJPs) producing rapid oscillations in membrane potential (at 2 Hz) in the circular muscle, lasting 20–30 s ($n = 5$). To characterize the temporal firing properties of myenteric neurons during these rapid oscillations in the muscle, the calcium indicator, Fluo-4 was loaded into the myenteric plexus. Calcium imaging at 35oC revealed each CMMC was associated with a repetitive discharge of synchronized calcium transients in large populations of Dogiel Type I and Type-II neurons across multiple rows of myenteric ganglia and internodal strands ($n = 9$). The frequency of the repetitive calcium transients in myenteric ganglia (2.0 ± 0.1 Hz) and internodal strands (2.2 ± 0.1 Hz) was the same as the cholinergic EJP's recorded at the same time in the smooth muscle. All synchronized calcium transients across multiple ganglia were abolished by tetrodotoxin. This is the first demonstration that Dogiel Type II (intrinsic sensory neurons) participate in a dynamic neuronal firing pattern with interneurons and motor neurons, during the complex colonic motor pattern. We suggest Dogiel type II may perform multiple functions in the enteric nervous system.

WTH18-17

Age-related changes in the central auditory system of rat

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Aging is accompanied in mammals mostly by the deterioration of hearing function, which is based on defects occurring not only in the receptors situated in the inner ear, but also in the central part of the auditory system. We described previously changes that occur in the auditory system during the last several months of life in both the normally aging rat strain Long Evans and the fast aging strain Fischer 344. Using histological and immunocytochemical methods as well as Western blot we found differences between young and old animals of both strains in the labeling and protein levels of GAD 65 and 67, parvalbumin, calbindin, calretinin, phosphate-activated glutaminase, and the potassium-chloride cotransporter KCC2. In most cases, aging was accompanied by a decrease in the labeling and protein levels of all investigated structures, i.e. the inferior colliculus, medial geniculate body and auditory cortex. In the present experiments we were interested in the extent to which these changes are accompanied by neuronal loss. Therefore, we used an unbiased stereological method to determine the number of all neurons in Nissl stained sections of the three mentioned auditory structures in young and old rats of both strains. With aging, we found a rather mild and statistically non-significant decline in the total number of neurons in all three analyzed auditory regions in both rat strains. Our results demonstrate that presbycusis in rats is not likely to be primarily associated with a decrease in the total number of neurons in the central auditory system. Hearing loss results from deterioration of the peripheral hearing organ accompanied by changes in the function of specific neuronal networks, e.g.

inhibitory interneurons containing calcium binding proteins, in the central auditory system.

WTH18-18

Non-invasive tactile feedback from artificial sensors using a biomimetic code

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The loss of a hand or arm is a devastating event that has inspired a significant effort to improve prostheses for upper limb amputees. Current commercially-available hand prostheses offer a limited level of motor control but none incorporate tactile feedback. Feedback requires a mechanism to efficiently convey information from an artificial sensor to the nervous system of the user. We describe a novel method of providing tactile feedback that employed sensors whose outputs were passed to algorithms that converted them into physiologically-plausible action potential (spike) trains. The spike trains were then delivered to intact peripheral afferents using non-invasive stimulation. All experimental protocols were approved by the UNSW Human Research Ethics Committee. Four virtual neurons were built using a noisy leaky integrate and fire model and trained on non-human primate data obtained from fingertip afferents (two FA1 and two SA1). Signals from an artificial tactile sensor responding to mechanical stimuli that moved in either a proximal or distal direction were input to virtual neurons and their spiking output pattern recorded. Healthy young adult subjects had these spike patterns delivered to their upper arm by a pair of tactile stimulators (electrical or pulsatile mechanical) to determine their ability discriminate the direction of movement. A single training session of ~ 10 min combined skin stimulation with a visual representation of the movement for 60 presentations of each afferent type. During the subsequent testing phase, subjects were presented with spike patterns they had been trained on as well as novel spike patterns, with no visual cues. Several subjects were able to discern movement direction with greater than 90% accuracy for both SA1 and FA1 signals with electrotactile or mechanical stimulation. There was no significant difference between novel and non-novel stimuli, indicating subjects could generalise stimulus sensations across specific instances of firing patterns.

