JNC
The Official Journal of the International Society for Neurochemistry

Journal of Neurochemistry

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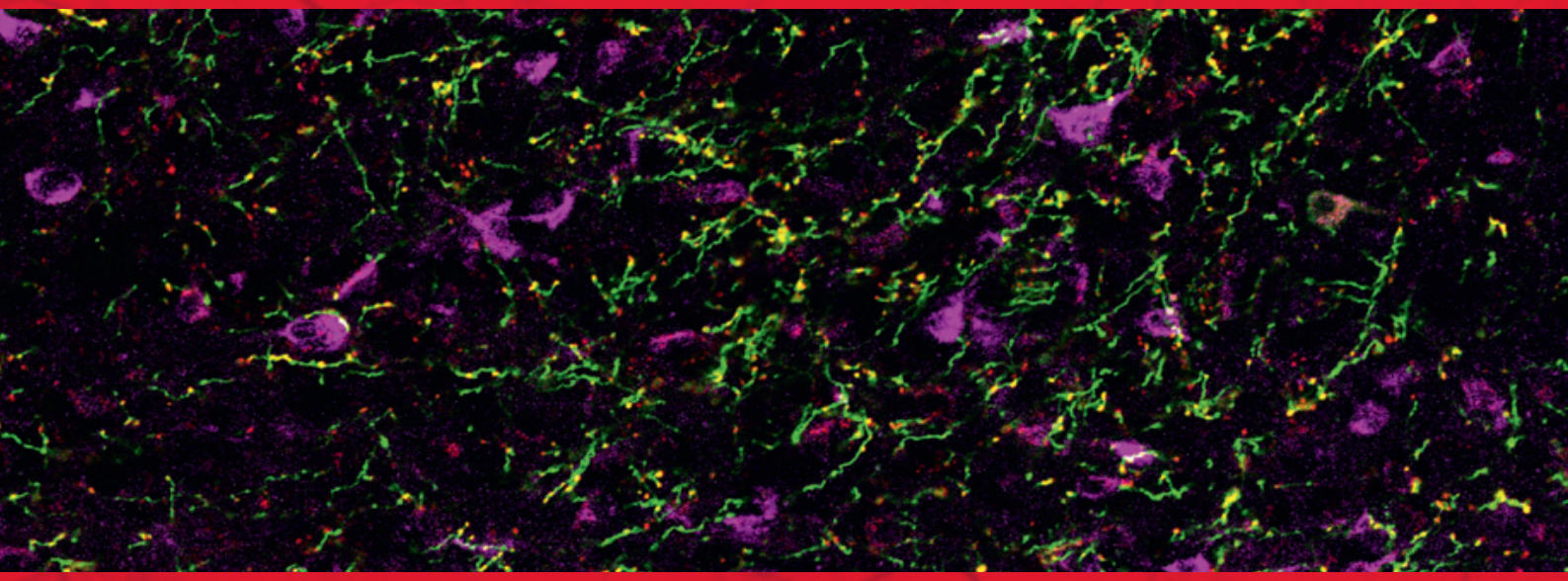

ISN-ESN 2017 Meeting
Paris, France
20–24th August 2017

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Journal of Neurochemistry

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Aims & Scope

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

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Front cover

Medial preoptic neurons projecting to the ventral tegmental area (magenta) receive input from the dorsal BNST (green/red/yellow). Cholera toxin B was injected into the VTA and AAV-hSYN-FLEX-mGFP-synaptophysin-mRuby was delivered into the dorsal BNST of RXFP3-Cre mice for retrograde and anterograde labelling respectively. Sections were prepared and photographed by Sarah Ch'ng using a Zeiss Axio Observer LSM 780 confocal microscope.

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Journal of Neurochemistry (2017), **142** (Suppl. 1), 5–9 doi: 10.1111/jnc.14086

Plenary Lectures

PL01 Plenary Lecture 1 – Professor Reinhard Jahn

PL01

Exocytosis of synaptic vesicles – lessons from biochemistry and functional reconstitution

R. Jahn

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Neurotransmitter release from presynaptic nerve endings is mediated by Ca^{2+}-dependent exocytosis of synaptic vesicles. Exocytotic fusion between the vesicle membrane and the presynaptic plasma membrane is carried out by the SNARE proteins synaptobrevin/VAMP, syntaxin 1, and SNAP-25. Upon membrane contact, the three SNARE proteins assemble into a complex that consists of a helical bundle. Complex formation proceeds from the N-terminal end towards the C-terminal membrane anchors, thus pulling the membranes together and initiating fusion ('zipper' hypothesis of SNARE function). The steps of SNARE assembly are controlled both by members of conserved protein families such as the SM- and CATCHR-proteins that include the synaptic proteins Munc18 and Munc13, and they are tightly controlled by specialist proteins responsible for calcium regulation such as the calcium sensor synaptotagmin and complexins.

In our own work, we have focused on understanding the mechanisms of SNARE assembly and SNARE-induced fusion using structural and biochemical approaches and in-vitro fusion reactions with native and artificial membranes. Our recent results lend strong support to the zipper hypothesis, showing that during SNARE complex formation the helical bundle extends into the membrane and that only few SNARE complexes may suffice for effective fusion of bilayers. Furthermore, we have studied intermediate states of the SNARE-dependent fusion pathway. In addition, we have investigated how regulatory proteins such as the SM-protein Munc-18 and the calcium sensor synaptotagmin 1 interact with SNAREs and how they affect their reactivity. Our results lend support to the view that fusion is driven by the energy liberated during SNARE zippering. Moreover, they indicate that there are several energy barriers in the fusion pathway that the SNAREs are well suited to overcome.

PL02 Plenary Lecture 2 – Professor Maiken Nedergaard

PL02

The nightlife of astrocytes

M. Nedergaard

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

We have recently described a macroscopic pathway in the central nervous system – the glymphatic system that facilitates the clearance of interstitial waste products from neuronal metabolism. Glymphatic clearance of macromolecules is driven by cerebrospinal fluid (CSF) that flows in along para-arterial spaces and through the brain parenchyma via support from astroglial aquaporin-4 water channels. The glymphatic circulation constitutes a complete anatomical pathway; para-arterial CSF exchanges with the interstitial fluid, solutes collect along para-venous spaces, then drain into the vessels of the lymphatic system for ultimate excretion from the kidney or degradation in the liver. The glymphatic system is only active during sleep. As such, this circulation represents a novel and unexplored pathway for understanding the biological necessity for sleep.

PL03 Plenary Lecture 3 – Professor Tamas Horvath

PL03

Hypothalamic AGRP neurons in control of brain development and adult function

T. Horvath

Yale University, Comparative Medicine, New Haven, USA

A small set of neurons in the hypothalamus, which produce Agouti-related peptide (AgRP), acts as master regulator of energy utilization by all tissues, and hence, these hypothalamic neurons are in a unique position to coordinate complex behaviors with appropriate peripheral tissue functions. These neurons are located outside of the blood-brain barrier and respond to multitude of peripheral signals by internal mitochondrial plasticity affecting their afferents and efferents. AgRP neurons in the adult are indispensable for eating and survival, and, they directly and forcefully affect higher brain regions, including the pre-frontal cortex, during development as well as in adulthood. Recent advancements in our understanding of these and other hypothalamic neurons provide novel insights for cellular biological- and neuronal circuit adaptations in support of healthy and diseased brain and peripheral tissue functions.

PL04 Plenary Lecture 4 – Professor Yoshio Hirabayashi

PL04

Glia-neuron interaction: at the crossroad of sugar, lipids and amino acids

Y. Hirabayashi

Riken BSI, Molecular Membrane Neuroscience, Wako, Japan

Lipid glycosylation is a highly conserved system in living organisms, indicating it's critical role in life. It is well established that glycolipids, such as glycoshingolipids (GS), are important components of membrane microdomains/lipids rafts and are highly enriched in brain tissue. GS metabolism is literally at the crossroad of the key cellular nutrients, namely carbohydrates, lipids and amino acids, as the sphingoid base backbone biosynthesis of GS is based on the condensation of L-serine with acyl-CoA. Carbohydrates do not only form the head group of GS, but also cerebral glucose metabolism is strongly linked to L-serine levels. Although L-serine is classified as a non-essential amino acid, neuronal L-serine strongly depends on glial supply and is essential for neuronal development and survival (1).

Great improvements in mass spectrometry significantly fueled lipidomics advances during the last decade. It not only revealed the enormous complexity of brain lipids but also enabled the discovery of novel glycolipid classes in CNS tissues, such as glucosylated sterols (2) and glucosylated phosphatidic acid (PtdGlc) (3). PtdGlc for example is exclusively expressed in developing glia cells but not neurons. It forms distinct lipid domains on the plasma membrane and its metabolite lysoPtdGlc, produce by PLA2, is released into the extracellular matrix from radial glia cells during neuronal development. lysoPtdGlc is detected by its specific receptor GPR55 on extending nociceptive axons of dorsal root ganglion neurons and repels neuronal extension. Function blocking anti-lysoPtdGlc antibodies and GPR55-knock out mice confirmed the in vivo role of this lyso-glycolipid mediated glial-neuron signaling axis in the spinal cord (3). The significance of this emerging lipid mediated glia-neuron interaction and its pathophysiological role will be discussed.

References:

1) Esaki *et al.* (2015) "L-Serine deficiency elicits intracellular accumulation of cytotoxic deoxysphingolipids and lipid body formation" doi: 10.1074.
2) Akiyama H, *et al.* (2016) "Aglycon diversity of brain sterylglucosides: structure determination of cholesteryl- and sitosterylglucoside" doi: 10.1194.
3) Guy AT, *et al.* (2015) "Glycerophospholipid regulation of modality-specific sensory axon guidance in the spinal cord" doi: 10.1126.

Acknowledgments:

Riken Integrated Lipidology Program, Program of Lipid AMED-CREST.

PL05 Plenary Lecture 5 – Professor Giovanna Malluci

PL05

Neurodegeneration: from molecules to medicines

G. Mallucci

University of Cambridge, Clinical Neurosciences, Cambridge, UK

This talk will cover our recent progress in understanding mechanisms of neurodegeneration and how this is informing new therapeutic approaches. The central concept is the identification of common pathways across the spectrum of these disorders (which include Alzheimer's and related diseases) that are relevant for both mechanistic insights and therapy. These include both 'toxic' processes that can be targeted to prevent neuronal death, and regenerative processes that can be harnessed for repair. I will discuss our data in mouse models targeting both of these aspects to prevent neurodegeneration and their relevance for human disease. I will focus on modulating the Unfolded Protein Response pharmacologically and the recent discovery of repurposed drugs ready for clinical trials. I will also discuss the strategy of harnessing pathways that drive synapse regeneration, as occurs in hibernation, for the therapy of neurodegeneration.

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ESN Bachelard Lecture

EBL01 ESN Bachelard Lecture – Professor Ferdinand Hucho

EBL01-01

The neurochemistry of pain – what limits the contribution of biochemistry to sensory perception?

F. Hucho

Free University Berlin, Institute of Chemistry & Biochemistry, Berlin, Germany

Receptor > signal transduction > signal conduction to the CNS > signal perception: this is the standard sequence of events in sensory perception. Do we understand this sequence in the case of pain? An overview will be given summarizing the state-of-the art of pain research on the molecular level. Emphasis will be focussed on the problem of the conversion of *nociceptive* pain into *neuropathic* pain, the former being termed 'Good Pain', the latter 'Bad Pain'. *Good* and *bad* are no biochemical terms. But then what can we biochemists contribute to its understanding? A summary will be given listing the open questions and the tasks for future research.

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Young Scientist Lectures

YSLA01 ISN Young Scientist Lecture 01 – Xinglong Wang

YSLA01

Targeting TDP-43 in mitochondria to treat neurodegenerative diseases

X. Wang

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Dominant missense mutations in TAR DNA-binding protein 43 (TDP-43) cause amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), and the cytoplasmic accumulation of TDP-43 represents a pathological hallmark in ALS, FTD and Alzheimer's disease (AD). We have found that accumulated cytoplasmic TDP-43 in degenerating neurons of patients with ALS, FTD or AD mainly resides inside of mitochondria. Within mitochondria, TDP-43 preferentially bind mitochondria-transcribed messenger RNAs (mRNAs) encoding respiratory complex I subunit ND3 and ND6, impair their expression and specifically cause complex I disassembly. Based on identified motifs critical for TDP-43 mitochondrial localization, we have synthesized a competitive inhibitory peptide that can prevent the accumulation of TDP-43 in mitochondria and abolish TDP-43-induced mitochondrial dysfunction and neuronal loss. Excitingly, suppression of TDP-43 mitochondrial localization by this synthetic inhibitory is sufficient to prevent and even reverse ALS or FTD-related phenotypes in TDP-43 transgenic mouse models after symptom onset. Thus, our study suggests mitochondrial TDP-43 as a promising novel therapeutic target for TDP-43-linked neurodegenerative diseases.

YSLA02 ESN Young Scientist Lecture 01 – Clévio Nobrega

YSLA02

Machado-Joseph disease: from pathogenic mechanisms to therapeutic strategies

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Machado-Joseph disease (MJD), or spinocerebellar ataxia type 3 (SCA3), is a dominant neurodegenerative disorder of adult onset, associated with an abnormal expansion of a CAG tract in the coding region of the MJD1/ATXN3 gene. MJD1/ATXN3 encodes ataxin-3, a protein of still unknown biologic function. Like the others polyglutamine disorders, MJD is fatal, with no therapeutic option available to stop or delay disease progression.

Despite important progresses that helped generate genetic models of the disease, the pathogenic mechanisms involved in MJD are still largely unknown. Several questions remain elusive for researchers: (i) are the intranuclear aggregates, which are a hallmark of the disease, protective or deleterious to cells?; (ii) why only selected regions of MJD patient's brain seem to be affected, despite ataxin-3 being ubiquitously expressed?

In the last years, our group and others have identified several molecular mechanisms and cellular pathways implicated in MJD pathogenesis. Dysfunctions in autophagy, proteolysis or posttranslational mechanisms were identified as relevant in the neurodegenerative process. All these mechanisms provide a new view on the high complexity of polyglutamine disease pathogenesis, having contributing to a better understanding of MJD and allowing the identification of new molecular targets for therapeutic intervention.

The current presentation will provide a brief overview of different mechanisms involved in MJD pathogenesis – including autophagy, translation, proteolysis and post-translational modifications – and focus on rational therapeutic strategies for MJD that target those systems.

YSLA03 ISN Young Scientist Lecture 02 – Patricia Garcez

YSLA03

Cellular and molecular mechanisms of primary microcephaly

P. Garcez[1], E. Loiola[2], J. Minardi[2], R. Madeiro[2], P. Trindade[2], S. Rehen[2]

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Reports of Primary Microcephaly have increased significantly associated with Zika virus infection. Recently, the causality between the Zika (ZIKV) viral epidemic and brain malformations was confirmed using *in vitro* cerebral organoids as a model to study microcephaly as well as *in vivo* models of vertical transmission. We examine the effects of ZIKV infection in human neural stem cells growing as neurospheres and brain organoids. Using immunocytochemistry and electron microscopy, we show that ZIKV targets human neural stem cells, reducing their viability and growth as neurospheres and brain organoids. Using proteomics and mRNA transcriptional profiling, we found more than 500 proteins and genes differentially expressed in the infected tissue. These genes and proteins provide an interactome map and suggest that ZIKV impairs in the molecular pathways underpinning cell cycle and neuronal differentiation. These results point to biological mechanisms implicated in brain malformation, which are important to further the understanding of this congenital syndrome.

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Symposia
S01 History of Neurochemistry

S01-01

Early neurochemists in the United States of America

A. Boullerne

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It was not until after World War II that neurochemistry emerged as a distinct discipline in the United States with new technologies – chromatography; electrophoresis; electron microscopy; subcellular fractionation; lipid extraction; immuno-cytochemistry; mass spectrometry; nuclear magnetic resonance; autoradiography; X-ray crystallography. Other essential developments in the 1950s were significant funding, training young investigators, and emergence of neurochemical societies. Eugene Roberts (Russian) discovered in 1950 gamma-Aminobutyric acid, main inhibitory CNS neurotransmitter, and organized in 1959 one the first neurochemical symposia. Among the International Society for Neurochemistry (ISN) founders, Alfred Pope (McLean Hospital, Massachusetts) developed chemical testing of very small human brain samples in 1952 that led to therapeutic administration of anticholinesterases in dementia. The American Society for Neurochemistry (ASN) was founded by three pioneers: Jordi Folch-Pi, Donald Tower and Wallace Tourtellotte. Tower (NIH) showed neurotransmitter abnormalities in epilepsy, and in 1952 compared the brains of great whales and elephant. ASN held its first meeting in spring 1970, and in 1972 sponsored the first *Basic Neurochemistry* textbook under the aegis of Bernard Agranoff, who elucidated enzymatic synthesis of crucial signal transduction inositol lipids, and pioneered studies in memory and neuroplasticity. Folch-Pi (Spanish) enjoyed a quite extraordinary career. In 1944 he headed research at McLean Hospital, and in 1956 became the first Professor of Neurochemistry in Harvard. He discovered many lipids and was one of the most influential leaders, editor of Journal of Neurochemistry and founding member of both ISN and ASN. Folch-Pi's charismatic personality contributed to formal recognition of these organizations and to neurochemistry standards of excellence. Folch-Pi, together with the first neurochemist woman Marjorie Lees, purified in 1951 myelin protein Proteolipid and developed in 1957 a method for brain lipid extraction still used worldwide. In 1964 Elizabeth Roboz-Einstein (Stanford University) and Marian Kies (NIH), both active in ASN, purified Myelin Basic Protein. In 1971 Earl Sutherland won the Nobel prize for cyclic AMP as second messenger. In 1973 William Norton (Albert Einstein College) devised a sucrose gradient to purify myelin, and Richard Quarles (NIH) identified Myelin-Associated Glycoprotein (MAG) with radioactive fucose. The United States has proved a formidable force driving neurochemistry and hopefully will continue.

S01-02

Celebrating neurochemistry: the anniversary years 1956–1976 and the influence of UK neurochemists

A. Turner

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The period 1956–1976 spanning the first publication of Journal of Neurochemistry (JNC) and the foundations of the International and European Societies for Neurochemistry (ISN, ESN) was a golden age for the development of neurochemistry and its global dissemination with notable contributions from UK neurochemists in this period. Henry McIlwain, with his pioneering work on cerebral metabolism, established a neurochemistry focus at the Institute of Psychiatry in London from which emerged the first comprehensive neurochemistry textbook (*Biochemistry and the Central Nervous System*, 1955). He was involved in organising the first international neurochemical symposia in the 1950s together with Derek Richter and others, which, in turn, led to the formation of ISN and its first meetings. Richter, a leader in the catecholamine and monamine oxidase fields alongside Hermann Blaschko, was encouraged by another neurochemical pioneer, Marthe Vogt, to establish a journal focusing on neurochemistry (JNC). McIlwain published the first ever paper in JNC (Thomas & McIlwain (1956) *JNC* **1**, 1–7) and Richter (ISN President 69–71) became the first, and longest-serving, "Eastern Hemisphere" Chief Editor followed by a succession of UK chief editors up to 2011. While McIlwain introduced the tissue slice to neurochemistry, Victor Whittaker in Cambridge developed the synaptosome preparation as *in vitro* neurochemistry rapidly developed in the 60s and 70s, and he served as JNC editor and was also ISN Secretary (83–87). Two of the UK-based JNC Chief Editors, Alan Davison and Herman Bachelard, alongside European colleagues, were instrumental in establishing the ESN and hosting its first biennial meeting integrating basic and clinical neurochemistry in Bath, U.K. in 1976. Subsequent meetings oscillated between Western and Eastern Europe at that time. Given that the establishment of ESN was also, in part, a political project to unite and support European neurochemists during the time of the Cold War, it is now ironic that the UK should have voted in 2016 to leave the European Union but European neurochemistry remains united.

S01-03

Early neurochemists in Europe

P. Foley

Independent scholar, None, Sydney, Australia

Initial investigations of the chemistry of the central nervous system formed part of broader efforts to understand the chemistry of life. Investigators in France and Germany surveyed and cataloged the chemical constituents of brain tissue, essentially viewing nervous tissue chemistry as a subdivision of general biochemistry. Although the term *Nervenchemie* was introduced in 1856 by the Tübingen physiologist Julius Eugen Schlossberger, many prominent pioneers, including Johann Ludwig Wilhelm Thudichum (a German biochemist working in London), rejected attempts to integrate brain chemistry and physiology as overly speculative. At the start of the 20th century, a new approach was motivated by the recognition that acetylcholine and epinephrine were employed by peripheral nerve cells as messengers. Neuroanatomists Cécile and Oskar Vogt in Berlin further proposed that understanding localized brain function required knowledge not only of neural architecture, but also of local chemical features; in Munich, pathologist Hugo Spatz nominated such a feature (iron concentration) as the basis for defining the

extrapyramidal motor system. The first department specifically devoted to neurochemistry was established at the *Kaiser-Wilhelm-Institut für Psychiatrie* in Munich in 1928 by the American Irvine Page, who regretted that such a laboratory was not yet possible at home. Chemical transmission in brain tissue proved more difficult to study than in the periphery, but considerable progress had been achieved by the end of the Second World War, including work by Peter Holtz in Rostock and Ulf von Euler in Stockholm. European researchers continued to make key contributions during the new era of 'neurochemistry' that began with the introduction of the term in the United States in 1944, and Europeans from various fields were instrumental in the founding of the Journal of Neurochemistry in 1956, a highly significant development in the establishment of neurochemistry as a distinct field of laboratory and clinical research, and of the International Society for Neurochemistry in 1963, the first three meetings of which were held in Europe. The European Society for Neurochemistry was established in 1975, and its first meeting hosted by Bath the following year.

S01-04

Early neurochemists in the Asia-Pacific

G. Johnston

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Neurochemistry in Asia can be traced back to the late 1920s in Japan when psychiatrists began chemical analyses of autopsied brain tissues for lipids and carbohydrates looking for chemical disorders in the patients with mental diseases. Western neurochemistry had progressed considerably by this time and Japanese researchers 'eagerly sought enlightenment from researchers in the Western tradition of neuroscience" (Tsukada, 1987). A number of 'research teams' were formed in Japan targeting neurochemistry that led eventually to the foundation of the first neurochemistry society in the world.

The Japanese Neurochemical Society was founded in 1958 out a series of "Symposium of Neurochemistry", formally becoming a learned society in 1962 under the leadership of Yasuzo Tsukada, predating the founding of the ISN in 1967. JSN is the largest neurochemical society in terms of membership.

The Society for Neurochemistry, India was created in 1979 in Hyderabad. Japan and India are the only countries in the Asia-Pacific that have separate societies for neurochemistry. In other countries neurochemistry usually incorporated into neuroscience societies. Thus for example Australia and New Zealand have the Australasian Neuroscience Society and Singapore has the Singapore Neuroscience Association. Neurochemistry in the region received a boost by the formation of the Asian Pacific Society for Neurochemistry at the Sydney meeting of the ISN in 1991. It is modelled on the American and European regional neurochemistry societies, holding meetings every 2 years in the years when an ISN meeting is not held. Membership of the APSN is open to individual scientists, scientific societies with a significant interest in neurochemistry and to corporations based in the Asian Pacific region.

The Asia-Pacific region holds special challenges given the extreme breadth of cultural, economic and scientific diversity in the region. Countries already involved with the APSN include Australia, China – Beijing, China – Taipei, Hong Kong, India, Japan, Korea, Malaysia, New Zealand, Philippines, Singapore, and Thailand.

Tsukada Y, A long way from the wilderness: thirty memorable years of the Japanese Neurochemical Society, *Neurochemical Research,* 1987, 12, 759–765.

S01-05

Early ISN officers, councillors and the journal of neurochemistry

P. Beart

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ISN was established in the UK (1967), although previously attention was given to neurochemical themes at national and international meetings of biochemists, neurologists and psychiatrists on both sides of the Atlantic and in Japan. A meeting in Oxford (1952) was the first truly international neurochemical forum. Key players in the founding of ISN were Jordi-Folch-Pi, Henry McIlwain, Derek Richter and Heinrich Waelsch. Roger Rossiter, first ISN Chairman, was a true international – an Australian, Oxford trained, who became Professor of Biochemistry, University of Western Ontario, Canada. The term President was adopted in 1999 and there have been 27 Presidents. The early formation and recruitment of ISN members was not without controversy, given its early base was strongly biochemical – apparently there was initial opposition to inclusion of other scientists and clinicians with active interests in neurochemistry. ISN Council also has misgivings about formation of regional societies, even though the ASN (created 1969) was flourishing, and ESN was only founded in 1976 and APSN much later (1991). Financial support of regional societies was formalized in 2007 and a professional Secretariat was established as late as 2008. Elisabeth Bock was the 1st female ISN President (1995–7) and over the last decade women have increasingly played roles in ISN. The Journal of Neurochemistry (JNC, 1956) pre-dates ISN, although early Chief Editors and members of Editorial Board were active in early years of ISN. Marthe Vogt played an instrumental role in founding of JNC, which was initially published by Permagon Press (1st Chief Editors, Derek Richter and Heinrich Waelsch). ISN took possession of JNC in 1970 and derived little financial benefit until the Raven Press (1980) took over its publication, with Blackwell who became Wiley being publishers since 2001. There have been 18 distinguished Chief Editors of JNC, with recent changes including around one-third of handling editors being female, move to a single editorial office (2013) and on-line publication (2014). Income from JNC has ensured financial stability of ISN and allowed growth of its international programmes.

S02 The actions of sugar and fat on the brain reward system: Implications for food addiction

S02-01

Neuroimmune basis of dietary-induced palatable food cravings

S. Fulton

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Metabolic dysfunction elicited by excessive fat intake can contribute to anhedonia and mood impairments. Increased craving of palatable, energy-rich food is a common concurrent feature that can serve to offset the negative emotional state. Results will be presented showing that long-term consumption of diets enriched with saturated lipids facilitate compulsive sucrose seeking in mice in a manner that relies on neuroinflammatory processes. On the other hand, unsaturated lipids can have acute actions to inhibit motivation for palatable food, in part via direct actions on the mesolimbic dopamine system. These findings are discussed in the context of the neuropsychiatric consequences of diet-induced obesity and potential treatment strategies.

S02-02

Circuit logic for sugar sensing

I.D. Araujo

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Sugars exert their potent reinforcing effects by activating both gustatory and post-ingestive sensing pathways. However, because nutritional content reinforces behavior independently of taste, distinct reward circuitries must exist to compute taste and nutrient values separately. We have established that two distinct portions of striatum, ventral vs. dorsal, respectively, mediate the gustatory and nutritional values of sugar. This wiring logic allows animals to prioritize nutritional value over taste quality. Our current studies support a model in which metabolic cues act on dopamine-excitable neurons in dorsal striatum, which in turn release brainstem oral-motor centers from tonic inhibition by basal ganglia output stations. Our model thus implicates a descending pathway from ventral midbrain to the premotor reticular formation that links nutrient sensing in basal ganglia to the execution of feeding motor programs.

S02-03

Triglycerides sensing in the mesolimbic system and the regulation of food reward

S. Luquet[1], C. Berland[1, 3], C. Cansell[1], Y. Nakamura[4, 5], G. Gangarossa[1], J. Castel[1], F. Darlot[6, 7], M. Cador[6, 7], S. Caille[6, 7], M.C. Burke[1], X. Sun[4, 5], F. Marti[8], S. Tolu[8], P. Faure[8], M. Tschöp[2, 3], T. Hnasko[9], D.M. Small[4, 5]

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Circulating triglycerides (TGs) normally increase after a meal but are altered in pathophysiological conditions, such as obesity in both human and rodent. Several brain structures express enzymes that process TG enriched particles, including mesolimbic structures suggesting that TGs might directly act as a reward signal in both rodent & humans.

Using brain TG delivery in rodent we found that small amounts of TG rapidly reduced both spontaneous and amphetamine-induced locomotion, abolished preference for palatable food and reduced the motivation to engage in food-seeking behavior. Conversely, targeted disruption of the TG-hydrolyzing enzyme lipoprotein-lipase (Lpl) in various pre and post synaptic structure of the dopamine circuitry revealed that Lpl-mediated sensing of TG regulate reward-seeking behavior. Using fMRI in human we found that TG excursion in response to a meal induced changes in brain response to food versus nonfood cues in genetic context of the TaqIA polymorphism, a mutation known to affect D2R signaling and susceptibility to addiction in human. Among these the ventromedial prefrontal cortex (vmPFC) presented a correlation with plasma TG that was independent of other energy-related signals. Collectively, these findings reveal new mechanisms by which dietary TG may alter mesolimbic circuit function and reward seeking behavior.

S02-04

High fat/high sugar foods, diet-induced obesity and glutamatergic plasticity

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There is increasing evidence that the pathological overeating of high fat/high sugar foods which often underlies obesity is compulsive in nature and therefore contains elements of an addictive disorder. However, direct physiological evidence linking exposure to high fat/high sugar foods with synaptic plasticity akin to that occurring in addiction is lacking. Moreover these hallmark synaptic impairments in the glutamatergic input to the nucleus accumbens core have not been examined in the context of obesity.

We have investigated these questions using an outbred model of diet-induced obesity. Sprague-Dawley rats are allowed free access to a palatable diet (moderately high fat/sugar) and then separated by weight gain into diet-induced obese and resistant groups. Unlike rats fed standard chow, a deficit in the ability to form a classical form of synaptic plasticity was found at excitatory synapses in nucleus accumbens core brain slices prepared from these animals regardless of their propensity for weight gain. This LTD deficit was even more pronounced in rats that became obese on the diet. Rats that became obese on the diet also displayed 'addiction-like' behaviour when assessed in operant conditioning and conditioned suppression tasks. Recordings taken from animals that had been through the operant conditioning protocol revealed further changes at glutamatergic synapses in the nucleus accumbens core: increased potentiation at these synapses as measured by the ratio of AMPA/NMDA currents and slower decay of the NMDA current. These hallmark synaptic impairments have been observed in the brains of animals that have self-administered drugs of abuse such as cocaine, nicotine and heroin. Thus, high fat/high sugar foods cause changes in plasticity at excitatory synapses in a key brain reward area and this potentially underlies their addictive potential.

S03 Role of astrocytes in formation and remodeling of synaptic circuits

S03-01

HOMEOSTATIC CONTROL OF DOPAMINE BY ASTROCYTES IN THE POSTNATAL MATURATION OF THE PREFRONTAL CORTEX

Paola Bezzi[1], F. Petrelli[1], L. Pucci[1], G. Dallérac[2], C. Calí[1,3], S. Sultan[1], T. Zehnder[1], C. S. Asensio[4], V. Gundersen[5], N. Toni[1], G. Knott[6], F. Magara[7], F. Kirchhoff[8], N. Déglon[9,10], B. Giros[11,12], R. H. Edwards[4], J. Mothet[2]

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Over the last 20 years, accumulating evidence has shown that astrocytes can influence many aspects of synaptic transmission, network activity, and cognitive functions by controlling the extracellular homeostasis of ions and neurotransmitters. However, whether and how astrocytes participate in regulating the homeostasis of dopamine (DA) has never been investigated in detail. Most interestingly, recent advances indicate that astrocytes also express proteins involved in DA uptake and metabolism such as mitochondrial monoamino oxidase B (MAOB) enzyme [Zhang et al., *Neuron*, 2016] and, importantly, vesicular monoamine transporter 2 (VMAT2) [Romero-Calderon et al., *Plos Gen*, 2008; Zhang et al., *Neuron*, 2016], an integral vesicular membrane protein that directly controls vesicular storage of monoamines in neurons and neurosecretory cells [Edwards et al., *Neuron*, 2007]. Here, we find that a subset of cortical astrocytes is crucial in maintaining an efficient DA homeostasis in the developing prefrontal cortex (PFC) through expression of VMAT2. Astrocytes start to express VMAT2 during the early stage of postnatal development preceding adolescence, i.e. when the establishment of DA connectivity in the PFC occurs. At subcellular level VMAT2 in astrocytes is responsible for sequestering DA in intracellular organelles and, thus, for regulating the amount of cytosolic DA available for metabolism through MAOB activity. By using *in vivo* conditional gene inactivation and viral-mediated gene replacement we also find that extracellular levels of DA in the developing PFC can be potently controlled through modulation of VMAT2 expression in astrocytes. Interestingly, we show that dysfunction of the VMAT2-dependent homeostatic control of DA by astrocytes alters synaptogenesis of pyramidal neurons in PFC and finally, prevents an efficient acquisition of behavioral and cognitive performances.

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S03-02

A role for astrocytes in shaping visual cortex circuits

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Brain information processing is commonly thought to be a neuronal performance. However recent data point to a key role of astrocytes in controlling synapse formation, maturation, activity and elimination. The visual cortex is a hallmark brain region of experience-dependent developmental shaping of synaptic circuits during the critical period of enhanced plasticity that follows eyes opening. Physical removal and/or shaping of synapses implies morphological alterations, which may well be influenced by astroglial perisynaptic processes. Strikingly, we have recently shown that the gap junction protein connexin 30, besides forming channels, regulates synaptic transmission *via* an unconventional non-channel function, by preventing astrocytes to penetrate synaptic clefts. We found that the developmental expression of Cx30 in the visual cortex coincide with the closing of the critical period. We therefore hypothesized that Cx30 regulates the ability of astrocytes to eliminate excess synapses, thereby seizing up experience dependent modeling of cortical networks. We will here present molecular and functional data suggesting that astroglial Cx30 controls maturation and plasticity of visual cortex circuits during critical period of development.

S03-03

Astrocyte regulation of neuronal glutamate receptors

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Astrocytes secrete factors that regulate the formation of excitatory glutamatergic synapses, including factors that increase the number of synaptic AMPA glutamate receptors (AMPARs). The levels of AMPARs at a synapse determine the size of the synaptic response, and the regulated addition and removal of AMPARs at synaptic sites is a molecular mechanism underlying learning and memory. AMPARs are tetramers and there are four subunits, GluA1-4, and functional

receptors are composed of combinations of these e.g. GluA1 homomers or GluA2/3 heteromers. The functional properties of the AMPAR are determined by its subunit composition, for example the presence of the GluA2 subunit makes the channels impermeable to calcium, while GluA1 homomers are calcium permeable. We found that astrocytes secrete factors that increase the surface levels and synaptic accumulation of all AMPAR subunits in neurons by three-fold, strongly regulating neuronal activity. We identified the astrocyte-secreted proteins, glypican 4 and 6 (Gpc4 and 6), as sufficient to increase both the frequency and amplitude of glutamatergic synaptic transmission by recruiting the GluA1 subunit of the AMPAR to synaptic sites. These studies showed that Gpc4 specifically regulates GluA1 AMPARs, and that there are additional unknown astrocyte-derived signals that regulate the trafficking of other AMPAR subunits, i.e. GluA2/3 and GluA4. We have now identified the molecular pathway that astrocyte-secreted glypican 4 regulates in neurons in order to cluster GluA1 AMPARs and induce excitatory synapse formation. In addition, we have identified a novel astrocyte-secreted factor that regulates GluA2 recruitment to synapses and induces synapse maturation. Identifying how astrocytes regulate synaptic AMPARs has important implications for understanding how synaptic strength is normally regulated during development, in learning in the adult, and in disease.

S03-04

Astrocytes control the synaptic integration of neurons generated in the adult hippocampus

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Adult neurogenesis results in the continuous formation of new neurons that integrate into, and remodel the pre-existing network. The question is: "How do new neurons find their appropriate synaptic partners and do astrocytes participate to this process?"

Using viral approaches to birthdate and identify new neurons and controllable transgenic mice to manipulate astrocytes, we identified that astrocytes locally control the synaptic integration of immature neurons. More specifically, vesicular release from astrocytes is required for the production of D-serine, that enables spine formation on new neurons and regulates their survival. These results support a crucial role for astrocytes in one of the most drastic form of adult brain plasticity. Furthermore, since vesicular release from astrocytes is regulated by neuronal activity, this mechanism may enable the integration of new neurons in specific places of the network of increased computational demand. Of gene transfer and microscopy approaches, we examined the role of hippocampal astrocytes in the integration.

S04 How neurons influence oligodendrocyte behavior and their myelination?

S04-01

Neuronal regulation of remyelination

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Myelin is essential for normal brain function, as it enables fast information transmission and trophic support for axons. Its importance is evident in diseases, such as spinal cord injury and multiple sclerosis, where it is lost or damaged, as this leads to mental and/or physical disability. During development, oligodendrocyte precursor cells (OPCs) differentiate into myelinating oligodendrocytes, which myelinate axons. In white matter diseases, adult OPCs rapidly respond to demyelinating lesions and differentiate to replace lost myelin sheaths to recover fast axonal transmission; however, this repair process often fails. To promote endogenous myelin repair it is essential to understand the mechanism that can regulate remyelination.

Glutamate and electrical activity influence OPC differentiation and myelination in normal development. OPCs receive synaptic input from unmyelinated axons, possibly to initiate myelination, and both OPCs and mature oligodendrocytes respond to glutamate via AMPA and NMDA receptors. However, little is known about the role different OPCs' glutamate receptors play in remyelination.

Therefore we examined the role of glutamate signalling in remyelination following experimental demyelination in the adult rodent CNS. We voltage-clamped OPCs in brain-slices of adult rat cerebellar peduncle containing focal areas of primary demyelination and post-identified these by NG2-immunolabelling. OPCs recruited to the demyelinated lesion expressed AMPA/Kainatereceptors at the peak of the OPCs proliferation (5–10 days post-lesion) but lacked NMDA receptor expression at this time point.

The demyelinated axons continued to propagate action potentials, but with latencies similar to those in unmyelinated axons during development. Critically, the demyelinated axons established synapses with OPCs expressing voltage-gated sodium currents. These synaptic inputs had identical decay times to those recorded during developmental myelination. Pharmacological manipulation of neuronal activity or glutamate signalling blocked remyelination by affecting OPCs differentiation.

These findings reveal how neuronal activity and release of glutamate instruct OPCs, via AMPA receptors, to differentiate into new oligodendrocytes that restore myelin and recover lost function.

S04-02

Neuronal activity promotes proliferation of normal and neoplastic glial cells

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Neuronal activity regulates the proliferation and differentiation of myelin-forming precursor cells during development and in adulthood. In the healthy brain, this results in activity-regulated plasticity of myelin microstructure and subsequent modulation of neural circuit function evident in oligodendrogenesis-dependent behavioral changes. The robust mitogenic effect of neuronal activity on normal neural precursor and oligodendroglial precursor cells, the putative cellular origins of high-grade glioma (HGG), suggests that dysregulated or "hijacked" mechanisms of myelin plasticity might similarly promote proliferation in this devastating group of brain cancers. Using *in vivo* and *in situ* optogenetic techniques together with patient-derived high-grade glioma cell cultures and xenograft models, we have demonstrated that active neurons similarly promote proliferation and growth of both pediatric and adult high-grade glioma subtypes. Crucial mechanisms mediating activity-regulated high-grade glioma growth include secretion of Brain Derived Neurotrophic Factor (BDNF) and the synaptic protein neuroligin-3 (NLGN3), which induces PI3K-mTOR pathway activity and feed-forward expression of NLGN3 in glioma cells. Nlgn3 is necessary for the growth of high-grade glioma xenografts in the mouse brain, and *NLGN3* expression levels in human HGG negatively correlate with patient overall survival. Thus, neuronal activity not only modulates the structure and function of the brain's myelinated infrastructure, but active neurons also play an important role in the brain tumor microenvironment, with activity-regulated secretion of NLGN3 emerging as an unexpected mechanism underlying axo-glioma interactions and promoting neuronal activity-regulated cancer growth.

S04-03

Regulation of central myelination by neuronally expressed neurotrophin signals

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Communication between neurons and oligodendrocytes are central to myelin formation in the developing and the adult CNS. This has profound implications for life-long plasticity and learning. However, the molecular cues that instruct myelin wrapping around CNS axons have yet to be identified. Brain-derived neurotrophin factor (BDNF) has been implicated in controlling CNS myelination via its tropomyosin related kinase (Trk) B receptors, however the precise cellular and molecular mechanisms by which BDNF/TrkB signalling regulates CNS myelination remains unclear. TrkB is expressed by both neurons and oligodendrocytes. We have previously identified that BDNF activated oligodendroglial TrkB to exert specific influence upon myelin wrapping. Here we investigated the influence that BDNF/TrkB signalling in neurons exerts upon oligodendroglial cells and myelination via generating a neuronal specific TrkB knockout mouse. Our unpublished data show that neuronal deletion of TrkB exerts a profound influence upon oligodendrocyte development and the initation of myelination in multiple CNS regions *in vivo* throughout development and into adulthood, suggesting that neuronal-expressed TrkB is crucial for myelination during CNS development, revealing a novel role of neuronally-expressed neurotrophin signals in potentiating oligodendrocyte development *in vivo*.