WTH18-19

Binocular neurons in marmoset lateral geniculate nucleus: contrast sensitivity and response summation

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Purpose: Although most neurons in marmoset lateral geniculate nucleus get dominant excitatory input from one eye, some koniocellular (K) neurons respond almost equally strongly to stimuli presented through either eye. Here we compared sensitivity and timecourse of K and parvocellular (P) cell responses to monocular and binocular stimuli.

Methods: We made extracellular recordings from two Sufentanil-anaesthetized marmosets (*Callithrix jacchus*). Responses to a large (12–20°) uniform field of varying contrast were measured and fit to a saturating hyperbolic ("Naka-Rushton") equation: $A + R_{max} \cdot c/(c_{50} + c)$, where A is a constant and c is contrast, to estimate response amplitude (R_{max}) and gain (c_{50}). Response amplitude and timecourse (spike counts in 100 ms bins) for large 200 ms pulse stimuli were measured.

Results: R_{max} values for dominant and non-dominant eye stimulation were correlated for K cells ($r^2 = 0.86$, $p < 0.01$, $n = 10$) but not P cells ($r^2 = 0.05$, $p = 0.67$, $n = 6$). For K cells, contrast gain (R_{max}/c_{50}) was greater for dominant (mean 3.03 ± 4.25 imp $s^{-1\%-1}$) than for non-dominant eye stimulation (mean 0.31 ± 0.53 imp $s^{-1\%-1}$) and was weakly correlated for the two eyes ($r^2 = 0.44$, $p = 0.04$). Spike rate correlations for monocular vs. binocular pulse presentation were high (mean r^2: 0.49 ± 0.16, $p < 0.01$, $n = 8$) for most K and P cells. Correlations for three K cells exhibiting binocular responses were higher for monocular (r^2: 0.67, 0.37, 0.27) than binocular (r^2: 0.53, 0.00, 0.00, $p < 0.01$) stimulus presentation.

Conclusion: Responses of many K cells are similar for each eye but the dominant eye inputs show higher contrast sensitivity. Binocular K cells show sub-additive binocular integration.

WTH19 Limbic and other Systems

WTH19-01

Hippocampal influence on the respiratory and cardiovascular system

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The hippocampus is a critical part of the limbic system involved in memory and learning and also contains the biological global positioning system. The ventral hippocampal CA1 field in particular is suggested to be involved in the mediation of stress and anxiety. Expression of stress and anxiety require changes to the cardiorespiratory tone. The midbrain periaqueductal gray (PAG) has been shown to function as a critical relay of the limbic brain in integrating cardiorespiratory and emotional/behavioural responses (Subramanian and Holstege, 2014). One important limbic command to the PAG could be from the ventral hippocampus in regulating cardiorespiratory response to stress and anxiety. However, it is not known whether the ventral hippocampus has any influence on the cardiorespiratory system. It is also not known whether the ventral hippocampus has direct connections with the PAG. Thus, in urethane anesthetised (1.5 g/kg I.P) adult Sprague-Dawley (SD, $n = 40$, of either sex) rats, we stereotaxically mapped the hippocampus, both the dorsal and the ventral areas for cardiorespiratory modulation. Excitatory Amino Acid (EAA), D, L, Homocysteic acid (DLH; 50 mM, pH 7.4) was used for chemical stimulation. Homeostatic parameters such as changes to blood pressure, heartrate, respiratory frequency, inspiratory, post-inspiratory and expiratory durations were investigated. In another set of experiments ($n = 10$) we investigated the neuroanatomical pathways between the hippocampus and the PAG using anterograde tracer microinjections (biotinylated dextran amines (BDA)) into the hippocampus. DLH stimulation of the CA1 field triggered significant modulation to cardiorespiratory parameters. Neuroanatomical tract-tracing also revealed pathways that may have a pronouncement in the limbic inputs to the PAG involved in cardiorespiratory modulation The results and the functional implications of the hippocampus-PAG network on homeostatic regulation during emotional behaviour are further discussed.

WTH19-02

5-HT1A receptor in sex-specific neonatal hippocampal development and later-life mood disorders: possible cooperation with GPR30

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The neurotransmitter serotonin (5-HT) plays an important role in mood disorders and multiple studies show that its synthetic rate and signaling activity are different in the brains of men and women. Intriguingly, mood disorders are twice as common in women than men. We have demonstrated that 5-HT signaling through 5-HT1A receptor (5-HT_{1A}-R) is crucial for early postnatal hippocampal development and later-life behavior. Although 5-HT signaling through 5-HT_{1A} receptors (5-HT_{1A}-R) regulates early brain development, the mechanistic underpinnings have remained unclear. We have shown that *supra*-basal 5-HT_{1A}-R signaling in postnatal day 6 (P6) mice through protein kinase Cε (PKCε) and extracellular receptor activated kinase 1/2 (Erk1/2) in the dentate gyrus (DG) boosts neonatal neuroblast proliferation in both sexes. However, the basal 5-HT_{1A}-R signaling exerts a female-specific effect and neuroproliferation is severely impaired in P6 female but not male 5-HT_{1A}-R(−/−) (KO) mice. Confirming the developmental importance of sex-specific early neuroproliferation, the KO female but not male mice showed significantly elevated anxiety-like behavior at P60. Intrahippocampal infusion of the selective PKCε stimulator DCP-LA at P6 partially rescued neuroblast proliferation in the KO females and repeated systemic treatment from P6 to P14 corrected the later-life anxiety-like behavior. To investigate the mechanism of this sex-specific effect of 5-HT_{1A}-R signaling, we analyzed the involvement of the estradiol receptor GPR30, which has been reported to associate with the 5-HT_{1A}-R and also influence its signaling activity. GPR30 is believed to locate to the post-synaptic density, may therefore associate with the 5-HT_{1A}-R and the coupled signaling molecules to influence the downstream pathway. Intriguingly, 5-HT_{1A}-R-mediated neuroproliferation in the P6 hippocampus was eliminated in the presence of the GPR30 antagonist G15 and the GPR30 agonist G1 also elicited a dramatic increase in neuroproliferation. Our studies will investigate the influence of sex in the functional interactions between GPR30 and 5-HT_{1A}-R.

WTH19-03

Sensory drive for medial olivocochlear efferent control of hearing balance depends upon type II spiral ganglion neuron innervation

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The objective was to test the hypothesis that the subpopulation of (type II) spiral ganglion neurons (SGN) that innervate the cochlear outer hair cells (OHC) provides sensory input to the medial olivocochlear efferent reflex pathway balancing hearing between the ears. The physiological role of type II SGN has not been established. Type II SGNs are distinguished by their expression of peripherin, a type III intermediate filament known to drive neurite outgrowth. Results: The peripherin knockout (PrphKO) mouse lacks type II SGN innervation of the OHC, based on immunofluorescence for Neurofilament 200 kDa protein, and Serial Block Face SEM tomography. When the medial olivocochlear efferent reflex pathway innervating the OHC was activated by applying noise to the opposite ear, quadratic distortion product otoacoustic emissions (f2-f1 DPOAE), which measure the OHC-based cochlear amplifier, failed to show the suppression (reduced hearing sensitivity) observed in wildtypes. Auditory brainstem responses (ABR) showed normal hearing sensitivity in the absence of noise. Conclusion: Hearing balance is a closed-loop neural reflex, with the OHC acting as both the sensory and motor elements for dynamic regulation of hearing between the ears that underlies sound localization, focused hearing in noise, and oto-protection from acoustic overstimulation. Protocols approved by the UNSW Australia Animal Care and Ethics Committee; Primary Funding: NHMRC APP1052463.

WTH19-04

Outcome-history dependent effects of blocking anterior insular dopamine and serotonin on risky decision making