S04-04

THE REGULATION OF CNS MYELINATION BY NEURONAL ACTIVITY

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Increasing evidence from human neuroimaging studies and animal models suggests that neuronal activity can influence myelination in an adaptive manner, potentially allowing for strengthening or synchronization of specific connections and circuits. The degree to which dynamic changes to myelin really serve as a physiologically relevant form of neuroplasticity in the nervous system is unknown, however. In particular, it is not clear whether neural activity can modulate myelination at the level of individual axons, which would presumably be a requirement for adaptive modulation of circuitry. We investigate this by manipulating neural activity in the postnatal mouse brain using a pharmacogenetic approach (the DREADDs). Enhancement of neural activity in a small subset of collosally projecting neurons increased the proliferation and subsequent differentiation of OPCs within the white matter of both the developing and adult CNS, albeit with slower kinetics in the adult. In addition to these relatively broad lineage changes, neural activity resulted in selective changes to the myelination of activated axons, within increased thickness of the myelin surrounding DREADD expressing axons and preferential myelination of these axons by newly formed oligodendrocytes. These results underscore that highly specific changes to myelin are a feasible form of neuroplasticity in the intact adult nervous system.

S05 Probing synapse structure and pathology with advanced imaging

S05-01

Coordinated spine pruning and maturation mediated by inter-spine competition for cadherin/catenin complexes

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Dendritic spines are postsynaptic compartments of excitatory synapses that undergo dynamic changes during development, including rapid spinogenesis in early postnatal life and significant pruning during adolescence. Spine pruning defects have been implicated in developmental neurological disorders such as autism, yet much remains to be uncovered regarding its molecular mechanism. Here, we showed that spine pruning and maturation in the mouse somatosensory cortex are coordinated via the cadherin/catenin cell adhesion complex and bidirectionally regulated by sensory experience, i.e. accelerated by environmental enrichment (EE) and blocked by whisker deprivation. We further demonstrated using live imaging in cultured neurons that locally enhancing cadherin/catenin-dependent adhesion or photo-stimulating a contacting channelrhodopsin-expressing axon stabilized the manipulated spine and eliminated its neighbors, an effect requiring cadherin/catenin-dependent adhesion and depending on the inter-spine distance. Importantly, when we overexpressed β-catenin in a subset of presynaptic axons in the mouse somatosensory cortex, thus differentiating the level of cadherin/catenin-dependent adhesion between neighboring spines of the same postsynaptic neuron *in vivo*, we observed enhanced survival of the spine contacting the β-catenin overexpressing axon, at the expense of its β-catenin deprived neighbor. Thus, we demonstrated, both *in vitro* and *in vivo*, that inter-spine competition for cadherin/catenin complexes biased spine fate. Finally, both EE-induced acceleration of spine pruning and spine maturation were abolished in the absence of endogenous β-catenin. Together, these results suggest that activity-induced inter-spine competition for β-catenin provides specificity for concurrent spine maturation and elimination, and thus is critical for the molecular control of spine pruning during neural circuit refinement.

S05-02

Phosphorylation signals in hippocampal and striatal synapses

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Protein phosphorylation is a major post-translational modification in eukaryotic cells that plays a critical role in various cellular processes. More than 500 protein kinases are encoded in the human genome and are classified into 7 major groups based on their catalytic domain sequences. Although several kinases have been well-characterized and demonstrated to be physiologically important, the functions of a number of kinases remain to be elucidated. For example, more than 200 000 human phosphorylation sites are registered in PhosphoSitePlus, a large database of post-translational modifications, but only 8000 sites are linked to their respective kinases. To identify the specific substrates for the specific kinases, we have recently developed an *in vitro* approach termed the kinase-interacting substrate screening (KISS) method (Amano et al. J Cell Biol 2015) and an *in vivo* approach termed kinase-oriented substrate screening (KIOSS) method (Nagai et al. Neuron 2016; Nagai et al. Trend Pharmacol Sci 2016).

Ca-activated calmodulin-dependent protein kinases (CaMKs) play pivotal roles in controlling neurotransmitter release, synaptic plasticity, morphological changes in dendritic spines and transcription of specific genes when Ca influx is induced through ligand-gated or voltage-dependent Ca channels in neurons. Although major efforts have been paid to identify the CaMK substrates to understand their modes of actions, a few of the *in vivo* substrates including synapsin, NR1, GluR1 and Dlg1 have been reported. We here performed comprehensive phosphoproteimic analyses to search for the novel substrates of CaMKs including CaMKII from the hippocampal and striatal slices treated with *N*-methyl-D-aspartate (NMDA) or elevated K^+, and identified more than a hundred putative substrates such as GEF-H1 (RhoGEF). We also found that CaMKII phosphorylated GEF-H1 in response to NMDA, and enhanced its guanine neucleotide exchange activity on RhoA and translocation from the dendritic shafts into the dendritic spines to regulate spine morphologies.

We also constructed an on-line database system, named "KANPHOS (Kinase-Associated Neural PHOspho-Signaling), that provides the phosphorylation signals identified by our methods as well as those previously reported in the literature. We here discuss how we explore phosphorylation signals in synapses by phosphoproteomics approach and KANPHOS.

S05-03

Super-resolution imaging of the interstitial fluid to reveal the nano-anatomical organization of live brain tissue

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All brain cells are surrounded by a narrow extracellular space (ECS), which is filled with interstitial fluid and the extracellular matrix. The ECS is a reticular structure that forms a reservoir for extracellular ions and corridor for nutrients from the blood stream. It is critical for brain homeostasis and metabolite clearance and serves as a communication channel for extrasynaptic volume transmission. Because its spatial structure is extremely dense and convoluted, the ECS has so far defied all attempts at visualization by conventional light microscopy, which makes it appear like a featureless and homogenous mass.

We present a method to directly visualize the ECS and cellular structures in living brain tissue. It is based on 3D-STED microscopy and fluorescent labeling of the interstitial fluid. This new approach, termed 'super-resolution shadow imaging' (SUSHI), allows visualization of dense biological tissue at sub-micron resolution and comes with minimal photobleaching and phototoxicity as inherent benefits. It hinges on the very high resolution of 3D-STED

microscopy, which undercuts the volume resolution of 2-photon microscopy by about three orders of magnitude, breaking the 1 attoliter barrier.

We provide proof-of-concept of this method by applying it to living organotypic hippocampal brain slices. It yields strikingly detailed and rich images of the complex structure of the fluorescently labeled ECS, revealing the anatomical organization of the tissue and all its resident cellular structures in sharp relief. By adding a second color channel, positively labeled cells can be imaged in the context of their neuropil, allowing the anatomical identification of synaptic connections.

In summary, SUSHI represents a new paradigm for nanoscale imaging of dense biological tissue, which complements and extends traditional approaches based on intracellular labeling, potentially bridging the gap between brain connectomics approaches based on serial section electron microscopy and MRI-based diffusion-tensor imaging. As a versatile fluorescence imaging technique, it opens up many experimental opportunities to study the structure and mechanisms of brain ECS.

S05-04

Superresolution imaging of synaptic function in psychiatric disease models

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It is becoming clear that synapses are a major cellular substrates of psychiatric disorders, including autism and schizophrenia, but the underlying mechanisms are only beginning to be uncovered. I will present novel data generated using superresolution imaging approaches, including structured illumination microscopy (SIM), to image the function of psychiatric risk molecules in mouse disease models and human induced pluripotent stem cell (iPSC)-derived neurons.

S06 Mechanisms of cellular stress in neurodegenerative diseases

S06-01

New mechanisms regulating neuronal resilience in neurodegeneration

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Alzheimer's disease (AD), the most common form of dementia, is characterized by accumulation of protein aggregates and progressive cognitive decline. Chaperones and co-chaperones play an important role in regulating neuronal resilience, protein misfolding and aggregation. We have used genetic mouse models to investigate the roles of a critical co-chaperone of the Hsp70/Hsp90 chaperone machinery, Stress-inducible phosphoprotein 1 (STI1, STIP1 or HOP for Hsp-organizing protein), in neuronal resilience in neurodegenerative diseases. STI1 function as a co-chaperone to transfer clients from Hsp70 to Hsp90, but it also works as a scaffold to link Hsp90 to a number of regulatory proteins. STI1 can also be secreted by cells acting as a cytokine to trigger neuronal protection signals via the prion protein/alpha7 nicotinic receptor complex. We find that secreted STI1 can protect neurons in culture by shifting amyloid beta toxic signalling from prion protein/mGluR5 complexes to prion protein/alpha7 nicotinic acetylcholine signal pathways. To further understand the roles of STI1 *in vivo*, we generated several mouse lines targeting this gene. Elimination of STI1 in mice causes embryonic lethality at E10.5, which can be rescued by crossing knockout mice with mice expressing a STI1 BAC transgene. Interestingly, mice with 70% reduction in STI1 levels (STIP1ΔTPR1) survive and this hypomorphic STI1 allele can also rescue STI1 knockout mice. Cells with reduced levels of STI1 are less resilient to stress, whereas increased STI1 levels can improve cellular resilience. We have crossed different STI1 mouse models to an AD mouse model and discovered new roles of extracellular STI1 in amyloid plaques. Our results suggest that both extracellular and intracellular STI1 can regulate multiple aspects of neuronal function and point to STI1 as a potential regulator of amyloid toxicity in Alzheimer's disease.

S06-02

Cholinergic dysfunction may explain the link between obstructive sleep apnea and sporadic Alzheimer's disease

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Neuronal death, leading to overall brain atrophy, is one of the fundamental characteristics of Alzheimer's disease. Epidemiological studies have shown that obstructive sleep apnea (OSA) is a risk factor for Alzheimer's disease. Although the mechanisms are unclear, human studies suggest that intermittent hypoxia rather than disrupted sleep is the cause. We have developed an OSA mouse model in which lesions of cholinergic mesopontine tegmentum (cMPT) neurons result in altered breathing during sleep and intermittent hypoxia. Furthermore, the lesions produce a subsequent and selective degeneration of basal forebrain cholinergic (cBF) neurons, which are characteristically lost in Alzheimer's disease and are vulnerable to ischemia/hypoxia-induced degeneration. Furthermore, in genetically susceptible mice, cMPT lesion results in a decline in spatial memory and exacerbation of pathological features of Alzheimer's disease. Importantly, cBF neuronal degeneration can be rescued by provision of a high oxygen environment during sleep (mimicking standard continuous positive airway pressure therapy for OSA), or by cholinergic-specific genetic deletion of the p75 neurotrophin gene. Cholinergic dysfunction is correlated with, and may precede, amyloid burden in elderly humans, and has been shown to induce and exacerbate amyloid pathology in animal models. Therefore, our findings suggest that intermittent hypoxia-induced cBF neuron dysfunction provides a causal link between obstructive sleep apnea and Alzheimer's disease, which may be targetted by current therapies.

S06-03

Regulation of cell stress responses and the proteostasis network in aging and neurodegenerative disease

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The health of the proteome is orchestrated by the proteostasis network (PN) comprised of molecular chaperones, ubiquitin-dependent proteasomes, and autophagy pathways. The PN responds to metabolic challenge and diverse forms of acute and chronic cell stress conditions to ensure balanced synthesis, function, and degradation in the face of proteotoxic stress and aging. To achieve an understanding of the biology of proteostasis, we have examined the properties of the PN throughout the lifespan of *C. elegans*. The transition from development through reproduction into adulthood and aging places a tremendous demand on quality control processes and protein biogenesis. To address this, we have examined how HSF-1, the stress responsive transcription factor of the heat shock response (HSR), regulates the functional properties of the PN in different tissues. In early development HSF-1 is essential and co-regulates target gene expression together with the cell cycle factor E2F, to control anabolic growth and suppress degradation pathways. Upon entry into adulthood, the HSR is blunted at reproductive maturity by signals from the germ line stem cells that downregulates the jumonji demethylase resulting in corresponding epigenetic changes in H3K27me3 occupancy at stress gene loci, repressing the HSF-1 binding that leads to the collapse of proteostasis. These signals, however, can be reversed such that overexpression of the jumonji demethylase or inhibition of germ line signaling prevents proteostasis collapse in aging to prevent protein aggregation. These observations lead us to propose that the regulation of molecular chaperones and other components of the PN by cell stress response pathways has profound consequences on the risk for misfolding and aggregation as occurs in neurodegenerative diseases.

S06-04

Molecular connections between Alzheimer's disease and type 2 diabetes

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Neurodegenerative disorders linked to aging populations and unhealthy diets are emerging medical challenges worldwide, with increases in Alzheimer's disease (AD) and type 2 diabetes (T2D) of particular concern. Significantly, T2D is a risk factor for AD and vice versa, and epidemiological, pathological and molecular studies show that T2D and AD are connected both in terms of cognitive failure and metabolic dysregulation. While available evidence indicates a neurological connection between AD and T2D, the instigating mechanisms common to the two diseases remain largely unknown. This critical gap stands in the way of optimal mechanism-based therapeutics. We have investigated pathogenic neurological mechanisms common to AD and T2D to determine the origin and impact of the brain metabolic stress in AD and to identify new targets for therapeutics that rescue cognition in AD. We described an inflammatory pathway that causes brain insulin signaling dysfunction and endoplasmic reticulum stress in AD experimental models. Acting in the hypothalamus, this pathway leads to peripheral glucose intolerance. This same pathway acting in the hippocampus leads to synapse degeneration and memory impairment. We further found that specific hormones act in the brain to ameliorate metabolic stress and to prevent cognitive decline in AD models. Elucidation of diabetes-like CNS mechanisms may thus provide knowledge that will result in new drug discovery strategies to preserve brain health.

S07 Do strengthened synapses account for memory storage?

S07-01

Synaptic strengthening and weakening: links to memory processes?

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Ever since the discovery of the phenomenon of long-term potentiation (LTP) of synaptic transmission in hippocampus, scientists have debated the question of its relationship to the mechanisms of learning and memory. This issue became even more complex following the identification of multiple forms of activity-dependent synaptic plasticity, including long-term depression. The elucidation of the molecular/cellular mechanisms of various forms of synaptic plasticity has provided a variety of tools to further address the links between changes in the strength of synapses and their relationship to learning and memory. There is a wealth of data using genetic or pharmacological manipulations that have shown that enhancing or inhibiting LTP is associated with enhancement or impairment of learning and memory, respectively. These results strongly suggest that modifying the strength of synaptic contacts participate in memory formation. While weakening of synaptic strength has also been shown to take place with various types of manipulations, the relationship between these synaptic modifications and learning and memory processes have only recently been addressed. We will use examples from our own experiments with mutant mice and with drugs modifying the features of LTP and LTD to show how changes in synaptic strength in field CA1 of hippocampus and in the cerebellar cortex are directly related to various forms of learning and memory. In addition, we will discuss recent findings indicating that different patterns of activity can trigger different signaling cascades, and that cross-talks between different molecular pathways provide for a rich repertoire of synaptic modifications. Moreover, the spatio-temporal relationships between these signaling pathways might provide new clues to link changes at the synaptic level to changes at the dendritic and neuronal levels.

S07-02

Neuroscience of memory storage and retrieval

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Our study (Ryan et al., Science 2015) suggested that while a rapid increase of synaptic strength is crucial for encoding of a memory, a newly formed pattern of connectivity between the upstream and downstream engram cell ensembles may serve as the primary means for long-term memory storage. Supporting this concept is the fact that a mouse suffering from retrograde amnesia can be induced to express the full level of memory by optogenetic stimulation 1 day after encoding, despite the fact that these engram cells are without enhanced synaptic strength, and that these amnesic engram cells (in hippocampal DG) retain preferential connectivity with downstream engram cells (in CA3 and BLA) (Ryan et al.). We have further investigated this hypothesis from several different angles and have shown that the engram-to-engram connectivity is stable in both control and amnesic mice, lasting for at least 8 days after encoding, and that optogenetic activation of the engram in amnesic mice is stimulus strength-dependent. These and other recent studies on memory engram cells are generating the concept of "silent engram cells" that hold memory information but are not susceptible to reactivation by natural cues for recall. These silent engram cells are found not only in retrograde amnesia but also in mouse models of early Alzheimer's disease (Roy et al., Nature 2016). Furthermore, our recent study revealed that remote episodic memory is in a silent state during recent time points, and that hippocampal episodic memory engram cells that are active in recent time points are converted to the silent state over time. The common structural and physiological features of these silent engram cells, compared to active engram cells, are reduced spine density and reduced synaptic strength. We have also showed that a silent engram could be converted to an active engram by repeated optogenetic enhancement of synaptic strength (Roy et al.). Based on these observations, we propose that the primary purpose of molecular consolidation of memory is to make memory engram cells accessible to natural recall cues.

S07-03

Protein synthesis at neuronal synapses

E. Schuman

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An individual neuron in the brain possesses approximately 10,000 synapses, many of which are hundreds of microns away from the cell body, which can process independent streams of information. During synaptic transmission and plasticity, remodeling of the local pool of neuronal proteins occurs via the regulated synthesis and degradation of new proteins. I will discuss previous and current studies aimed at understanding how local protein synthesis contributes to synaptic function and plasticity.

S07-04

Time units for learning and their implementation through system-wide cFos + neuronal assemblies

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An isolated experience can produce long-lasting memories, but learning often involves multiple interactions with related information. The outcome of these interactions could be integrated incrementally, independent of when individual interactions occur. Alternatively, integration might occur within dedicated periods of time, breaking down learning processes into discrete time units.

We will provide evidence that related information acquired within time windows of 5 h (time units for learning) is combined to determine whether and what mice learn. We detected elevated cFos expression during 5 h after initial acquisition throughout specific system-wide neuronal assemblies. Locally inhibiting network activity or cFos function at any time during the 5 h time window disrupted system-wide cFos assemblies and time unit function. Activation of learning-related cFos assemblies was sufficient and necessary for time unit function. Therefore, learning processes consist of discrete 5 h time units implemented through maintenance of specific system-wide neuronal assemblies through network activity and cFos expression.

S08 Mechanisms of myelin disorders

S08-01

TSC1 controls homeostasis and survival of myelinating oligodendrocytes by regulating perk- eIF2α signaling

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Tuberous sclerosis complex (TSC) is an autosomal dominant disorder caused by mutations in either TSC1 or TSC2. Patients exhibit white matter abnormalities, including myelin deficits in the central nervous system, however, underlying mechanisms are not fully understood. TSC1/2 are upstream negative regulators of mammalian-target-of-rapamycin (mTOR), which is required for oligodendrocyte differentiation. Here we report that, unexpectedly, constitutive activation of mTOR signaling caused by *Tsc1* deletion in the oligodendrocyte lineage in mice results in severe myelination defects and oligodendrocyte cell death, despite an initial increase of oligodendrocyte precursors during early development. Expression profiling analysis reveals that *Tsc1* ablation induces prominent endoplasmic reticulum (ER) stress responses through a PERK-eIF2α dependent signaling axis and activates Fas-JNK apoptotic pathways. Enhancement of PERK-eIF2α signaling by inhibition of Gadd34-PP1 phosphatase with guanabenz protects oligodendrocytes and partially rescues myelination defects in *Tsc1* mutants. Thus, our studies suggest that TSC1-mTOR signaling acts as an important checkpoint for maintaining oligodendrocyte homeostasis and point to a previously uncharacterized ER stress mechanism that contributes to hypomyelination in TSC disease.

S08-02

NF-κB activation protects myelinating oligodendrocytes against inflammation

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Transcription factor Nuclear Factor κ B (NF-κB) plays a critical role in inflammatory diseases, including immune-mediated demyelinating diseases, by regulating inflammation and cell viability. Activation of NF-κB has been observed in oligodendrocytes in immune-mediated demyelinating diseases. Although *in vitro* studies suggest that NF-κB activation promotes oligodendrocyte survival in response to inflammatory mediators, the effects of NF-κB activation on oligodendrocytes in immune-mediated demyelinating diseases remain unknown. We generated a mouse model that expresses IκBαΔN, a super-suppressor of NF-κB, specifically in oligodendrocytes and found that IκBαΔN expression had no effect on oligodendrocytes under normal conditions. Multiple pathological features of immune-mediated demyelinating diseases are recapitulated in the developing CNS of mice that ectopically express IFN-γ in the CNS, including inflammation, oligodendrocyte death, and myelin loss. Interestingly, we showed that oligodendrocyte-specific expression of IκBαΔN blocked NF-κB activation, exacerbated myelinating oligodendrocyte death and myelin loss, but did not alter inflammation in the developing CNS of IFN-γ-expressing mice. Moreover, we showed that NF-κB inactivation in oligodendrocytes aggravated IFN-γ-induced remyelinating oligodendrocyte death and remyelination failure in the cuprizone model. These findings imply the cytoprotective effects of NF-κB activation on myelinating oligodendrocytes in immune-mediated demyelinating diseases.

S08-03

Suppressing N-acetyl-L-aspartate synthesis prevents spongiform leukodystrophy and neuron loss in a murine canavan disease model

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Canavan disease, a recessively inherited spongiform leukodystrophy caused by deficient aspartoacylase (ASPA) enzymatic activity, is characterized clinically by loss of motor and intellectual milestones and, because of the need for ASPA to cleave N-acetyl-L-aspartate (NAA) to aspartate and acetate, a marked elevation in the brain concentration of NAA ($[NAA_B]$). Affected infants demonstrate myelin lamellar and astroglial vacuolation in superficial cerebellar, forebrain, and upper brainstem white matter and adjacent gray matter, and there is brain neuronal loss later in the course. Thus far, attempts at *ASPA* gene therapy have not yielded substantial benefits in this progressive neurodegenerative disorder. Mice homozygous for an *Aspa*-nonsense mutation ("*Aspa*^{nur7} mice") lack immunochemically demonstrable Aspa, and develop spongiform leukodystrophy closely resembling that in Canavan disease. Suppression of activity of the neuronal NAA synthesizing enzyme N-acetyltransferase 8-like (Nat8 l) in these Aspa-deficient "Canavan" mice prevents $[NAA_B]$ elevation, spongiform leukodystrophy, and neuronal loss. These results strongly support the hypothesis that brain spongiform degeneration and neuronal loss in Canavan disease are neurotoxic consequences of elevated $[NAA_B]$, and suggest that interventions to prevent elevated $[NAA_B]$ in infants and children with Canavan disease would have therapeutic value.

S08-04

mTORC2 and ILK signaling in oligodendrocyte differentiation and myelination

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The differentiation of oligodendrocyte precursor cells (OPCs) into mature, myelinating oligodendrocytes is an important process involving numerous intracellular signaling cascades. Two interacting pathways, mammalian target of rapamycin (mTOR) and integrin-linked kinase (ILK), can impact oligodendrocyte development, potentially through cytoskeletal changes. Extensive studies on CNS myelination demonstrate the impact of mTOR, a protein kinase

that exists in two complexes defined by the presence of adaptor proteins: Raptor-containing mTOR complex 1 (mTORC1) and Rictor-containing mTOR complex 2 (mTORC2). Both mTORC2 and ILK regulate actin cytoskeleton in other cell types, and an important question is whether they act to regulate the cytoskeleton during CNS myelination. In earlier studies, little impact was noted when Rictor was conditionally deleted using the 2′,3′-Cyclic-nucleotide 3′-phosphodiesterase (CNP) promoter. However, this promoter has significant early embryonic expression, which could impact development beyond oligodendrocytes. Additionally, ILK deletion using the CNP promoter was embryonic lethal, again suggesting early effects. In the current studies, Rictor was deleted from later oligodendrocyte progenitor cells, using the platelet-derived growth factor receptor alpha (PDGFRa) promoter (PDGFRa-Cre), and ILK was deleted with the Olig1-Cre promoter. Both conditional knockout (cKO) mouse lines had reduced oligodendrocyte development and myelination. cKO mice with OPC-specific deletion of Rictor exhibited deficient oligodendrocyte differentiation and hypomyelination, particularly in the corpus callosum. ILK cKO deletion reduced the number of oligodendrocytes, and there were fewer myelinated axons, with thinner myelin in corpus callosum. The signaling pathways that are affected by either mTORC2 or ILK signaling may intersect to alter postnatal CNS myelination. Both complexes phosphorylate AktS473, and in both cKO lines, AktS473 was reduced. Additionally other downstream pathways were affected, including several mTOR pathway targets. In Rictor cKO mice, cytoskeletal targets important for myelination were downregulated. Interestingly in Rictor cKO mice, ILK phosphorylation was reduced, while in ILK cKO mice, Rictor was downregulated. How these pathways interact and whether they regulate the cytoskeleton in differentiating oligodendrocytes is currently under investigation. These studies supported by NIH NS080223 and NMSS.

S09 Insulin and IGF Signaling in the Adult Brain: New Functions in Stem Cells, Plasticity, Aging and Neurodegeneration

S09-01

Insulin-like growth factor signaling in maintenance and protection of neurons

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IGF signaling controls aging and affects age-related neurological diseases. Downregulation of IGF pathways promotes longevity in mice, with cellular stress resistance and tissue homeostasis playing major roles therein. To test the long-term effects of IGF on neuronal replacement in the aging brain, we suppressed IGF1R specifically in adult neural stem cells (NSC) using conditional mutagenesis and performed cell lineage tracing. We showed that IGF1R knockout maintained youthful characteristics of olfactory bulb (OB) neurogenesis within the aging brain: Blocking IGF signaling in NSCs increased cumulative neuroblast production and enhanced neuronal integration into the OB. This resulted in neuro-anatomical changes and improved olfaction, together efficiently preventing age-related decline. These results were sustained by mathematical modeling, predicting that diminished growth stimulation is indeed optimal for tissue aging. Thus, inhibiting the longevity gene IGF1R in adult NSCs induced a gain-of-function aging phenotype, marked by optimized cell renewal and enhanced sensory function. A similar IGF dependent mechanism also controls adult neurogenesis in the recently discovered hypothalamic niche. Alike many aging processes, experimental Alzheimer disease in mice responds positively to decreased IGF signaling. We showed that rendering adult neurons completely resistant to IGF protects from APP/PS1-induced amyloid pathology. Mutants exhibited improved memory, fewer plaques, less Aβ and diminished neuroinflammation. Importantly, neurons undergoing IGF1RKO reduce their apical soma and develop leaner dendrites, indicative of structural plasticity entailing condensed forebrain neuroarchitecture. Our data indicate that neuronal IGF-1R ablation, *via* preserved autophagic compartment and enhanced systemic elimination, generates lifelong protection from Alzheimer pathology by clearing Aβ. Thus, neuronal IGF1R, and possibly other cell size-controlling pathways are promising targets for AD treatment. Functional analysis using microarray analysis for comparing neurons in early-stage AD with IGF1R KO neurons revealed strongly convergent signatures, notably involving neurite growth, cytoskeleton organization, stress response and neurotransmission. We showed that neuronal defenses against AD rely on an endogenous gene expression signature similar to the neuroprotective response activated by genetic disruption of IGF1R. Collectively, our studies highlight neuronal IGF1R signaling as a relevant target for developing AD prevention and anti-aging strategies.

S09-02

Brain glucose handling and insulin peptides

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Insulin, the main regulator of glucose handling in the periphery, is not considered to intervene in brain glucose metabolism even though the brain widely expresses insulin receptors. We now report that insulin does stimulate glucose uptake by cortical astrocytes acting in concert with the closely related insulin-like growth factor I (IGF-I). Both IGF-I and insulin receptors are required for the concerted stimulatory action of their ligands on glucose transporter 1 (GLUT1), the main facilitative glucose transporter in astrocytes. Indeed, blockade of either one reduced astrocytic glucose uptake in response to sensory stimulation, and hindered recovery of cortical neuronal activity after hypoglycemia. In addition, previous findings have shown that reduction of insulin-like growth factor I receptor (IGF-IR) levels protects against different brain pathologies. Accordingly, we also found that knocking down IGF-IR in somatosensory cortex increases astrocytic glucose uptake and brain glucose metabolism upon sensory stimulation. This is because in the absence of IGF-IR, GLUT1 is constitutively active. Underlying mechanisms show that IGF-IR retains GLUT1 inside the cell and the combined action of IGF-I and insulin translocates it to the cell membrane through multiple protein-protein interactions involving various known modulators of glucose transport.

Our observations identify insulin-like peptides as physiological modulators of brain glucose handling and explain previously considered paradoxical actions of these peptides on brain function. Declining brain glucose use during normal aging as well as severe brain glucose dysregulation associated to neurodegenerative diseases such as Alzheimer´s dementia (AD) is therefore likely related to a combined brain insulin and IGF-I resistance, a condition found in the aged brain and exacerbated in AD.

S09-03

IGF-1 receptor: from synaptic transmission to Alzheimer's degeneration

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The insulin-like growth factor-1 receptor (IGF-1R) signaling is a key regulator of lifespan, growth and development. While reduced IGF-1R signaling delays aging and Alzheimer's disease progression, whether and how it regulates information processing in central neural networks remains elusive. Moreover, whether it is involved in maintaining stability of neural circuits' activity remains unknown. To target this question, we have developed an integrative approach to study the relationships between ongoing spiking activity of individual hippocampal neurons and neuronal populations and signaling processes at the level of individual synapses. I will describe the basic relationships between IGF-1R activity, Ca^{2+}

dynamics, ATP levels, mitochondrial function and presynaptic vesicle recycling. Moreover, I will discuss how excessive IGF-1R tone may contribute to hippocampal hyperactivity associated with Alzheimer's disease.

S09-04

IGF-II and insulin receptor signaling in adult neural stem cell homeostasis

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Our prior studies demonstrated that IGF-II acting through the insulin receptor (IR-A) promotes self-renewal of mouse neural stem/progenitor cells (NSPs) *in vitro*. IGF-II is produced within the adult brain by cells that contribute to neural stem cell niches including the choroid plexus, hippocampal progenitors and endothelial cells. The goal of these studies was to test the hypothesis that IGF-II/IR is an essential niche signaling system for adult NSPs in the subventricular zone (SVZ) of the lateral ventricles and sub-granular zone (SGZ) of the hippocampus. We used a floxed *Igf2* mouse line with a tamoxifen inducible Rosa 26 Cre driver to delete *Igf2* in the adult mouse. To delete the IR specifically in NSPs we used a floxed *Insr* mouse line carrying a tamoxifen inducible Cre transgene driven by the nestin intron 2 promoter (ΔNSC-IR KO). Loss of *Igf2* in adult mice reduced the slowly dividing, label-retaining cells in the SVZ and SGZ by ~50% with alterations in the numbers of newly generated neurons in the olfactory bulb, accompanied by hyposmia, impairments in learning and memory and increased anxiety. Similarly, the number of label-retaining cells in the ΔNSC-IR KO mice was decreased by 50% in the adult SVZ with altered olfactory bulb neurogenesis and hyposmia. Flow cytometry analyses revealed 3.5 fold fewer SVZ neural stem cells and a corresponding increase in multipotential and bipotential progenitors in the ΔNSC-IR KO mice. In contrast to the SVZ, NSC numbers in the hippocampus were unchanged compared to controls. These data support an essential function for IGF-II in the maintenance of multiple adult neural stem cell niches and suggest that IGF-II acts through the IR in the SVZ whereas its function in the hippocampal SGZ is independent of the IR.

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S10 Neuronal activity, Myelination, and Higher Brain Function

S10-01

Adaptive myelination in health and disease

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Cancer chemotherapy frequently results in long-term neurological and motor dysfunction, such as slowed information processing, deficits in attention, concentration, working memory and learning, and fine motor skills. The etiology of this syndrome remains to be fully elucidated, but much of the pathology is thought to be due to chemotherapy-induced damage to neural precursor cells. In particular, the depletion or dysfunction of the oligodendroglial precursor cells (OPCs) may play an important role in the long-term neurological consequences of cancer chemotherapy. OPCs are necessary for developmental myelination, as well as for ongoing myelin remodeling throughout life, known as adaptive myelination in which neuronal activity can increase OPC proliferation, differentiation, and myelination. Cancer therapy-induced damage to oligodendrocyte lineage cell dynamics would be particularly devastating for children who have not yet completed developmental myelination. We find a persistent depletion of OPC lineage cells in frontal lobe white matter but not grey matter regions in both children exposed to early-life chemotherapy treatments and in a newly developed juvenile mouse model of chemotherapy-induced neurotoxicity. Concomitant with the OPC depletion, OPCs exhibit an accelerated but incomplete differentiation into immature, non-myelinating oligodendrocytes in this mouse model of 'chemobrain'. Syngeneic transplantation of healthy, non-chemotherapy exposed OPCs into the microenvironment of previously chemotherapy-treated brains exhibit accelerated differentiation compared to those transplanted into vehicle control-treated brains. These data suggest that the accelerated differentiation is associated with a chemotherapy-induced microenvironmental perturbation. We find that juvenile mouse chemotherapy treatment results in persistent activation of white matter microglia and altered microglial:OPC TGFb signaling. Additionally, the proliferation and differentiation of OPCs in response to neuronal activity is abrogated in the previously chemotherapy-treated brain, suggesting an impaired adaptive response to neuronal activity following chemotherapy treatment. Collectively, these data suggest that the long-term changes to the glial microenvironment following chemotherapy treatment and the associated impaired oligodendrocyte lineage cell dynamics and adaptive response to neuronal activity may underlie the long-term deficits associated with chemotherapy-induced neurological decline.

S10-02

Powering axons: novel functions of oligodendrocytes in energy metabolism

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Oligodendrocytes, which make myelin for rapid impulse propagation, are also required for the function and long-term survival of axons, independent of myelin itself. The two functions of glia in myelination and axon support can be genetically uncoupled in mutant mice. We later found that oligodendroytes must provide lactate to the axonal compartment for the generation of ATP. Trying to understand how lactate supply is matched to axonal energy needs, we found that activation of oligodendroglial NMDA receptors (presumably a proxy for axonal spiking activity) causes the rapid redistribution of glucose transporter GLUT1, leading to enhanced glucose import and lactate release. In mice lacking oligodendroglial NMDA receptors, the functional GLUT1 expression in the myelin compartment is reduced, which perturbs axon conduction under metabolic stress. Reduced axonal ATP levels can be measured by metabolic imaging in real time using transgenic mice with neuronally expressed FRET sensors. Loss of metabolic support by oligodendrocytes is likely a contributing factor for axon loss in a range of myelin diseases, and is also relevant for neurodegenerative diseases, since long axons in white matter tracts are a bottle neck of neuronal integrity (Supported by MPG, DFG and ERC).

S10-03

Metabolic coupling between exercise and dietary fat regulates myelin in the adult CNS

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Diet is an intrinsic aspect of everyday life and is emerging as an important regulator of brain function and plasticity. In particular, consumption of high levels of saturated fats is considered detrimental for CNS function; however, based on the rich lipid content of the brain, how to manage dietary fats for optimal CNS health is controversial. Myelin membranes have a very high lipid-to-protein ratio, with cholesterol availability rate limiting for myelin formation. These considerations along with strong evidence that activity promotes myelin plasticity form the basis of our studies to assess the effects of exercise training alone, or in the context of high dietary fat, on parameters of myelin formation in the spinal cord of adult mice. Mice were provided a regular diet, or one high in saturated fat and sucrose, with or without access to voluntary exercise for 7 weeks. Results suggest that consumption of a diet high in saturated fat in the setting of a sedentary lifestyle leads to reductions in oligodendrocyte progenitors and mature myelinating cells and that coordinate exercise can prevent these deleterious effects. In addition, results point to an important interplay between high dietary fat and exercise training that increases the predominant myelin proteins, proteolipid protein and myelin basic protein. Exercise in conjunction with high fat consumption also boosted spinal cord IGF-1, its high affinity receptor (IGF-1R), and activated AKT, a key signaling partner known to promote myelinogenesis. Coordinate elevations in SIRT1, PGC-1α, 4-HNE, GPx1 and SOD2 suggest the dietary- and exercise-mediated changes in spinal cord myelinogenesis observed may be linked to changes in mitochondrial function, energy metabolism, lipid peroxidation, and levels of antioxidant enzymes. These findings provide new evidence that exercise can modulate the action of diet on the CNS through

significant metabolic coupling. Altogether, results suggest that the beneficial effects of exercise on CNS function may be mediated in part by regulating energy metabolism, reducing oxidative stress and stimulating myelinogenesis, and importantly that the availability of dietary saturated fat can play an important role in this process.

S10-04

Diet and metabolic preconditioning of brain function and plasticity

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Diet is one the most crucial needs for species survival and adaptation, and for maintaining overall health of individuals. I will discuss how diet builds resistance to neurological disorders by acting centrally and peripherally, and involving a cascade of molecular events around the action of cell energy metabolism involving glial and neuronal cells. We are using fructose consumption as a model of metabolic perturbation, with detrimental effects on mitochondria bioenergetics, myelination, synaptic plasticity, and cognitive function, and aggravating the outcome of brain trauma. Using a systems nutrigenomics approach, we have found that metabolic perturbations carried by fructose promote selective transcriptomic and epigenomic alterations in the hypothalamus (control of metabolism) and hippocampus (critical for cognitive functions), engaging neuronal and glial cells and affecting inflammation, immune response, neuronal signaling, and cognition. These molecular alterations in rodents converge with genes conferring genetic risks of metabolic and neuropsychiatric disorders in human genome-wide association studies. Furthermore, metabolic perturbation aggravates the effects of brain trauma. Single cell analysis using drop-seq shows that the brain responds to TBI by coordinating the interaction between glia cells and neurons to maintain the energetic demands of the brain. In separate studies, we have found that early exposure to foods has a long-term impact on brain plasticity by building an "epigenetic memory" that provides resistance to neurological challenges. Early life exposure to the omega 3 fatty acid DHA promotes long-term protection against the detriments of adult exposure to a western diet by involving DNA methylation (most stable form of epigenetic variability). The interplay between the effects of fructose and DHA consumption on brain pathogenesis highlights the impact of diet on determining resilience to neurological disorders. These studies are significant on the context of the health risk posed by the contemporary elevated fructose consumption on the current epidemic of metabolic and brain disorders.

S11 Synaptic plasticity in health and disease

S11-01

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 briefly review experiments that identified a unique assembly of SNARE proteins required for the exocytosis of AMPA receptors during LTP in hippocampal CA1 pyramidal neurons. In the majority of the lecture, I will present evidence that blocking postsynaptic expression of both synaptotagmin-1 and synaptotagmin-7 *in vivo*, but not of synaptotagmin-1 and synaptotagmin-7 alone, prevents LTP assayed in acute hippocampal slices. LTP was rescued by wild-type but not by Ca^{2+}-binding-deficient mutant synaptotagmin-7. Several assays in acute hippocampal slices and cultured hippocampal neurons revealed that blocking postsynaptic synaptotagmin-1/7 expression did not impair basal synaptic transmission nor the levels of synaptic or extrasynaptic AMPA receptors. Furthermore, NMDA receptor-dependent long-term depression and retinoic acid dependent synaptic scaling were not prevented by postsynaptic synaptotagmin-1/7 depletion. Expression of a dominant-negative mutant synaptotagmin-1, which inhibited Ca^{2+}-dependent presynaptic vesicle exocytosis, also blocked Ca^{2+}-dependent postsynaptic AMPA receptor exocytosis, thereby abolishing LTP. These results suggest that postsynaptic synaptotagmin-1 and synaptotagmin-7 act as redundant Ca^{2+}-sensors for Ca^{2+}-dependent exocytosis of AMPA receptors during LTP.

S11-02

Ampa receptor trafficking during health and disease

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Neurotransmitter receptors mediate signal transduction at synaptic connections between neurons in the brain and the regulation of receptor function is critical for synaptic plasticity. My laboratory has been elucidating the molecular mechanisms underlying the regulation of AMPA receptors, the major excitatory neurotransmitters receptors in the central nervous system. We have found that AMPA receptors are extensively posttranslationally modified by phosphorylation, palmitoylation and ubiquination. Protein phosphorylation is a major form of AMPA receptor regulation and the receptors are phosphorylated on serine, threonine and tyrosine residues by many different protein kinases. We have shown that phosphorylation of the receptor regulates its ion channel properties and membrane trafficking and that receptor phosphorylation is critical for the expression of several forms of synaptic plasticity and for learning and memory. We have also identified a variety of AMPA receptor interacting proteins, including GRIP1/2, PICK1, GRASP1, SNX27, KIBRA, and SynGAP that interact with AMPA receptors and are necessary for their proper subcellular trafficking. This AMPA receptor complex is important for several forms of synaptic plasticity and learning and memory. These studies indicate that the modulation of receptor function is a major mechanism for the regulation of synaptic transmission and is a critical determinant of animal behavior. Recent evidence has indicated that AMPA receptor function may be disrupted in several neurological and psychiatric disorders. Specifically, mutations in SynGAP, GRIP1 and GRASP1 have been found to be associated with cognitive disorders including intellectual disability, autism, and schizophrenia. We have been characterizing some of these disease-associated mutations to examine their effect on protein function, AMPA receptor trafficking, synaptic plasticity and behavior. These studies may help develop novel therapeutics for these devastating disorders.

S11-03

Acute stress triggers constitutive activation of kappa opioid receptors

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Dopaminergic neurons in the ventral tegmental area (VTA) are an important locus for the convergent effects of stress and drugs of abuse. We previously identified a long-term potentiation of GABAergic synapses on these neurons (LTP_{GABA}) that is blocked by stress through activation of kappa opioid receptors (KORs, Graziane et al., Neuron, 2013). A brief swim stress blocks LTP_{GABA} for 5 days. Blocking KORs with norBNI even after stress restores LTP_{GABA} and prevents reinstatement of cocaine self-administration (Polter et al., Biol. Psychiatry, 2014).

We find that the block of LTP_{GABA} by stress is caused by constitutively active KORs. While bath application of an inverse agonist (norBNI, 100 nM) rescues LTP_{GABA} in slices from stressed animals, a neutral antagonist (6-β-naltrexol, 10 μM) does not. These results suggest that LTP_{GABA} is blocked by constitutive activity of KORs rather than by persistently elevated dynorphin. NorBNI rescue of LTP_{GABA} in slices was blocked by the JNK inhibitor SP600125, supporting a non-competitive effect of norBNI.

In contrast to norBNI, which rescues LTP_{GABA} after stress, we find that *in vivo* 6-β-naltrexol only rescues LTP_{GABA} when administered prior to stress. These data suggest that activation of the KOR by dynorphin during stress leads to persistent, dynorphin-independent activation of the receptor. Moreover, treatment of rats with norBNI, but not 6-β-naltrexol, 24 h after stress prevents reinstatement of cocaine self-administration. Our results show that a single exposure to acute stress causes long-lasting changes in inhibitory plasticity through constitutive activity of KORs. These studies demonstrate a novel mechanism of KOR regulation and highlight a potential target for treatment of stress-induced drug seeking behavior.

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S11-04

Synaptic pathology in Alzheimer's disease

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Identifying the pathological mechanisms causing Alzheimer's disease is a crucial challenge of the 21st century as by 2050 we expect 1/85 persons living with the disease worldwide and no cure or effective palliative therapies are currently available. Genetic and pre-clinical evidence led to a major hypothesis regarding the etiology of the disease, which centers on the toxic effects of amyloid-beta (Aβ) peptide assemblies at synapses within the hippocampus. This Aβ peptide originates from amyloid precursor protein (APP) cleavage and accumulates into oligomers, peptide assemblies that perturb synapse plasticity processes such as long term potentiation and long term depression. Yet, several human clinical trials show that removing Aβ aggregates from brain areas controlling memory processes, such as the hippocampus, did not provide the expected protection against cognitive decline. What if Aβ was not the only APP fragment perturbing synapse function in the context of Alzheimer's disease? I will present results demonstrating that other fragments originating from APP cleavage also modulate the function of hippocampal synapses, arguing for a role of these fragments as neuromodulators. These findings shed new light on the biological relevance of APP processing at synapses in physiological and pathological conditions.

S12 The local integrator: astrocyte mitochondria couple signaling and metabolism and modulate synaptic transmission

S12-01

Regulation of activity-depenedent mitochondrial dynamics in astrocytes

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Regulated trafficking of mitochondria in astrocytes is important for providing energy where it is required, and for providing calcium buffering at sites of calcium entry or release. Indeed the regulation of mitochondrial distribution, morphology and function have recently emerged to play an important role in astrocyte function and signalling but the regulatory mechanisms are not yet fully understood. Miro family proteins contain a transmembrane domain locating them to the outer mitochondrial membrane, along with two GTPase domains and two calcium-sensing EF-hand domains that face into the cytosol. They play a key role in regulating mitochondrial transport by linking mitochondria to kinesin and dynein motor proteins. Miro proteins are also targets for the Parkinson's Disease associated PINK1/Parkin mitophagy pathway and are therefore implicated in altered mitochondrial dynamics during mitophagy. Here I will present our work demonstrating that the calcium-sensing EF-hand domains of Miro1 are important for regulating mitochondrial trafficking in astrocytes and required for activity-driven mitochondrial confinement near synapses. How, activity-dependent mitochondrial positioning by Miro1 reciprocally regulates the levels of intracellular calcium in astrocytic processes will also be discussed. Finally the role of the PINK1/Parkin pathway for regulating mitochondrial turnover in astrocytes during mitochondrial damage will also be explored.

S12-02

Characterization of two-photon imaging-induced astrocytic microdomain calcium transients

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Recent advances in the cell-type specific expression of genetically encoded calcium indicators combined with two photon excitation fluorescence (2PEF) imaging have allowed imaging spontaneous microdomain calcium (Ca^{2+}) activity in fine astrocytic processes. However, up to date, the origin and physiological function of these spontaneous transients are still under debate. Here we show that 2PEF imaging of fine astrocytic processes in acute brain slices with standard imaging parameters (920 nm, 83 fs pulses, 80 MHz repetition rate, some 10 mW average laser power) inevitably leads to an increase in the frequency of microdomain Ca^{2+} transients, and we investigate the dependence of this photodamage effect on the laser pulse length.

We imaged Ca^{2+} transients in a single equatorial section of sparsely distributed GCaMP6f-expressing cortical astrocytes and analyzed their frequency, amplitude, duration and localization using a custom-written program. Optimal pre-compensation for the pulse dispersion introduced by the beam delivery optics was achieved using a motorized pre-chirp prism compensator and custom, objective-dependent lookup tables allowed us finding the shortest pulse lengths in the sample plane. Unexpectedly, photodamage was due to cumulative one-photon rather than two-photon absorption, and continuous-wave 920-nm illumination was equally damaging than 920-nm laser pulses. We conclude that direct infrared absorption results in the spatial and temporal accumulation of a damage-promoting factor modifying Ca^{2+} homeostasis in cortical astrocytes. Our study calls for a careful consideration of photodamage effects on 2PEF-imaging of astrocytic microdomain Ca^{2+} transients.

S12-03

Co-compartmentalization of glutamate transporters and mitochondria in fine astrocyte processes

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Unlike most other neurotransmitters, glutamate (Glu) is recycled through astrocytes. The first step in this recycling is mediated by two Na^+-dependent transporters called GLT-1 and GLAST (or EAAT2 and EAAT1) that are located on fine astrocyte processes. Several years ago, we showed that GLT-1 or GLAST form immunoprecipitable complexes with several different mitochondrial proteins and that mitochondria overlap with these transporters more often than would occur by chance, consistent with a physical interaction. We used biolistic transfection to selectively transduce astrocytes in organotypic hippocampal slices with fluorescent proteins that outline the cell membrane, accumulate in mitochondria, or sense changes in Ca^{2+} (genetic Ca^{2+} indicators, GCaMPs). In a subset of experiments, we used AAV-based vectors to selectively express these proteins in astrocytes and analyzed mitochondrial distribution and mobility *in vivo*. We find that mitochondria in fine astrocyte processes are mobile in organotypic slices and *in vivo*. We find that inhibitors of glutamate uptake or reversed Na^+/Ca^{2+} exchange reduce basal Ca^{2+} and increase mitochondrial mobility. We also find that dominant-negative variants of Miro proteins increase mitochondrial mobility. Based on these and other data, we propose that mitochondrial positioning near glutamate transporters/synapses is controlled by neuronal activity/release of glutamate, glutamate uptake into astrocytes, and subsequent activation of reversed Na^+/Ca^{2+} exchange. We find that dominant-negative variants of Miro,

impairment of mitochondrial function, or photo-ablation of mitochondria increase Ca^{2+} signals in astrocyte processes. Finally, we find that transient oxygen/glucose deprivation (30 min) causes a delayed loss of these mitochondria. This loss is associated with increased mitophagy and is blocked by inhibitors of glutamate uptake or reversed Na^{+}/Ca^{2+} exchange. This loss of mitochondria is also associated with dramatic increases in Ca^{2+} signals in these astrocyte processes. We propose that physical and functional coupling between glutamate transporters and mitochondria has profound implications for the control of Ca^{2+} signaling under physiologic and pathologic conditions.

S12-04

The role of glutamate uptake and metabolism in purinergic agonist-enhanced neuroprotection after brain injury

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Stroke is a leading cause of patient disability and mortality in the world. Research is generally focused on preserving and repairing the cells of the penumbra. Immediate ischemic events throughout this area are loss of oxygen and glucose, which leads to energy depletion and failure of ATP-dependent processes. One of the major consequences of this energy loss is disruption of ion homeostasis, increasing neuronal excitability, cell swelling (edema), and lysis if homeostasis is not restored. Previous work in our lab has shown that the purinergic agonists 2MeSADP and MRS2365 increase mitochondrial ATP production in astrocytes, and this, in turn, promotes the survival of both astrocytes and neurons within the penumbra. Our current study investigates the role of Glutamate Dehydrogenase (Glud1) in purinergic stimulated energy production and neuroprotection. Glud1 is a mitochondrial enzyme that converts glutamate into α-ketoglutarate, a substrate for the tri-carboxylic acid (TCA) cycle. We hypothesized that purinergic agonists increase glutamate metabolism through Glud1 in astrocytes, which in turn increases astrocytic energy production to promote survival of cells within the penumbra. Our work demonstrates that these agonists in C8D1A cells, an immortalized mouse astrocyte cell line, increases ATP levels within the first 20 min of application, and that this effect is lost in cells transfected with shRNA to Glud1. These results are observed when glucose in the media is replaced with galactose, forcing cells to use oxidative phosphorylation, but are blocked when treated with oligomycin, an ATP synthetase inhibitor. Preliminary results in an *in vitro* model of stroke, oxygen glucose deprivation (OGD), suggest Glud1 levels increase following OGD. *In vivo*, we are using a photothrombotic model of stroke to study the effect of astrocytic loss of Glud1 on purinergic agonist-enhanced neuroprotection. Preliminary studies indicate knockout of Glud1 in astrocytes alone reduces cortical expression of Glud1 by about 50%, and baseline lesion sizes are not changed after stroke. Ongoing studies are examining the dependence of Glud1.

S13 Adult neurogenesis - New roles in normal and pathological conditions

S13-01

Revealing the potential of postnatal neural stem cells

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The adult mammalian brain contains neural stem cells (NSCs), which provide new neurons in neurogenic regions, such as the olfactory bulb and dentate gyrus (DG) of the hippocampus. Accumulating evidence has demonstrated reduced cell proliferation and neurogenesis in the DG of the adult hippocampus following psychosocial or physical stressors. Indeed, we have demonstrated that the number of NSCs is reduced after chronic psychiatric stress, which is sustained without treatment but reversed by antidepressant administration. Adult neurogenesis could be involved in the pathogenesis of mood affective disorders based on the observations that mood stabilizers, which are used to treat bipolar disorder patients, enhance the self-renewal of NSCs and increase the neurogenesis in postnatal rodent brains and that oligodendrocyte precursor cells are reduced in the frontopolar cortex of major depression patients. Especially, the latter observation prompted us to generate a primate model of mood affective disorders because the frontopolar cortex is poorly developed in rodents. In this symposium, I would like to introduce our recent attempt to establish a macaque (*Macaca fascicularis*) model of major depression by chronic administration of interferon-α, which is used to treat patients with hepatitis and cancers and often causes aversive effects of depression. Some behavioral changes are observed in this macaque model, which might be related to reduced cell genesis in the frontal cortex and to altered epigenetics status.

S13-02

Activating endogenous stem cells to promote brain repair and cognitive recovery

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Endogenous neural repair strategies based on activation of neural precursor cells and leading to functional recovery have shown great promise. We have isolated and characterized two distinct populations of neural stem cells in the developing and adult mouse brain. Herein I will discuss our work exploring the potential of small molecules to activate endogenous neural precursor cells and promote self-repair of the injured brain. We have shown that drugs commonly used in the clinical setting can lead to dramatic increases in neurogenesis and oligodendrogenesis in the stroke injured brain. Most interesting, we demonstrate functional recovery in motor/sensory tasks and cognition recovery in brain injured mice. Further, we have demonstrated sex-dependent effects of drug treatment on cognitive performance, a finding that correlates with differential effects of activation strategies on the neural precursor cells in the subventricular zone and dentate gyrus. These findings highlight the promising therapeutic potential of stem cell based therapy for neurorepair as well as limitations to be considered.

S13-03

Neuronal migration for maintenance and repair of adult brain

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The migration of olfactory bulb (OB) interneurons, generated in the ventricular-subventricular zone (V-SVZ), provides an excellent model to study the mechanisms that control neuronal migration in the adult brain. These new neurons form chain-like aggregates, in which they are making contact with a large number of similar new neurons, from the V-SVZ towards the OB. Upon approaching their destinations, new neurons decrease their speed and extend processes to terminate the migration. The timing of neuronal migration termination affects the final positioning, dendritic patterns, and functions of new neurons in the OB. After brain injury, the new neurons generated in the V-SVZ migrate toward the lesion. These new neurons also form chain-like aggregates and migrate along blood vessels wrapped by astrocytes. The migration of new neurons depends on several signaling mechanisms that control neuron-astrocyte interaction. Moreover, artificial scaffolds promote neuronal migration toward the injured areas. These findings suggest that appropriate neuronal positioning, which is achieved by precise control of neuronal migration, is critical for the maintenance and repair of adult brain.

S13-04

Neural progenitor migration in the adult brain under physiological and pathological conditions

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While neural stem cells divide within their niche, the resulting progenitor cells migrate towards their target sites to undergo neuronal differentiation. Hippocampal progenitor cells migrate only a short distance into the granule cell layer; however, progenitor cells within the subventricular zone migrate a substantial distance along the rostral migratory stream towards the olfactory bulb. The ERM (ezrin/radixin/moesin) family of proteins connects the actin cytoskeleton to the extracellular matrix through transmembrane proteins. We were able to demonstrate that radixin appears to play a specific role in neuronal migration and differentiation in the adult RMS.

In rodents, progenitor cells will deviate from the rostral migratory stream only under lesion conditions, such as after stroke, and migrate towards the lesion site. The migration is long-lasting, and we have observed significant migration to cortical stroke areas up to 1 year after stroke lesion. However, neuronal differentiation in the lesioned cortex appears to be very limited.

As lesion-induced migration, is to some extend triggered by local release of cytokines, such as CCL2, the migration may be limited by the distance of the lesion site to the subventricular zone. In order to stimulate migration to more remote areas of the brain, we used injectable peptide biomaterial that polymerizes in the presence of physiological Ca^{2+} concentrations. The polymerized biomaterial carries a biologically active peptide sequence of tenascin-C, an integrin receptor-binding protein also found in the rostral migratory stream. We were able to demonstrate that neural progenitor cells can be deviated from the rostral migratory stream to more remote cortical areas through this novel biomaterial.

S14 Exploring the molecular basis of progressive MS

S14-01

NEW INSIGHTS INTO THE PATHOGENESIS OF PROGRESSIVE MULTIPLE SCLEROSIS

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Multiple sclerosis is a chronic inflammatory disease, which leads to focal confluent lesions of primary demyelination in the brain and spinal cord. In the majority of patients the disease starts with relapses and remissions and converts after 10 to 15 years into a chronic progressive course. In the progressive stage of the disease new focal white matter lesions become rare, but extensive cortical demyelination, slow expansion of pre-existing white matter lesions and diffuse injury of the normal appearing white and grey matter are the characteristic pathologoical features. Recent data show that brain and spinal cord damage in the progressive stage of the disease is invariably associated with inflammation. MHC Class I restricted CD8+ T-lymphocytes and B-lymphocytes dominated the inflammatory reaction. B-cells appear to play a major role and to trigger demyelination and neurodegeneration by soluble factors. The nature of the soluble factors is so far unknown, potential candidates are specific pathogenic autoantibodies or B-cell specific cytokines. Demyelination and tissue destruction is associated with oxidative burst activation of microglia, resulting to mitochondrial injury. Mitochondrial injury includes functional blockade of the respiratory chain as well as deletion of mitochondrially encoded DNA. The consequence of mitochondrial injury is energy failure ("histotoxic hypoxia") and its downstream consequences of cell stress, ionic imbalance and cell degeneration. This mechanism of tissue injury and neurodegeneration is further amplified by age factors, the amplification of oxidative injury by iron liberation in the lesions and by vascular comorbidities.

S14-02

Dissecting novel therapeutic targets for progressive multiple sclerosis

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Multiple sclerosis (MS) is a chronic disease affecting young individuals with long-term detrimental impact. Although treatments are available, predicting treatment efficacy and outcomes remains a significant challenge. Genetic risk factors, including HLA status, alter susceptibility to the development of MS, but their functional impact remains largely unknown. Prior work from our laboratory has focused on the family of receptor tyrosine kinases, TYRO3, AXL and MERTK, known as the TAM family. The TAM family has been shown by us and others to be important in regulating the course and outcome of demyelinating disease in both humans and animals. In particular, we have published work linking polymorphisms within the *MERTK* gene with MS susceptibility, and more recently have completed work using a next-generation sequencing approach, with the aim of identifying the causal variants within the MERTK gene that contribute to MS susceptibility. Our further work investigating MS risk variants as drivers of altered gene expression identified MS-associated variants in *MERTK* that correlated with MERTK expression changes in innate immune cells. This work also led to the discovery that patients with high risk HLA genotype and low MERTK expression are not only at increased risk of developing MS, but are also more prone to progressive disease. In combination, these intriguing results require further investigation into the genetic drivers of MERTK expression, and the functional consequences for innate immune cells on the basis that MERTK is a viable therapeutic target for progressive MS.

S14-03

Detangling molecular mechanisms of progression in ms using positron emission tomography

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There is an essential need for imaging techniques specific for the main candidate mechanisms underlying neurodegeneration and clinical progression in multiple sclerosis (MS). This goal is not fully met by available MRI technologies and will be a key step towards the development of novel and effective neuroprotective strategies. Positron Emission Tomography (PET), an imaging technique based on the injection of radiotracers directed against specific targets within brain tissues, allows to quantify selective biological mechanisms of MS pathophysiology.

Several stilbene and benzothiazole derivatives have been repurposed for the imaging of myelin dynamics by PET and robust non-invasive quantification techniques were validated and recently allowed to perform longitudinal studies *in vivo*. A great heterogeneity in individual remyelination potential that strongly influence neurological disability was showed. Work in progress will allow to disseminate myelin PET technology by using fluorinated tracers in larger cohorts of patients and in early therapeutical trials of remyelination.

Imaging innate immune cells by PET can be achieved using tracers that bind to the TSPO macromolecular complex, which is drastically up-regulated in activated microglial cells. PET with ^{11}C-PK11195 identified diffuse microglial activation in the normal appearing tissue of patients with a progressive form of MS. As this tracer showed important limitations, a range of improved second-generation TSPO ligands has been developed. Using ^{18}F-DPA-714, we recently showed that the clinical progression of MS was linked to a strong activation of microglia both in white matter lesions and in normal appearing white and grey matter. At the individual level ^{18}F-DPA-714 PET allowed to reproduce the histological classification of lesions.

PET could also provide early markers of neurodegeneration by targeting neuronal specific receptors such as the benzodiazepine receptor, that can be assessed using ^{11}C-Flumazenil, or by developing synaptic tracers. This will soon open the perspective to regionally map the neuronal component of grey matter damage in MS.

Overall the combination of PET imaging probes with MRI sequences should enable to detangle pathophysiologic mechanisms that drive disability progression in MS, and will contribute to the development of new therapies.

S14-04

Dissecting novel therapeutic targets for progressive multiple sclerosis

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Multiple sclerosis (MS) is a chronic disease affecting young individuals with long-term detrimental impact. Although treatments are available, predicting treatment efficacy and outcomes remains a significant challenge. Genetic risk factors, including HLA status, alter susceptibility to the development of MS, but their functional impact remains largely unknown. Prior work from our laboratory has focused on the family of receptor tyrosine kinases, TYRO3, AXL and MERTK, known as the TAM family. The TAM family has been shown by us and others to be important in regulating the course and outcome of demyelinating disease in both humans and animals. In particular, we have published work linking polymorphisms within the *MERTK* gene with MS susceptibility, and more recently have completed work using a next-generation sequencing approach, with the aim of identifying the causal variants within the MERTK gene that contribute to MS susceptibility. Our further work investigating MS risk variants as drivers of altered gene expression identified MS-associated variants in *MERTK* that correlated with MERTK expression changes in innate immune cells. This work also led to the discovery that patients with high risk HLA genotype and low MERTK expression are not only at increased risk of developing MS, but are also more prone to progressive disease. In combination, these intriguing results require further investigation into the genetic drivers of MERTK expression, and the functional consequences for innate immune cells on the basis that MERTK is a viable therapeutic target for progressive MS.

S15 Inflammatory Mechanisms in Neurodegeneration

S15-01

Innate immune mechanisms drive Alzheimer's disease pathogenesis

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Generation of neurotoxic amyloid-ß peptides and their deposition along with neurofibrillary tangle formation represent key pathological hallmarks in Alzheimer's disease (AD). Recent evidence suggests that inflammation may be a third important component, which, once initiated in response to neurodegeneration or dysfunction actively contributes to disease progression and chronicity. Microglia is being activated by binding of aggregated proteins or aberrant nucleic acids to pattern recognition receptors, which elicit an innate immune response. The latter is characterized by the release of inflammatory mediators including complement activators and inhibitors, chemokines, cytokines, radical oxygen species and enzyme systems. Exogenous as well as endogenous factors may promote and facilitate neuroinflammation in the AD brain. Thus, degeneration of aminergic brain stem nuclei including the locus ceruleus and the nucleus basalis of Meynert may drive inflammation in their projection areas given the antiinflammatory and neuroprotective action of their key transmitters norepinephrine and acetylcholine. Inflammation may not just occur secondary to degeneration, but actively drive amyloid beta aggregation and APP processing. Modulation of the microglia driven innate immune response at key signalling steps may provide protection. Therefore, antiinflammatory treatment strategies should be considered. Data on microglial activation in AD along with suggestions to modify and alter the pro- into an antiinflammatory phenotype will be reviewed and discussed.

S15-02

Importance of amyloid-β conformation in microglial interactions

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Inflammation is a significant component of Alzheimer's disease (AD) and microglia play a key role in this neuroinflammatory environment. Neuritic plaques composed primarily of fibrillar amyloid-β peptide (Aβ) are a focal point of inflammatory pathology in the AD brain. Although microglia tend to cluster around the plaques, our *in vitro* studies indicate that these cells are highly sensitive to soluble Aβ species such as protofibrils. These findings are intriguing when considering the recent observations that plaque composition is complex and contains a halo of soluble Aβ aggregates around the fibrillar core. Our investigations determined that soluble Aβ42 protofibrils were much stronger stimulators of microglia than insoluble Aβ42 fibrils or Aβ42 monomer and induced cytokine production in a Toll-like receptor/MyD88-dependent manner. Extended incubation of monomeric Aβ42 was necessary to activate similar microglia inflammatory pathways. Characterization of protofibrils by electron microscopy revealed classic curvilinear structures for protofibrils with lengths less than 100 nm. Dynamic and multi-angle light scattering measurements of the protofibrils revealed a mean hydrodynamic radius of 21 nm and a molecular weight range of roughly 200–2500 kD (45–550 monomers). Confocal microscopy studies showed significant binding of protofibrils to the microglia surface with little binding observed for fibrils and monomers. Microglial internalization of Aβ was also sensitive to conformation. We found that primary murine microglia internalized Aβ42 protofibrils within minutes and in significant amounts compared to Aβ42 monomers or fibrils. Internalized Aβ42 protofibrils were found widely dispersed in the cytosol with some lysosomal accumulation but little degradation. Binding studies with Aβ and microvesicles shed from microglia yielded a substantial interaction with protofibrils and only a small interaction with monomers. Immunization of rabbits with Aβ42 protofibrils produced an antibody with strong affinity for protofibrils and selectively other Aβ monomers and fibrils. The cumulative data indicate that microglia are particularly responsive to soluble Aβ42 oligomers such as protofibrils. Thus, it is likely that multiple Aβ conformations will influence the neuroinflammatory component in AD.

S15-03

Microglia-synapse interactions in health and disease

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A series of discoveries spanning the last decade has challenged our view of microglia, the brain's immune cells, showing their essential but previously unexpected contribution to the experience-dependent remodeling of neuronal circuits. My research program aims to determine how this newly-defined fundamental mechanism could be implicated in the loss of synapses that best correlates with the impairment of learning and memory across chronic stress, depression, schizophrenia, aging, and Alzheimer's disease. In my presentation, I will discuss about an ultrastructurally distinct microglial phenotype that is predominantly associated with pathological states. These cells are rare under steady-state condition, but become prevalent upon chronic stress, aging, or Alzheimer's disease pathology. They exhibit several signs of oxidative stress including a condensed, electron-dense cytoplasm and nucleoplasm giving them a 'dark' appearance, accompanied by endoplasmic reticulum dilation, mitochondrial disruption, and nuclear heterochromatin remodeling. Dark microglia appear extremely active at synapses, even more than the normal microglia, suggesting their implication in the pathological/traumatic remodeling of neuronal circuits, through synaptic stripping, extracellular digestion, or phagocytosis.

S15-04

Microglia behavior with aging and cerebral amyloidosis

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Microglia cells are essential for brain homeostasis and have crucial roles in neurodegenerative diseases. We have analyzed the morphology and dynamic behavior of microglia in their physiological environment in aging mice using *in vivo* 2-photon microscopy. Additionally, mouse models of cerebral amyloidosis were studied. Our results show changes in microglial soma and process movement with aging and an unexpected longevity of individual microglia throughout the mouse lifespan. Aging is the main risk factor for most neurodegenerative diseases; therefore, age-related changes in function as well as turnover and maintenance of microglia are likely to be important in order to understand their contribution to neurodegenerative diseases.

S16 Synaptic Proteostasis: Regulation and dysregulation of synaptic protein turnover

S16-01

Molecular mechanism of presynaptic integrity

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The mechanisms regulating turnover of synaptic proteins and vesicles are not well understood. Recent studies point to a contribution of autophagy to synaptic function, yet its cellular targets and molecular mechanisms remain enigmatic. Autophagy is an essential cellular program, allowing cells to manage protein repertoires as well as clear aggregated proteins or damaged organelles arising by genetic or environmental insults. Dysregulation of autophagy is seen in many neurodegenerative diseases, yet it remains unclear whether in defects are cell-wide or initiated within specific sub-compartments. Intriguingly, synaptic dysfunction and loss are early hallmarks of neurodegenerative diseases but the molecular mechanisms are unknown. Our analysis of presynaptic active zones reveals that two structural components, Piccolo and Bassoon, are critical regulators of synapse integrity, as synapses disintegrate in their absence. This presentation will discuss how mechanistically, they appear to regulate presynaptic proteostasis by scaffolding and modulating the activity of the ubiquitin, proteasome, endo-lysosomal and autophagy systems and their relationship to neurodegenerative disorders.

S16-02

Protein turnover at synapses: implications for parkinson's disease

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Neurodegeneration is characterized by misfolded proteins and dysfunctional synapses. However, how synaptic compartments normally cope with proteopathic stress resulting from synaptic activity is not understood. We show that the most commonly mutated protein in Parkinson's disease, LRRK2, and a recently identified protein mutated in Parkinson's disease Synaptojain both regulate macroautophagy at presynaptic terminals by regulating the function of EndophilinA. EndophilinA is a synapse enriched protein that deforms membranes, is phosphorylated by LRRK2 and binds tightly to Synaptojanin. This unexpected function of EndophilinA is evolutionary conserved from flies to embryonic stem cell-derived human neurons and is independent of its well-studied role in synaptic endocytosis. Specific targeted dysregulation of synaptic EndophilinA, Synaptojanin and LRRK2-dependent autophagy causes age-dependent degeneration of dopaminergic neurons that are also vulnerable in Parkinson patients. Our work shows the existence of a previously unknown, evolutionary conserved synapse-specific branch of autophagy critical for neuronal survival and relevant to mechanisms of Parkinson's disease.

S16-03

Synaptic proteostasis: beyond one molecule or another

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Imaging studies, carried out over the last decade or so indicate that synapses are not truly structures in a strict sense, and are better thought of as dynamic assemblies of molecules that move in, out and between synapses. Nevertheless, synaptic molecules have finite lifetimes and are ultimately degraded. Until recently, life spans of synaptic proteins or the roles of specific catabolic pathways in their constitutive degradation have not been analyzed systematically, resulting in a somewhat fragmented understanding of basal synaptic protein turnover.

We have used dynamic, pulsed and multiplexed SILAC (Stable Isotope Labeling with Amino acids in Cell culture), mass spectrometry (MS), immunohistochemistry to systematically measure the metabolic half-lives of synaptic proteins and the dependence of synaptic protein degradation on unperturbed proteasomal function. These studies reveal that the half-lives of most synaptic proteins are relatively long (on the order of 3–5 days in primary cell culture). The degradation of several synaptic proteins, previously implicated in glutamate receptor trafficking, was observed to strongly depend, directly or indirectly, on unperturbed proteasomal function, yet the degradation of most synaptic proteins was not significantly slowed in the presence of effective proteasomal inhibitors. By combining information on protein turnover rates with information on protein degradation rates, a simple quantitative relationship emerged that supported conclusions regarding the role of proteasome-based degradation of synaptic proteins. At the same time, however, it pointed to inherent limitations in the use of pharmacological approaches for studying synaptic protein catabolism.

S16-04

Roles of the RAB35/ESCRT pathway in synaptic protein degradation and neurodegenerative mechanisms

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Synaptic vesicle (SV) pools must maintain a functional repertoire of proteins in order to support stable neurotransmission. The breakdown of SV protein turnover mechanisms has been linked to synaptic dysfunction and neurodegenerative disease etiology, yet these mechanisms have remained poorly understood. We have identified a molecular pathway that mediates the activity-dependent degradation of SV membrane proteins at mammalian glutamatergic synapses. This pathway requires the SV-associated GTPase Rab35 and the endosomal sorting complex required for transport (ESCRT) machinery. In particular, we show that neuronal activity induces the

activation of Rab35, which in turn binds to its effector Hrs, the initial ESCRT component. These actions stimulate the recruitment of the ESCRT machinery to SV pools, thereby initiating the formation of multivesicular bodies to transport SV proteins to somatic lysosomes for their degradation. More recently, we have discovered that this pathway also mediates the degradation of the axonal microtubule-associated protein Tau. Furthermore, we find that Rab35 directly regulates Tau pathomechanisms (i.e. Tau accumulation, hyperphosphorylation, and synaptic missorting) that induce synaptotoxicity and neurodegeneration in diseases such as Alzheimer's and frontotemporal dementia. Our findings implicate the Rab35/ESCRT pathway in the degradation of both soluble and integral membrane proteins, and suggest that this pathway could be a promising therapeutic target for the treatment of tauopathies.

S17 Cortical progenitor biology, cell cycling versus neurogenesis

S17-01

Novel mechanisms of neurogenesis

M. Goetz

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The mechanisms of dynamic phylogenetic and ontogentic expansion of different brain regions are still poorly understood. I will present unpublished work about the role of a novel centrosomal protein which is crucial for formation of the subventricular zone and retention of cells therein, a key event in cerebral cortex evolution. I will further present new data on the molecular function of Trnp1, a novel nuclear protein, with key roles in cerebral cortex folding and seeding of the basal radial glial cells into the SVZ in ferret (Stahl et al., Cell 2013; Martinez-Martinez et al., Nature Comm. 2016). Both these proteins highlight that previously considered general cell biological functions are rather cell type specific and their modulation even in subtypes of neural stem cells exerts key roles during neurogenesis and expansion of the cerebral cortex.

S17-02

Zika virus-associated microcephaly is caused by stress-induced unfolded protein response

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Accumulating evidence support a causal link between Zika virus (ZIKV) infection during pregnancy and congenital microcephaly. However, the mechanism of ZIKV-associated microcephaly remains unknown. We combined analyses of ZIKV-infected human and mouse fetal samples to understand how ZIKV induces microcephaly. We show that, by triggering endoplasmic reticulum stress, ZIKV deregulates a physiological unfolded protein response (UPR) that controls neurogenesis and promotes apoptosis of some projection neurons that are settling into the cerebral cortex. Remarkably, we show that inhibiting UPR counteracts these pathophysiological mechanisms and prevents microcephaly in mouse embryos.

S17-03

Wrinkling of human brain organoids on a chip driven by mechanical instabilities

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Wrinkling appears during growth and development of soft natural systems including marine invertebrates, flowers, lungs and the brain. The underlying mechanical instabilities have so far been studied mainly in polymer model systems. Here, we report the emergence of surface wrinkles during *in vitro* development and self-organization of a human neuroepithelium in a micro-fabricated compartment, which supports long-term culturing and *in situ* whole organ imaging. Surface convolutions emerges at a critical cell density, and exhibits linear scaling of wavelength with epithelium thickness. The scaling prefactor increases two-fold in an elastically softer LIS1 mutant cell line, associated with the smooth brain disease (lissencephaly). We identified two opposing active forces, which contribute to differential tissue growth: cytoskeleton contraction at the tissue core, and nuclear expansion during cell-cycle at the tissue perimeter. Collectively, our data suggest that the *in vitro* neuroepithelium wrinkling emerges as a mechanical instability, which is universal for differentially swelling systems, and may have implications for early brain development.

S17-04

Cortical expansion in the development of complex mammalian brains

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Rapid expansion of brain size and complexity is a hallmark of mammalian evolution. The rodent dorsal brain, which is typically lissencephalic, forms a single primary germinal zone, the ventricular zone (VZ), which faces the ventricle on the apical side during development. In the VZ, self-renewing neural progenitors called radial glia undergo interkinetic nuclear movement and divide asymmetrically at the apical surface to give rise to a pair of daughter cells of distinct fates: another radial glial cell and an intermediate progenitor that divides once to generate a few neurons at the adjacent subventricular zone (SVZ). During the development of the complex brain, such as in ferret or primate, however, huge numbers of neurons are generated in the formation of the complex organization of the folded cortical structure. In such gyrencephalic brains, a new germinal zone, the outer SVZ (OSVZ), is formed during neurogenesis, and is thought to play important roles in the expansion of the neuronal population and fortmation of gyrencephaly.

To gain a better understanding of the processes by which the OSVZ is formed from the VZ, we have used the ferret brain as a model of the complex brain in studies using long-term time-lapse imaging of brain slices, lineage analysis, and genetic perturbations (based on CRISPR/Cas9). We found that the cerebral cortex develops in a similar manner to the ganglionic eminence (the ventral side of the telencephalon) in ferret, unlike in rodent models. We discuss recent results from our group in light of this model of OSVZ formation.

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S18 Regulatory pathways of organelles affecting CNS and PNS pathophysiology

S18-01

Regulation of extracellular vesicle sorting of alpha-synuclein

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A large body of evidence supports Braak's hypothesis of toxic α-Synuclein propagation in Parkinson's disease due to intercellular spreading of α-Synuclein pathology. The release of misfolded α-Synuclein by diseased neurons with extracellular vesicles, followed by transfer to so far unaffected neurons where aggregation of soluble α-Synuclein is induced in a prion-like manner, has been proposed as one pathway of disease propagation. However, it remained unclear, whether such transfer occurs *in vivo* in the CNS and which molecular mechanism governs the release of α-Synuclein with extracellular vesicles.

Here we show that extracellular vesicles can deliver functionally active proteins within the central nervous system (CNS) and demonstrate that α-Synuclein is present in extracellular vesicles in the CNS *in vivo*. Importantly, we demonstrate that cerbrospinal fluid (CSF) exosomes from patients with Parkinson's disease and dementia with Lewy bodies contain a pathogenic α-Synuclein species which serves as a seed to induce the aggregation of soluble α-Synuclein in a target reporter cell line. We further characterized sorting determinants and regultors of extracellular vesicle release of α-Synuclein. We find that α-Synuclein sorting to extracellular vesicles depends on the ESCRT machinery (endosomal sorting complex required for transport) which depends on SUMO modification of α-Synuclein. So far, ubiquitination has been regarded as an exclusive, necessary and sufficient signal for ESCRT interaction of proteins. We show that SUMOylation can target proteins to the ESCRT complex via protein-lipid interaction. Taken together, our study sheds light on the mechanism of α-Synuclein transmission between cells and the induction of pathological aggregates in recipient neurons.

S18-02

The autophagosome and lysosome pathway in motoneuron diseases

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The intracellular organelles autophagosomes and lysosomes are the essential components of a high capacity degradative pathway. Autophagosomes have two double layer membranes and are formed by engulfing damaged, misfolded and aggregated proteins or even organelles to be cleared from cells. Autophagosomes fuse with lysosomes, and the entire content of the newly formed autophagolysosomes is digested by the lysosomal enzymes. The entire process is named autophagy, an essenzial component of the protein quality control (PQC) system in cells. The PQC system also requires chaperones and the proteasome that work synergistically to autophagy degradative pathways.Autophagy is altered in motorneuronal diseases (MNDs), like Spinal bulbar muscular atrophy (SBMA) and Amyotrophic lateral sclerosis, in which specific mutant or aberrant misfolded proteins accumulate causing autophagic flux blockage, possibly leading to neuronal death.We found that a peculiar chaperone, the small heat shock protein (HSP) B8, is able to revert the autophagy flux blockage by facilitating the autophagic removal of misfolded proteins prone to aggregate in MNDs. HSPB8 is induced in response to several neuronal stresses such has proteotoxic and oxidative stresses. We found that HSPB8 is highly induced in the two main targets of misfolded protein toxicity in tg mice models of SBMA and ALS, the motorneurons and the muscle. HSPB8 acts in conjunction with BAG3 to bind the HSC70-CHIP mediator of degradation, and the pharmacological or genetic induction of HSPB8 expression is protective in MNDs, while its silencing has opposite effects. We also showed that HSPB8 protects from a misfolded protein induced aberrant phenotype in fly models of ALS.By increasing the HSPB8-mediated selective targeting of misfolded proteins to autophagy neurons and muscle reduce their proteasome-mediated clearance limiting its possible overwhelming.Therefore, pharmacological approached which potentiate the HSPB8-BAG3 autophagic pathway could contribute to maintain a correct proteostasis in motorneuron and muscle cells and might have therapeutic implication in MNDs.

S18-03

The Er-mitochondria axis in fronto-temporal dementia and related amyotrophic lateral sclerosis (FTD/ALS)

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FTD/ALS is characterised by damage to a diverse number of seemingly disparate cellular functions. However, many of these damaged functions are regulated by signaling between mitochondria and the ER. This signaling involves close physical interactions between the two organelles; the regions of ER in contact with mitochondria are termed mitochondria-associated ER membranes (MAM). The mechanisms by which regions of ER form contacts with mitochondria are not properly understood but we identified the integral ER protein VAPB and the outer mitochondrial membrane protein PTPIP51 as molecular scaffolds that interact to tether the two organelles. The VAPB-PTPIP51 tethers regulate IP3 receptor mediated delivery of Ca^{2+} to mitochondria which is a physiological readout of ER-mitochondria contacts. We have shown that Tar DNA binding protein-43 (TDP-43) and Fused in Sarcoma (FUS) which are genetically and pathologically linked to FTD/ALS, both disrupt

ER-mitochondria contacts. This disruption perturbs IP3 receptor mediated delivery of Ca^{2+} to mitochondria. Moreover, we demonstrate that the TDP-43 and FUS induced loosening of ER-mitochondria contacts involves activation glycogen synthase kinase-3beta and reduced binding of VAPB to PTPIP51. Our findings reveal a new molecular target for damage in FTD/ALS.