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To survive against competition higher animals sometimes take a risk for a higher gain although it can produce a significant loss. The point is the balance between risk taking and avoiding. The two major neuromodulators, dopamine and serotonin have long been implicated in this risky decision making (Cools *et al.*, 2011; Rogers, 2011; Takahashi, 2012). A recent study found that dopamine and serotonin have different influences on risky decision making in terms of feedback from previous outcome of choice; dopamine is involved in feedback from positive experience whereas serotonin is involved in feedback from negative experience (den Ouden et al., 2013). Here, we found that blocking dopamine and serotonin signals in their target region also caused outcome-history dependent change in risky decision making (Ishii et al., 2015). We examined the effects of locally blocking dopamine and serotonin receptors in the anterior insular cortex (AIC) on risk preference of the rats in a gambling task. The task required water deprived rats to choose between a sure option which guaranteed 2 drops of water versus a risky option which possibly provided 4 drops of water but in 50% (no water in the other 50%). The rats normally preferred the risky option in this task. Both intra-AIC injection of the D_2R blocker (eticlopride) and 5-$HT_{1A}R$ blocker (WAY100635) increased this risk preference. However, further analysis revealed that the D_2R blocker increased risk preference particularly after winning in a risky choice whereas the 5-$HT_{1A}R$ blocker increased risk preference after losing. We previously found that inactivation of AIC by locally injecting muscimol and baclofen decreased risk preference, suggesting that intact AIC promotes and risk taking (Ishii *et al.*, 2012). Given these evidences, the present results could be interpreted as follows; dopamine released after wins may, via D2 receptors, suppress risk-seeking function of the AIC and prevent repeated risky choices after wins. Meanwhile, serotonin released after losses may suppress AIC function via 5-HT1A receptors and prevent repeated risky choices after losses.

WTH19-05

Control of fear expression by manipulation of infralimbic cortex during extinction retrieval

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Evidence from rodent and human studies implicates the ventromedial prefrontal cortex (vmPFC), specifically infralimbic cortex (IL), in the regulation of fear expression after extinction. However, lesions or pharmacological inhibition of vmPFC before extinction reportedly has no effect on extinction or even facilitates extinction. Thus, the role of IL activity in expression of fear extinction memory remains unclear. To address this question, we examined the real-time contribution of IL neurons to expression of fear extinction using optogenetic approaches. Our results reveal that inactivation of neurons in infralimbic but not prelimbic cortex impairs expression of fear extinction. Conversely, activation of infralimbic excitatory neurons enhances expression of fear extinction but has no significant effect on expression of unextinguished conditioned fear. Fear extinction returned to normal after halting these optogenetic manipulations. Therefore, our results demonstrate that the activity of IL is necessary and sufficient for inhibition of fear expression after extinction.

WTH19-06

Synaptic gating of viscerosensory signals in the solitary tract nucleus

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Viscerosensory signals enter the brain at the nucleus of the solitary tract (NTS). These signals enable the brain to control and coordinate internal organ function via autonomic reflexes. We hypothesise that synaptic gating of vicersoensory signals results in the strengthening or weakening of autonomic reflex gain. For example, the baroreceptor reflex governs short term blood pressure and operates under different rules at rest as compared to during stress. Here we utilized optogentics to activate both local and long range inhibitory interneurons in/to NTS. To target local interneuons we used a transgenic mouse line in which ChR2 was under control of somatisation (Sst) expression in neurons. To target long range inhibitory neurons an adeno-associated virus expressing ChR2 under control of a ubiquitous promoter (CAG) was microinjected into the central nucleus of the amygdala (CeA) of transgenic mice expressing GFP in tyrosine hydroxylase-expressing neurons. Whole cell measurements in horizontal brain stem slices containing both the NTS and solitary tract (ST) were recorded. Shocks to the ST evoked low jitter EPSCs that identified second order NTS neurons. Optical activation of CeA efferents evoked large amplitude IPSCs (76 ± 14 pA), that rarely failed, exhibited frequency-dependent depression and relatively low input convergence (10 inputs across 5 neurons). In contrast, ChR2 activation of local Sst-neurons evoked lower amplitude IPSCs (35 ± 12 pA), though increased pulse durations resulted in summated IPSCs indicating greater input convergence (> 5 inputs per neuron). These results indicate synaptic gating of viscerosensory signals occurs via direct inhibition of second order NTS neurons to impact reflex performance and organ function.