S18-04

Endogenous fatty acid synthesis is required to maintain peripheral nerve structure and function

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Myelin is a membrane characterized by high lipid content to facilitate impulse propagation. Changes in myelin fatty acid (FA) composition have been associated with peripheral neuropathy (PN), but the specific role of peripheral nerve FA synthesis in myelin formation and function is poorly understood. We explored the extent to which lack of the key regulator of FA synthesis as *Sterol Regulatory Element Binding Factor-1c* (*Srebf-1c*) could result in the development of PN.

Functional, morphological/morphometric analyses were used to evaluate the development of PN in *Srebf-1c* null mice. Genes and metabolites related to PN were identified by transcriptomic and metabolomic approaches.

We found that *Srebf-1c* null mice display a neuropathic phenotype consisting in hypermyelinated small caliber fibers, the result of changes in myelin periodicity. Unexpectedly, transcriptomics and metabolomics revealed activation of peroxisome proliferator activated receptor α (Pparα) signaling in *Srebf-1c* null peripheral nerve as a result of increased levels of two distinct phosphatidylcholine-based Pparα ligands, PC-C16:0/C18:1 and PC-C18:0/C18:1. Pparα is a nuclear receptor that directs uptake, utilization and catabolism of FAs. As a consequence of abnormal local Pparα activation, *Srebf-1c* null peripheral nerve exhibit increased fatty acid utilization, a detrimental condition leading to PN. Treatment with a Pparα antagonist rescues the neuropathy of *Srebf-1c* null mice.

These findings reveal the importance of FA synthesis to sustain peripheral nerve structure and function.

Work supported by Fondazione Cariplo (2014-0991).

S19 Novel mechanisms in Alzheimer's diseases

S19-01

Presenilins-parkin molecular dialogue unravels a functional interplay between Alzheimer's and parkinson's diseases

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Parkin is associated to autosomal recessive early-onset Parkinson's disease and acts as an E3-ubiquitin ligase involved in the proteasome-mediated degradation of various substrates. It has been suggested that pathogenic mutations of parkin, abolishing its ubiquitin-ligase activity, could explain the accumulation of proteins and lead to neuronal death by apoptosis. However, besides this function, additional parkin-dependent cellular pathways exist. We demonstrated that parkin is a direct transcriptional repressor of the tumor suppressor p53. p53 regulates the expression and functions of presenilin-1 (PS1) and presenilin-2 (PS2), two members of the gamma secretase complex involved in the production of the amyloid β peptide (Aβ) and parkin could control the homeostasis of intracellular Aβ. These findings prompted us to investigate whether parkin could control presenilins and if so, whether it is via a direct transcriptional control of PS promoters or indirectly, via p53.

We show that parkin controls presenilin 1 and 2 expressions, promoter activity, and mRNA levels *ex vivo* and in mouse brains. This regulation impacts on PS-dependent γ-secretase activity and presenilin-mediated control of cell death. This control is independent of parkin ubiquitin-ligase activity, does not involve p53 and is not affected by PS1 and PS2 functional interplay. Parkin binds to presenilins promoters via a consensus binding sequence that we identify and validate by functional analysis.

This study delineates a putative interplay between Parkinson's and Alzheimer's diseases. Furthermore, we identified a common putative parkin responsive element on p53 that should help identifying novel transcriptional targets of parkin and unravel putative additional functions functions.

S19-02

Selective filtering defect at the axon initial segment in Alzheimer's disease mouse models

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Axon pathology has been widely reported in Alzheimer's disease (AD) patients and AD mouse models. We now report that miR-342-5p is upregulated in APP/PS1, PS1ΔE9 and PS1-M146V transgenic AD mice that is mechanistically linked to elevated β-catenin, c-Myc and interferon regulatory factor (IRF)-9. The increased miR-342-5p downregulates the expression of ankyrin G (AnkG), a protein known to play a critical role in establishing selective filtering machinery at the axon initial segment (AIS). Furthermore, decreased AnkG expression led to defective AIS filtering in cultured hippocampal neurons from AD mouse models, as monitored by selective exclusion of large macromolecules from the axons. Furthermore, AnkG-deficiency impaired AIS localization of Na_v 1.6 channels and confined NR2B to the somatodendritic compartments. The expression of exogenous AnkG improved the cognitive performance of 12 months old APP/PS1 mice. Thus, our data suggested that AnkG and impairing AIS filtering may play important role in AD pathology, indicating the presence of early developmental defects of neurons in familial AD mutation carriers.

S19-03

New functions of the Alzheimer protease bace1 in inhibitory neurotransmission

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Objectives: BACE1 is a key drug target in Alzheimer's disease. However, it remains unclear whether therapeutic BACE1 inhibition is safe, because BACE1 has an increasing number of physiological substrates and functions. Here, we identify a new phenotype of BACE1-/- mice and a contributing substrate.

Methods: We used electrophysiology, proteomics, primary neuronal cultures and *in vitro* BACE1 assays.

Results: Using electrophysiology we identified a new phenotype in BACE1-/- mice, namely an altered transmission at inhibitory synapses. Using proteomics we identified known BACE1 substrates, such as APP, contactin-2 and CHL1, as well as new BACE1 substrates, including synaptic cell adhesion proteins, from the brain of BACE1-deficient mice. One of the newly identified immunoglobulin-domain containing cell adhesion proteins was found to interfere with the interaction between neurexins and neuroligins at inhibitory synapses, thus explaining the phenotype in BACE1-deficient mice. Cleavage of the Ig-domain-containing protein was strongly reduced in BACE1-/- mice and in BACE inhibitor-treated primary murine neurons. Cleavage occurred directly by BACE1 as shown in an *in vitro* assay. Furthermore, we demonstrate that several of the identified proteins, including SEZ6, can be detected in murine CSF and that their levels are strongly reduced in BACE1-/- mice.

Conclusions: We have identified a new physiological function for BACE1 in controlling synaptic strength in the murine brain, which may cause potential side-effects of BACE1 inhibitors in patients. Additionally, we demonstrate that several BACE1 substrates are excellent markers to monitor BACE1 activity and target engagement *in vivo* in mice.

S19-04

A novel ad-associated PS1 mutation: new insights into ad pathogenesis and drug development

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Deposition of amyloid-beta protein (Aβ) to form neuritic plaques is the unique neuropathological hallmark of Alzheimer's disease (AD). Aβ is derived from amyloid-beta precursor protein (APP) through sequential cleavages by β- and γ-secretase. Presenilin 1 is

the the catalytic subunit of the γ-secretase complex. Pathogenic mutations in presenilin 1 gene (PSEN1) account for the majority of the autosome-dominantly inherited familial AD (FAD) cases. PSEN1 mutations not only impair APP processing and Aβ generation, but also reduce the cleavage of Notch, one of the most prominent substrates of γ-secretase, leading to Notch signaling disruption. In this study, we found that a novel PSEN1 mutation significantly impairs APP processing and Aβ generation. We generated a transgenic mouse strain carrying this mutation. This PSEN1 mutation promotes neuritic plaque formation, and learning and memory deficits in the AD transgenic mice. However, the mutant PSEN1 undergoes normal endoproteolysis, and displays normal functions on Notch cleavage and Notch signaling *in vitro* and *in vivo*, sparing the impairment of Notch signaling. Taken together, we first demonstrate that a novel mutant PSEN1 functions separately on APP processing and Notch signaling pathway. Furthermore, our study provides a novel insight into developing γ-secretase modulators for AD treatment by specifically modulating APP processing, not affecting Notch cleavage to avoid the severe side effects of γ-secretase inhibitors on Notch signaling.

S20 NMDA receptors: from molecular structure to synaptic regulation and disease

S20-01

Structure and pharmacology of NMDA receptors

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N-methyl-D-aspartate receptor (NMDAR) belongs to the large family of ionotroic glutamate receptors (iGluRs), which are critically involved in basic brain functions and development as well as brain diseases and disorders. They are large heterotetrameric membrane protein complexes of ~550 KDa with heavy glycosylation. The extracellular domains recognize neurotransmitters, antagonists, metals, and allosteric compounds and regulate activity of the transmembrane ion channel. Here, I will talk about how the NMDA receptor subunits are shaped and organized to form an ion channel and how their modular domains may move to mediate specific functions.

S20-02

Interrogating NMDA receptor functional diversity using optochemical tools

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NMDA receptors (NMDARs) are glutamate-gated ion channels that play crucial roles in brain development and function. In particular, they exert control over many forms of synaptic plasticity that underlie learning and memory. NMDARs are also targets of therapeutic interest since their dysfunction is associated to numerous neuropsychiatric disorders such as schizophrenia, mental retardation and epilepsy. At the molecular level, NMDARs form large tetrameric complexes composed of two GluN1 subunits and two GluN2 or GluN3 subunits encoded by six different genes (GluN2A-D, GluN3A-B). This broad molecular heterogeneity translates into a wide variety of receptor subtypes, each with distinct biophysical, pharmacological and signaling properties. This is further diversified by their differential location between brain regions, developmental stages, and even subcellular localizations, supporting the idea that each receptor subpopulation is tailored to match the strict requirements of specific neuronal functions. Understanding the physiological relevance of this diversity on normal and diseased brain function is currently a major challenge. For this purpose, there is great need for novel approaches allowing the manipulation and interrogation of individual receptor subtypes in a direct manner and with high temporal resolution.

By providing precise ways to control endogenous signaling proteins, optopharmacological approaches open new avenues for deciphering the molecular basis of neuronal excitability and brain function. Optopharmacology combines the power of optics, endowing high spatiotemporal resolution, with that of genetics and pharmacology, to achieve unique photocontrol at the molecular receptor level. Recently, we set out to develop a set of NMDAR subunits that can be precisely controlled by light using a variation of receptor engineering methodologies. Light sensitivity was successfully achieved by either attaching photoswitchable ligands, both at orthosteric and allosteric sites, or by directly encoding light-sensitive amino acids by means of the genetic code expansion technology. Our results demonstrate the feasibility, utility, and general applicability of these approaches to probe the structure and biophysics of a key neurotransmitter receptor. We are now aiming at implementing these innovative optochemical tools in more native situations for *in vivo* optogenetic dissection of specific neuronal receptor functions.

S20-03

Molecular mechanisms regulating synaptic expression of NMDA receptors

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The synaptic expression of NMDA receptors is exquisitely regulated by subunit-specific phosphorylation and protein-protein interactions. In particular, we have found that phosphorylation of the GluN2B C-terminal domain on two nearby residues plays a critical role in regulating binding to the PSD-95 family of proteins (S1480) or the endocytic machinery (Y1472). The phosphorylation of these sites has opposing effects on NMDA receptor stability on the plasma membrane and at synapses. Specifically, the tyrosine phosphorylation of Y1472, which falls within a strong endocytic motif, disrupts binding to clathrin adaptor proteins and enhances surface expression. STriatal-Enriched protein tyrosine Phosphatase, $STEP_{61}$, is a member of the family of intracellular tyrosine-specific phosphatases and GluN2B Y1472 is dephosphorylated by $STEP_{61}$, thereby increasing endocytosis of GluN2B-containing NMDARs. Therefore, $STEP_{61}$ is a strong regulator of the surface expression of NMDA receptors. We have recently demonstrated that PSD-95 binds directly to $STEP_{61}$, whereas other PSD-95 family members do not. Furthermore, PSD-95 expression triggers the degradation of $STEP_{61}$. Thus PSD-95 stabilizes NMDA receptors via direct binding and also through this novel mechanism of degrading the negative regulator $STEP_{61}$. In addition, our studies reveal that $STEP_{61}$ specifically regulates extrasynaptic NMDA receptors, because PSD-95 limits the localization of STEP at the PSD. Finally, we find that $STEP_{61}$ has a robust effect on AMPA receptors as well as NMDA receptors pointing to a central role for STEP in organizing glutamate receptors at synapses.

S20-04

Suppression of NMDA receptor expression enhances synaptic inputs to prefrontal cortex and increases motivated escape behaviour

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A single, low dose of the NMDA receptor (NMDAR) antagonist ketamine elicits long-lasting antidepressant effects in patients suffering from treatment-resistant depression (TRD). Developing a detailed mechanistic understanding of how NMDAR antagonism alters synapses and circuits is pivotal to developing translatable therapies for TRD and other neuropsychiatric conditions associated with amotivation. We are using viral vectors, anatomical tracing, fMRI, and optogenetic-assisted circuit analysis to assess the consequences of suppressing NMDAR function in driving cellular, synaptic, and circuit-level changes in medial prefrontal cortex (mPFC). Our experiments demonstrate that selective, post-developmental deletion of the NMDAR subunit GluN2B, from pyramidal neurons in mPFC, increases synaptic drive onto these neurons and enhances their output. Interestingly, we found that this genetic manipulation alters functional connectivity into mPFC in an input specific manner. Our findings identify a novel and potentially targetable circuit for promoting motivated behaviour.

S21 Perineuronal nets: from molecular assembly to plasticity in neurological diseases

S21-01

The hyaluronan and proteoglycan link proteins: organizers of the brain ecm and key molecules for neuronal function and plasticity

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Perineuronal nets (PNNs) are pericellular coats of condensed matrix that enwrap the cell bodies and dendrites of certain neurons in the adult central nervous system. PNNs are completed at the end of developmental critical periods for experience-dependent plasticity and contribute to the stabilization of specific connection patterns, thus limiting plasticity. PNNs further modulate synaptic activity, and play a neuroprotective role.

The hyaluronan and proteoglycanbinding link protein (Hapln) is a key molecule in the formation and control of hyaluronan-based condensed perineuronal matrix in the adult brain. Hapln4/Bral2-deficient mice have attenuated PNNs mainly in the brainstem and cerebellum, and are markedly affected in the localization of brevican in all of the nuclei tested, whereas no effect was seen on aggrecan. The mutant mice showed impairment in the auditory assessment in accordance with an increase of maturation of PNN.

In our previous report, the number of synapses at the deep cerebellar nuclei (DCN) was reduced. A variety of excitatory and inhibitory projections, representing several streams of sensory-motor information, converge at DCN. It remains unclear whether Hapln4/Bral2 deficiency affects both types of synapses or either one. To elucidate the functional roles of Hapln4/Bral2 at synapse, we investigated the properties of synaptic transmission at DCN in Hapln4/Bral2 KO mice. We applied slice patch-clamp technique to acute cerebellar slices around postnatal day 14 (P14). We found that the evoked IPSC amplitudes were reduced in Hapln4/Bral2 KO mice whereas the evoked EPSC amplitudes were relatively similar to wildtype mice. In support of this finding, the number of immunolabeled GABAergic Purkinje cell terminals was significantly reduced at the DCN. These results provide strong evidence that Hapln4/Bral2 is important for the functional connectivity of inhibitory synapses at the DCN.

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S21-02

Perineuronal nets: from molecular assembly to plasticity enhancement

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Perineuronal nets (PNNs) are aggregated extracellular matrix (ECM) structures formed at the end of the critical period, a time when the central nervous system (CNS) is plastic and adaptive to environment changes, and are crucial in controlling plasticity. The basic composition of PNNs includes members from four families: chondroitin sulphate proteoglycans, hyaluronan and its synthases, tenascins and hyaluronan binding link proteins. These ECM molecules in the CNS assemble hierarchically and condense into a dense layer of matrix structures on the surface of neurons. The formation of PNNs closes the critical period and consolidates the neuronal circuits during late development. Our recent finding suggests that there is a high heterogeneity of PNN composition in different areas of the brain and in the spinal cord. The different molecular composition further fine-tunes the precise functions of the PNNs. In this talk, I shall take a molecular approach and address the interplay between PNN composition and its function.

S21-03

On the role of the perineuronal net (PNN) enveloping fast spiking (FS) neocortical interneurons in long-term memory

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In the neocortex, stimulation of Fast Spiking (FS) Parvalbumin (PV) interneurons triggers gamma oscillation, an electrical expression of long-term memory. Recently two classes of FS-PV interneurons in the S1 region of the mouse cerebral cortex were described. One class co-expressed PV with Somatostatin and was not surrounded by PeriNeuronal Net (PNN). The other class was enwrapped by PNN and contained specific proteases (Adamts) known to reshape the PNN. This observation is important since modifying the structure of PNN affects plasticity, critical period and long-term memory. Recently taxonomic data base of the Allen Institute data bank of RNA-Seq of 1600 individual brain cells have revealed similar subclasses in V1 cerebral cortex of the mouse. The subclass of FS-PV interneurons containing Adamts expresses also aggrecan, an important component of PNN. The subclass of FS-PV interneurons expressing Somatostatin did not express aggrecan nor Adamts.

Rossier, J et al. Cortical fast-spiking parvalbumin interneurons enwrapped in the perineuronal net express the metallopeptidases Adamts8, Adamts15 and Neprilysin. Molecular Psychiatry (2015) 20, 154–161; https://doi.org/10.1038/mp.2014.162

S21-04

Perineuronal nets: current findings in psychiatric disorders

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Rapidly emerging evidence points to extracellular matrix (ECM) abnormalities as a key component of the pathophysiology of several psychiatric disorders. Human genetic studies have identified key ECM molecules, including chondroitin sulfate proteoglycans (CSPGs), Reelin, semaphorin 3A, integrins and ECM remodeling enzymes (e.g. metalloproteinases), as potential risk factors for a number of disorders such as schizophrenia, bipolar disorder, depression and autism spectrum disorder. Other studies in humans have shown decreased Reelin expression in the prefrontal cortex, hippocampus, and cerebellum, as well as in blood, of subjects with bipolar disorder or major depression. Similarly, involvement of ECM molecules has been reported in Fragile X syndrome and Rett Syndrome, this latter also shown to have PNN abnormalities. Our human postmortem investigations have focused on organized ECM structures, such as perineuronal nets (PNNs), one of the most well studied ECM structures, as well as ECM 'clusters' expressing a CS-6 sulfated form of CSPG (CS-6 clusters). We show marked decreases of PNNs in the amygdala, entorhinal cortex and prefrontal cortex of subjects with schizophrenia and bipolar disorders. These abnormalities were observed by immunolabeling PNNs with several of their components, thus suggesting a profound disruption of these structures. Notably, PNN decreases are accompanied by altered CSPGs expression in glial cells, a significant finding because these cells represent the main contributors to the ECM/PNNs molecular building blocks. CS-6 clusters were also found to be markedly decreased in the amygdala of subjects with schizophrenia and bipolar disorder.

We note that ECM/PNN molecules, and their cell surface receptors, mediate a broad range of synaptic regulatory functions impacting dendritic spine and synapse structure and plasticity, glutamatergic and GABAergic transmission. These functions resonate well-established evidence for a disruption of synaptic functions in several psychiatric disorders. For instance, loss of dendritic spines, pre- and postsynaptic regulatory elements have been reported in several of the disorders mentioned above. We put forth the hypothesis that ECM/PNNs abnormalities may contribute to synaptic abnormalities in these disorders.

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Young Investigator Colloquia

YIC01 Circuitry, Plasticity and Development

YIC01-01

Uncovering early molecular drivers of TDP-43 proteinopathy using novel transgenic mouse models of disease

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Frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) are progressive neurodegenerative diseases that ultimately lead to death, with no effective disease-modifying treatments. Although with seemingly disparate symptoms, most cases of both ALS and FTD are characterised pathologically at autopsy by accumulation of phosphorylated TAR DNA binding protein of 43 kDa (TDP-43) in affected neurons. An RNA/DNA-binding protein normally located primarily in the nucleus, TDP-43 aggregates and forms cytoplasmic inclusions in disease. However, the early drivers of disease pathogenesis have remained largely unclear in part due to a lack of valid mouse models that recapitulate the key features of human disease. To address this problem, we have characterised in detail new doxycycline-inducible transgenic mouse models in which cytoplasmically-targeted TDP-43 is expressed under the control of the *neurofilament heavy chain* (*NEFH*) promoter. In these mice, TDP-43 is expressed broadly in the brain and spinal cord upon removal of doxycycline. This leads to pathological disease features (accumulation of insoluble phosphorylated TDP-43, neuron loss in the brain and spinal cord, muscle atrophy and denervation) in conjunction with a progressive motor phenotype reminiscent of ALS (limb weakness, loss of coordinated movement, weight loss, and paresis leading to death). Advanced quantitative mass spectrometry (SWATH-MS) screening of the brains, spinal cords, and blood of these mice over the time-course of disease reveals more than 200 proteins with significantly altered levels compared to non-transgenic controls. Along with confocal fluorescence imaging, biochemical and RNA analyses, and *in vivo* testing of targeted potential disease-modifying compounds, we are investigating how these molecular changes contribute to disease onset and progression. These studies are revealing the most upstream pathological events in TDP-43 proteinopathies, and thereby offer possibilities for identification of new therapeutic avenues for these devastating diseases.

YIC01-03

Neuroprotective functions for the histone deacetylase SIRT6

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The histone deacetylase SIRT6 promotes DNA repair, while its activity declines with age, with a concomitant accumulation of DNA damage. Furthermore, SIRT6-KO mice exhibit an accelerated aging phenotype and die prematurely. Here we report that brain-specific SIRT6 deficient mice survive, but present behavioral defects with major learning impairments by 4 months of age. Moreover, the brains of these mice present increased signs of DNA damage, cell death and hyperphosphorylated Tau, a critical mark in several neurodegenerative diseases. Mechanistically, SIRT6 regulates Tau protein stability and phosphorylation through increased activation of the kinase GSK3α/β. Finally, we found that SIRT6 mRNA and protein levels are reduced in patients with Alzheimer's disease. Together, our results suggest that SIRT6 is critical to maintain genomic stability in the brain and its failure leads to toxic Tau stability and phosphorylation. Therefore, SIRT6 and its downstream signaling could be targeted in Alzheimer's disease and age related neurodegeneration.

YIC01-04

Environmental and pharmacological modulation of molecular pathogenesis in Huntington's disease

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Huntington's disease (HD) is a devastating neurodegenerative genetic disorder involving the progressive development of psychiatric symptoms, cognitive deficits and motor impairment. An estimated 50% of HD patients develop clinical depression which is often diagnosed decades prior to motor onset. The R6/1 mouse model of HD expresses a mutant human huntingtin transgene and provides an accurate disease model exhibiting strong construct and face validity. Our group was the first to show that female R6/1 HD mice display depression-related behaviours, prior to cognitive and motor deficits, consistent with the progressive development of clinical symptoms in HD.

While there is currently no cure for HD, we previously found that environmental enrichment and enhanced physical activity, were able to slow down the progression of the disease. In addition, we recently published beneficial effects of N-Acetylcysteine (NAC), a cysteine donor (glutathione precursor), on mitochondrial function and motor deficits in HD mice. We then demonstrated that HD mice had lower basal levels of cystine, and showed depressive-like behaviours in the forced-swim test. Administration of NAC reversed these behaviours. This effect was blocked by co-administration of the system xc- and GLT-1 inhibitors CPG and

DHK, showing that glutamate transporter activity was required for the antidepressant effects of NAC. NAC was also able to specifically increase glutamate in HD mice, in a glutamate transporter-dependent manner. Furthermore, NAC was able to rescue changes in key glutamate receptor proteins related to excitotoxicity in HD, including NMDAR2B. Altogether, our data suggest an undiscovered key role of system xc- in both the pathogenesis of HD and the mechanism of action of NAC. These findings have implications for the development of new therapeutic approaches for HD and depressive disorders.

YIC02 Mechanisms of Glial Function, including Inflammation

YIC02-01

Human genetics uncover fundamental principles of cellular recognition in wiring circuits during development and disease

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Developing neurons integrate into functional circuits through a series of cell recognition events that lead to neurodevelopmental disorders when they go awry. These cell recognition events include neuronal sorting, axon and dendrite recognition, and synaptic selection, among others. To determine the molecular mechanisms that specify neuronal connectivity, we performed whole exome sequencing in patients with a brain-wiring syndrome, Diencephalic Mesencephalic Junction Dysplasia, characterized by failed cortical axonal projections. Here we identify mutations in non-clustered protocadherin *PCDH12,* a cell-surface recognition molecule that mediates interactions between neurons during the process of circuit assembly. Clustered protocadherins provide combinatorial cell surface diversity required for neuronal self-recognition and self-avoidance, but little is known about the role of non-clustered protocadherins. Loss of *PCDH12* in human or mouse resulted in abnormal white matter tracts. Patient-derived neurons were defective in cell-cell contact dependent neurite growth and showed dysregulated actin signaling. PCDH12 mediated strict homophilic cellular recognition, but unlike clustered protocadherins, mismatches with other non-clustered members did not prevent recognition. The results reveal a role for PCDH12 in human brain wiring, and suggest that non-clustered and clustered protocadherin families use distinct mechanisms to determine cellular identity. This is an example on how using human genetics reveals fundamental principles of cellular recognition in wiring circuits and contribute to our understanding of how neuronal dysfunction and neurobehavioral disorders arise from abnormalities during the development of the human brain.

YIC02-02

NEURAL AND BEHAVIOURAL CHANGES IN MALE PERIADOLESCENT MICE AFTER PROLONGED NICOTINE - MDMA TREATMENT

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The interaction between MDMA and Nicotine affects multiple brain centers and neurotransmitter systems (serotonin, dopamine and glutamate) involved in motor coordination and cognition. In this study, we have elucidated the effect of prolonged MDMA, Nicotine and a combined Nicotine-MDMA treatment on motor-cognitive neural functions. In addition, we have shown the correlation between the observed behavioural change and neural structural changes induced by these treatments in BALB/c mice. We observed that MDMA (2 mg/Kg body weight; subcutaneous) induced a decline in motor function, while Nicotine (2 mg/Kg body weight; subcutaneous) improved motor function in male periadolescent mice. In combined treatment, Nicotine reduced the motor function decline observed in MDMA treatment, thus no significant change in motor function for the combined treatment versus the control. Nicotine or MDMA treatment reduced memory function and altered hippocampal structure. Similarly, a combined Nicotine-MDMA treatment reduced memory function when compared with the control. It is noteworthy to mention that a combined treatment increased the rate of lipid peroxidation in brain tissue.

YIC02-03

Pacsin regulates the dynamics of AMPA receptor trafficking and synaptic plasticity

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The AMPA-type ionotropic glutamate receptor mediates the majority of excitatory synaptic transmission in the mammalian central nervous system. The dynamic trafficking of AMPA receptor is one of the major mechanisms controlling synaptic plasticity, a cellular correlate of learning and memory. Previously, we have identified a functional interaction between two BAR domain containing proteins, PACSIN/syndapin and PICK1, in regulating AMPA receptor internalisation and cerebellar long-term depression (Anggono et al., 2013, *Proc. Natl. Acad. Sci. USA*). However, the molecular mechanism by which PACSIN regulates the dynamics of AMPA receptor trafficking remains elusive. Here I will describe the use of the pH-sensitive green fluorescent protein, pHluorin, tagged to the extracellular domain of the GluA2 subunit (pH-GluA2) to track the internalisation and recycling of AMPA receptors in living hippocampal neurons following the activation of NMDA receptors. Structure and function analysis reveals a requirement for the PACSIN1 F-BAR and SH3 domains in controlling these NMDAR-dependent processes. Interestingly, the variable region, which binds to PICK1, is not essential for NMDAR-dependent GluA2 internalisation and is required only for the correct recycling of AMPARs. Overall, our data demonstrate a critical role for the membrane deformation protein, PACSIN1, in controlling the dynamic trafficking of AMPA receptors by linking the endocytic and recycling machineries in neurons.

YIC02-04

Dopamine and DARPP-32 regulate the development of gabaergic system in zebrafish larvae brain

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Imbalance in dopamine-mediated neurotransmission, as well as neurodevelopmental abnormalities, are both features of schizophrenia. It is well known that dopaminergic receptors regulate DARPP-32 and Akt in the adult brain, which play a key role in the regulation

of transcriptional factors and kinases. Several articles have reported decreased levels of DARPP-32 and Akt in the prefrontal cortex of schizophrenic patients. In addition, the main target of antipsychotics – the dopaminergic receptor D2 – modulates the activity of both DARPP-32 and Akt. However, it is possible that dopaminergic signalling also plays an important role in the development of the brain and behavior. To address this hypothesis, we used zebrafish larvae as experimental model. First, we demonstrated that dopamine regulates Akt signaling in the developing brain of zebrafish. We also showed that zebrafish DARPP-32 gene sequence is approximately 46% similar to human DARPP-32 and that dopamine regulates DARPP-32 phosphorylation in the developing brain. We then investigated if alterations in dopaminergic signaling in the 3 to 5 dpf developmental window could affect the development of GABAergic system and of motor behavior. We showed that dopamine has an inverted U-curve function in the development of GABAergic system. Finally, we demonstrated that DARPP-32 knockdown decreases the number of GABAergic neurons only in the pallium. Thus, we observed that dopamine regulates Akt and DARPP-32 in the developing and that it is involved in the development of GABAergic system. These results will help shape our understanding of the role of dopamine in brain development and provide new mechanistic insights for further assessing the neurodevelopmental origin model of schizophrenia.

YIC03 Behavior, Addiction and Psychobiology

YIC03-01

Activation of hippocampal neurogenesis and reversal of cognitive deficits in Alzheimer's rat model by ethosuximide by PI3k/Akt/Wnt

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Neurogenesis is a process of generation of new neurons through neural stem cell" s (NSC) proliferation, differentiation, and integration into existing neuronal circuitry in the neurogenic niche regions such as dentate gyrus (DG) of the hippocampus and sub-ventricular zone (SVZ). Adult hippocampal neurogenesis is found to be altered in several neurodegenerative disorders including Alzheimer" s disease (AD). Ethosuximide (ETH), is an anticonvulsant drug and used for the treatment of epileptic seizure. However, the effects of ETH on adult hippocampal neurogenesis and associated cellular and molecular mechanism(s) are not known yet. Therefore, we studied the effects of ETH on rat NSC proliferation and neuronal differentiation in vitro, and adult hippocampal neurogenesis in an amyloid beta (Aβ) induced rat model of AD. ETH potently increased proliferation of NSC and induced neuronal differentiation in the hippocampal derived NSC. ETH induced NSC proliferation (increased $BrdU^+$ and BrdU/Nestin+ co-labeled cells) and neuronal differentiation (increased $BrdU/DCX^+$, $BrdU/NeuN^+$ co-labeled cells), leading to behavioral recovery in rat AD model. ETH also inhibited Aβ mediated suppression of neurogenic and Akt-Wnt/β-catenin pathway genes expression in the hippocampus. ETH activated the PI3K/Akt and Wnt/β-catenin transduction pathways that are known to be involved in the regulation of neurogenesis. Inhibition of the PI3K/Akt and Wnt/β-catenin pathways effectively blocked the mitogenic and neurogenic effects of ETH. *In silico* molecular target prediction docking studies suggest that ETH interacts with Akt, Dkk-1 and GSK-3β. Our findings suggest that ETH stimulates NSC proliferation and differentiation *in vitro* and adult hippocampal neurogenesis *via* the PI3K/Akt and Wnt/β-catenin signaling.

YIC03-02

A transcriptional code for experience-dependent plasticity

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Our brain is wired to learn from experience and improve our capacity to interact with the world. This fundamental process of 'experience-dependent plasticity" has mostly been studied electrophysiologically (AMPAR/NMDAR ratios), which provides an important conceptual framework for understanding the circuit modifications that are involved in encoding a particular experience. However, measurement of synaptic plasticity is lacking in that different experiences, often with opposing valence, appear to be encoded through similar modification of synaptic strength at the same synapses. In addition, this measure provides little information regarding the molecular mechanisms underlying the circuit modification.

In this talk, I will present recent evidence we have accumulated in my lab, demonstrating a transcriptional code for experience-dependent plasticity. We studied 15 well defined and carefully calibrated salient experiences ranging from rewarding: contingent development of a sucrose habit – following the initial exposure or 10 days of repeated exposures to 10% sucrose; cocaine experiences (acute cocaine administration, chronic cocaine administration and acute cocaine in mice abstinent from chronic administration) to aversive experiences: intraperitoneal LiCl injections, footshock, as well as a host of control conditions. For each condition, we studied the dynamics of immediate-early gene transcription at 1hr, 2hrs and 4hrs following the experience in up to 8 brain structures from each mouse (prefrontal cortex, anterior cingulate cortex, nucleus accumbens, dorsal striatum, amygdala, lateral hypothalamus, hippocampus and ventral tegmental area) using high-throughput microfluidic qPCR and RNAseq.

We observe very low variation between individual mice in the transcriptional response to a given experience, whereas each experience had a very robust and reliable transcriptional signature associated with it. The transcriptional patterns we observed were clear enough to enable the decoding of the recent experience of each individual mouse with above 90% accuracy. Furthermore, a small subset of the transcriptional response is sufficient for this decoding.

We believe this new approach to the investigation of experience-dependent plasticity, which we coin "behavioral transcriptomics" is a widely applicable approach to investigating the encoding of experience in the brain, as well as the formation of habits and compulsions.

I will describe our observations into how repeated aversive and rewarding experiences are differently processed in relevant brain structures, leading to habituation or habit formation. Furthermore, I will demonstrate how the 'behavioral transcriptomics" approach provides a powerful handle, enabling identification of neuronal ensembles encoding aversive and rewarding experiences, and our work into mechanistic investigation of the function and connectivity of these ensembles using pharmacogenetics, optogenetics and virus-based circuit mapping in transgenic mice. As this approach provides information regarding the identity of particular gene products induced in defined brain nuclei which potentially encode features of a given experience, I will describe the steps we are making, using single-molecule flurorescent in-situ hybridization, cell-specific ribosomal profiling, CRISPR and shRNA in order to investigate the function of specific gene products in the development of experience-dependent plasticity at the molecular and behavioral level.

It is our conviction that these avenues of investigation will lead to a new level of understanding of the mechanisms underlying the encoding of experience, and how encoding could go awry in neuropsychiatric disorders.

YIC03-03

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. However, it was not known how the m^6A RNA methylome is regulated by experience. In this study, we aimed to investigate how m^6A is regulated during associative fear learning and whether it may be important for learning and memory processes.

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 medial prefrontal cortex (mPFC) following behavioural training. Transcriptome-wide profiling of m^6A revealed learning-specific patterns of RNA methylation, with the highest enrichment positioned near the stop codon. In primary cortical neurons, *in vitro*, modulation of m^6A by the RNA demethylase FTO influenced learning- and plasticity-related mRNA stability.

Finally, the impact of modulating m^6A on learning was tested *in vivo*. The expression of the m^6A methyltransferase, *Mettl3* and the m^6A demethylase, *Fto* correlated with behavioral training–induced increases in m^6A levels. Targeted knockdown of FTO in the mPFC led to enhanced consolidation of cued fear memory, without affecting basal anxiety level. Taken together, these findings provide the first demonstration of m^6A role in learning and memory.

YIC03-04

MGLU5 receptors are necessary for extinction of drug associated cues and contexts

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Drug-associated cues and contexts are strong predictors of relapse. We used complex behavioural preparations to examine whether extinction of such cues reduces their capacity to trigger drug-seeking. We also examined whether the mGlu5 receptor is necessary for extinction learning. In Experiment 1, rats were trained to lever press for cocaine. Once stable responding was established, the context was extinguished by replacing the rats in the chambers, but with no opportunity to respond (levers were retracted). Control group remained in their home cage. An mGlu5 receptor negative allosteric modulator (MTEP) or vehicle was administered immediately after context extinction sessions. During subsequent drug-induced reinstatement, rats responded less if they had received context extinction; however, this effect was attenuated where MTEP had been applied. In Experiment 2, rats were trained to lever press for cocaine, now paired with a cue light. To extinguish the cue, half of the rats were placed in the chambers and given non-reinforced presentations of the cue, but with the levers retracted. Control rats remained in home cage. All rats received either MTEP or vehicle 20 minutes prior. Cue-induced reinstatement was tested the following day by re-pairing the lever with the light. Rats gave fewer drug-seeking responses following cue extinction. This effect was attenuated by MTEP. Experiment 3 followed the same protocol as Experiment 2, except that a positive allosteric modulator CDPPB or vehicle was administered 20 minutes before CS extinction. At reinstatement, cue-elicited cocaine seeking was lower for the animals that had previously been administered CDPPB, regardless of extinction condition. This study highlights the important role cues and contexts play in driving drug-seeking behaviour during reinstatement. It also shows that mGlu 5 signalling is necessary for extinction of drug-cue associations, and that mGlu5 positive allosteric modulators are promising targets for treating cocaine addiction.

YIC04 Disease, Neurodegeneration and Therapy

YIC04-01

Nitrosative stress-induced disruption of baroreflex neural circuits in a rat model of hepatic encephalopathy: a DTI study

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Background: In acute hepatic failure, the onset of hepatic encephalopathy (HE) is an indication for liver transplantation, without which is associated with 50–90% mortality. Noting that defunct baroreflex is a clinical marker of brain death, we assessed the hypothesis that nitrosative stress in nucleus tractus solitarii (NTS), the terminal site of baroreceptor afferents in brain stem, or rostral ventrolateral medulla (RVLM), the origin of sympathetic innervation of blood vessels, underpins the high mortality associated with HE by inducing defunct baroreflex.

Methods: A thioacetamide (TAA)-induced acute liver failure model of HE employing Sprague-Dawley rats was used. Rats received intraperitoneal injection of TAA for 3 consecutive days. MRI/diffusion weighted imaging (DWI)/diffusion tensor imaging (DTI) of the brain stem was performed daily, together with blood pressure, heart rate and indices of baroreflex recorded by radiotelemetry.

Results: Intraperitoneal administration of TAA increased HE severity and mortality. DWI showed a progressive reduction in apparent diffusion coefficient in the brain stem. DTI further revealed that the connectivity between the NTS and nucleus ambiguus (NA), the origin of the vagal innervation of the heart, was progressively disrupted though sustained, concurrent with impaired but persistent cardiac vagal baroreflex. On the other hand, the connectivity between NTS and RVLM was progressively disrupted until its disappearance, coincidental with the abolition of baroreflex-mediated sympathetic vasomotor tone that signifies brain death clinically. Furthermore, superoxide, nitric oxide, peroxynitrite and ammonia levels in the NTS or RVLM were elevated, alongside swelling of astroctytes. A scavenger of peroxynitrite delivered intracisternally reversed all these events.

Conclusions: Nitrosative stress because of augmented peroxynitrite related to accumulation of ammonia and swelling of astrocytes in the NTS or RVLM, leading to cytotoxic edema in the brain stem and severance of the NTS-RVLM connectivity, underpins defunct baroreflex-mediated sympathetic vasomotor tone that accounts for the high mortality associated with HE.

YIC04-02

HDAC Inhibitors as a tool for chemical preconditioning of the brain: similarity of underlying mechanisms with hypoxic precondition

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Severe hypobaric hypoxia that occurs at atmospheric pressure of 180 mm Hg is a harmful factor producing extensive injury of susceptible brain neurons. Mechanistic basis of the injury is associated with changed expression of many genes, implying the involvement of epigenetic processes, in particular acetylation/deacetylation of histones. Inhibitors of histone deacetylases (HDAC) facilitate acetylation of histones resulting in chromatin relaxation followed by activation of genes and thus might be considered as promising tool to reduce the post-hypoxic injury. The aim of the present study was to examine effects of HDAC inhibitors trichostatin A (TSA) and sodium butyrate (SB) on post-hypoxic brain expression of glucocorticoid receptor (GR) which is known to play roles in regulation of neuronal death/survival and endocrine adaptive responses, and other neuroprotective proteins, such as erythropoietin (EPO) and hypoxia-inducible factor (HIF-1). As was expected, injections of SB and TSA enhanced the acetylation processes in forebrain neurons in total and particularly up-regulated the levels of acetylated H3 histone (H3K9) in response to hypoxia. At the same time, both HDAC inhibitors greatly potentiated neocortical and hippocampal expression of GR in response to severe hypoxic challenge. Smaller changes were observed for EPO and HIF-1. In our earlier studies it has been shown that strong up-regulation of GR, as well as EPO and HIF-1 contributes in development of brain hypoxic tolerance induced by hypoxic preconditioning. Findings of the present study lead to several important follow-up conclusions. First, HDAC inhibitors enable the same protective mechanisms as the hypoxic preconditioning, at least to a significant extent. Second, up-regulation of GR in tolerant brain appears to be dependent from acetylation of histones, whereas other epigenetic modifications might contribute to the up-regulation of EPO and HIF-1. Finally, HDAC inhibitors can be used as chemical preconditioning to increase brain tolerance although lower efficacy might be expected as compared to hypoxic preconditioning since underlying mechanisms overlap significantly but not completely.

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YIC04-03

PROTECTIVE ROLES OF SELENIUM & ZINC AGAINST NEUROCHEMICAL ALTERATIONS LEADING TO COGNITIVE DEFICITS IN PROTEIN-UNDERNOURISHED RATS

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Protein undernutrition (PU) is a severe problem worldwide particularly among the children. High birth rates in some countries have actually led to an increase in the number of severely malnourished children in some geographical areas such as East Africa and other part of Africa, Asia, Latin America and many other developing countries. Attention has been given to the effect of PU on brain because it results in morphological, neurochemical and functional changes. Moreover, oxidative stress has been implicated in a number of neurological disorders such as stroke, Alzheimer" s disease, Parkinson" s disease etc. This is due to the high lipid content, poor antioxidant defense, high metabolic rate and an abundant supply of the transition metals which make the brain

susceptible to free radical attack leading to impaired neuronal function, and ultimately leading to neuronal death. However, there are increasing evidence that antioxidants supplementation can fight and inhibit oxidative damage in the brain. Selenium (Se) and zinc (Zn) are essential trace elements with antioxidant properties that are important in maintaining optimal brain functions. Selenium has been shown to be neuroprotective against trauma and epilepsy, acute ischemia and Alzheimer" s disease. Also, Zn is a micronutrient that is essential for optimal function of human body especially the brain. Zinc protects against malnutrition-induced brain developmental impairments. It plays critical roles as a cofactor both to stabilize protein structurally and facilitate enzymatic catalysis.

My research activity is focused majorly on understanding the perturbations in antioxidants defense system and oxidative stress and the underlying mechanisms involved in cognitive deficits arising from PU and the possible preventive role of selenium and zinc. PU leads to cognitive deficits in rats, exhibited by poor performance in cognitive tests including impairment in learning and memory and increased locomotor and exploratory behavior. In an attempt to understand the mechanisms of these neuronal dysfunctions, I postulated that low dietary protein leads to intracellular calcium accumulation and inhibition of calcium transport enzymes presumably via free radical generations resulting in calpain and caspase-3 activation. Also, my study showed that PU causes imbalance in mitochondrial antioxidants and electron transport chain enzymes. PU increases the rate of free radical generation and affects mitochondrial permeability transition pore resulting in mitochondrial swelling. In addition, my research activity shows that PU alters synaptosomal lipid packaging, fluidity and integrity of membranes. All these factors contribute to neurodegenerations. I successfully used Se and Zn to reverse many of the anomalies observed in all the parameters analyzed and thus postulated that Se and Zn might be beneficial antioxidants in protecting against neuronal dysfunctions arising from PU. My future work is aimed at molecular study of the effect of PU on inflammation and apoptosis-related proteins; N-methyl- D- aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and some signaling pathways and the role of Se and Zn on them.

YIC04-04

Copper oxide nanoparticles modulate the astrocytic vitality and metabolism

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Copper oxide nanoparticles (CuO-NPs) recently raised the industry's interest due to their interesting chemical and physical properties. The continuous increase of products containing CuO-NPs and the unintentional generation of CuO-NPs by technical processes establish an increased risk of human exposure. Since nanoparticles can reach the brain upon exposure, it is of high interest to evaluate the uptake and potential adverse effects on brain cells. In this context astrocytes are of special interest due to their central role in the brain homeostasis.

A synthesis method for CuO-NPs was established and after a detailed analysis of the physico-chemical properties of those CuO-NPs, primary astrocytes cultures were exposed. The accumulation and uptake mechanism of CuO-NPs by cultured astrocytes as well as the resulting effects on the cell vitality and metabolism were investigated. It was shown that cultured astrocytes strongly accumulated CuO-NPs in a time-, concentration-, temperature- and media-dependent manner. Pharmacological inhibition of different endocytotic pathways suggested that clathrin-mediated endocytosis as well as macropinocytosis are involved in the uptake of CuO-NPs. Accumulated CuO-NPs exerted a strong toxicity when the specific cellular copper contents reached values above 10 nmol copper/mg protein. The mechanism of toxicity was assigned to a strong increase in reactive oxygen species in the treated cells. Cultured astrocytes treated with subtoxic concentrations of CuO-NPs over a time period of 24 h strongly increased their gycolytic flux, their glutathione content as well as the levels of the copper storage protein metallothioneine. The observed increase in glycolytic flux and metallothioneine levels was prevented in presence of the cell permeable copper chelator tetrathiomolybdate. This chelator was also capable of preventing the strong toxicity and the increased generation of reactive oxygen species in acute exposure scenarios where high concentrations of CuO-NPs were applied to cultured astrocytes. The presented data reveals that CuO-NPs can have severe deleterious effects on astrocytes, which otherwise are very robust against several toxins.

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Young Members' Symposia

YMS01 Young Members' Symposia 1

YMS01-01

Clarity visualization of the fluorescent Cl-/PH-sensor expression in transgenic mouse brain

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Genetically encoded biosensors are widely used in cell biology for non-invasive imaging of concentrations of ions, the activity of enzymes, distribution of small molecules, proteins and organelles, and the protein interactions in living cells. These fluorescent molecules are used either at the transient expression in cultured cells or organisms or at stable expression producing transgenic animals possessing heritable and functional biosensors. Using the mouse Thy1 mini-promoter, we generated two lines of transgenic mice for the monitoring of intracellular chloride (Cl^-) and for the simultaneous measurements of intracellular $Cl^-$and pH. The first line expresses a CFP-YFP-based Cl probe called Cl-Sensor and exhibited a good biosensor expression in neurons of the hippocampus and cortex. The second line expresses ClopHensor, which consists of a pH and chloride sensitive variant of enhanced green fluorescent protein (E^2GFP) fused with red fluorescent protein (DsRedm). To reveal the ClopHensor expression pattern across the brain of transgenic mice we obtained transparent brain samples using the advanced CLARITY method and imaged them with confocal and light-sheet microscopy. Then we developed a semi-quantitative approach to identify brain structures with high intrinsic fluorescence of the sensor. These include layer V of the cortex, the pyramidal layer of CA1-3 fields, dentate gyrus, olfactory bulbs, pons, and several thalamic, midbrain and cerebellar nuclei. This approach allowed us to assess cell morphology and protrusion direction tracking, as well as to confirm E^2GFP and DsRedm fluorescence colocalization. This analysis also provides the map of the brain areas available for non-invasive monitoring of intracellular Cl^-/pH in normal conditions and models of pathological conditions.

YMS01-02

Modulation of ligand binding to melanocortin 4 receptors by bivalent ions

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Melanocortin 4 (MC_4) receptors have an important role in the central nervous system in the regulation neuroprotective actions in a variety of neurodegenerative disorders [1]. Ligand binding process to these G protein-coupled receptors (GPCRs) is controlled by several steps of dynamic regulations [2], which affects signal transduction by these receptors. Ligand binding kinetic studies gives additional parameters to find novel drug candidates with optimal binding mechanism, increased selectivity and reduced side effects.

Fluorescence anisotropy method has been successfully applied in kinetic studies of ligand binding to several GPCRs including MC_4 receptors [3]. However the slow dissociation of the fluorescently labelled ligand NDP-α-MSH limits its use in kinetic studies with MC_4 receptors [4]. Therefore we have designed two novel red-shifted fluorescent ligands UTBC101 and UTBC102, which exhibit nanomolar level affinities towards MC_4 receptors [5]. These ligands have relatively different kinetic properties, as UTBC101 has approximately 1.4 times and UTBC102 approximately 30 times faster dissociation in comparison with Cy3B-NDP-α-MSH ($\tau_{1/2}$ = 224 min) [4, 5]. Binding of these ligands to MC_4 receptors is modulated by bivalent metal ions. There is a clear requirement of Ca^{2+} for the binding process, whereas other bivalent ions also modulate the binding of these ligands, but not with the same extent. The exact mechanism of the role of these ions and their relevance in drug development and efficacy of signal transduction requires further studies.

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YMS01-04

Acute and chronic exposure to ritonavir stimulates GSH export from cultured astrocytes via the multidrug resistance protein 1

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Treatment of human immunodeficiency virus (HIV) infection with various types of antiretroviral drugs often leads to neurocognitive side effects. To screen for potential long-term consequences of different HIV drugs on the metabolism of brain cells, cultured primary astrocytes were incubated with 10 μM of these substances for up to 4 days. None of the applied drugs impaired the viability of astrocytes under these conditions. However, 8 out of 13 substances significantly lowered the cellular glutathione content. Among those compounds, the protease inhibitor ritonavir showed the strongest effect lowering the cellular glutathione content after 4 days of incubation by more than 50% compared to that of control cells. Incubation of astrocytes with MK571, an inhibitor of the glutathione

exporter multidrug resistance protein 1 (Mrp 1), significantly enhanced the specific cellular glutathione content compared to untreated control cells, irrespective of the absence or presence of ritonavir and completely prevented the loss of cellular glutathione observed for control and ritonavir-treated cells. These results suggest that the stimulation of glutathione export from viable astrocytes by ritonavir is mediated via Mrp 1. Short-time experiments confirmed a significant decrease in the cellular glutathione content already within 3 h of incubation with 20 μM ritonavir, which was accompanied by a matching increase in the extracellular glutathione content. The stimulatory effect of ritonavir on the acute export of glutathione from astrocytes was found to be time- and concentration-dependent and was completely prevented by the Mrp1 inhibitor MK571. Since ritonavir and other protease inhibitors are commonly used drugs for the treatment of HIV infection, it should be considered that a chronic treatment with such compounds may affect cellular glutathione metabolism.

YMS01-05

'Communication' of extracellular and intracellular glycine receptor domains - how does this lead to severe startle disease?

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The dysfunction of glycinergic neurotransmission is the underlying mechanism in the neuromotor disorder hyperekplexia also called Startle disease. Familiar hyperekplexia is a hypertonic movement disorder with seizures caused by sudden tactile or auditory stimuli.

Mutations in the human *GLRA1* gene, coding for the glycine receptor (GlyR) alpha 1 subunit are the most common cause for hyperekplexia, which can follow either a dominant or recessive trait. The underlying pathomechanisms of this inhibitory ion channel are, however, not completely understood. Since mice, carrying a mutation in GlyR subunit genes, result in a similar neuromotor phenotype compared to humans, mouse models are excellent tools to study the molecular mechanisms of this disease. Known mouse mutants are oscillator, spastic and spasmodic.

Here, we used a novel spontaneous hyperekplexia mouse model "*shaky*" to investigate the role of the affected extracellular loop betta8-betta9 of the GlyRa1 subunit for ion channel function. The recently published GlyR structures suggest a role of this extracellular loop in the GlyR signal transduction pathway, e.g. molecular transitions of the receptor following ligand binding resulting in ion channel opening and closing.

The underlying missense mutation Q177K of this spontaneous mouse model resulted in an enhanced expression level of mutated GlyRa1 *in vivo* although synaptic integration of GlyRs was decreased. The remaining portion of synaptic heteromeric GlyRs generated decreased current amplitudes with significantly faster decay times. Ligand binding was not affected. We propose that the largely reduced GlyR function is attributed to lack of translation processes from ligand binding to channel opening. Thus, the observed functional disruption associated with the neurological phenotype illuminates for the first time the key role of loop F for initiating rearrangements within the extracellular-transmembrane GlyR interface *in vivo* required for inhibitory signaling. Further structural analysis suggests a disorganized hydrogen bond network as a consequence of the betta8-betta9 loop mutation. These data sharpen our molecular view of startle disease with structural elements significant for synaptic clustering and function.

YMS02 Young Members' Symposia 2

YMS02-01

BAG1 prevents misfolded proteins accumulation when autophagy flux is blocked in neurodegenerative disorders

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Different disease associated proteins, as SOD1 and TDP-43 in familial and sporadic amyotrophic lateral sclerosis and frontotemporal dementia, or androgen receptor (AR) in spinal and bulbar muscular atrophy, tend to misfold and accumulate into aggregates in neurons. Protein quality control system prevents their aggregation and toxicity by enhancing their degradation via proteasome and/or autophagy. An efficient dynein mediated transport of misfolded proteins to the site of degradation is required as key point to control their aggregation and degradation. HSPB8 is a protective protein that reduces disease associated proteins aggregation by autophagy facilitation. Here we evaluated the HSPB8 effects on the recently discovered RAN translated poly-di-peptides (DPRs) from C9ORF72 gene. Using filter trap and western blot we observed that HSPB8 over-expression facilitates DPRs clearance even when proteasome is blocked. when we blocked the dynein retrograde transport by EHNA we found an alteration of SQSTM1/p62 and LC3 expression and localization. However, dynein inhibition reduced SQSTM1/p62 and LC3 levels induced by trehalose and drastically reduced the number of autophagosome per cell. Moreover, EHNA reduced the PBS insoluble fraction of mutated misfolded proteins and DPRs also when autophagy is blocked. This effect was counteracted by proteasome inhibition. Notably, EHNA selectively increased BAG1 mRNA (responsible for misfolded protein degradation via proteasome) in NSC34 and motoneuron derived from iPS cells, while exogenous BAG1 overexpression reduced misfolded species aggregation and BAG1 down-regulation blocked the EHNA effect. Moreover, EHNA increased mRNA and protein levels of chaperone mediated autophagy receptor Lamp2A, suggesting that CMA can restore the degradation of misfolded proteins with KFERQ-like motif that are internalized into lysosome by Lamp2A. Collectively, these data suggest that when autophagy flux is blocked, misfolded proteins can be re-routed by BAG1 to alternative degradative pathways.

YMS02-02

A novel avenue for protection against tauopathy: ADNP/NAP dramatically increase microtubule end-binding protein-tau interaction

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Background: Activity-dependent neuroprotective protein (ADNP), vital for brain formation and cognitive function, is mutated in autism and linked to neurodegenerative/psychiatric diseases. An eight-amino-acid peptide snippet of ADNP, NAP (NAPVSIPQ), identified as a smallest active fragment, includes the SxIP microtubule (MT) end-binding protein (EB) association motif, and enhances ADNP-EB3 interaction. Depletion of EB1 or EB3 abolishes NAP protection against zinc intoxication. Furthermore, NAP enhances Tau-MT interaction, and Tau regulates the localization and function of EB1 and EB3 in developing neuronal cells.

Aims: To reveal how NAP (ADNP) enhances Tau-MT interactions and whether this is mediated by EBs.

Methods: NAP impact on the EB's morphology and dynamics was estimated by immunofluorescence and time-lapse imaging, EB-Tau and EB/Tau-MT interactions - by Co-Immunoprecipitation and Polimeryzed vs. Solible tubulin assay in the differentiated N1E-115 cells.

Results: We showed, for we believe the first time, that NAP augmented endogenous EB1 comet density in the N1E-115 neuroblastoma neuronal model. This finding was substantiated by cell transfection with fluorescent EB1 and live cell imaging. NAP increased comet amounts, length and speed. At the molecular level, NAP enhanced EB3 homodimer formation, while decreasing EB1-EB3 heterodimer content and driving EB1- and EB3-Tau interactions (dramatic 20-fold increases), leading to recruitment of EB1/EB3 and Tau to MTs under zinc intoxication. NAP did not protect NIH3T3 cells against zinc intoxication, unless these cells were transfected with Tau.

Conclusions: EB-Tau interaction is identified as a novel target for endogenous ADNP neuroprotection, and a future target for drug development, with NAP as a prototype. (Molecular Psychiatry, 2017; https://doi.org/10.1038/mp.2016.255).

YMS02-03

Innate immune response protein lactoferrin directly binds amyloid-beta precursor protein to promote amyloidogenic processing

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Background: The cellular balance of iron and response to inflammation (e.g. through innate immunity) are closely linked, and disruption of both are features of early Alzheimer's disease (AD). Amyloid-β precursor protein (APP) has a role in neuronal iron homeostasis through stabilizing the iron efflux pore protein ferroportin in a functional location on the cell surface. The iron transporter lactoferrin (LF) contributes to alterations in iron metabolism during iron loading, infection and inflammation and has other immunomodulatory properties. In AD, neuronal LF levels are elevated and present within amyloid plaques.
Methods: Sedimentation velocity analysis and immunoprecipitation identified an interaction between APP and LF. LF endocytosis was investigated with biotinylated-LF on APP siRNA pretreated neurons whereby internalized biotin-LF was detected with streptavidin-HRP after the removal of extracellular biotin by 2-mercaptoethanesulfonate. Membrane-bound APP was detected by cell surface biotinylation assay and flow cytometry, while amyloidogenic processing of APP was by Western detection of sAPPβ and Aβ.
Results: As well as ferroportin, we now discover LF as another iron-associated protein that binds to APP. This interaction only occurs with the iron-bound form of LF. Recently, we have identified that amyloidogenic processing of APP increases neuronal iron retention and susceptibility to oxidative damage. Evidence suggests that holo-LF promotes APP endocytosis as neuronal surface expression of endogenous APP is reduced and processed through the endocytic amyloidogenic processing pathway resulting with increased extracellular sAPPβ and Aβ production.
Conclusion: The binding of holo-LF to APP and subsequent shedding of APP from the cell surface may provide an efficient acute neuroinflammatory response aimed at preventing iron availability to invading pathogens. However, chronically sustained exposure to holo-LF culminates in excess amyloidogenic processing of APP. Subsequent Aβ production and intraneuronal iron retention increase neuronal susceptibility to oxidative stress.

YMS02-04

Altered neuronal endoplasmic reticulum-mitochondria coupling in a transgenic rat model of Alzheimer's disease

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Neuronal bioenergetic failure has been suggested as an early event leading to cognitive impairment and ultimately to Alzheimer's disease (AD), a neurodegenerative disorder characterized by intraneuronal amyloid β (iAβ) accumulation. Several metabolic neuronal functions are regulated by close physical communications that mitochondria make with the endoplasmic reticulum (ER), involving distances of approximately 10–30 nm. In this context, it is relevant to evaluate the impact of iAβ on such distances by an accurate method. To address this question, we employed hippocampal primary neurons from embryonic transgenic McGill-R-Thy1-APP rats, which mimic AD, and wild-type (control) animals. Neurons were transfected with plasmids coding drug-inducible synthetic interorganellar linkers targeting outer mitochondrial membrane or ER fused to fluorescent proteins that form a FRET pair upon addition of rapamycin. Live imaging data recorded by multi-colour epifluorescence microcospy revealed that ER-mitochondria distance is altered in transgenic neurons as early as 7 days *in vitro* as compared to controls. Complementary studies showed iAβ accumulation and bioenergetic impairment, assessed by super-resolution microscopy and high-resolution respirometry, respectively. These results may suggest that iAβ accumulation disrupts ER-mitochondria coupling which in turn alters proper mitochondrial function. Understanding the mechanisms that lead to neuronal bioenergetic failure may be useful to develop new specific therapeutic strategies.

YMS02-05

The accumulation of amyloid-β and the dopaminergic neurotransmission form a cyclical feedback loop in Alzheimer's pathology

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Increasing evidence suggests that dopaminergic neurotransmission is impaired in Alzheimer's disease but this impairment has mostly been associated to non-cognitive symptoms. Dopamine is a key neuromodulator of synaptic plasticity and it has been shown that the enhancement of its activity improves memory in animal models and Alzheimer's patients. Additionally, evidences have shown that dopamine and dopamine structure-related molecules can interact with amyloid-beta (Aβ), inhibiting aggregation and disaggregating Aβ *in vitro*. We hypothesized that the pathological accumulation of Aβ and the dopaminergic neurotransmission form a cyclical feedback loop in the pathology of AD. We established a novel

relation between dopaminergic neurotransmission and Aβ-induced alterations in the plasticity of cortical synapses and its relation to memory impairment. We found that early Aβ deposition in a transgenic mouse model of AD (3xTg-AD) and also the administration of Aβ oligomers in WT mice, impaired synaptic plasticity, not only to the point of flattening changes by inhibiting Long-term potentiation (LTP), but by displaying actual long-term depression (LTD). The restoration of cortical dopamine levels induced LTP and restored memory. Then, we found that intra-cerebral administration of dopamine and its core structure molecule, catechol, decreases Aβ accumulation 24 h after the infusion in the cerebral cortex and hippocampus of 3xT-AD mice. Accordingly, the systemic administration of levodopa, the precursor molecule in the biosynthesis of dopamine, restored dopamine release, improving cognitive performance and lessened Aβ accumulation in 3xTg-AD mice. Additionally we *in silico* studied the protective effect of dopamine on the accumulation of Aβ that is mediated by its catechol core, which interacts with Aβ aggregates, favoring its degradation. Together, these results further support the notion that therapy based on dopaminergic stimulation might have beneficial effects at two levels, on the cognitive function of AD patients and on the progression of this pathology.

YMS03 Young Members' Symposia 3

YMS03-01

The tyrosine kinase inhibitor tyrphostin 23 activates glycolysis and citric acid cycle of cultured astrocytes

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Tyrphostin 23 (T23) is a well-established inhibitor of protein tyrosine kinases, is used as endocytosis inhibitor and is considered as potential anti-cancer drug. To investigate acute effects of T23 on the viability and the glucose metabolism of brain cells, we have exposed cultured primary rat astrocytes to T23. The viability and the morphology of these cells were not acutely affected by the presence of T23 in concentrations of up to 300 μM. However, already within the first 60 min of exposure, T23 caused a time- and concentration-dependent increase in glucose consumption and lactate release, resulting within 2 h in a doubling of glycolytic flux for incubations with 100 μM T23. The stimulation of glycolytic flux by T23 was completely abolished upon removal of the compound and not found in presence of the tyrosine phosphatase inhibitor vanadate, which prevented the T23-induced stimulation of astrocytic lactate production in a concentration dependent manner. In contrast to T23, structurally related compounds such as tyrphostin 25 and catechol, modulators of AMP-kinase activity or other endocytosis inhibitors did neither affect the basal nor the T23-stimulated lactate production in cultured astrocytes. Incubations of astrocytes with [U-^{13}C]glucose followed by analysis of ^{13}C-metabolite labelling patterns using gas chromatography coupled with mass spectrometry confirmed that T23 stimulates glycolytic flux but also demonstrated that T23 strongly stimulates the carbon labeling of intermediates of the citric acid cycle and the citric acid cycle activity. These data indicate that T23-sensitive phosphorylation/dephosphorylation events are involved in the regulation of the energy metabolism of astrocytes.

YMS03-02

GM1 neurotrophic properties are related to gm1 oligosaccharide - TrkA interaction in mouse neuroblastoma cells

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Experimental evidence, both *in vitro* and *in vivo,* highlight the neurotrophic and neurodifferentiative effects of ganglioside GM1. So far, the molecular mechanism underlining the GM1 neuro activities is still unknown. In order to clarify the molecular mechanism by which GM1 exerts its neurotrophic action we decide to investigate the importance of its oligosaccharide portion. Here, we describe the specific role of the GM1 oligosaccharide in promoting differentiation in neuron like cells.

GM1 oligosaccharide was added to the culture medium of murine neuroblastoma N2a cells. To study the neurodifferentiation, cells were followed over the time to assess both morphological parameters and biochemical markers.

In murine neuroblastoma cell lines we found that the oligosaccharide chain of GM1 is directly involved in the processes of neuronal differentiation by inducing TrkA-MAPK pathway activation. By biochemical and structural analysis we demonstrated a direct interaction at the plasma membrane level between the GM1 oligosaccharidic chain and TrkA receptor.

We surmise that the neurotrophic effect of GM1 is due to a direct interaction between the oligosaccharide chain and TrkA receptor. This evidence leads us to consider both the *trans-* and *cis* - interaction via head-to-head and side-by-side interaction, respectively.

YMS03-04

Functional differentiation of primed hips-derived glial cells into mature oligodendrocytes following transplantation into the CNS

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Induced pluripotent stem cell-derived neural or glial precursor cells (iPS-NPCs, iPS-GPCs) may represent the ideal autologous cell source for cell-based therapy to promote remyelination and neuroprotection in myelin diseases and can serve as suitable tools to model myelin disorders in order to test the potential of pharmacological compounds. The therapeutic potential of these cells was mainly approached in neonatal conditions. However, the repair efficacy and safety of these cells in the demyelinated adult central nervous system (CNS), a condition associated with decreased cell plasticity and scaring, remains to be well addressed. Our previous data revealed the prominent capacity of survival, safe integration, migration and timely differentiation of mouse iPS-NPCs into mature *bona fide* oligodendrocytes following transplantation in the adult models of demyelination (Mozafari et al., 2015, JCI). However, the re/myelination potential of iPS-derived human cells remains unclear.

Using a very efficient and rapid technique our collaborators succeeded in by-passing the poor and slow differentiation process of human pluripotent cells into oligodendrocytes, *in vitro*. To validate the functionality of these cells *in vivo*, the cells were transplanted in newborn and adult models of dys/demyelination.

Our data showed widespread migration, integration and extensive generation of functional oligodendrocytes ensheathing host axons, forming compact myelin while reconstructing nodes of Ranvier both in newborn grafted and adult demyelination contexts. This unique cellular tool will help better understand the biology of human

oligodendrocytes in pathological conditions. These novel insights into the biology of reprogrammed cells in myelin-affected conditions should help establishing the pertinence of their use for i) regenerative biomedicine of myelin diseases affecting the CNS or ii) modeling myelin disorders in order to achieve personalized preclinical therapies for complex disorders of CNS myelin such as multiple sclerosis. Supported by MS Alliance, MSIF, Fondation des Treilles, ENP, EDF foundation, SFB-TR128-B07, IZKF, KuT3/006/11.

YMS04 Young Members' Symposia 4

YMS04-01

Agmatine induces NRF2 and protects against corticosterone effects in hippocampal neuronal cell line

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Hyperactivation of the hypothalamic-pituitary-adrenal axis is a common finding in major depression; this may lead to increased levels of cortisol, which are known to cause oxidative stress imbalance and apoptotic neuronal cell death, particularly in the hippocampus, a key region implicated in mood regulation. Agmatine, an endogenous metabolite of L-arginine, has been proposed for the treatment of major depression. Corticosterone induced apoptotic cell death and increased ROS production in cultured hippocampal neuronal cells, effects that were abolished in a concentration- and time-dependent manner by agmatine. Interestingly, the combination of sub-effective concentrations of agmatine with fluoxetine or imipramine afforded synergic protection. The neuroprotective effect of agmatine was abolished by yohimbine (α2-adrenoceptor antagonist), ketanserin (5-HT2A receptor antagonist), LY294002 (PI3K inhibitor), PD98059 (MEK1/2 inhibitor), SnPP (HO-1 inhibitor), and cycloheximide (protein synthesis inhibitor). Agmatine increased Akt and ERK phosphorylation and induced the transcription factor Nrf2 and the proteins HO-1 and GCLc; induction of these proteins was prevented by yohimbine, ketanserin, LY294002, and PD98059. In conclusion, agmatine affords neuroprotection against corticosterone effects by a mechanism that implicates Nrf2 induction via α2-adrenergic and 5-HT2A receptors, Akt and ERK pathways, and HO-1 and GCLc expression.

YMS04-02

Identification and characterisation of a novel mirror movement gene

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Objective: To determine the molecular basis of disease in a family with isolated mirror movements (MM), partial agenesis of the corpus callosum (pACC) and mild intellectual disability.

Background: TUBB3 is required for neuronal migration and axon guidance. *TUBB3* mutations cause CDCBM1:OMIM#614039 and CFEOM3A:OMIM#600638. Both syndromes are associated with dysgenesis of the corpus callosum but not MM:PS#157600.

Methods: Clinco-genetic approaches were utilized to identify the disease-causing gene. MRI tractography was used to assess brain structure and corticospinal tract (CST) wiring. *In silico*, *in vitro* and *in vivo* analyses were employed to delineate the pathogenicity of the mutation.

Findings: A novel missense mutation in *TUBB3* was identified in both affected individuals (the mother and her offspring), who presented with MM and pACC. The mutation segregated with disease and was not identified in any unaffected family members tested. *In silico* analyses predicted the mutation to be destabilizing. *In vitro* and *in vivo* studies of the mutant protein showed decreased steady-state levels, a lower rate of heterodimer incorporation and perturbed microtubule dynamics. Tractography identified decreased crossing of descending CST axons at the pyramidal decussation.

Interpretation: We describe a family with MM and pACC with a novel *TUBB3* mutation. Tractography revealed a failure of corticospinal axonal decussation at the midline, consistent with what we (Marsh, *et al*. 2017) and others have previously observed in individuals with MM. Our analyses showed the mutation disrupted normal TUBB3 function. This study highlights the importance of microtubule dynamics for commissural axon guidance and lateralization of the human central nervous system.

Conclusion: This study represents the first disease modelling of a MM gene and provides novel evidence that *TUBB3* mutations can cause MM.

YMS04-03

Epigenetic regulation of synaptic plasticity and associativity by G9a/Glp complex in hippocampal CA1 pyramidal neurons

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Epigenetic regulations play an important role in regulating the learning and memory processes. G9a/G9a-like protein (GLP) lysine dimethyltransferase complex controls a prominent histone H3 lysine9 dimethylation (H3K9me2) that results in transcriptional silencing of the chromatin. We observed that the inhibition of G9a/GLP complex by either of the substrate competitive inhibitors UNC 0638 or BIX 01294 reinforces protein synthesis-independent long-term potentiation (early-LTP) to protein synthesis-dependent long-term potentiation (late-LTP). Surprisingly, the reinforced LTP by G9a/GLP complex inhibition could associate with a weak plasticity event from nearby independent synaptic populations, resulting in synaptic tagging/capture (STC). Altered epigenetic mechanisms are implicated in the cognitive decline associated with neurodegenerative studies like Alzheimer's disease (AD). To assess the role of G9a/GLP complex in cognitive impairment, we further investigated the involvement of G9a/GLP complex in alleviating the effects of Amyloid-b oligomer on neuronal plasticity. Late-LTP and STC were studied in the CA1 area of hippocampal slices from 5 to 7 week old

male Wistar Rats. Amyloid-b oligomer impair both LTP and STC. Our findings demonstrate that the pharmacological inhibition of G9a/GLP complex activity reverses the amyloid-b oligomer-induced deficits in late-LTP and STC. We also identify BDNF as a critical plasticity protein that mediates the restoration of plasticity and associativity by G9a/GLP complex inhibition. Our study reveals an epigenetic mechanism for promoting plasticity and associativity by G9a/GLP complex inhibition, and it may engender a promising epigenetic target for enhancing memory in neural networks.

YMS04-04

FNDC5/irisin corrects memory deficits in animal models of Alzheimer's disease

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by brain amyloid deposition, synaptic failure and memory loss. Mounting clinical and experimental evidence has indicated that regular physical exercise reduces AD risk in the elderly and attenuates cognitive decline in AD patients. Still, neuroprotective actions of physical exercise have not been fully elucidated. FNDC5/irisin is a recently described exercise-derived protein found to be expressed in the brain, where it induces neurotrophic factors. Here we aimed to determine if FNDC5/irisin levels are altered in AD, and whether restoring FNDC5/irisin could protect against memory dysfunction in experimental models of AD. Our experimental models consisted of cultured hippocampal neurons exposed to amyloid-β oligomers (AβOs), neurotoxins associated to memory failure in AD, wild-type mice given intracerebroventricular injections of AβOs, and the APP/PS1 transgenic mouse model of AD. Biochemical and morphological outcomes were determined by immunofluorescence, ELISA and Western blotting. Memory tests, such as novel object recognition and fear conditioning, were used in mice. We initially found that FNDC5/irisin levels were reduced in the brains and cerebrospinal fluid of AD patients, and in the brains of APP/PS1 mice. AβOs reduced FNDC5/irisin in cultured hippocampal neurons, and in the hippocampus of AβO-injected mice. Furthermore, knockdown of brain FNDC5/irisin induced memory impairment in wild-type mice. Interestingly, restoring brain FNDC5/irisin levels, either pharmacologically, through adenoviral expression or through regular physical exercise, corrected memory failure in mouse models of AD. Our findings uncover new potential protective actions of FNDC5/irisin against memory impairment in AD models, likely contributing to identify novel therapeutic targets in AD and to help explain neuroprotective actions of physical exercise against dementia.

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Workshops

W01 Light-regulated drugs for therapeutic applications

W01-01

Synthetic physiology - remote control of cellular signals

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"What I cannot create, I do not understand." (R. Feynman, 1988). Our research lies at the interface of synthetic biology and physiology with a focus on understanding and manipulating cellular signaling and cell-cell communication in health and disease. In the past five years, we developed innovative methods to remotely control cellular signals with high spatio-temporal precision (e.g. using light or ultrasound). In the next five years, we will address major problems in signaling and tissue regeneration using our synthetic approaches. Because our research program encompasses the development of new molecular methods and their application to physiology we call it 'synthetic physiology'.

W01-02

Photocontrolled peptidomimetics

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The optical control of biological functions with small photoswitchable molecules has gathered significant attention in the last decade by following two general approaches, viz. optogenetics and photopharmacology. While the first one relies on the genetic introduction of light-responsive proteins, the latter describes the exogenous use of small photochromic molecules that interact with a specific target. The advantage of photopharmacology is the precise control of cell signaling through native receptors, without necessarily introducing foreign genes. Recently, we synthesized photoswitchable mimetics of the specific glucagon-like peptide-1 receptor (GLP-1R) agonist liraglutide and the human atrial natriuretic peptide (ANP) by incorporating azobenzene photoresponsive elements into the peptide backbones. Whereas the incretin-mimetic LirAzo allows isomer-biased optical control of GLP-1R signaling due to differential activation of second messenger pathways upon irradiation, the photochromic natriuretic peptide receptor A (NPR-A) ligand offers reversible, repeated manipulation of *c*GMP synthesis and remote control of vasoactivity in explanted murine aortic ring preparations can be afforded, as well as pancreatic beta cell function in islets of Langerhans. Thus, our studies extend the toolbox of photoswitchable molecules for enzyme-dependent signaling processes and demonstrate the broad applicability of photopharmacology to induce receptor activation on a molecular level.

W01-03

Photopharmacology: from basic concepts to proof of principle, and beyond

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Pharmacotherapy is severely limited by poor drug selectivity, resulting in side-effects, and the emergence of resistance. Lack of selectivity is caused by the inability to control drug activity in time and space. **Photopharmacology**[1] aims at solving these issues at a molecular level by incorporating photoswitchable groups into the molecular structure of bioactive compounds.[2] This allows for the use of light as an external control element for pharmacological activity, which can be delivered with very high spatiotemporal precision.

We have applied the concept of photopharmacology, *inter alia*, for the photocontrol of antibiotic activity[3] and chemotherapy.[4] In the latter case, we have developed photoswitchable histone deacetylase inhibitors as potential antitumor agents. Analogues of the clinically used chemotherapeutic agents were designed with a photoswitchable azobenzene moiety incorporated into their structure. The most promising compound exhibits high inhibitory potency in the thermodynamically less stable *cis* form and a significantly lower activity for the *trans* form, both in terms of HDAC activity and proliferation of HeLa cells.

The presentation will describe several aspects of photopharmacology: i) basic principles behind the concept; ii) design principles for photopharmacological agents; iii) recent examples from our group and the field and iv) the main challenges for the future and the outlook on the possible applications in clinics.

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W01-04

In vivo optical manipulation of nicotinic acetylcholine receptors

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Nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels widely expressed in the brain. nAChRs play a key role in nicotine addiction and in high-level behaviors such as learning, motivation, decision-making, exploration or reward processing. In the central nervous system, 12 different subunits can be found (α2–9

and β2–4), which self-assemble into homo- or hetero-pentameric combinations. The various nAChR combinations differ in their pharmacological and kinetic properties, in their permeability for calcium and in their distribution throughout the brain. The ability to affect the natural patterns of ACh release or the exogenous effects of nicotine, by activating or blocking a specific nAChR subtype at a specific time and place, would greatly aid studies aimed at clarifying their diverse roles. To this aim, we designed a strategy, called optogenetic pharmacology, that enables endogenous mammalian receptors to be optically and repeatedly switched on and off with great temporal precision and in targeted subsets of neurons (1). This strategy relies on the attachment of a photoswitchable tethered ligand onto a genetically-encoded, cysteine-ready receptor. We have notably developed a series of light-regulated neuronal nAChRs (LinAChRs) that can be conjugated with photoswitchable agonists or antagonists, allowing for powerful bidirectional control (activation and inhibition) of receptor activity (2, 3). Using a lentiviral vector strategy, we expressed β2*LinAChRs selectively in the ventral tegmental area (VTA), a dopaminergic nucleus involved in the reinforcing properties of nicotine. Patch clamp recordings of VTA dopamine neurons showed that LinAChRs could optically inhibit nicotinic inputs to the VTA. Furthermore, using in vivo extracellular recordings, we showed that β2*LinAChRs could rapidly and reversibly modulate not only the spontaneous phasic activity of dopamine neurons, but also the bursts of action potentials induced by intravenous injection of nicotine. Our findings establish a causal role for β2*nAChRs in regulating the excitability of VTA dopamine neurons.

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2) Tochitsky et al., Nat. Chem. 2012.
3) Lemoine et al., Meth. Mol. Biol. 2016.

W02 Opening Black Boxes in Academia and Beyond

W02-01

How to choose a good scientific problem

R. Bansal

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We will address this issue that is faced by people in the research field at different stages in their academic progression. First, as graduate students, they need to choose a good scientific problem for their Ph.D. work. Next comes the question about the scientific project to pursue when one starts their postdoctoral training. Finally, the biggest decision one needs to make is when they establish their own laboratory and begin to lead a team of junior researchers. No matter where one is in their scientific carrier the question of what is a "good" scientific problem to pursue will always remain. Strategies for finding the best options for accomplishing one's mission will be discussed.

W02-02

Building a motivated research group around a good scientific problem

D. Feinstein

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Establishing a research group to work on a shared scientific goal requires effort on the part of the PI, as well as on the part of the team members. Many factors play a role in determining the success of a project, ranging from scientific merit to the day-to-day interest of investigators working to accomplish study goals. Both personal and inter-personal factors influence a project;'s trajectory, but maintaining a strong motivation to work on a project is one of the factors that can lead a project to success or to failure. This talk will briefly cover some of those factors, derived from personal experience as well as from reaching out to other PIs and lab members for their insights. This will cover practical considerations needed to carry out a good scientific project, various ways a PI can help motivate lab members, ways that research projects can be designed to benefit all lab members, and a discussion of lab practices that should be avoided. At the end of the talk we hope to generate a list of 'Do's and Don'ts' that can be shared with other PIs and lab members.

W02-04

Alternative careers outside academia

F. Orfaniotou

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Career aspirations and paths for people in academia have been evolving over the last few decades and the number of less conventional options beyond the traditional university career trajectory has increased. Given the rising number of post-graduate scientists, the ever decreasing amount of research funding and number of positions in academia make alternative career options all the more valuable – and highly competitive. One alternative career option is the pharmaceutical industry, with a variety of opportunities ranging from basic research and bench work to more business-oriented commercial careers. Considering the different skill sets and expertise developed during an academic training period, how could one explore, use and develop the skills needed to continue a career outside academia? This session will provide insights into what type of opportunities exist outside academia, within the pharmaceutical industry and how one might achieve career aspirations beyond academia.

W02-05

Road from science to patent world

P. Bhasin

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Field of neurosciences is a highly interdisciplinary field and the knowledge gained can be channeled into many fields including medicine, psychology, law and education. A number of graduates from the field of neuroscience often engage in a complex thought process of our next move. Should the next move be to stay in neurosciences and engage in research/education/medicine or should we change the course of our career. One of the field where a neuroscience graduate can make a smooth transition is merging neuroscience background into another fascinating field of law, which is to become a part of the patent world. The field is now enjoying its share of limelight when compared to some 15–20 years ago. However, the information generating awareness on law career choices and how to make a move is still deficient. Discussion will be focused on what are the career options available for neuroscience graduates and how to explore and work on those options.