WTH19-07

Activation of habenula nucleus elicits thermogenesis in brown adipose tissue and vasoconstriction in cutaneous vascular bed

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The habenula, a phylogenetically ancient nucleus in the diencephalon, is part of the neural circuitry mediating behavioral interactions with the external environment essential for survival and reproduction. The neurons in the lateral habenula are activated by adverse environmental events and contribute to appropriate modification of behavior. In adverse situation, animals increase their body and brain temperature, referred as emotional hyperthermia, and promotes cardiovascular function to prepare for the behavioural action. So far it is not known whether the LHb neurons regulates these autonomic-physiological responses. To address this issue, we investigated whether activation of neurons in the lateral habenula elicits the autonomic-physiological responses. Male Sprague–Dawley rats (350–450 g) were implanted with ultrasound Doppler flow probes around the tail artery and thermocouple probes were placed in interscapular brown adipose tissue (BAT) and rectum (body) for temperature measurements. To activate neurons, we injected bicuculline (1 nmol/100 nl) into the lateral habenula in anesthetized studies, or clozapine-N oxide (CNO, 1 mg/kg, s.c.) in conscious studies in which adeno-associated virus vectors were pre-injected into the lateral habenula to express Gq-coupled modified human M3 muscarinic receptors (hM3Dq) in neurons. In both anesthetized ($n = 9$) and conscious rats ($n = 3$), pharmacological stimulation of neurons in the lateral habenula increased temperatures of BAT (from 37.3 ± 0.2 to 37.9 ± 0.2, $p < 0.01$ in anesthetized studies, from 38.3 ± 0.2 to 40.3 ± 0.3, $n < 0.01$ in conscious studies) and decreased tail blood flow to approximately 30% of pre-injection level ($p < 0.01$, in both anesthetized and conscious studies). The responses of BAT thermogenesis and cutaneous vasoconstriction elicited by activating neurons in the lateral habenula are similar to those observed during stress-induced emotional hyperthermia, suggesting that the lateral habenula may have an important role in this response.

WTH19-08

Claustrum projections to cingulate and medial prefrontal cortex of the marmoset (*Callithrix jacchus*)

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The claustrum is a thin, sheetlike nucleus situated between the insular cortex and putamen. It is notable for its widespread cortical and subcortical connectivity, but its function(s) are currently unknown. In this study, we examined claustrum projections to the posterior cingulate and medial prefrontal cortex of the marmoset monkey. Under surgical anaesthesia, neuroanatomical tracers were placed in cortical areas 23 and 31 (posterior cingulate) and in area 32 (medial prefrontal) of 4 adult marmosets ranging in age from 1.5 to 14 years at injection. All procedures were performed in compliance with Monash Animal Ethics Committee protocol and NHMRC guidelines. Detailed maps and 3-D reconstructions of the resulting tracer distribution were generated, and the claustrum connectivity of each of the targeted areas was compared using quantitative and classical neuroanatomical methods. Dense claustrum projections were evident in each of the target cortical areas. Afferents to the posterior cingulate were concentrated in the dorsal (insular part of the claustrum, and extended along the entire rostral-caudal axis. Afferents to the medial prefrontal cortex were largely confined to 2 restricted patches in the mid-ventral and caudal ventrolateral parts of the insular claustrum. In the posterior cingulate, there was also evidence of age-related decline in claustrum connectivity. Our findings have implications for understanding the role of networks based on functional connectivity, e.g. via imaging or electrophysiological measures, in the primate brain under both normal and pathological conditions.

WTH19-09

A novel treatment approach for obesity and over-weight - targeting brain nicotinic receptors with Champix

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Obesity is a growing epidemic worldwide. It is estimated that the annual costs arising from obesity-related illnesses exceed $56.6 billion dollars in Australia alone, with 80% of the Australian population predicted to be overweight and obese by 2025. Sugar directly contributes to a significant amount of weight gain that leads to obesity. Sugar is as addictive as alcohol and nicotine and our lab has recently shown that it directly targets brain nicotinic receptors. We have shown that sugar modulates neuronal nicotinic receptors in the reward centre of the brain in exactly the same pattern as we have shown for nicotine and alcohol. The main goals of our research are to translate this finding into novel treatments for patients. Therefore we went on to show that the FDA approved nicotine cessation medication, Champix™, a modulator of neuronal nicotinic acetylcholine receptors, reduces sugar addiction in animals by targeting the mesolimbic reward pathway of the brain. This is a completely novel approach for the treatment of obesity. As the compound is already FDA approved, we have started planning small scale proof of concept studies in humans. We have also demonstrated the morphological change in neurons at the level of the accumbens as a result of sucrose consumption. Taken together, our results demonstrate a completely novel treatment strategy for weight control associated with sugar addiction but equally importantly highlight the brain reward center as a promising consideration in developing treatment approaches for obesity.