W03 Tubulin and Microtubule Diversity in the Nervous System

W03-01

Tubulin and microtubule diversity in the nervous system

S. Brady

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Neuronal microtubules (MTs) exhibit remarkable functional and biochemical diversity that can be related to tubulin isoforms, posttranslational modifications and associated proteins. Recent studies have begun to illuminate the molecular basis of these diverse functions for neuronal MTs, but many questions remain about how this diversity contributes to neuronal functions and neurological diseases. Microtubules provide the structural basis for neuronal morphologies and contribute to neuronal changes during development and even in learning and memory. They are necessary for extension of neurites during development, as well as providing the structural basis for maintaining axons and dendrites. MTs define the polarity of neurons, distinguishing between axon and dendrites as well as establishing the polarity of the neuronal cytoplasm. This polarity makes them suitable to serve as the tracks for transport of both membrane-bounded organelles and cytoskeletal elements. MTs define and maintain the integrity of multiple intracellular compartments in the neuron. Finally, they provide a scaffold for various signaling pathways. The diversity of these functions is reflected in differences in the kinetic properties, biochemistry and metabolic stability of different MT populations. This workshop will provide an up-to-date overview of molecular basis for the diverse functions of MTs in the nervous system.

W03-02

Microtubule stability in health and disease

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Microtubules (MTs) are structural components vital for important neuronal functions such as neurite outgrowth and maintenance, as well as for axonal trafficking and synaptic remodeling. Neuronal MTs are particularly stable compared with those in other cell types and often exist for years before they get degraded and recycled. They are mostly synthesized in the somatodendritic compartment and must be transported along axons as much as a meter or more in humans to reach the terminals where synaptic proteins are delivered and synaptic transmission occurs. A significant portion of neuronal MTs stay polymerized when challenged with cold temperature, Ca^{2+} and antimitotic drugs (e.g. Nocodazole), all of which destabilize MTs effectively in test tubes and various non-neuronal cell lines, suggesting that baseline MT stability is high in neurons. Such stability increases during development and maturation, but decreases with axonal injury and neurodegeneration. This leads to several intriguing questions: 1) What is molecular mechanism for the exceptional stability of axonal MTs especially in the central nervous system? 2) Can stabilizing MTs facilitate neurite outgrowth during early development or restore axonal integrity and function upon injury? To address these questions, we have characterized the temporal and spatial changes of MT stability *in vitro* and *in vivo*, a novel posttranslational modification on tubulin which contributes to this stability, and effects of a group of MT stabilizing drugs (Epothilone D, Epothilone B, Ixabepilone, Taxol and Synstab) on neurite growth and regeneration. These results may provide useful information for understanding not only neuronal MT dynamics and stability in health and disease, but also for determining the therapeutic value of MT stabilizers in axonal injury and neurodegeneration where loss of neuronal MT integrity may exacerbate disease pathology.

W03-03

Microtubules and neuronal polarity

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Tau, the best-known microtubule-associated protein, is concentrated in the axon, where it binds to microtubules, presumably regulating their properties, thereby contributing to the polarity of the neuron. Hundreds of primary research papers, as well as a great many text books and educational resource materials claim that tau's main function is to stabilize microtubules. This claim is based on the fact that excess tau can indeed stabilize microtubules in the test tube, and overexpression of tau in cells leads to hyper-stabilization of its microtubules. Disease researchers are so invested in the idea of tau as a microtubule stabilizer, they are seeking to use microtubule-stabilizing drugs to treat diseases in which tau loses its association with microtubules. However, there are reasons to doubt whether tau really stabilizes microtubules in the axon. We established years ago that individual microtubules in the developing axon consist of two distinct domains: a stable domain toward the minus end of the microtubule and a labile domain toward its plus end. Microtubule stability becomes more complex in adult axons, but the importance of the labile domains of microtubules persists throughout the life of the neuron. In fact, in light of so many potential stabilizers of microtubules in the axon, the more perplexing question may be what prevents that labile domain from becoming stable. We posit that tau binds at a higher density to the labile domain of the microtubule than to the stable domain, and thereby limits access to the factors that stabilize the stable domain. According to this hypothesis, tau is a preventer of microtubule stability in the functional context of the axon, and in this manner, is crucial for maintaining the domain structure of the axonal microtubule. In the oral presentation, data will be presented showing that tau depletion from cultured rat neurons does not result in microtubule destabilization, but rather results in a shortening of the labile domain of the microtubule, and eventually, a degradation of its normal domain structure. As tau is lost from the microtubules, MAP6 is able to bind more avidly to them, thus accounting for at least some of these observations.

W03-04

Tubulin polyglutamylation - a novel player in neurodegeneration

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Microtubule functions are regulated by a multitude of factors and pathways. One factor that directly modulates the microtubule lattice are posttranslational modifications of tubulin. While the modifications of tubulin have been discovered decades ago, functional insight is only recently emerging. We have studied the role of tubulin polyglutamylation, a modification prevalent in neurons, and found that deregulation of this posttranslational modification leads to neurodegeneration. To demonstrate the specificity of this mechanism, we have generated a series of combinatorial knockout mice in which we can specifically induce or avoid degeneration of certain neuron populations in the nervous system. We have further investigated the potential mechanisms of the hyperglutamylation-induced degeneration and found a strong impact on axonal transport. Our data suggest that alternations in polyglutamylation could be a biochemical mechanism linked to a range of neurodegenerative disorders in human.

W04 Publishing Biomedical Research in the Modern Era

W04-01

Translating basic research into clinical practice

J. Schulz

RWTH Aachen, Department of Neurology, Aachen, Germany

Despite a vast amount of very promising basic research findings, these failed to successfully translate into the clinical practice so far. In other words, a lot of resources spent on research seems wasted, and we are still on the quest for efficacious treatments to modify the course of most societally relevant diseases, let alone bringing them to a halt. The reasons for this "data reproducibility crisis" are numerous, and certainly root in insufficient experimental design, conceptual flaws, incorrect statistical planning and evaluation, incomplete model system that do not adequately reproduce the human physiology, and further problems that we will discuss with the aim to present practical solutions that can be implemented by researchers, journals editors and reviewers.

W04-02

Publication ethics and referee responsibilities

L. Hausmann

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

Scientific misconduct constitutes a major problem not only because faulty data mislead readers and cause a waste of resources, but also because they may ultimately harm patients, as we will discuss in part iii) of our workshop. For Journal of Neurochemistry's editorial office, dealing with allegations of misconduct, plagiarism, data manipulation, authorship issues and other types of ethical violations are part of the daily routine. Not all of these are committed with fraudulent intent, but often result from unawareness of standards or from pitfalls that result in "honest errors". We will outline frequent types of violations, and how these are handled by the Journal. Reviewers are often the ones who draw attention to ethical issues in the manuscripts, and with the plenary we will discuss this demanding task, including hands-on advice to create awareness for scholarly publication ethics in the context of the reviewers' ever increasing workload.

W04-03

Pursuing and publishing science

M. Robinson

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A scientific study begins by testing a hypothesis that is based on an earlier observation(s). Assuming one is appropriately following the literature (new and old), an unexpected observation that leads one in a new direction is more likely to be novel and potentially interesting. As soon as one is convinced that the study is likely to lead to a publication, one should design appropriately controlled and powered experiments that will be used to produce figures that ultimately form the basis for an outline to the paper. We will discuss strategies for preparing a clear and compelling manuscript for journal submission, the factors that are being evaluated in peer review, and approaches to responding to these reviews.

W04-04

The challenge of soliciting good review articles

M. Cookson

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Review articles serve to summarize, critically discuss, and interpret original research in the context of the existing body of literature. They should identify gaps in knowledge, point out open questions and how these could be addressed, and they should weigh potentially opposing paradigms against each other based on substantiated experimental evidence. Readers find them useful because of the synthesis of large amounts of information, authors have the opportunity to resolve potential controversies in their field and both journals and authors gain high citations. However, Reviews are not a way to manipulate 'impact factors' or other measures of success, rather they are an important scholarly contribution to the scientific literature in their own right. Writing Review articles therefore requires both the author's in-depth knowledge of the respective area and the ability to communicate it in a constructively critical viewpoint. Therefore, it is important to outline the qualities that are important for a 'good' review. First, the coverage of content needs to be as complete as is feasible and up to date without digressing into long historical overviews. Second, the work should synthesize more than list. I have argued that a personal opinion is fine in a review, so long as it is clearly stated and acknowledges all relevant work, whether easy to fit into the author's worldview or not. Third, the piece needs to be readable if it is ever to be read. These principles may be useful to those considering when to write a review and how to approach journal editors.

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Poster Sessions Monday/Tuesday
MTU01 Glia

MTU01-01

Astrocytic transforming growth factor beta 1 protects synapses against Aβ oligomers in Alzheimer's disease model

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Alzheimer's disease (AD) is characterized by progressive decline of cognitive functions, mainly due to neuronal/synaptic dysfunction induced by amyloid-β peptide oligomers (AβOs). Although the effects of AβOs in neurons have been extensively studied, if and how AβOs impact astrocytes remain largely unknown. Given the key role of astrocytes in synaptic formation and plasticity, we investigated the effect of AβOs on astrocytes and how it impacts synapse formation and function. Here, we show that murine hippocampal astrocytes are able to bind and internalize AβOs in vitro. Astrocyte conditioned medium (ACM) reduced AβOs binding to neurons, preventing AβO-induced synaptic loss. This 'synaptic protection' ability of astrocytes was impaired by prior stimulation of astrocytes with AβOs. Also, protection provided by ACM was severely inhibited by blocking the signaling of TGF-β (Transforming growth factor-β), previously identified as a neuroprotective and synaptogenic factor secreted by astrocytes (Diniz et al., 2012; 2014). Intracerebroventricular injection of AβOs led to synaptic loss and memory deficit, followed by reduction in the levels of TGF-β1 in the hippocampus. Injection of TGF-β1 in the brain of these mice rescued synaptic/memory déficits caused by AβOs. Thus, we show that astrocytes and their soluble factors, particularly TGF-β1, can repress synaptic loss and AD progression. Further, we show that astrocytes are targets for AβOs, shedding light into a new mechanism underlying AβOs synaptotoxicity, indirect through glial cells. The protocol of this study was approved by the Committee for Animal Research of the Federal University of Rio de Janeiro.

MTU01-02

The role of lysophosphatidic acid in the microglia-gliblastoma interaction

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The microglial activation is a key event in the nervous parenchyma defense against ischemia, neurodegenerative diseases and inflammation. However, it can be controlled by the tumor cells. Microglia in this context supports progression and tumor invasion. Glioblastomas (GBMs) are characterized by their high proliferation index, aggressiveness, invasiveness, insensitivity to chemotherapy and short survival of patients. Lysophosphatidic acid (LPA) is a lysophospholipid that act also as bioactive signaling molecules that play important roles in diverse biological processes, including migration of tumor cells. It was previously described that microglia is the glial cell that over expresses the mRNA of Autotaxin (ATX), a multifunctional phosphodiesterase which produces LPA. In the context of microglia-GBM interaction, the present study aimed to investigate the influence of LPA on tumor growth and invasion. Highly pure cultures of microglial cells from neonatal mice and cultures of tumor cells from GBM02 human cell line, established in our lab, were performed. We verified by Thin Layer Chromatography analysis, that both conditioned media from microglia (MG CM) and GBM02 (GBM02 CM) were able to secrete LPA. In addition, the GBM02 CM cells induced an increase of ATX and LPA_1 (receptor of LPA) expressions in microglia. On the other hand, the MG CM, promoted migration and proliferation of GBM02 cells, but failed when Ki16425, a LPA receptor antagonist with selectivity for LPA_1 and LPA_3, was added to conditioned medium. These results suggest that microglia-GBM interaction through LPA is important for microglial recruitment and also in tumor progression. Better understanding of this interaction as well as factors implicated in it can lead to the development of new therapeutic strategies in the treatment of GBMs. Supported by: FAPERJ, CNPq, CAPES, Cancer Foundation RJ, INCT-INNT.

MTU01-03

Reduction of the water-soluble tetrazolium salt 1 as indicator for substrate metabolization by cultured brain astrocytes

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The water-soluble tetrazolium salt WST-1 is frequently used to determine the vitality of cells, as the formation of its colored formazan product depends on intracellular reduction equivalents and can easily be quantified by photometry. Incubation of astrocytes with WST-1 in a glucose-containing medium in the presence of a membrane-permeable electron cycler caused an almost linear increase in the absorption of extracellular WST-1 formazan. The extracellular reduction of WST-1 strongly depended on the presence of glucose in the medium and was almost completely abolished during incubation in the absence of glucose. Formation of WST-1 formazan increased with the concentration of glucose applied and maximal formazan production within 30 and 60 min incubation was already observed in the presence of 0.5 mM glucose and not accelerated by an increase in glucose concentration up to 10 mM. Application of potential alternative substrates that may replace glucose as cellular substrate to deliver electrons for WST-1 reduction revealed that mannose was able to fully replace glucose. In contrast, neither the hexoses fructose and galactose nor the mitochondrial substrates pyruvate, lactate or β-hydroxybutyrate were able to substitute for glucose to provide electrons for WST-1 reduction in viable astrocytes. The glucose-dependent WST-1 reduction by viable astrocytes was strongly lowered by the alkylating substance 3-bromopyruvate (3-BP), a known inhibitor of astrocytic glycolysis, in a concentration-dependent manner causing half-

maximal inhibition of WST-1 reduction at a concentration of 147 ± 37 μM 3-BP. These data demonstrate that the reduction of WST-1 to its formazan product can be used as a tool to study the ability of cultured cells to take up and metabolize extracellular substrates.

MTU01-04

Functionalized lipidic nanocapsules internalization by oligodendrocytes in vitro

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Lipidic nanocapsules (LNC) could serve as putative vector for targeted cell therapy. We have studied their potential to reach specifically oligodendocytes (OL). Intracellular penetration of LNC vectorized with NFL-TBS.40-63 peptide was studied. This peptide is known to penetrate into OL through endocytosis, and has pro-remyelinating effects (Fressinaud and Eyer, 2015). Secondary OL cultures from newborn rat brain (Fressinaud, 2005) grown in chemically defined medium, were treated with various concentrations of LNC (Anton et al., 2010) labelled with DiD (dialkyl aminostyryl analog), and adsorbed or not with NFL-TBS. Intracellular location of LNC (100 nm diameter) was determined by confocal microscopy, and the number of DiD labelled OL was quantified.

The effects of LNC on OL development were characterized by double/triple immunocytochemistry using known markers of OL progenitors (A2B5), differentiated OL (CNP), or mature OL (MBP).

Experiments were run in triplicate. LNC did not penetrate significantly into astrocytes grown in the same conditions than OL. Around 29% OL incorporated 'blank' DiD-LNC, while NFL-TBS vectorized LNC were observed in around 83% OL. Colocation of DiD-LNC and OL markers, and the intracellular location of LNC were confirmed by confocal microscopy. LNC were abundant in the perinuclear region and sometimes in large processes of OL. Persistance of LNC into OL up to 4 days did not alter their differentiation or maturation. Highly differentiated OL with numerous processes, ramifications and membranous extension were present as well as in controls. LNC internalization is specific to OL. Internalization of LNC with appropriate diameter and concentration does not alter OL development. This endocytic process is strongly upregulated (+180%) by the adsorption of NFL-TBS.40-63 on LNC. LNC fonctionalized by NFL-TBS.40-63 could represent efficient vectors to deliver targeted therapeutics in MS. Internalization of LNC vectorized with NFL-TBS.40-63 into OL in vitro suggests their potential as cargos to deliver specifically therapeutic molecules during MS.

MTU01-05

Astrocytes protect dopaminergic neurons from synaptic loss induced by alpha-synuclein oligomers in Parkinson's disease model

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Parkinson's disease (PD) is one of the most common neurodegeneration in the world. Its features include the preferential loss of dopaminergic neurons in the substantia nigra and changes in the basal ganglia circuit. α-Synuclein (α-syn) aggregation is a common characteristic of sporadic and familial forms of PD. Considerable evidence suggest that α-syn oligomers (αSO), formed as pre-fibrillary intermediates, may contribute to dopaminergic neurons toxicity in PD. Astrocytes are closely associated with synapses contributing significantly to the regulation of synapse formation and neurotransmitters levels in the synaptic cleft. Although the direct effects of αSO have been evaluated in neurons, their effects on astrocytes have not. The main objective of this work is to evaluate the direct effect of αSO on astrocyte synaptogenic ability and on the regulation of glutamate-glutamine metabolism. Using primary cultures of mesencephalic astrocytes and animals injected with αSO, we demonstrated that αSO-treated astrocytes exhibited an increase in the levels of the glutamate transporters, GLAST and GLT1, and in the levels of the glutamine synthase enzyme. These alterations resulted in increased astrocytic intracellular glutamate levels and higher rates of glutamate uptake in astrocyte cultures. Additionally, we observed that treatment of cultured astrocytes with αSO led to enhancement in their synaptogenic capacity. Moreover, we verified that animals injected with αSO showed higher numbers of astrocytes and excitatory synapses in caudate-putamen. Together, our data demonstrate that alterations in astrocytic functions is present in PD progression models and corroborate the recent evidence that astrocytes are correlated with the increase of the activity of the striatal glutamatergic system in PD. Furthermore, we describe a new endogenous astrocyte molecule involved in increasing striatal glutamatergic synaptic density.

MTU01-06

Reactivation of astrocytes correlated to recovery from blood-brain barrier breakdown in the mouse brain injury

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Astrocytes and microglial cells will be activated when the brain had injury or inflammation; however, the biological significance is yet to be clarified. We have been used stab wound injury to the mouse cerebral cortex as a brain injury model to examine the functional role of reactive astrocytes and microglial cells. Our study with stab wound injury to the mouse brain induces blood-brain barrier (BBB) breakdown but it will be recovered in a week. We

have previously reported that extracellular matrix molecule tenascin-C might concerned in activation of astrocytes and have the functional role for recovery from BBB breakdown. In this study, to know the relationship between astrocyte activation and BBB recovery from breakdown after the brain injury, we analyzed IgG leakage using immunofluorescent staining with anti-mouse IgG antibody for evaluation of recovery from BBB breakdown. IgG leakage was highest at 1 day after the stab wound injury but it diminished by 7 days after that. We used bromo-deoxy uridine (BrdU) incorporation with drinking water for mice to analyze the proliferation rate of astrocytes using anti-BrdU antibody for the brain sections. At the same time, anti-GFAP antibody was used to examine activation of astrocytes by co-immunostaining. The number of GFAP-positive astrocyte was highest at day after 3 of injury, and then decreased by day after 7. RT-qPCR method was performed to study the expression level of the genes related to the BBB integrity and astrocyte activation, and found that most of the genes concerned in BBB integrity were down regulated just after the BBB breakdown, while they recovered to basal level within 7 days after the brain injury. These results indicated that astrocyte activation might be correlated to the BBB recovery from breakdown caused by stab wound brain injury.

MTU01-07

Copper accumulation and acute toxicity in C6 glioma cells after application of copper oxide nanoparticles

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The rat C6 glioma cell-line is frequently used as an experimental model for glial tumors. To investigate the potential use of copper oxide nanoparticles (CuO-NPs) as a therapeutic drug for glioma treatment, the consequences of an application of CuO-NPs on the cellular copper content and cell viability of C6 glioma cells was investigated. CuO-NPs were synthesized by a wet-chemical method and were coated with dimercaptosuccinic acid and bovine serum albumin to improve colloidal stability in physiological media. Application of these protein-coated nanoparticles (pCuO-NPs) to C6 cells caused a strong time-, concentration- and temperature-dependent copper accumulation. This cellular copper accumulation was accompanied by severe toxicity as indicated by the loss in cellular MTT-reduction capacity, the loss in cellular LDH activity, and by an increase in the number of propidium iodide-positive cells. Toxicity of pCuO-NPs to C6 cells was only observed for incubation conditions that increased the specific cellular copper contents above 20 nmol copper per mg protein. Despite severe toxicity, no obvious formation of reactive oxygen species was found in pCuO-NP-treated C6 cells. Unexpectedly, C6 glioma cells were less vulnerable to pCuO-NPs than cultured primary brain astrocytes. Both cellular copper accumulation and pCuO-NP-induced toxicity in C6 cells were prevented by application of cell membrane-permeable and -impermeable copper chelators, but not by frequently used endocytosis inhibitors. These data suggest that uptake of copper ions liberated extracellularly from the pCuO-NPs, rather than uptake of intact pCuO-NPs, leads to the observed toxicity of pCuO-NP-treated glioma cells.

MTU01-08

Noradrenergic modulation of cerebellar glial activity during nociception

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Cerebellar activation and increase in metabolic changes during pain processing have been reported in previous human brain imaging studies. However, it is still unknown whether and how the cerebellar Bergmann glia (BG) is involved in noxious information processing. To monitor the calcium activity of BG in intact cerebellar cortex lobule IV/V, we performed *in vivo* two-photon calcium imaging in anesthetized mice. Various noxious electrical stimuli were delivered to the mouse hind paw during calcium imaging and pharmacological manipulation. Formalin was also injected to the hind paw to monitor BG calcium responses under an acute spontaneous pain condition. We show that 1) noxious electrical stimulation (ES) in anesthetized mice results in norepinephrine release and subsequent activation of BG network in the cerebellum, 2) the ES-induced BG calcium response was completely blocked by prazosin, an alpha1-adrenergic receptor blocker, and 3) the formalin injection induces strong BG calcium responses during the early phase (~10 min) rather than the late phase (20~50 min) of the formalin test. Taken together, we suggest that noradrenergic signaling mediates the activation of the glial network during noxious information processing in the cerebellum.

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MTU01-09

Ischemic tolerance mediated by glia-neuron interactions

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A mild ischemic episode (preconditioning; PC) induces resistance to a subsequent severe ischemic injury. This phenomenon, known as ischemic tolerance, is an endogenous process that provides robust neuroprotection. We have worked on glia-neuron interaction, and found that phenotypical changes glia could affect a big variety of brain functions, which include both beneficial and hazardous phenomena. We previously showed that astrocytes become a neuroprotective phenotype in response to PC, which was essential for induction of ischemic tolerance. Such an astrocyte-mediated ischemic tolerance requires activation of astrocytes and subsequent upregulation of P2X7 receptors and expression of HIF-1α in astrocytes. Although PC also increased HIF-1α in neurons, this was not involved in ischemic tolerance. Here, we show the difference in mechanism of HIF-1α increase between neurons and astrocytes, and answer why astrocytic HIF-1α is more important. It is well-known that an increase in HIF-1α in neurons was dependent on hypoxia/PHD2. In fact, neurons *in vitro* caused a transient HIF-1α increase in response to hypoxia, but, interestingly, astrocytes did

not. Astrocytes did not express even PHD2, an oxygen-dependent HIF-1α degrading enzyme or constitutive HIF-1α. Instead, they showed persistent increase in P2X7 receptor by PC, which was a main mechanism for upregulation of HIF-1α in astrocytes. Such novel hypoxia-independent machinery for HIF-1α increase would allow astrocytes to cause persistent HIF-1a and subsequent strong ischemic tolerance. We also discuss a possible mechanism of P2X7 receptor activation in this phenomenon.

MTU01-10

The role of parenchymal cellular prion protein during glioblastoma progression

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Glioblastoma (GBM) is the most aggressive primary glial tumor that affects the central nervous system (CNS). These tumors are highly heterogeneous, angiogenic and insensitive to radio and chemotherapy. The cellular prion protein (PrP^c) is highly expressed in the CNS and plays a key role in the differentiation of neural cells during development. Here we have investigated the tumor growth produced by the injection of cells from the human GBM cell lineage GBM95, established in our laboratory, into the brain parenchyma of wild type (WT), PrP^c knockout (KO) and PrP^c over-expressing (TG20) mice. In this context, the role of PrP^c was investigated during tumor progression. During 2 weeks, the animals were submitted to magnetic resonance imaging (MRI) and histopathological analysis. Our data showed that after this period, the xenografted tumor was similar to a human GBM and was able to produce reactive gliosis in the adjacent parenchyma, angiogenesis and an intense recruitment of macrophage and microglial cells. MRI showed that the tumor mass had enhanced contrast suggesting a blood brain barrier disruption. In addition, analyzing the tumor volume (mm^3), we have observed that tumors produced in KO animals were bigger than those produced in WT mice. Whereas tumors produced in TG20 animals were the smallest ones. Interestingly, in *in vitro* cell migration assays, when GBM95 cells were cultured with conditioned medium (CM) obtained from brain primary cultures, these cells migrated almost twice less when compared to the GBM95 cells that were cultured with CM obtained from WT primary cultures, confirming our results *in vivo*. Our recent results suggest that the parenchymal PrP^c should play a protective role in a dose-dependent manner from tumor invasion. Supported by: FAPERJ, CNPq, CAPES, Cancer Foundation RJ, INCT-INNT.

MTU01-11

Dopamine attenuates LPS-induced cytokine expression by inhibiting the nuclear translocation of nf-kb p65 in microglial BV-2 cells

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It has been reported that inflammatory cytokines and nitric oxide (NO) produced by microglial cells mediate neuronal cell death in brain ischemia/reperfusion injury and traumatic brain injury. Release of dopamine in the brain has been known to be accelerated in cerebral ischemia and trauma. We recently reported that dopamine attenuated lipopolysaccharide (LPS)-induced NO production in mouse microglial cell line BV-2. In this study, we investigated the effect of dopamine on LPS-induced mRNA expression of cytokines in BV-2 cells. The mRNA levels of cytokines were determined by RT-PCR and real-time RT-PCR, and the levels of NF-κB p65 and IκBα were determined by Western blotting. LPS (10 μg/mL) increased mRNA levels of IL-1β, IL-6 and TNF-α. Pretreatment with dopamine (1–30 μM) for 24 h concentration-dependently attenuated the LPS-induced mRNA expression of these cytokines. Neither SCH23390 nor sulpiride, D_1-like and D_2-like dopamine receptor antagonists, respectively, affected the attenuation of LPS-induced mRNA expression of cytokines by dopamine. N-acetylcysteine (NAC), a free radical scavenger, inhibited the attenuation of LPS-induced mRNA expression of cytokines by dopamine. On the other hand, hypoxanthine/xanthine oxidase, a super oxide generating system, did not affect the LPS-induced mRNA expression of cytokines. Dopamine concentration-dependently increased the level of quinoproteins, and the increase was inhibited by NAC. LPS increased the levels of NF-κB p65 in nuclei of BV-2 cells, and decreased the levels of IκBα in the cytosol. Although dopamine did not affect the LPS-induced decrease of IκBα, dopamine attenuated the increase in the levels of NF-κB p65 in the nuclei. NAC inhibited the effect of dopamine on the levels of NF-κB p65. These results suggest that dopamine attenuates LPS-induced expression of cytokines by inhibiting the nuclear translocation of NF-κB p65 through the formation of quinoprotein in microglial cells.

MTU01-12

Mitotic defects in neural cells after aberrant nuclear entry of neurofibromin

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Neurofibromin is a regulator of cellular proliferation, yet its actions as a tumor suppressor may not be solely explained on its ability to de-activate Ras. Recent evidence has established that tumor suppressors enter the nucleus and regulate microtubule dynamics for mitotic spindle integrity and proper capturing of chromosomes. Our most recent data postulate that neurofibromin belongs in this functional category. Specifically, we have now established that neurofibromin controls the pivotal function of chromosome congression**.** Through a functional NLS on its primary sequence, neurofibromin accumulates in the nucleus at G2 phase, and its nuclear entry depends on intense, PKCε-dependent

phosphorylation on Ser2808, a C-terminus residue, adjacent to NLS. Moreover, we discovered that depletion of neurofibromin by RNAi causes a striking phenotype of aberrant chromosome congression with daughter cells exhibiting increased aneuploidy. We now show that overexpression of the C-terminal domain (CTD) of neurofibromin, led to nuclear accumulation of the recombinant protein in a dose-dependent manner. Moreover, when Ser2808 was mutagenized to the phosphomimetic Asp (CTD-D), nuclear accumulation was almost as quick as translation. Interestingly, its phosphoablating mutation showed the highest affinity of binding to cytosolic tubulins in co-immunoprecipitation assays, when compared to the affinities of wild-type CTD or CTD-D. Next, we examined the effect of CTD constructs, wild-type or mimicking patient mutations, on the proliferation rate of glioma cells. We found that some CTD constructs increased the mitotic index, some produced mitotic catastrophe, while other constructs induced spindle and chromosome congression and/or segregation defects. Most importantly, we observed such mitotic phenotypes in similarly transfected primary astrocytes. Thus, our results show that neurofibromin, through its nuclear entry and tubulin-binding abilities, actively participates in the mitotic process at least in astrocytic backgrounds, as also implied by the cell type-specific abnormalities in proliferation and frequent formation of benign and/or malignant tumors in patients with Neurofibromatosis 1 (NF-1).

MTU01-13

Ablation of NG2 glia in the CNS induces anxiety-like behaviour in adult mice

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NG2 glia, also known as oligodendrocyte progenitor cells (OPCs), are mitotically active cells known primarily for their role in producing myelin-forming oligodendrocytes in the central nervous system (CNS). Additional roles of NG2 glia in adult brain physiology, particularly the modulation of neural processing, have been suggested but the underlying mechanisms remain elusive. Attempts to investigate the function of NG2 glia by targeted cell ablation in the adult CNS have been limited by methodological challenges resulting in in only partial and transient OPC ablation. To overcome these limitations we have developed a novel transgenic mouse model of conditional NG2 glia ablation. By crossing *Pdgfrα-CreER^{T2}* mice with a Cre-conditional cell ablation line called *Sox10-DTA* mice, tamoxifen-mediated Cre recombination resulted in both the deletion of GFP cassette in the recombined PdgfRα^{+} cells and the expression of a suicide gene (diphtheria toxin fragment A, DTA), which rendered NG2 glia selectively sensitive to DTA-mediated apoptosis. In combination with intracisternal infusion of the antimitotic drug cytosine-ß-D-arabinofuranoside (AraC), tamoxifen-administered *Pdgfrα-CreER^{T2}: Sox10-DTA* mice exhibit complete ablation of NG2 glia throughout the entire brain for up to 10 days post AraC infusion. To determine the functional consequences of NG2 glia ablation, we assessed cohorts of animals using a range of behavioural tests. Our data reveal that ablation of NG2 glia precipitates anxiety-like behaviours consistent with a possible role for these cells in modulating neuronal function in the CNS.

MTU01-14

Docosahexaenoic acid maintains GFAP levels of developing rat brain via PI3K-dependent FABP7-pparg interaction in astrocytes

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Introduction: The omega-3 fatty acid, docosahexaenoic acid (DHA), supports brain growth and is known to be neuroprotective. Glial fibrillary acidic protein (GFAP) is a key component of brain astrocytes. However, very few reports are available regarding the effect of DHA on GFAP levels in astrocytes, particularly during GFAP-suppressed conditions.
Objective: Here, we examined whether DHA could have a protective effect on the astrocytes, and if it may involve GFAP restoration.
Method: We fed pregnant and lactating rats with DHA and co-treated with troglitazone (TZ), an agonist of peroxisome-proliferator-activated-receptor-gamma (PPARγ) that suppresses GFAP and induces astrocyte damage. Primary astrocytes were cultured and treated with DHA and TZ. Western-blotting, immunohistochemistry, EMSA, ChIP, luciferase assay, FRET and CoIP analysis were done to confirm our objectives.
Results: We observed an augmentation of DHA levels in the rat brain, which inhibited the TZ-mediated suppression in GFAP levels. Investigating the mechanism revealed a DHA-mediated up-regulation in phosphatidylinositol-3-kinase (PI3K)/AKT levels in the astrocytes. We also observed elevated levels of fatty acid-binding protein-7 (FABP7), a molecule responsible for fatty acid uptake, transport, and metabolism. We detected that PI3K/AKT and FABP7 participated in GFAP augmentation, as evident from GFAP attenuation by PI3K/AKT-inhibitor or FABP7-siRNA in TZ-treated cultured astrocytes. Furthermore, the FABP7 expression was found to be PI3K/AKT dependent. Examining the participation of PPARg revealed that DHA-mediated PPARg binding to response-elements (PPRE) within the FABP7-gene was actually responsible for the FABP7 expression. FABP7 then underwent protein-protein interaction with PPARg, which suppressed the cyclin-dependent-kinase-5 (CDK5)-PPARg-complex. Therefore, DHA attenuated the reported CDK5-PPARg-dependent phospho-PPARg(Ser112) pathway that reduces GFAP expression.
Conclusion: Overall, we demonstrate that DHA is capable of protecting astrocytes via restoration of GFAP. Mechanism unearthed is a PI3K/AKT-dependent increase in FABP7, which stimulates FABP7-PPARg at the cost of CDK5-PPARg, causing GFAP augmentation in the astrocytes.

MTU01-15

Multiple origins of perilesional nestin-expressing reactive astrocytes following closed-head injury

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The expression of a neural stem cell marker, nestin in perilesional reactive astrocytes is common in brain injuries, and the multipotency of reactive astrocytes for producing neurons is suggested by culturing cells from brain lesion. These neural stem cell-like behaviors of reactive astrocytes are assumed to reflect the injury-

induced reprogramming of astrocytes or the migration of SVZ or RMS cells to lesion. In order to address this issue, the present study have determined the origin and fate of nestin-expressing reactive astrocytes in a mouse model of closed-head injury. For this purpose, nestin-creERT2/CAG-CAT$^{f/f}$-GFP mice were used for labelling nestin-expressing cells. As results, GFP+ reactive astrocytes were found to originate from SVZ, as well as from lesion core. GFP+ reactive astrocytes from SVZ increased the expression of astrocyte markers during their migration to lesion, and this astrocyte differentiation was suppressed by ablating STAT3 gene. Thus, the induction of astrocyte differentiation and pathological activation of neural stem cells in SVZ by brain injury were indicated. The generation of reactive astrocyte from SVZ correlated with the deposit of IgG and microglial activation in SVZ, suggesting the activation of SVZ by hemorrhage and following inflammation. The GFP+ reactive astrocytes were also derived from NG2 + and partly laminin+ cells in lesion core, indicating the generation of reactive astrocytes from NG2 glia and/or pericytes. GFP+ reactive astrocytes from SVZ and lesion core were indistinguishable after incorporated in the perilesional reactive astrocytes. Nestin-creERT2 failed in the GFP-labeling of perilesional reactive astrocyte derived from pre-existing astrocytes, which is the majority of nestin-expressing reactive astroytes during the early period of injury. These results indicate the multiple origins of perilesional reactive astrocytes, which form glial scar. The number perilesional GFAP+ reactive astrocytes reduced drastically after wound healing, and this resolution of gliosis accompanied by the disappearance of most GFP+ reactive astrocytes. Thus, reactive astrocytes were likely eliminated, rather than de-activated to normal astrocyte. The present study has excluded the multipotency of reactive astrocytes and revealed multiple layers of astrogliosis.

MTU01-16

Role of serotonergic signaling in regulation of astrocytes morphology

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Serotonin is an important neurotransmitter regulating various brain functions via activation of specific serotonin receptors. In neurons, serotonin receptors can modulate multiple signaling pathways including activation of small GTPases of the Rho family which determine cell morphology. Interestingly, serotonin receptors are also expressed by astrocytes. These glial cells possess a unique morphology allowing single astrocytes to modulate thousands of synapses over defined anatomical regions. It is also known that astrocytes' Ca^{2+} signaling is implicated in these functions. Properties and propagation of Ca^{2+} signals depend on diffusion and therefore on astrocyte morphology, which is dynamic itself. Therefore, it is important to understand which signaling cascades are involved in controlling astrocyte morphology. We investigate molecular mechanisms by which serotonin receptors regulate small GTPases of the Rho family to control astrocyte morphology and astrocyte Ca^{2+} signaling.

We show that knockdown of defined serotonin receptors leads to a more ramified morphology in cultured mouse hippocampal astrocytes. Furthermore, transient expression of constitutively active variants of the small GTPase RhoA results in drastic morphological changes with decreased size and perimeter of the cells. Sholl analysis also reveals impact of RhoA on the arborization of mouse hippocampal astrocytes. Moreover, our data suggest that astrocytes Ca^{2+} dynamics correlate with their morphology. Together, these data indicate that serotonin receptors are critically involved in regulation of astrocyte morphology and Ca^{2+} signaling.

MTU01-17

The endoplasmic reticulum of astrocytes constitutes a dynamic glucose pool

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Glucose-6-Phosphatase (G6PC) is an ER luminal enzyme that in glucose-releasing cells catalyses the dephosphorylation of glucose-6-phosphate, generating free glucose inside the ER and allowing it to be released by the cell. G6PC3 is the ubiquitous isoform of G6PC, expressed throughout most cell types and tissues, yet its role in non glucose-releasing cells remains unknown. However, mutations in the G6PC3 gene cause severe congenital neutropenia and about half of the patients suffer from developmental brain defects. A previous study reported that G6PC3 is highly expressed in rodent astrocytes, despite the fact that astrocytes do not release glucose. We investigate the function of G6PC3 in astrocytes, using both human cells and rodent tissue.

Using immunohistochemistry in cortical brain slices from rats we confirm previous reports that G6PC3 is strongly expressed in rat astrocytes. Using qPCR and immunocytochemistry we show that the protein is also highly expressed in the ER of primary cultures of human astrocytes. In order to test whether G6PC3 is functional in astrocytes, we generated lentiviral versions of previously published glucose nanosensors targeted to the ER lumen. We show that the ER of human astrocytes is able accumulate glucose. To investigate the spatial dynamics of this luminal glucose pool, we developed a culturing protocol for growing primary human astrocytes in a microfluidic device that allows separation of soma and processes, with the processes growing throughout a channel of 500um length. We demonstrate that the ER can extend in form of a single tube throughout astrocytic processes of 500um length. Combining glucose nanosensors and microfluidics, we are currently investigating the existence of a diffusive transport of glucose from the distal end of the astrocytic processes to the soma.

We propose that the ER constitutes a dynamic pool of glucose that in astrocytes facilitates a more efficient uptake of glucose, and allows free glucose to diffuse throughout the cell inside a protected compartment that lacks enzymes able to metabolize it.

MTU01-18

The novel dual-action prodrug Q-PAC targets glutathione and LSD1 to trigger apoptosis in glioblastoma cells

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Glioblastoma (GBM) is a highly aggressive cancer of the brain with a poor prognosis for patients and few treatments. Targeting epigenetic mechanisms has shown promising results against several cancers, but has so far been unsuccessful against GBM. Altered

histone 3 lysine 4 methylation and increased lysine-specific histone demethylase 1A (LSD1) expression in GBM tumours nonetheless suggests that epigenetic mechanisms are involved in gliomagenesis and progression. We therefore engineered a dual-action prodrug, which is activated by the high hydrogen peroxide levels associated with GBM cells. The quinone-methide-phenylaminecyclopropane (Q-PAC) prodrug combines the LSD1 inhibitor properties of 2-phenylcyclopropylamine with the glutathione (GSH) scavenging properties of *para*-quinone methide to trigger apoptosis in GBM cells. Q-PAC selectively impaired key GBM cell behaviours, such as migration, proliferation and invasion, and triggered cell apoptosis through its hybrid action in several primary and immortal GBM cell cultures. These results support our double-hit hypothesis of inhibiting LSD1 and quenching GSH, in order to impair and ultimately kill GBM cells whilst sparing healthy astrocytes. Together our data suggest that such a strategy is effective at selectively targeting GBM and potentially other types of cancers.

MTU01-19

Glutamine synthetase translational control in cerebellar bergmann glia cells

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Glutamate is the major excitatory transmitter of the vertebrate brain. Exerts its actions through the activation of specific plasma membrane receptors expressed both in neurons and glial cells. Recent evidences have shown that glutamate uptake systems, particularly enriched in glia cells, trigger biochemical cascades in a similar fashion as receptors. A tight regulation of glutamate extracellular levels prevents neuronal over-stimulation and cell death and it is critically involved in glutamate turnover. Glial glutamate transporters are responsible of the majority of the brain glutamate uptake activity. Once internalized, this excitatory amino acid is rapidly metabolized to glutamine *via* the astrocyte enriched enzyme glutamine synthetase. A coupling between glutamate uptake and glutamine synthesis and release has been commonly known as the glutamate/glutamine shuttle. Taking advantage of the established model of cultured Bergmann glia cells, in this contribution, we explored the gene expression regulation of glutamine synthetase. A time and dose dependent regulation of Glutamine synthetase protein and activity levels was found. Moreover, glutamate exposure resulted in the transient shift of glutamine synthetase mRNA from the monosomal to the polysomal fraction. These results demonstrate a novel mode of glutamate-dependent glutamine synthetase regulation and strengthen the notion of an exquisite glia neuronal interaction in glutamatergic synapses.