WTH19-10

Anatomy and physiology of the central extended amygdala

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Anxiety is a sustained state of apprehension to distal and potential threat, which can become extremely debilitating in disease states. Anxiety disorders represent the most common of psychiatric disorders, affecting nearly one in four adults in the population. Accumulating evidence suggests that amygdala and extended amygdala, brain regions important for emotional processing, have a central role in anxiety. In particular, central nucleus of the amygdala (CeA) and its forebrain target the central sublenticular extended amygdala (SLEAc), two key parts of central extended amygdala, have been identified as critical elements of anxiety processing. We have studied the neuronal types and synaptic connections within this anxiety circuit using tract tracing and optogenetics. Injection of retrograde beads or retrograde lenti-virus into the SLEAc in C57BL6 mice revealed that the most substantial projections from the CeA to SLEAc originate from the lateral division of the CeA (CeL). 78% of the retrogradely-labelled cells in CeL were somatostatin-positive (SOM+) neurons. To test the properties of this CeL to SLEAc connection, an adeno-associated virus (AAV) expressing Channelrhodopsin-2 (ChR2) was injected in the CeL and whole-cell recordings were obtained in the SLEAc. Stimulation of CeL input evoked GABAergic synaptic currents in SLEAc neurons. To test reciprocal connections, ChR2 was expressed in SLEAc neurons. After AAV transduction of the SLEAc with ChR2, we found SLEAc sent feedback projections to CeL, which was mainly GABAergic, as revealed by the currents induced by light reversing at around −70 mV and blocked by picrotoxin. These results show the CeL SOM+ neurons send a robust inhibitory projection to the SLEAc and the CeL receives a reciprocal inhibitory connection. In future studies we will determine the types of neurons in the SLEAc projecting to the CeA and the physiological role of these bidirectional connections.

WTH19-11

The essential role of baf53b in learning-related synaptic plasticity and long-term fear memory formation in the lateral amygdala

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Epigenetic regulation of gene expression has been implicated in long-lasting memory formation. However, little is known about the role of chromatin remodeling mechanism in learning and memory. Recent study reports that BAF53b, a post-mitotic neuron-specific subunit of Brg/Brm-associated factor (BAF) chromatin remodeling complex is involved in hippocampus-dependent memory formation. However, whether BAF53b plays a role for long-term fear memory formation in lateral amygdala (LA), and its underlying mechanisms remain unknown. To address these issues, we used viral vectors to manipulate expression level of BAF53b specifically in LA and investigated its effects on long-term memory formation and synaptic structural plasticity using auditory fear conditioning paradigm. First, we found that BAF53b knockdown in LA neurons impaired long-term, but not short-term, fear memory formation, proving that BAF53b function in LA is essential for long-term fear memory formation. Second, transient BAF53b overexpression in LA enhanced long-term memory up to 1 month with no effect on short-term memory. Third, at the synapse level, confocal imaging of synaptic spines, visualized with GFP or dye injection in LA after fear learning revealed that the density of thin-type spine was specifically increased in BAF53b-overexpressing neurons one hour after learning, while head volume of mushroom-type spine in BAF53b knockdown neurons was significantly decreased compared to that in control neurons 24 hours after learning. In parallel to this result, our RT-PCR analysis showed that BAF53b knockdown blocked activity-dependent upregulation of miR132, which is known to be involved in regulation of activity-dependent spine enlargement. Taken together, our results demonstrate that BAF53b in LA is essential for long-term fear memory formation of auditory fear conditioning and reveal novel underlying mechanisms of BAF53b during long-lasting memory formation.