MTU01-20

The glutamate-cystine exchanger (XCT) is expressed in a subpopulation of astrocytes at significant levels compared to EAAT3

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The glutamate-cystine antiporter (xCT, Slc7a11) mediates cystine uptake and glutamate release. This is thought to promote glutathione synthesis and increase extracellular glutamate possibly to toxic levels in certain pathological situations. There is, however, a great deal of uncertainty, at least in part, due to unresolved questions concerning xCT localization and expression levels. Here, we determined the localization of xCT in the mouse brain immunohistochemically using xCT-deficient tissue to validate antibody specificity. Our results show that xCT is expressed in a subpopulation of astrocytes and is highly present in the leptomeninges and along larger blood vessels. We did not detect xCT in oligodendrocytes and neurons. Further, we neither detected xCT in resting microglia nor in reactive microglia induced by glutamine synthetase deficiency. All main brain regions express xCT with the lowest levels in the cerebellum and brain stem. Using a chimeric xCT-EAAT3 protein to normalize the differences in antibody binding, we compared the levels of xCT and EAAT3 by Western blotting and found that mouse hippocampus contains similar amounts of xCT and EAAT3. Thus, the estimated quantities of xCT are sufficient to support the hypothesized physiological roles.

MTU01-21

Trkb receptor mediates BDNF protection of astrocytes

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Brain-derived neurotrophic factor (BDNF) is a neurotrophin that promotes neuronal survival and inhibits apoptosis. Since little is known about BDNF action in astrocytes, we examined the effect of BDNF on astrocyte viability and the involvement of TrkB in this action. BDNF treatment for 24 h increased astrocyte viability by reducing apoptosis induced by serum deprivation (SD). BDNF also reduced p53 and active caspase-3 expression induced by SD. Next, we determined TrkB participation by using the selective and potent TrkB antagonist ANA-12 (which inhibits TrkB- full length (TrkB-FL) and TrkB-Truncated1 (TrkB-T1) isoforms) or K252a which is a tyrosine kinase inhibitor of TrkB-FL receptors. The presence of each inhibitor blocked the decrease in cell death induced by BDNF. In order to identify which TrkB isoform is involved in BDNF protective effect we analyzed mRNA expression levels of TrkB-FL and TrkB-T1 by RT-qPCR, both isoforms TrkB-FL and TrkB-T1 are expressed in cultured astrocytes although TrkB-FL is expressed at lower levels than TrkB-T1. Western Blot of TrkB showed that only TrkB-T1 protein is present in cultured astrocytes. Thus,

although astrocytes express mRNA for TrkB-FL, this is not translated into detectable protein levels in these cells. However, BDNF induced ERK activation in astrocytes and blocking this pathway abolished BDNF protection from SD-induced apoptosis. Finally, we evaluated if BDNF could protect astrocytes from 3-nitropropionic acid (3NP)-induced cell death, being 3NP a toxin widely used as an *in vitro* model of Huntington's disease. We found that BDNF prevented astrocyte death induced by 3NP and this effect was blocked by both TrkB and ERK inhibitors. In conclusion, our results indicate that TrkB mediates BDNF antiapoptotic effect on astrocytes through ERK activation. Since astrocytes are key players in neuroprotection, understanding BDNF protective mechanisms in these cells may help develop new strategies for treating neurodegeneration.

MTU01-22

Transcription factor MAFB mediates activation of spinal microglia relating to neuropathic pain after peripheral nerve injury

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Microglia as a pathological effector and amplifier in the central nervous system undergo various form of activation. Some kinds of microglial activation alters neuronal environment leading to pathological symptom, but the critical determinants for the spectrum of microglial activation is not fully understood. Among the well-studied microglia induced-pathological paradigms, it is known that spinal microglial activation following peripheral nerve injury is a key event for developing neuropathic pain. The factors critical for pain-inducing function of activated microglia are extensively studied, but the factors contributing the activation of microglia are also less uncovered.

Herein, we demonstrate that MafB, a known pivotal transcriptional regulator of macrophage differentiation, is involved in the activation of microglia in the mouse model of neuropathic pain. Peripheral nerve transection caused rapid and marked increase of MafB expression selectively in the spinal microglia that displayed activation phenotype represented by a proliferation and CD68. MafB knockdown by siRNA suppressed the expression levels of pain-related genes and alleviated development of tactile allodynia. CCL21, a chemokine involved in the onset of neuropathic pain and derived from injured neurons, enhanced microglial MafB expression *in vivo*. Taken together, we propose that MafB is a key mediator in the peripheral nerve injury-induced phenotypic alteration of spinal microglia which contributes to development of neuropathic pain.

MTU01-23

Location matters: the role of calmodulin-mediated AQP4 translocation in human astrocyte response to hypoxia and mild hypothermia

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Therapeutic hypothermia is one of the effective measures following brain injury. Astrocytes are the brain's most abundant cell type so it is essential to understand their stress response following hypothermia. One of the main mechanisms by which hypothermia attenuates oedema formation, is preserving the brain's water homeostasis. This is tightly regulated in astrocytes by a group of membrane proteins called aquaporins (AQPs). It is established that calmodulin is a key element in the regulatory mechanism of AQP translocation. In this study, we investigated the alterations in the expression profile of cerebral AQPs, with a particular interest in AQP4 membrane expression and calmodulin levels in astrocytes cultured under hypoxic conditions for 6 h at mild hypothermia (32°C).

The microarray data and subsequent KEGG analysis, suggested the involvement of MAPK and the wnt signalling pathways, which was confirmed by Profiler PCR Arrays (184 genes). All the investigated cerebral AQPs genes were expressed and RT-qPCR data showed significant upregulation of AQP4 accompanied by a significant down-regulation of AQPs 1, 5, 9 and calmodulin. ELISA results confirmed these findings for AQP4 and calmodulin.

Hypothermia has a well-known protective effect against brain ischemia and hypoxia. This upregulation of AQP4, at both the gene and protein levels, during this acute phase does not appear to fit well with the fact that hypothermia attenuates oedema formation by preserving the brain's water balance. This could be explained through the significant decrease in AQP4 surface expression after inducing hypothermia obtained from the CSB data. This finding indicates the involvement of impairment of AQP4 translocation; and hence its function, as one of the possible mechanisms in mediating the neuroprotection effect of mild hypothermia. This hypothermia effect could be mediated through the significant inhibition of calmodulin at both the transcriptional and translational levels.

Hypothermia has a complex effect and there is no single factor that could explain its neuroprotective effect. The data reported here reveals the involvement of inhibition of MAPK and wnt signalling pathways and impairment of AQP4 translocation that is mediated by calmodulin; could be one of the many mechanisms through which hypothermia mediates its neuroprotective effect.

MTU01-24

Regional differences in nitric oxide synthesis and HSP27 expression between spinal cord and cortical glia

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As a non-cell autonomous disease, motor neuron disease (MND) initiation and progression depends on both the molecular pathologies developed within motor neurons, and the subsequent reactivity of non-neuronal cell populations such as astroglia and microglia. Given that spinal cord motor neurons are the primary target of the disease over neurons in other regions in the brain, regional differences in cytoprotective glial stress responses to pathological stimuli might be responsible for the susceptibility of these motor neurons. Therefore, we compared inflammatory and heat shock responses (HSR) in glia from the cortex and spinal cord after treatment with lipopolysaccharide (LPS). Griess assay showed that spinal cord mixed glial cultures had a 2-4 fold greater synthesis of nitric oxide (NO) after LPS treatment compared to cortical glial cultures. However, there was no difference in upregulated iNOS protein expression or total number of $iNOS^{+ve}$ microglia in either CNS region with LPS stimulation. Immunoblot analysis of Hsp70 and Hsp27 expression in LPS treated cultures showed that these treatments were not sufficient to induce a heat shock response. However, flow cytometric analysis revealed that double the number of $Hsp27^{+ve}$ astroglia were present in the spinal cord compared to cortical cultures under basal conditions. We propose that greater numbers of $Hsp27^{+ve}$ astroglia in the wild-type spinal cord could play a role in supporting motor neuron growth and maturation, and/or provide a cytoprotective buffer in pathological conditions. On the other hand, enhanced NO synthesis in spinal cord glial cultures might suggest that these cells have a lower threshold regarding turning cell protective inflammatory reactions into destructive processes. This could lead to diminishing support and death of spinal cord motor neurons.

MTU01-25

5% O^2 improves oligodendroglial development in vitro as compared to 21% O^2

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Immature oligodendroglia and oligodendroglial precursor cells (OPCs) are very vulnerable to oxygen toxicity and oxidative stress. In the brain tissue, the physiological O_2 saturation of is at around 3–5%, while standard protocols foresee 21% O_2 for cell cultures. We hypothesized that the 21% O2 pose a hyperoxic challenge to OPCs and oligodendroglia which interferes with their development.

Primary rat OPCs and cells of the OLN93 cell line were incubated in culture wells for 48 and 96 h at 5% and at 21% O_2. Immunocytochemistry was performed with antibodies against A2B5 + and O^{4+}. Proliferating cells were labeled using Ki67 antibodies, apoptosis was measured by TUNEL staining. The expression of oligodendroglial transcription factors Olig1, Olig2, Sox9, Sox10, and also of maturation markers MBP and CNP were determined by realtime qPCR. Oxidative challenge in the cultures was described by gene expression analysis of SOD2 and NRF2 and by Western blot quantification of nitrotyrosine. A potential relevance of HIF1a pathways was investigated by comparison to cells with HIF1a knockdown.

As a result, the morphology of OPCs and of oligodendroglia was between the two oxygen culture conditions. After 48 h, O^{4+} oligodendroglia commonly showed complex process formation at the lower 5% O_2, while they had a much less differentiated structure at 21% O_2, as revealed through Sholl analysis. Levels of MBP, CNP, Olig1, and Olig2 in the extracted RNA samples were significantly diminished, and the expression of antioxidant genes were significantly induced after 48 h at 21% O_2. Elevated nitrotyrosine pointed towards oxidative stress at 21% O_2. There was no difference in apoptosis. In OLN93 cells kept at 5% O_2, HIF1a knockdown induced a reduction in the expression of oligodendroglial transcription factors and maturation markers comparable to the one found at 21% O_2.

According to these data, the commonly used 21% O_2 for OPC cultures may impair oligodendroglial development *in vitro*. Oxidative stress and dysregulation of HIF1a pathways are underlying mechanisms of impaired development caused by oxygen.

MTU01-26

Major glial expression and cell population changes in the aging human brain

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The current main hallmarks of aging mainly include signalling and cellular pathways. However, the relative role of RNA, in particular in human brain aging, was hardly studied so far. Age is the major risk factor for neurodegenerative diseases and a better understanding of the underlying molecular processes will enable a better understanding of the leading diseases. In our recent study, we analysed a large expression data-set produced from human post-mortem brain samples of individuals from 16 or over 100 years old and overall 10 brain regions. The data was composed of microarrays (a total of 1231 samples), as well as massive direct cell quantification based on tailored analysis of data produced by high resolution imaging from cell specific stained brain sections (for neurons and oligodendrocytes). We applied machine learning analysis techniques as well as additional data mining techniques to analysed these extensive data-sets. We detected significant changes in glial cell marker genes, including brain-wide increased expression of microglia markers, in contrast to global down-regulation of neuronal markers. Other glial cell-type markers showed regional specific expression changes patterns, in particular in regions relevant to neurodegenerative diseases (e.g. Alzheimer's and Parkinson's disease). Additionally, glial genes expression could predict the biological age in greater sensitivity compared to neuronal cell markers. A decrease of both cell types was found in cortex from old compared to young individuals. As glial cells can be replenished in the brain (in contract to neurons), our findings may yield a better understanding of these diseases, as well as to novel future therapeutic approaches.

MTU01-27

Astrocytic reduction of menadione is catalysed by cytosolic NAD(P)H: quinone acceptor reductase 1

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Menadione (2-methyl-1,4-naphtoquinone, vitamin K3) is a synthetic derivate of vitamin K1 and an excellent redox cycler that can mediate the formation of reactive oxygen species. As astrocytes are known to contain a highly efficient enzymatic antioxidant defence system, which protects them as well as neighbouring cells against adverse effects of oxidants and toxins, it is not surprising that intact astrocytes are capable to reduce menadione at an impressive rate. The assumption that astrocytic menadione reduction is an enzyme catalysed process was validated by the investigation of cell lysates of astrocyte cultures. Menadione reduction was dependent on the lysate volume applied and was prevented by heat inactivation (5 min, 90°C) or filtration (size exclusion: 5 kDa) of the lysate. Determination of the kinetic parameters of the menadione reduction by astrocyte lysates revealed a K_m-value of 11 ± 4 μM and a V_{max}-value of 213 ± 27 nmol/(min*mg). Suitable electron donors to facilitate menadione reduction were both NADH and NADPH as demonstrated by similar K_m- and V_{max}-values. Digitonin lysis of astrocyte cultures and subsequent separation of the cytosolic and mitochondrial fractions allowed to demonstrate that the menadione-reducing capacity of astrocytes was localised almost exclusively to the cytosol. In addition, the menadione reduction activity of astrocyte lysates was extremely sensitive to the NAD(P)H: quinone acceptor oxidoreductase 1 (NQO1) inhibitor dicoumarol as demonstrated by the K_i-value of 1.2 ± 0.3 nM. These findings strongly suggest that the cytosolic NQO1 is the enzyme responsible for the efficient reduction of menadione in astrocytes.

MTU01-28

OLIG2-lineage astrocytes: a subtype of astrocytes differs from GFAP-positive astrocytes

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Accumulating evidence revealed that astrocytes modulate synaptic activities by promoting neurotransmitter uptake from synaptic clefts and/or by releasing gliotransmitters such as glutamate, D-serine, and ATP to synaptic clefts. The tripartite synapse that consists of pre-, post-synaptic neurons, and astrocytic fine processes functions in various brain regions.

During lineage-tracing studies of Olig2 expressing cells using Olig2^{creER}: ROSA-GAP43-EGFP mice, we found that Olig2-lineage mature astrocytes preferentially cluster in some regions, for example, the globus pallidus (GP). Taking advantage of membrane-targeted GAP43-EGFP, which can visualize the morphology of Olig2-lineage cells in detail, we performed morphometric analyses of astrocytic fine processes that underwent plastic changes in response to overall running activities in the GP. We suggested that astrocytes actively modulate neuronal activities in the GP that play pivotal roles in motor control.

Given the fact that Olig2-lineage astrocytes clustered in specific brain nuclei other than the GP, we further mapped distribution of Olig2-lineage astrocytes in the whole brain. We then compared the distribution pattern with that of GFAP-positive astrocytes visualized in GFAP^{cre}: ROSA-GAP43-EGFP mice. The brain regions rich in Olig2-lineage astrocytes often lacked GFAP-positive astrocytes and vice versa. In a single brain nucleus, Olig2-lineage astrocytes and GFAP astrocytes tended to occupy different territories. These findings implied that the Olig2-lineage astrocyte is a subtype of astrocyte playing different roles from those of the GFAP-positive astrocyte in the adult brain. Interestingly, the brain nuclei rich in Olig2-lineage astrocytes strongly expressed GABA-transporter 3 (GAT-3) and vesicular GABA transporter (vGAT), suggesting that Olig2-lineage astrocytes may be involved in inhibitory neuronal transmission.

MTU01-29

Extracellular GAL-3 induces oligodendroglial differentiation through the modulation of ERK and cytoskeleton pathways

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Galectin-3 (Gal-3), a chimeric protein structurally composed of unusual tandem repeats of proline and short glycine-rich segments fused onto a carbohydrate recognition domain, possesses multifaceted roles in physiological processes including the regulation of innate and adaptive immune responses. Our studies have previously demonstrated that recombinant Gal-3 (rGal-3) treatment accelerates oligodendrocyte (OLG) differentiation, and that a permissive glycophenotype to Gal-3 binding is only found in immature OLG. The cytoskeleton has been shown to play a key role in OLG maturation and the morphological changes necessary to create fully mature OLG capable of myelination. Recent studies demonstrate that the initial stage of OLG process extension requires actin filament assembly, while subsequent myelin wrapping coincides with upregulation of actin disassembly proteins which are dependent on MPB expression. The aim of our work is to elucidate the mechanism by which rGal3 expedites OLG maturation, giving special attention to the actin cytoskeleton. Our results show that, in primary rat OLG cultured *in vitro* in the presence of rGal-3 and with rGal-3 renewal every 48 h, the total area of polymerized actin at 15′, 30′ and 1 h of treatment was greater than the area measured in OLG cultured in the absence of rGal-3, accompanied by a concomitant decrease in pERK at all times evaluated. At day *in vitro* 1 (DIV1), a decrease was observed in the polymerized actin area and in the number of PDGFRalpha+ cells in rGal-3-treated OLG, and an increase was detected in the number of CNPase+ cells, with no changes in the number of NG2 + and MBP+ cells. At DIV5, a decrease was observed in the polymerized actin area, accompanied by an increase in the number of MBP+ cells at the expense of a decrease in the number of PDGFRalpha+ and NG2 + cells. Results indicate that rGal-3 may favor OLG maturation by mediating the necessary changes in the actin cytoskeleton.

MTU01-30

Noradrenaline protects neurons against H_2O_2-induced cell death by increasing the supply of glutathione from astrocytes

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Hydrogen peroxide (H_2O_2) has been implicated in a variety of neurodegenerative disorders, such as Alzheimer's and Parkinson's disease. Astrocytes express many types of functional neurotransmitter receptors, and play a significant role in maintaining survival of neurons by supplying antioxidants such as glutathione (GSH) to neurons. Recently, we found that noradrenaline increased GSH in astrocytes via β_3-adrenoceptor stimulation. Thus, noradrenaline may protect neurons from oxidative stress-induced death by increasing the supply of GSH from astrocytes to neurons via the stimulation of β_3-adrenoceptor in astrocytes. In this study, we investigated the neuroprotective effect of noradrenaline against H_2O_2-induced neurotoxicity using the mixed cultures of neurons and astrocytes prepared from the E14 mouse embryonic cerebellum of C57BL/6 mice. To assess the viability of neurons, we carried out immunostaining with anti-neuronal nuclei (NeuN) antibody and counted the number of NeuN-positive cells. Pretreatment with noradrenaline (10 μM) for 24 h protected neurons against H_2O_2-induced death in the mixed cultures, but not in purified neuronal cultures. SR 59230A, a selective β_3-adrenoceptor antagonist, inhibited the cytoprotective effect of noradrenaline in the mixed culture. CL316243, a selective β_3-adrenoceptor agonist, attenuated H_2O_2-induced neuronal cell death in the mixed culture. DL-buthionine-[S, R]-sulfoximine, a GSH synthesis inhibitor, negated the cytoprotective effect of noradrenaline in the mixed cultures. MK-571, which inhibits the export of GSH from astrocyte mediated by multidrug resistance-associated protein 1, also prevented the cytoprotective effect of noradrenaline. These results suggest that noradrenaline protects neurons against H_2O_2-induced death by increasing the supply of GSH from astrocytes via β_3-adrenoceptor stimulation in mixed culture of neurons and astrocytes.

MTU01-31

Ovarian hormones induce the expression and release of astroglial mitochondrial-encoded rat humanin in vitro

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Ovarian hormones exert neuroprotective actions in part by direct effects on neurons but also indirectly by regulating the release of neurotrophic factors by astrocytes. Ovarian hormone loss during menopause is associated with brain hypometabolism, synaptic failure and increased risk of neurodegeneration. Humanin (HN) is a mitochondrial-derived peptide with cytoprotective, metabolic, and anti-inflammatory effects in multiple cell types and animal models; it is localized in tissues with high metabolic rates and its expression decreases with age. Our previous studies *in vivo* show that HN colocalizes with astrocyte markers and its expression decreases in the hippocampus of hormone-deprived female rats. Still, little is known about ovarian hormone regulation of HN expression and secretion by astrocytes and the effects of this peptide on neuronal function. The aim of this work was to study the direct actions of estradiol and progesterone on the expression and release of HN by hippocampal astrocytes *in vitro*. To this aim, cultured astrocytes were incubated with estradiol (E, 1 nM), progesterone (P, 1 μM), E+P or vehicle for 24 h. Intracellular HNr expression was evaluated by immunocytochemistry and secreted HNr was determined by ELISA in the conditioned media. Our results show that HNr is expressed in astrocytes in vitro and that ovarian hormones increased its levels. The incubation with E+P was the most effective treatment to induce HNr secretion by astrocytes. Our results indicate that ovarian hormones positively regulate HN expression and release by astrocytes. Further experiments will assess the effect of astrocyte HN on neuronal function. The knowledge of HN effects in brain cells and its regulation by ovarian hormones could help find new therapeutic targets for interventions that may promote a healthier lifespan for post-menopausal women.

MTU02 Gene Regulation and Genetics

MTU02-01

Transcriptional regulation of monoamine oxidase b under basal and dopamine-induced conditions: key roles of SP1, EGR1 and CREB

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Monoamine oxidase B (MAO-B) is a flavoenzyme, which is located on the outer mitochondrial membrane, involved in the catabolism of endogenous and exogenous monoamines. Hydrogen peroxide, one of the byproducts of MAO-B catabolic reaction causes oxidative stress, DNA damage and apoptosis of native and surrounding cells. Altered levels of MAO-B have been associated with several neurological, cardiovascular diseases and diabetes. However, the regulatory mechanisms of MAO-B expression remains incompletely understood. We systematically investigated the roles of putative transcription factors that might regulate MAO-B gene expression. Generation of varying lengths of deletion-reporter constructs of the promoter followed by transfection into various cell lines led to identification of the core promoter region (viz. -144 to +25 bp). Stringent *in silico* analysis of this promoter domain revealed putative binding sites for transcription factors Sp1, Egr1 and CREB. Co-transfection of MAO-B core promoter-reporter construct with Sp1/Egr1/CREB expression plasmid augmented the promoter-reporter activity, whereas down-regulation of endogenous Sp1/Egr1/CREB level diminished the promoter-reporter activity. Site-directed mutagenesis of putative Sp1/Egr1 binding sites in the MAO-B promoter drastically reduced the promoter-reporter activities leading to the identification of cis-elements on the promoter. Competitive electrophoretic mobility shift assays (EMSA) using labeled/unlabeled-wild-type/mutant MAO-B promoter oligonucleotides displayed formation of specific complexes. *In vivo* interaction of Sp1, Egr1 and CREB with the MAO-B promoter was confirmed by chromatin immunoprecipitation (ChIP) assays using MAO-B promoter specific primers. 8-Br-cAMP (cAMP analogue), and forskolin (activator of adenylyl cylase), augmented the MAO-B promoter-reporter activities, whereas PKI (PKA inhibitor alpha) decreased the MAO-B promoter-reporter activity. Dopamine dose-dependently enhanced the MAO-B promoter-reporter activities, mRNA and protein levels. Taken together, this study unraveled crucial roles of the transcription factors Sp1, Egr1 and CREB in MAO-B gene regulation and provides insights into the mechanism of dopamine-induced activation of MAO-B gene expression. This study has implications for pathological conditions involving dysregulated catecholamine homeostasis.

MTU02-02

A novel genetic screen identifies modifiers of age-dependent amyloid β toxicity in the drosophila brain

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Accumulation of amyloid β peptide (Aβ) is one of the major hallmarks of Alzheimer′s disease (AD) and its accumulation begins many years before clinical onset. Such process has been proposed to be pathogenic through the toxicity of Aβ soluble oligomers leading to synaptic dysfunction, phospho-tau aggregation and neuronal loss. Yet, massive accumulation of Aβ can be found in approximately 30% of aged individuals with preserved cognitive function. Therefore, compensatory mechanisms and additional neurotoxic or protective factors are the main issues to be elucidated. We carried out a modifier genetic screen in *Drosophila* designed to identify genes that modulate toxicity of Aβ42 in the CNS on aged flies. Expression of Aβ42 led to its accumulation in the brain and a moderate impairment of negative geotaxis at 18 days post-eclosion (d.p.e) as compared with genetic or parental controls. These flies were mated with a collection of lines carrying chromosomal deletions and negative geotaxis was assessed at 5 and 18 d.p.e. 199 deficiency lines accounting for ~6300 genes were analyzed. 6 lines, including the deletion of 52 *Drosophila* genes with human orthologs, significantly modified Aβ42 neurotoxicity at 18 d.p.e. We have validated *CG17249* and *CG11796* (whose human orthologs are *PRCC* and *HPD*, respectively) by using RNAi or mutant hemizygous lines. *PRCC* encodes proline-rich protein-PRCC of unknown function associated with papillary renal cell carcinoma. *HPD* encodes 4-hydroxyphenylpyruvate dioxygenase, a key enzyme in tyrosine degradation whose deficiency causes autosomal recessive Tyrosinemia type 3, characterized by mental retardation. Our screen is the first to take into account features relevant to sporadic AD: pan-neuronal expression of wild-type Aβ42; a quantifiable complex behavior; Aβ neurotoxicity associated with progressive accumulation of the peptide and improvement or worsening of climbing ability only evident in aged animals. These new modifiers of Aβ42 neurotoxicity in *Drosophila* warrant further study to validate their possible role and significance in sporadic AD.

MTU02-03

Polymorphisms of ENT4 modulate the behavioral phenotypes of autism spectrum disorder (ASD) in male subjects

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ASD is a group of behaviorally defined neurodevelopmental disorders having genetic origin. Recent decade witnessed alarming rise in prevalence besides having male predominancy. Serotonin (5-HT), monoamine neurotransmitter that modulates behavior is elevated in the platelets of a subset of autistic probands and it

formed the basis to suggest involvement of serotonergic system dysfunction in ASD behavioral phenotypes. Several possibilities may underlie the serotonergic dysfunctions, which include the defect of *ENT4* function. ENT4 is encoded by *SLC29A4,* located at chromosome 7p22 and functions in the reuptake of 5-HT into presynaptic neurons. ENT4, also known as plasma membrane monoamine transporter (PMAT) is a low affinity, high capacity, pH dependent transporter and its dysfunction affects the 5-HT neurogenesis. As ENT4 regulates the serotonergic function and the gene location being on chromosome 7, which is a QTL for autism language and speech problems, *SLC29A4* is considered as a potential susceptibility gene for autism. Therefore, the objective of the present study is to investigate the genetic association of *SLC29A4* with behavioral problems of ASD. Study covered analysis of three markers (rs4724512-intron1, rs6965716-intron5 and rs6971788-3′UTR) in West Bengal cohort of 181 ASD subjects and 240 controls. PCR-based RFLP/sequencing was adopted for genotyping analysis and behavioral severity was measured using childhood autism rating scale (CARS). Online available software was used for population-based association and genetic correlation of the variants with ASD and associated behaviors. All the three markers conformed to HWE. Case-control association analysis revealed significant effect for rs6971788 on ASD. Quantitative trait (QT) analysis to test the genetic effect of the markers on the behavioral severity revealed significant male-specific effect on total CARS score ($p = 0.010$), listening response ($p = 0.001$), verbal communication ($p = 0.001$), nonverbal communication ($p = 0.007$), visual response ($p = 0.005$) and general impression ($p = 0.028$). Results of the present study suggest likely involvement of *ENT4* markers on the behavioral phenotypes of male ASD subjects in the Indian population.

MTU02-04

Disease-associated mutations in RP58 disrupt its neuronal functions through a mechanism involving transcriptional regulation

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During the development of the mammalian cerebral cortex, appropriate numbers of neurons, glial cells, and oligodendrocytes must be generated and functionally integrated to form appropriate neuronal circuitry. The regulation of gene expression by DNA-binding transcription factors is crucial for this development, and abnormal brain development can result in intellectual disability. The transcription factor RP58 has been reported to regulate cerebral cortical development by suppressing the transcription of target genes. Human genetic association studies have recognised the importance of RP58 for human neuronal development, with genetic mutations to *RP58* associated with abnormal brain development and intellectual disability in humans. However, the causative nature of genetic mutations to *RP58* remains to be clarified. This study investigates the possible pathological consequences of two individual *de novo,* missense mutations in *RP58* (N461S and R495G), detected in two unrelated patients diagnosed with intellectual disability. Immunolocalisation studies revealed that the subcellular localisation of the two mutated proteins differs to the wild-type protein. Strikingly, a luciferase reporter assay revealed a unique outcome for the R495G mutation, which exhibited transcriptional activation rather than repression. The N461S mutation was also observed to disrupt the transcriptional regulatory activity of RP58. Thirdly, *in utero* electroporation experiments show that both missense mutations have different capacities to restore the defective migration of Rp58 shRNA-treated cells. Altogether, the findings demonstrate that these disease-associated mutations alter the transcriptional regulatory function of RP58, and impair its capacity to control radial migration during cerebral cortex development.

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MTU02-05

Investigation of GLI2 functions in regulating primary cilia and cell cycle re-entry using CRISPR/Cas 9 technology

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The central nervous system arises from the neural tube, consisting of neural stem cells, which give rise to neurons and glia cells. Interestingly, many neural stem cells contain the primary cilium, a microtubule-based organelle projecting from the plasma membrane. Primary cilia are critical in numerous functions ranging from mechanosensation, proliferation, and differentiation. Importantly, primary cilia participate in patterning of the central nervous system by functioning as cellular antennae for transmitting molecular signals, such as Sonic Hedgehog (SHH) signaling. Gli2, a fundamental player in the SHH signaling, is known for regulating cell cycle progression. Interestingly, Gli2 is also involved in cell cycle re-entry from G0 in many cell types, including neural progenitors. However, unlike Gli2-dependent cell cycle progression, how Gli2 regulates cell cycle re-entry is not fully elucidated. Notably, numerous studies demonstrated a negative correlation between ciliary length and cell cycle re-entry. Therefore, we aim to investigate the impacts of Gli2 on the regulation of ciliary length. Here, we generated a Gli2-knockout cell line by CRISPR/Cas9 technology to investigate the potential function of Gli2 in regulating the primary cilium. We validated that cells depleted of Gli2 possess longer primary cilia by immunostaining and live cell imaging of ciliary markers. Meanwhile, we found a delay in cell cycle re-entry in Gli2-knockout cells by flow cytometry. Surprisingly, ablation of the primary cilium by Kif3a knockdown promotes cell cycle re-entry in Gli2-knockout cells, suggesting a potential role of primary cilia in this process. We are currently investigating how primary cilia and Gli2 together regulate cell cycle progression and re-entry. This line of investigation may provide insights into how primary cilia regulate the development of the nervous system through cell cycle regulation.

MTU02-06

Monoamine oxidase b gene polymorphisms revealed association with male ADHD probands

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Attention deficit hyperactivity disorder (ADHD) is a childhood-onset neuropsychiatric disorder characterized by age-inappropriate symptoms of inattention, hyperactivity, and impulsivity. A male predominance was reported in the probands. Influence of monoamine neurotransmitter (such as dopamine, serotonin, and epinephrine/norepinephrine) in ADHD associated symptoms is well accepted. Monoamine oxidase B (MAOB) partially mediates degradation of these neurotransmitters thus regulating the circulating level. Few *MAOB* variants showed association with ADHD in different populations. In this pilot study, we have tested association of three *MAOB* polymorphisms (i.e., rs2283728, rs6324 and rs3027440) in the Indo-Caucasoid families with ADHD probands (N = 190) recruited following the Diagnostic and Statistical Manual for Mental Disorders-4th edition. Comparative analysis was carried out with ethnically matched control individuals (N = 156). Genotyping was performed by amplification of target sites followed by DNA sequencing and data obtained were analyzed by population based statistical methods and validated by family based statistical methods. rs2283728 '*C*' (p = 2.38E-07), rs3027440 '*T*' (p = 0.002) alleles and rs2283728-rs6324 '*C-C*' (p = 1.53E-08), rs2283728-rs3027440 '*C-T*' (p = 7.73E-11), rs6324-rs3027440 '*C-T*' (p = 0.0004) haplotypes showed higher frequencies in ADHD probands as compared to controls. Gender based stratified analysis revealed higher frequencies of rs2283728 '*C*' (p = 4.38E-08), rs3027440 '*T*' (p = 0.0006) alleles and rs2283728-rs6324 '*C-C*' (p = 1.15E-09), rs2283728-rs3027440 '*C-T*' (p = 2.28E-13), rs6324-rs3027440 '*C-T*' (p = 5.42E-05) haplotypes in the male ADHD probands as compared to sex-matched controls. rs2283728 '*C*' (p = 0.0008), rs6324 '*C*' (p = 0.003), rs3027440 '*T*' (p = 0.0002) alleles and rs2283728-rs6324 '*C-C*' (p = 2.73E-05), rs2283728-rs3027440 '*C-T*' (p = 1.66E-05), rs6324-rs3027440 '*C-T*' (p = 0.0002) haplotypes also showed statistically significant maternal transmission to the male ADHD probands. In the female probands, no such biased occurrences were noticed. It may be inferred that these *MAOB* polymorphisms may contribute to the etiology of ADHD, more so in the male probands, warranting further in depth analysis.

MTU02-07

Estrous cycle-related changes in transient receptor potential vanilloid (TRPV) ion channels gene expression in mouse brain

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TRPV-subfamily of ion channels are expressed in the brain and serve as novel players in neurotransmission, Ca^{2+} signalling, synaptic plasticity, and behaviour. Although the TRPV-expressing elements are widely organized in the brain, their importance in CNS is poorly understood. Since TRPV ion channels are polymodal in nature and estradiol has emerged as potential regulator of these ion channels, we determined if TRPV1-6 genes contain estrogen receptor alpha binding sites and their expression in different compartments of the mouse brain is modulated during estrous cycle. Analysis of TRPV1-6 genes sequences showed the presence of putative functional estrogen response element in their promoter regions. Subjects were adult, male and female BALB/c mice. The estrous cycle stages were identified using vaginal smear cytology. The brains of male mice and mice during each stage of estrous cycle were dissected out and processed for qRT-PCR analysis. TRPV1-6 mRNA expression was observed in the olfactory bulb, cortex, hypothalamus, hippocampus, brainstem, and cerebellum. In these regions, compared to estrus, metestrus, and diestrus, while significant decrease was observed in TRPV1 and TRPV5 mRNA levels, expression of TRPV2 and TRPV6 mRNA were elevated during proestrus. Although lower levels of TRPV3 and TRPV4 mRNA levels were seen during estrus, higher expression of these ion channels was observed during metestrus and diestrus. TRPV2 mRNA was abundantly expressed during all stages of the estrous cycle. Reduced levels of TRPV5 and TRPV6 were observed during proestrus and other stages of the estrous cycle, respectively. Except TRPV4 expression in the hippocampus and TRPV6 expression in hippocampus and brainstem, the expression of ion channels in different brain regions of male mice were comparable to that in respective brain regions of female mice during metestrus and diestrus. We suggest that TRPV channels serve as direct target of estradiol and the hormone-ion channel cross talk may modulate neural substrates regulating reproduction.

MTU02-08

Microrna regulation in medial prefrontal cortex after nerve injury

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MicroRNAs are small non-coding RNA molecules that constitute post-transcriptional regulators of gene expression. Recently, it has been shown that besides their prominent role in the regulation of physiological mechanisms they are also important for the modulation of pain pathways. Nerve injury can lead to long lasting neuropathic pain, affecting a large proportion of the population and exerting major impacts on the patients' quality of life. A brain region involved in central processing and modulation of pain is the medial prefrontal cortex (mPFC), which shows alterations in chronic pain patients.

We employed a combination of in vitro electrophysiology, pharmacological stimulation and RNA sequencing, to explore the changes in the mPFC that underlie the transition to a neuropathic pain state on a neuronal and microRNA network level.

We found that the induction of neuropathic pain in a mouse model of sciatic nerve injury led to specific expression profiles of microRNAs and mRNAs in mPFC samples. Interactions between microRNA and mRNA expression are currently being analyzed.

MTU02-09

A critical role for the transcription coactivators in the regulation of the human tryptophan hydroxylase-2 gene expression

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Dysfunction of the central 5-HT system has been implicated in the etiology of the wide range of neurodevelopmental disorders. As the rate-limiting enzyme for the synthesis of central 5-HT, tryptophan hydroxylase-2(TPH2) is thus a promising therapeutic target for the treatment of neuropsychiatric disorders. However, the mechanism by which human (hTPH2) gene expression is activated remains unresolved. In the present study, we characterized how the hTPH2 promoter activity is regulated by cAMP-mediated signaling pathways. A 2-kb of the hTPH2 gene (−1850/+141) was cloned into pGL4-Basic and promoter activities were assessed by transient transfections into RN46A cells. Forskolin increased the hTPH2 promoter activity. Whereas PKA activators (Sp-cAMPS or N^6-phenyl-cAMP) increased the hTPH2 promoter activity, the specific EPAC (exchange protein directly activated by cAMP) activator (8-pCPT-2′-O-Me-cAMP) did not show any appreciable effects. Forskolin-induced increase in the hTPH2 promoter activity was reversed by the PKA specific inhibitor (H-89), but not by the EPAC specific antagonists (ESI-09 or HJC0197). Overexpression of CREB and PKA-alpha, either alone or in combination, only caused marginal effects. Overexpression of either CREB-regulated transcription coactivator CRTC1 or 3 with PKA-alpha and CREB remarkably increased the hTPH2 promoter activity. Forskolin- or PKA-alpha/CREB/CRTC-mediated increase in the hTPH2 promoter activity was abolished when the inverted CRE (cAMP response element) motif was mutated. CRTC-mediated increase in the hTPH2 promoter activity was attenuated either by overexpression of R314A-CREB (defective for interaction with CRTCs) or R301L-CREB (defective for interaction with CRE) instead of wild-type CREB. In contrast, overexpression of S131A-CREB (defective for phosphorylation by PKA) increased the hTPH2 promoter activity comparable to wild-type CREB, suggesting that CREB phosphorylation itself is not necessarily essential. Collectively, these results indicate that CRTCs play a critical role for positive transcriptional regulation of the hTPH2 gene via cAMP-mediated signaling pathways and interaction with CRE-bound CREB.

MTU02-10

Characterization of natriuretic peptides and their receptors in the avian brain

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The natriuretic peptide family includes structurally related peptides that have important roles in body fluid balance and cardiovascular homeostasis. In mammals, these peptides comprise a family of three structurally related molecules: atrial natriuretic peptides (ANP), B-type natriuretic peptide (BNP) and C-type natriuretic peptide (CNP). In birds, genomic analysis showed that the genes encoding BNP and CNP were retained, whereas the ANP gene was lost. Some members of NP family are expressed in both the mammalian and avian brain, but their functional roles in brain have not been fully elucidated. Three receptors for NPs have been identified in mammals, but the information concerning avian NP receptors is limited. To gain insight into the functional roles of NP systems in brain, we aimed to characterize the NPs and their receptors in chick brain, because the avian system has an advantage for studying early learning.

MTU02-11

Elucidating the transcriptional regulation of NUR77 in neurons

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Nur77 is a transcription factor encoded by an early gene that belongs to orphan members of the nuclear receptor superfamily. The expression of Nur77 is regulated by dopamine in brain nuclei as the striatum and prefrontal cortex, which are targets of dopaminergic projections from midbrain. Even though Nur77 has been implicated in stress response and drug addiction, the mechanisms regulating its expression have not been elucidated. Recently, it has been shown that the transcriptional repressor Lysine-Specific Histone Demethylase 1 (LSD1) plays an important role regulating the expression of early genes. LSD1 has four splice variants. Two of these splice variants include the micro exon 8a that encode 4 amino acids among them a phosphorylatable threonine. Neuro-LSD1, which is expressed only in neurons, has lower transcriptional repressive activity although displays similar demethylase activity compared with ubiquitous LSD1. The effect of LSD1 and neuro-LSD1 over early genes in the brain seems to be mediated by the Serum Responsive Factor (SRF). Here, we show that both LSD1 and neuro-LSD1 induce Nur77 expression in neurons. A phospho-mimetic but not a phospho-deficient mutant of neuro-LSD1 displays the same inductive effect over Nur77, suggesting that dephosphorylation of neuro-LSD1 limits its transactivation function. Reporter genes assays showed that SRF induces Nur77 expression in an independent way of LSD1. CArG-Box element present in the Nur77 proximal promoter is necessary for SRF mediated induction, but not for the effect of LSD1. Besides, PCR using exon-inclusion frequency by relative quantity fluorescent indicates that LSD1/neuro-LSD1 ratio in the striatum is regulated by ligands of the dopamine D2 receptor.