WTH20 Neuroengineering

WTH20-01

Optimizing CNS-delivery by lactyl stearate-coupled liposomes

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Brain drug targeting brings a healthy skepticism to the study of the BBB, which is the most frustrating obstacle for pharmacologists wishing to find treatments for brain disorders. The BBB restricts the brain uptake of many valuable hydrophilic drugs and limits their efficacy in the treatment of brain diseases because of the presence of tight junctions, high metabolic capacity, low pinocytic vesicular traffic and efficient efflux mechanisms. Meningitis is the inflammation of tissues which covers brain & spinal cord. The drug of choice is rifampicin which is highly lipophilic in nature. Thus lactyl stearate (LS) coupled liposomes bearing rifampicin were developed. LS was synthesized from stearic acid and lactic acid. LS coupled and uncoupled liposomes bearing rifampicin were prepared by Lipid cast film method. Formulations were characterized for vesicle shape, average vesicle size, drug entrapment efficiency, *in-vitro* drug release and *in-vivo* studies. The quantitative uptake of the formulations by the brain in albino rats was assessed by fluorescent microscopy. Brain uptake was increased about 2–3 times and accumulation was increased about 6–8 times with coupled liposomes in comparison to uncoupled liposomes and about 10–12 times higher compared to plain drug solution. Higher uptake of coupled liposomes can be explained as, that mono carboxylic acid transporters present on brain endothelial cells cross the BBB through carrier mediated transport mechanism. Fluorescence study clearly indicates that the preparation is crossing the basal carotid system and accessing to the nervous system. 6-CF was distributed in blood vessels and accumulated in cerebellum and cerebrum. This delivery system not only increased the brain uptake of the drug but it also reduces the administered dose and toxic effect of the drug. Hence it proves great potential in the delivery of the drug into brain for the treatment of the diseases associated with the brain where very limited drug are available for those diseases. Thus, Lactyl stearate coupled liposomes effectively delivers the drug to the brain and has great potential for brain targeting.

WTH20-02

The braincubator as a system to extend the viability of acute brain slices

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The lifespan of an acute brain slice is approximately 6–12 hours, limiting potential experimentation time. We have designed a new recovery incubation system capable of extending their lifespan to more than 36 hours. This system controls the temperature of the incubated artificial cerebral spinal fluid (aCSF) while continuously passing the fluid through a UVC filtration system and simultaneously monitoring temperature and pH. The combination of controlled temperature and UVC filtering maintains bacteria levels in the lag phase and leads to the dramatic extension of the brain slice lifespan. Brain slice viability was validated through electrophysiological recordings as well as live/dead cell assays. This system benefits researchers by monitoring incubation conditions and standardizing this artificial environment. It further provides viable tissue for two experimental days, reducing the time spent preparing brain slices and the number of animals required for research.

WTH20-03

Ultra-structural imaging in cleared mouse cns using nanoparticles

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Recent advances in tissue clearing methods such as CLARITY, whereby whole organs become optically transparent and permeable to macromolecules, have allowed us to visualise structural and functional relationships at the cellular level using traditional fluorescent antibodies, without the necessity of multiple slicing, staining, imaging and subsequent 3D digital reconstruction.

Although these methods have allowed us to visualise with higher resolution smaller, non-neuronal structures of the mouse brain, photo-bleaching and limited range of available emission wavelengths of organic-dye fluorophores, has limited our ability to multiplex using traditional immuno-fluorescent techniques.

Functionalized nanoparticles such as Nanodiamonds, Nanorubies and SuperDots™ are specialised inorganic particles (with tunable monodisperse sizes, such as 10 nm, 15 nm, 20 nm, up to a few microns) coated with a bio-molecule such as protein, that can then bind to specific targets within tissue and be visualised using scanning microscopy or fibre optics. The benefit of these nanoparticles in a biological setting include; enhanced luminescence signal, reduced background and are more stable than traditional organic-fluoro dyes. Furthermore, the broad emission spectra and lifetime barcodes of SuperDots™, which allows excitation of multiple SuperDot™ populations at a single wavelength at either 800 nm or 980 nm, far removed from their respective emissions, allows for multiplexing of up to 10 targets simultaneously. However, fluorescence labelling using nanoparticles has yet to be demonstrated in a biological system such as cleared mouse CNS tissue.

We aim to develop a technique allowing high resolution fluorescence imaging of proteins in cleared mouse brain and spinal cord tissue, using bare and functionalized Nanodiamonds, Nanorubies and SuperDots™ and visualised with specialised microscopy developed through the ARC Centre of Excellence for Nanoscale BioPhotonics.

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