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MTU02-12

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

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Epigenetic mechanisms such as DNA methylation are well known regulators of genetic expression and they play key roles in the development of neurodegenerative diseases, mainly through a substantial transcriptional regulation of active and inactive

promoters and by modifying transcription elongation and splicing in CpG islands located intra and intergenically. It is also widely recognized that astrocytes, that are critical regulators of neuronal function, play a crucial role in neurovascular-related disorders like ischemic stroke. However, few studies have addressed at the molecular resolution the overall genetic and epigenetic changes of these complex phenomena, and in events like the reperfusion damage that occurs after ischemic stroke these processes are practically unknown. We performed RNA-seq and methylated DNA immunoprecipitation sequencing (MeDIP-seq) analysis of cultured human astrocyte-like cells derived from grade I non-tumorigenic glioblastoma subjected to oxygen and glucose deprivation (OGD), in order to establish a relationship between DNA methylation and gene expression under normoxia, OGD and recovery. We identified several genomic features including promoters and enhancers whose methylation levels change not only during OGD but also after 8 h of recovery that showed statistically significant differences in both; high (housekeeping and ubiquitous genes) and low (cell lineage-specific genes) CG promoters. Moreover, DNA methylation remodeling was correlated with gene expression of several genes under OGD and recovery, and the organization of the transcriptome and methylome resulted different under normoxia, OGD and recovery. These results can help to elucidate the overall transformation of cells in terms of transcription and DNA methylation in pathological occurrences involving ischemia and characterize the damage that occurs during reperfusion at the genomic scale that has been incompletely described until now. Supported by PAPIIT-DGAPA IN226617 and CONACYT 219542.

MTU02-13

Serotonin transporter gene, *SLC6A4* polymorphisms regulate SERT expression and 5-HT levels to influence severity of ASD symptoms

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Autism spectrum disorder (ASD) is a group of childhood onset neurodevelopmental disorders. Neurochemical, behavioral and pharmacological studies reveal serotonergic dysfunction in ASD. Platelet hyperserotonemia in ASD subsets and efficacy of selective serotonin reuptake inhibitors in reducing the behavioural symptoms suggest defects in serotonin (5-HT) reuptake as a possible cause for ASD-specific behavioural impairments. Serotonin transporter (SERT), a key determinant for 5-HT uptake is encoded by *SLC6A4,* which is a QTL for blood 5-HT levels. Therefore SERT has gained much attention as a target for hyperserotonemia. Here we report the genetic association of *SLC6A4* polymorphisms with ASD using case-control and family-based approaches and examined its genetic correlation with platelet 5-HT levels, SERT mRNA expression and severity of ASD behavioural symptoms. Results of genetic association analyses reveal that *SLC6A4* markers increase the risk for ASD. Its polymorphisms showed significant genetic effect on specific behavioural phenotypes as measured by CARS. Low expressing genotypes, C/C and S/S of rs6354 and 5-HTTLPR respectively and A/A of rs7224199 displayed reduced severity for certain behaviours such as activity level, adaptation to change, taste, touch & smell use, and body use. Haplotypes formed of L allele of 5-HTTLPR increased the severity for fear or nervousness. ASD children demonstrated higher 5-HT levels and SERT mRNA expression in platelets and lymphocytes respectively. When 12-repeat allele of STin2 showed association with elevated mRNA expression and platelet 5-HT levels, the C/C genotype of rs6354 showed correlation with low platelet 5-HT levels. Overall results suggest that *SLC6A4* markers influence the severity of ASD symptoms, through 5-HT modulation by SERT. Our findings imply its significance in pharmacogenomics research, as genotype-based personalised medication can be strategically implemented as a treatment mode for ASD.

MTU02-14

Lactic acid mediates the effects of physical exercise on learning and memory through Sirt dependent activation of hippocampal BDNF

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Exercise induces beneficial responses in the brain, which is accompanied by an increase in BDNF, a growth factor associated with cognitive improvement and the alleviation of depression and anxiety. However, the exact mechanisms by which physical exercise produces an induction in brain *Bdnf* gene expression are not completely understood. Here, we report that an endogenous molecule released after exercise is capable of inducing key promoters of the *Mus musculus Bdnf* gene. The metabolite lactate, which is increased after prolonged exercise in the blood, induces *Bdnf* expression and Trkb signaling in the hippocampus. Indeed, we find that lactate-dependent increases in hippocampal BDNF are associated with improved spatial learning and memory retention. We have discovered that the action of lactate is dependent on Sirt induction and activation potentially by affecting the activity of the transcriptional coactivator PGC1a. These results reveal an endogenous mechanism to explain how physical exercise leads to the induction of BDNF and identify novel targets that allow us to harness the therapeutic potential of exercise.

MTU02-15

Increased serum miRNAs as potential diagnosis and prognosis biomarker for human mild traumatic brain injury

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Objective: Circulating microRNAs (miRNAs) are emerging disease biomarkers. However, comprehensive characterization of the serum miRNA profile in patients with traumatic brain injury (TBI) has rarely been reported. Our study aims to investigate the value of novel serum miRNAs for diagnosing TBI, especially mTBI, and further predicting therapeutic efficacy and clinical outcome.
Methods: A TaqMan Low Density Array was initiated to analyzed the expression of 754 serum miRNAs in two pooled samples from 15 severe traumatic brain injury (sTBI) (Glasgow Coma Scale [GCS] score $\leq$ 8) patients with unfavorable outcome and 15 normal controls. Markedly upregulated miRNAs in sTBI cases were subsequently

validated by qRT-PCR in another cohort consisting of 85 with severe TBI, 85 with mild traumatic brain injury (mTBI) (Glasgow Coma Scale [GCS] score > 12) and 85 controls arranged in two stages. Clinical outcome was evaluated at 6 months using Glasgow Outcome Scale (GOS) score. An unfavorable outcome was defined as GOS of 1–3 and a favorable outcome was named as GOS of 4–5.

Result: Seven miRNAs including miR-103a, miR-219a, miR302d, miR-422a, miR-518f, miR-520d and miR-627 were significantly elevated ($p < 0.001$) in both sTBI and mTBI within 24 h post-injury compared with the controls. miR-520d was declined markly in post-treatment samples versus pre-treatment samples ($p < 0.05$). The levels of seven miRNAs were significantly higher in the patients with an unfavorable outcome than in those with a favorable outcome ($p < 0.05$).

Conclusion: The seven miRNAs identifed in our study represented potential diagnostic and prognostic biomarkers for TBI.

MTU02-16

Influence of placental allopregnanolone on adult brain transcriptome

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Allopregnanolone (AP), along with its precursor progesterone, is a neuroactive steroid primarily synthesized during pregnancy by the placenta, and later, the brain. AP exerts neurodevelopmental and neuroprotective actions through allosteric activation of the GABA-A receptor. In the fetal brain, AP notably promotes neurogenesis and myelination, and protects developing neurons and glial cells from damage. Preterm infants with very low birth weight are highly vulnerable to brain injuries, with possible long-term neurological consequences, including learning impairment, attention deficit hyperactivity disorder (ADHD) and cerebral palsy.

Since preterm delivery is associated with premature loss of placental AP and its accompanying neurotrophic and neuroprotective actions, we hypothesize that some of the neurological outcomes linked to premature birth are due in part to the early withdrawal of AP.

To test this hypothesis, we have generated a transgenic mouse model in which the gene encoding the synthesis enzyme of AP (AKR1C14) is specifically knocked-out in trophoblastic cells expressing Cyp19-Cre transgene. In these mice, named AKR1C14-Cyp19, the recombination may result in a dramatic reduction of AP production in the placenta only. We chose to use an unbiased RNA sequencing approach to analyze gene expression changes that may result from the lack of placental AP in the adult brain of $AKR1C14^{Cyp19}$ mice.

Our RNA sequencing analysis reveals that placenta AP withdrawal is associated with long term and sex-specific gene expression changes in the cerebral cortex, hippocampus, hypothalamus and cerebellum. The differentially expressed genes cover a variety of ubiquitous functions such as neural development and plasticity, neurotransmission and epigenetics, as well as region-specific functions such as energy homeostasis and body growth.

By providing new evidence of the importance of placental hormones in shaping and programing the developing brain, our data paves the way for future investigation in the field of neuroplacentology. Furthermore these findings may contribute to the development of novel therapeutic approaches to address the negative neurological outcomes of preterm birth.

MTU02-17

Region-specific microrna changes during avulsion-induced spinal motoneuron degeneration of adult rats following unilateral brachial plexus root avulsion: ipsilateral vs contralateral spinal cord

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Elucidation of the pathophysiological events underlying spinal root avulsion is expected to discover effective therapeutic methods. Currently, microRNAs are thought to play an important role in the gene changes involved in spinal cord injury. In the rats with right brachial plexus root avulsion, the spinal cords of all the animals were harvested 3 and 14 days after injury and divided into ipsilateral and controlateral ventral and dorsal horn, preparing for microarray analysis. The expression of 8 miRNAs (up, 6 miRNAs; down, 2 miRNAs) was significantly altered in Day 3 and the expression of 17 miRNAs (up, 16 miRNAs; down, 1 miRNAs) was significantly altered in Day 14 post-injury. It is worth to notice that miR-106b-3p was continuously upregulated in the ipsilateral ventral horn on both the 3rd and 14th days after the injury. Moreover, the upregulation of miR-106b-3p was also observed in the ipsilateral dorsal horn on the 14th days after the injury. Only miR-496-3p was continuously downregulated in both the ventral and dorsal horn of the affected spinal cord on the 14th day after injury. The present data revealed previously unknown region-specific alterations of a large set of miRNAs in affected spinal cord after root avulsion.

MTU02-18

Novel mutation in HTRA1 identified in a family with diffuse demyelination lesions

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Here we report on three siblings born to healthy Iranian parents with ataxia, behavioral and mood changes, dysarthria, dementia, low back pain and recurrent respiratory infections. Brain MRIs in the siblings showed diffuse demyelination lesions and also spinal stenosis was diagnosed. Based on the occurrence in siblings, no significant difference in phenotype between the siblings, absence of manifestations in parents, and several level consanguinity in the pedigree, we performed sought to discover possible genetic risk factors in this family. Using a combination of homozygosity mapping and whole exome sequencing, we identified an indel in High-Temperature Requirement A Serine Peptidase 1 (HTRA-1) gene, which showed complete segregation in the pedigree. The patient's clinical manifestations appear consistent with cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL), which has previously been reported to be associated with HTRA-1 mutations. Studies are currently underway to examine the functional effects of the novel HTRA-1 mutation we identified. As some aspects of the clinical presentation in this family deviate from those reported for CARASIL, our study expands the spectrum of clinical consequences of mutations in HTRA-1.

MTU03 Neuroinflammation

MTU03-01

Pioglitazone attenuates lipopolysaccharide (LPS) induced neuro-inflammation and depressive-like behaviour in experimental mice

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Depression is a stern neuro-psychiatric hitch with a lifetime prevalence exceeding 15% and has become the fourth leading cause of disability worldwide. According to a report by WHO mental illness affects around 450 million people globally of which 10–20 million commits suicide every year. Despite having several medications the disease is still a challenging with approximately a large number of populations do not respond to their first line medication. Hence there exists a necessity to explore new targeted drugs. Microglia's are resident immune defence cells of the brain and comprises ~ 12% of the total neurons. Upon activation, microglia undergoes proliferation, chemotaxis and morphological alteration to engender plethora mediators including cytokines, chemokines, reactive oxygen species (ROS) and reactive nitrogen species (RNS) which may alter the serotoninergic and glutamatergic neurotransmission. NF-κB(Nuclear factor kappa beta) is an important transcription factor is activated by LPS(lipopolysaccharide) causing transcription of many proinflammatory cytokine genes(IL-1β, TNF-α, IL-6) and thus microglia acts as a sensor for pathological events that occurs in the brain. Furthermore, LPS evoked brain alternation through hyperactivation of the hypothalamic–pituitary–adrenal axis (HPA axis) axis results in the rise of circulating serum corticosterone level. LPS evokes generation of free radicals causing ER stress and up-regulation of unfolded-protein response (UPR). LPS also alter the p-38MAP kinase and Nrf-2 signalling pathways causing an add on to neuroinflammation. Pioglitazone belongs to peroxisome proliferator-activated receptor gamma (PPAR-γ) agonist class regulates lipid metabolism, exerts potent central and peripheral anti-neuroinflammatory action and possesses neuroprotective effect. Several studies have also reported the protective role of Pioglitazone at a dose of 30 mg/kg body weight for 14 days inhibits the oxidative-nitrosative stress and other inflammatory cytokine markers. Thus, this has pushed us to explore the neuroprotective nature of Pioglitazone especially with psychiatric disorders associated with inflammation and oxidative stress.

MTU03-02

Extracellular CGMP normalizes TNF-α and membrane expression of AMPA receptors and spatial reference memory in hyperammonemic rats

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Patients with hepatic encephalopathy (HE) show working memory and visuo-spatial orientation deficits. Hyperammonemia is a main contributor to cognitive impairment in HE. Hyperammonemic rats show impaired spatial learning and learning ability in the Y maze. Intracerebral administration of extracellular cGMP restores learning in the Y-maze. The underlying mechanisms remain unknown. It also remains unknown whether extracellular cGMP improves neuroinflammation or restores spatial learning in hyperammonemic rats and if it affects differently reference and working memory. The aims of this work were:

a) assess whether treatment with extracellular cGMP reduces hippocampal neuroinflammation and restores spatial learning in hyperammonemic rats.

b) analyze the underlying mechanisms, including changes in membrane expression of NMDA and AMPA receptors.

Spatial working and reference memory were assessed using the radial and Morris water mazes and neuroinflammation by immunohistochemistry and western blot. Membrane expression of NMDA and AMPA receptor subunits was analyzed using the BS3 crosslinker. Extracellular cGMP was administered intracerebrally using osmotic minipumps.

Chronic hyperammonemia induces neuroinflammation in hippocampus, with astrocytes activation and increased IL-1b, which are associated with increased NMDA receptors membrane expression and impaired working memory. This process is not affected by extracellular cGMP. Hyperammonemia also activates microglia and increases TNF-α, alters membrane expression of AMPA receptor subunits (increased GluA1 and reduced GluA2) and impairs reference memory. All these changes are reversed by extracellular cGMP. These results show that extracellular cGMP modulates spatial reference memory but not working memory. This would be mediated by modulation of TNF-α levels and of membrane expression of GluA1 and GluA2 subunits of AMPA receptors.

MTU03-03

Melatonin attenuates cognitive impairment and neuroinflammation via interleukin-6 signaling pathway in hippocampus of aged mice

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Aging is a natural process that defined as progressive decline of biological functions and is the most risk factor for several neurodegenerative disorders. This process is accompanied by the impairment of cognitive functions and variety of neurobiological changes, one of which is neuroinflammation. Overproduction of inflammatory cytokines such as interleukin-6 (IL-6), a major cytokines in the central nervous system, can lead to neuronal dysfunctions. Several studies suggested IL-6 signaling cascade is not only associated with neuroinflammation but could also be associated with cognitive function especially in hippocampal-dependent memory. Futhermore, during aging the level of melatonin, neuronal hormone synthesized and secreted by the pineal

gland, is found to be significantly decline. Therefore, this study hypothesizes that the anti-inflammatory property of melatonin could help ameliorate the cognitive decline in aging and might be associated with the IL-6 signaling pathway. Mice were received melatonin (10 mg/kg body weight) in drinking water from 16 to 22 months of age (6 months). The Morris water maze (MWM) task was used to evaluate the cognitive function of animals, and the level of IL-6 and it signaling pathway were measured. The results revealed that aged mice show cognitive impairment in both learning ability and working capacity when compare with adult (2 months old) mice. However, aged mice which were received melatonin for 6 months show cognitive improvement when compare to aged without melatonin. Moreover, the IL-6 pathways are also activated in the aged mouse hippocampus which could be ameliorated by long-term administration of melatonin. The present data show melatonin supplement could be considered as a beneficial therapeutic strategy to prevent the cognitive decline and overproduction of inflammatory cytokines in aging. This work was supported by a Mahidol University Research Grant to SM, and a Ph.D scholarship from Burapha University to PC.

MTU03-04

A molecular characterisation of meningeal inflammatory infiltrates in the progressive multiple sclerosis brain

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Post-mortem tissue studies suggest that the presence of chronic inflammatory infiltrates in the leptomeninges may promote cortical pathology and thereby play a role in accumulating clinical disability in progressive multiple sclerosis. In a proportion of MS cases, these inflammatory infiltrates begin to approximate the composition and structure of tertiary lymphoid organs. However, we know little about the mechanisms by which they form and how they affect the underlying cortical tissue. In order to explore the upstream regulators as well as the molecular mechanisms and signaling pathways that might drive these pathological processes, we have studied a cohort of 40 SPMS patients and 10 non-neurological controls from the UK MS Society Tissue Bank. Cryosections were cut from five cerebral cortical blocks per case and meningeal tissue was dissected and RNA extracted for transcriptional profiling. Immunohistochemistry was used to determine the extent of lymphocytic infiltration and demyelination. Cases were then segregated into groups according to the level of inflammation. To interrogate all transcripts, a new high resolution array from Affymetrix, the GeneChip Human Transcriptome array, was used and data subsequently analysed using Affymetrix® Expression Console Software and Affymetrix® Transcriptome Analysis Console Software.

In those cases with the highest inflammation level, there were large increases in a significant number of immunoglobulin-related genes when compared to healthy controls or low inflamed MS cases. In addition, alterations were mainly found in gene expression of homing chemokines and receptors, such as CCL19, CXCR4, CCL5, CCR2, as well as cytokines that enhance B cell survival, proliferation and antibody and IFNg production, such as IL10 and IL18. Furthermore, modifications in genes that enact functions in the development of lymphatic vessels (e.g. LYVE1) and cell motility, survival and antigen presentation (e.g. HLA-B) were prominent.

We have demonstrated that the molecular cues mediating meningeal inflammation suggest a dysregulation of pathways that are critical for the trafficking and recruitment of B and T lymphocytes into the CNS. The resulting formation of self-maintaining ectopic lymphoid tissues would result in inflammatory tissue damage to the underlying grey matter of the progressive MS brain.

MTU03-05

Axo-glial pathology in multiple sclerosis and its effects on neurotransmission

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Saltatory conduction in the nervous system is enabled through the association between the axolemma and the leading loops of the myelin sheaths, which form the paranodal axo-glial junctions (PNJs) and define the nodes of Ranvier. Moreover, the proper clustering of voltage sodium channels (Nav) at the node and potassium channels (Kv1.2) at the juxtaparanode is crucial for correct conduction. Previous studies have identified changes to the structure of the nodes of Ranvier in the normal appearing white matter (NAWM) in the multiple sclerosis (MS) brain. In order to understand how these changes affect saltatory conduction in MS we have examined the spatial expression of Caspr, Nav and Kv1.2 in NAWM areas from post-mortem brains. $N = 20$ cases of neuropathologically confirmed multiple sclerosis, comprising 47 blocks with 470 NAWM areas, were analysed in the neuroanatomical part of our study. In order to determine axonal abnormalities such as channel dislocation, intensity profiles were measured from our imaging database for each axon and compared across all the cases. A significant increase in length of the PNJs was found in MS NAWM tissue and associated with stressed/damaged axons and activation of microglia. This underlying axonal pathology points to PNJ disruption as a crucial event in the cascade of events that may culminate in axon pathology and loss. Furthermore, we found a higher proportion of axons in MS NAWM with Kv 1.2 channels dislocated towards the PNJs, and a small proportion of axons with Nav channels also dislocated towards the PNJs. We have then integrated this axo-geometrical data into our computational model, and preliminary indications suggest a potential reduction in conduction velocity in the NAWM. We investigated also the reliability and metabolic cost of conduction. Overall, our results point to an ongoing disruption of the axonal-oligodendrocyte complex, which can affect greatly CNS neurotransmission and might contribute to non-lesion disease symptoms such as neurofatigue.

MTU03-06

Anti-inflammatory compound with sIPSC blocking potential, a promising therapeutic approach for neurological pain disorders

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Objective: The objective of present study is to explore multiple effects of the compound MG9 and relate them to achieve better therapeutic potential against neuroinflammation related disorders. We examined whether our compound is acting through regulating neuroinflammatory mediators and by blocking spontaneous inhibitory post synaptic currents (sIPSC) in brain hippocampal slice preparations.

Methods: Preliminary in-silico docking studies using glide and gold soft wares and behavioral screening studies using rodent models of peripheral nerve injury encouraged us to shortlist the derivatives and to extend our screening studies to explore the test compounds efficacy on other related peripheral neurological disorders such as Streptozotocin-induced diabetic peripheral neuropathy (DPN) and methyl mercury (MeHg) induced neurodegeneration in rats. Pro-inflammatory cytokines interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) were quantified with RT-qPCR studies and histopathology studies were performed taking tissue samples from MeHg induced neurodegeneration rats. sIPSCs were recorded from CA1 pyramidal cells using patch-clamp technique in a whole-cell configuration.

Results: The effect of MG9 was assessed on local and acute inflammation through carrageenan-induced rat paw edema model. We observed the reduction in nociceptive response in DPN rats. Pain threshold was reduced greater than 50% in various pain assessment modules. Upregulated pro-inflammatory cytokines which are thought to have the prominent role in neuroinflammation was controlled near to normal level quantified by RT-PCR studies. However, MG9 was able to regulate IL-6 and TNF-α but not IL-1β. sIPSCs were blocked more than 50% by MG9 which is very crucial to stop the continuous throbbing pain in neuropathic pain states.

Conclusion: Our results clearly suggest the beneficial potential of compound MG9 through regulation of proinflammatory cytokines release and also by blocking sIPSCs. MG9 could be an intriguing therapeutic approach in diabetes-related neuro-pathophysiological conditions.

MTU03-07

Intrathecal administration of a cannabinoid type 1 receptor agonist attenuates acute postoperative pain in rats

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The endocannabinoid system which includes endogenously synthesized cannabinoids like anandamide,cannabinoid receptors (type 1 & 2) and enzymes associated with their synthesis and breakdown contributes towards maintaining a basal antinociceptive tone in rodents.Besides,systemic administration of cannabinoid drugs produces a distinct antinociceptive effect.However,this is associated with psychomotor alterations and other side effects.One option to possibly avoid the side effects is to selectively activate the cannabinoid type 1 receptors (CB1r) in the spinal cord.In the present study,we aimed to evaluate the antinociceptive effect of arachidonylcyclopropylamide (ACPA),a specific CB1r agonist in the rat hind paw incision model,which is a preclinical model of postoperative pain.Sprague-Dawley rats were initially implanted with intrathecal catheters.Then,they were subjected to hind paw incision following preemptive one-time intrathecal administration of 1, 3 and 10 μg ACPA.Antinociceptive effect was investigated by the guarding behavior,mechanical allodynia and thermal hyperalgesia. These were compared to morphine (3, 10 and 30 μg). The antinociceptive effect was tested for reversibility by AM251,a CB1r antagonist.Motor coordination was examined by rotarod apparatus.Antinociceptive effect of ACPA was also evaluated by the formalin test.Spinal CB1r expression was investigated by immunohistochemistry.Both intrathecal morphine (3, 10 and 30 μg) and ACPA (1, 3 and 10 μg) significantly decreased guarding score in comparison to control group between 2 h to day 2 and 2 h to day 4 respectively. Significant decrease in allodynia was observed for ACPA (2 h-day 5) and morphine (2 h and days 3–5).Unlike guarding,in allodynia,significant difference between ACPA and morphine was absent.Thermal hyperalgesia was significantly decreased by 3 μg ACPA only (2 h, 8 h-day 5) and 3, 10 and 30 μg morphine (2 h) in comparison to control. Moreover,ACPA-induced antinociception was reversed by AM251.However,it did not affect formalin related flinching behavior.Immunohistochemistry showed selective CB1r expression in the superficial laminae of the spinal cord.Post-incision,ACPA increased at 2 h, but decreased thereafter.Thus, ACPA treatment likely activated the CB1 receptors to produce antinociception.This information could have clinical relevance.

MTU03-08

Rage mediates the increase in neurodegenerative markers and cognitive impairment following recovery from polymicrobial sepsis

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Patients that recover from sepsis have higher rates of central nervous system (CNS) morbidities associated with long-lasting impairment of cognitive functions, including neurodegenerative diseases. Here, we investigated the role of the receptor for advanced glycation endproducts (RAGE) in the neuroinflammation, neurodegenerative-associated changes and cognitive dysfunction arising after sepsis recovery in adult Wistar rats subjected to cecal ligation and perforation (CLP). Serum and brain (hippocampus and prefrontal cortex) samples were obtained at days 1, 15 and 30 after CLP for examination of systemic and brain inflammation, amyloid β peptide (Aβ) and phosphorylated tau at ser202 (p-tau^{ser202}) content, RAGE, RAGE ligands and RAGE intracellular signaling. The effect of RAGE immune neutralization in the hippocampus, using RAGE antibody (RAGE*ab*) injection at days 15, 17 and 19 after CLP, was

studied for the parameters of neuroinflammation, Aβ and p-tau^{ser202} accumulation and cognitive impairment. In the course of 30 days following CLP, a decrease in serum markers associated with the acute pro-inflammatory phase of sepsis (TNF-α, IL-1β and IL-6) was observed, concomitant with a progressive increase in RAGE ligands (S100B, Nε-(carboxymethyl)lysine, HSP70 and HMGB1). In the brain, the content of RAGE and TLR4, GFAP and nNOS, as well as Aβ and p-tau^{ser202} also increased following the acute phase of sepsis. RAGE*ab* inhibited Aβ and p-tau^{ser202} accumulation, Akt/mTOR signaling, Iba-1 and GFAP increases, and hindered behavioral changes associated to cognitive decline. These data demonstrate that brain RAGE is an essential factor in the pathogenesis of neurological disorders following an episode of acute systemic inflammation. Funding: CNPq, FAPERGS, FAPESC and CAPES.

MTU03-09

Physical exercise reverses pain and pathological changes in dorsal root ganglia induced by systemic lipoplolysaccharide in mice

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Background: Emerging research indicates that physical activity can ameliorate chronic pain, but the underlying mechanisms are still obscure. We have shown previously that systemic inflammation induced in mice by intraperitoneal (i.p.) injection of lipopolysaccharide (LPS) caused mechanical hypersensitivity and activated satellite glial cells (SGCs) in dorsal root ganglia (DRG). LPS injection also caused a large increase in gap junction-mediated coupling among SGCs and also between neurons. In the present work we asked whether physical exercise can reduce the pain by reversing the changes in SGCs induced by LPS.

Results: We assessed pain with von Frey filaments and characterized SGCs in L4,5 DRG using dye injection, measured respo3nses to the pain mediator ATP by calcium imaging, and immunostained for the activation marker glial fibrillary acidic protein (GFAP). Seven days post-LPS, SGCs were activated, as evidenced by GFAP upregulation, and dye coupling among SGCs increased 3–4.5-fold. Sensitivity of SGCs to ATP increased 2-fold. Pain threshold decreased 3.5 fold. Injecting gap junction blockers i.p. reduced pain behavior in LPS-treated mice, indicating a role for SGCs in pain. The results suggest that SGC activation plays a role in pain mechanisms. LPS-injected mice were given 1 week of free wheel running, after which we characterized the changes in SGCs in DRG. Pain threshold increased back to control level, dye coupling among SGCs and neurons was restored to control level and sensitivity of SGCs to ATP was reduced 2-fold. GFAP levels decreased back to control level.

Conclusions: Physical exercise reverses SGC activation caused by systemic inflammation, which may explain the amelioration in pain in the systemic inflammation model by exercise.

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MTU03-10

Linking inflammasome activation with mitochondrial alterations and oxidative stress, or not

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Neuroinflammation is mediated by protein complexes, inflammasomes, functioning as intracellular sensors. Once primed, purinergic receptors (PXR) or Nod-like receptor, pyrin domain 3 (NLRP3) respond to various stimuli (ATP) and assemble an inflammasome aggregate. The process involves potassium efflux, mitochondrial ROS generation, apoptosis-associated speck-like protein (ASC) aggregation, caspase-1 cleavage, and mature interleukin-1β (IL-1β) release. It is thought that mitochondrial DNA and mitochondrial ROS activate the NLRP3 inflammasome and that canonical inflammasome activators work via disrupting mitochondrial membrane potential (MMP). Recent work identified hundreds of environmental/industrial compounds that acutely decrease MMP. We hypothesize that many of these mitotoxicants may also activate the NLRP3 inflammasome. We examined the ability of two of these toxicants to serve as a secondary trigger for inflammasome activation in LPS primed (33 ng/mL; 3 h) RAW264.7 macrophages. We selected toxicants known to induce a macrophage response in vitro, trimethyltin; trimethyltin, and to cause selective damage to the hippocampus and myelin sheath, respectively. A 6 h exposure to TMT (10 μM) or TET (1.25 μM), resulted in an 80% viability/40% MMP disruption. Following priming with lipopolysaccharide (33 ng/mL; 3 h) TMT and TET induced inflammasome assembly in 20% of the cells as indicated by ASC aggregation, active caspase 1, and pyroptosis. These changes occurred in the absence of a nitric oxide production or evidence of ROS production. This was accompanied by IL-1β release, predominantly pro-IL-1β. Inflammasome activation was confirmed in primary bone marrow macrophages with the release of mature IL-1β in TET dosed cells at 6 and 16 h and in TMT dosed cells at 16 h. Overall the data showed a stimulation of IL-1 and an association between MMP disruption and inflammasome assembly with TET or TMT exposure; however, the robustness of the inflammasome response differed between the two compounds yet, with equivalent MMP disruption. The question now is if this is preceeded by alterations in mitochondrial function that can more closely distinguish between the two levels of induction and predict inflammasome activation.

MTU03-11

Role of microglial metabolism in perinatal neuroinflammation

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Inflammation within brain plays a central role in perinatal brain injury. Microglia (MG) cells, the immune cells of brain regulates both neuroinflammatory and normal brain development. They

acquire distinct phenotypes/activation states in response to maternal/foetal inflammation or infection and disrupt normal developmental process. To date metabolic features of MG in their different activation states have not been investigated. Previous findings in our lab points to early developing, robust pro-inflammatory activation of MG. Transcriptomic analysis of these MG linked their injurious activation state with disrupted fatty acid/phospholipid processing. Furthermore untargeted lipidomic profiling of these *ex-vivo* MG show distinct IL-1β profile.

Objective: Here we investigated the role of MG metabolism in inflammation and how altered lipid metabolism influences phenotype of MG.

Methods: MACS isolated CD11β $^{+}$MG, IL-1β model of perinatal brain injury, TRPS technology with qNANO, luminex cytokine assay, radioactive tracer studies.

Results: (i) Pro-inflammatory state of MG show upregulation in glycolysis and downregulation in mitochondrial β -oxidation, (ii) Studies conducted in *in-vitro* and *ex-vivo* MACS isolated CD11β $^{+}$MG shows that altered lipid metabolism plays important role in MG polarization and protects the neuron from toxicity induced by pro-inflammatory MG (iii) Extracellular vesicles derived from pro-inflammatory microglia alters dendritic spine density and morphology, however alteration in MG lipid metabolism has a beneficial effect. (iv) Extracellular vesicles derived from pro-inflammatory and anti–inflammatory MG effects OPC proliferation and differentiation.

Conclusion: Altered microglial lipid metabolism responsible for detrimental phenotype acquired by MG following prolonged inflammation. Acknowledgement:Supported by European Research Projects on Neuroinflammation Era-net neuron- MicroMet.

MTU03-12

Mitochondrial impairment amplifies NLRP3 inflammasome proinflammatory signaling in microglia in cell culture & animal models of PD

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The NLRP3 inflammasome signaling pathway has recently been recognized as a major contributor of neuro-inflammatory process in the CNS. Oxidative stress and mitochondrial dysfunction are long been recognized as key pathophysiological processes of many chronic neurodegenerative diseases, including Parkinson's disease (PD). However, the inter-relationship between mitochondrial defects and neuro-inflammation is not well understood. In the present study, we show that impaired mitochondrial function can greatly augment the NLRP3 inflammasome pro-inflammatory cascade in microglia. Primary mouse microglia treated with the common inflammagen, LPS induced NLRP3 and pro-IL1β expression. Interestingly, LPS-primed microglial cells exposed to the mitochondrial complex I inhibitory pesticides, rotenone and tebufenpyrad, specifically potentiated the NLRP3 inflammasome activation, and ASC Speck formation and pro-IL-1β processing to IL-1β in a time and dose-dependent manner, indicating that mitochondrial impairment heightened the pro-inflammatory response in microglia. The neurotoxic pesticide induced NLRP3 inflammasome activation was accompanied by bioenergetic defects, mitochondrial fission and autophagsome formation in microglia. Furthermore, neurotoxic pesticides enhanced mitochondrial ROS generation in primary microglia while amelioration of mitochondrial derived ROS by mito-targeted antioxidant mitoapocynin completely abolished IL-1β level, indicating mitochondrial ROS drives the potentiation of NLRP3 inflammasome in microglia. Additionally, co-culturing mitochondria impaired microglia with human dopaminergic neuronal cells (LUHMES) induced dopaminergic neurodegeneration. Notably, our in vivo results with rotenone neurotoxicity model of PD further supported the activation of NLRP3 inflammasome signaling due to mitochondrial dysfunction. Collectively, our results demonstrate that mitochondrial impairment in microglia can amplify NLRP3 inflammasome signaling to augment dopaminergic neurodegenerative process.

MTU03-13

Withania somnifera ameliorates neuroinflammation caused by high fat diet consumption in rat model of obesity

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The current century has witnessed enormous growth in technology which is, in part, responsible for sedentary lifestyle. Sub-optimal work schedules have made people more accustomed to consumption of junk or processed food items which has led to the disruption of energy balance. Both factors are the major contributors for obesity. Intake of high calorie diet has been linked to psychiatric and metabolic disorders which are intrigued by neuroinflammation. This study elucidates the potential beneficial effects of dry leaf powder of *Withania somnifera* (Ashwagandha) in amelioration of neuroinflammation caused by diet induced obesity. Young albino female Wistar rats were used for the study. The animals were divided into four groups: Low fat diet (LFD) rats fed with regular chow feed, High fat diet (HFD) rats maintained on feed with 30% fat by weight, Low fat diet plus extract (LFDE) rats fed with regular chow feed supplemented with dry leaf powder of *W. somnifera* 1 mg/g body weight (ASH) and High fat diet plus extract (HFDE) rats fed with high fat diet supplemented with ASH. All groups were maintained on respective feeding regimen for 12 weeks. ASH treated rats show reduction in anxiety-like behavior as compared to HFD group as evident from Elevated Plus Maze test. At molecular level, treatment with ASH led to the reduction in inflammation as seen by downregulation of JNK, phospho-MSK1, P38, Iba1, JAK2, COX2, PPARγ, IL-1β and IL-6 in both Western blotting and Real-Time PCR in piriform cortex and hippocampus regions of the brain. Further, ASH also inhibited apoptosis and promoted cell survival as indicated by downregulation of AP-1, phospho c-Jun and upregulation of Bcl-xL. ASH also improved leptin sensitivity as shown by upregulation of leptin receptor OB-Rb expression. Thus, ASH may be a potential candidate for mitigating neuroinflammation caused by consumption of calorie-rich diets and may serve as an effective dietary supplement for weight management and amelioration of obesity related pathological co-morbid conditions.

MTU03-14

Analgesic effects of bee venom and bee venom derived phospholipase A_2 in a mouse model of oxaliplatin-induced neuropathic pain

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Oxaliplatin, a chemotherapeutic drug, induces severe peripheral neuropathy. Bee venom (BV) is widely used in Korea to alleviate pain, and we assessed the curative and preventive effects of BV and BV derived phospholipase A_2 (bvPLA$_2$) on oxaliplatin (6 mg/kg, i.p.)-induced neuropathic pain in mice. BV (1 mg/kg, s.c.) alone or with morphine (2 mg/kg, i.p.) significantly attenuated peiripheral neuropathy. Furthermore, pretreatment of bvPLA$_2$ (0.2 mg/kg, i.p.) inhibited the development of allodynia, and suppressed the increase of macrophages and IL-1β level in the DRG. Such effects were shown to be mediated by regulatory T cells. Altogether, these results suggest that BV and bvPLA$_2$ may by effective in releiving oxaliplatin-induced neuropathic pain. *This work was supported by National Research Foundation of Korea grant funded by the Ministry of Education, Science and Technology (2016R1D1A1A02937335).*

MTU03-15

Encapsulated mesenchymal stem cells to modulate inflammation and facilitate functional recovery in spinal cord injury

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Encapsulation of mesenchymal stem cells (eMSC) in alginate facilitates cell delivery, survival, and modulates inflammation *in vivo*. However, the delivery of eMSC to spinal cord injury (SCI) rats is constrained because large (~0.5 mm) diameter capsules that are used widely are not suitable for intrathecal injection into the rat spine whereas sufficient quantities of small eMSC (~0.2 mm) for larger studies was not feasible. Therefore, we have prepared medium sized eMSC (~0.35 mm) that can be delivered into the lumbar rat spine. The MSC incorporated/capsule and total yield of eMSC for medium sized capsule was ~5-fold and ~20-fold greater than of the small capsules, respectively. Assays with all eMSC capsules suggested no major difference in their anti-inflammatory activity *in vitro*. The *in vivo* activity of the medium sized eMSC was tested after injecting them into the lumbar spine post-SCI day 1. Histological analyses post-SCI week 1 showed that eMSC reduced levels of activated macrophages (IB4 staining) and increased white matter sparing in similar regions adjacent to the SCI site. Retrieval of eMSC post-SCI week 1 showed ~50% survival of MSC, suggesting that the surviving MSC may have prolonged effects. We also found the facilitation in locomotor recovery and attenuation of mechanical allodynia after 4 weeks of eMSC transplantation. The data indicates that medium size eMSC reduced macrophage inflammation in regions where white matter was preserved during critical early phases and functional recovery in later phase after SCI. These techniques enable preparation of eMSC in sufficient quantities to perform pre-clinical SCI studies with much larger numbers of subjects that will provide functional analyses of several critical parameters in rodent models for CNS inflammatory injury.

MTU03-16

Glial fibrillary antigen protein (GFAP) expression in the hippocampal formation of mefloqine induced-seizured rats

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Luffa aegyptiaca mill normally known as sponge gourd, belong to the family called cucurbitaceous. The aim of this study was to investigate the antiepileptic and anxiolytic effects of aqueous leaf extract of *Luffa aegyptiaca Mill* on the hippocampus of the brain of Albino Wistar rats with Mefloquine induced seizure. Thirty albino wistar rats (190–250 g) were grouped into 6 groups of 5 rats each. Group 1 was control. Group 2 was induced with mefloquine only (4.28 mg/kg). Group 3 were given average dose of luffa extract only (800 mg/kg). Group four rats were induced with mefloquine (4.28 mg/kg) and treated with diazepam (5 mg/kg). Group 5 rats were induced with (4.28 kg/kg) with mefloquine and treated with low dose luffa aergyptiaca mill (400 mg/kg). Group 6 were induced with mefloquine (4.28 mg/kg) and treated with high dose luffa aergyptiaca mill (1200 mg/kg). The rats were then perfused transcardially and sacrificed. Brain sections were analyzed for histological (H&E) and immunohistochemical staining for glial fibrillary acidic protein (GFAP), marker for astrocytes were carried out. The histological results showed disruption of pyramidal cells layer in CA3 subfield of hippocampus and regional selectivity of pyramidal cell loss in seizured rats indicating induction of seizure with mefloquine. There was some restoration of pyramidal cells with the treated groups but no disruptions in the control group. There was less expression of GFAP positive cells in the control group and treated groups and more expression in the seizure rats. The expression of GFAP positive cells was an indication of different levels of neuroinflammation. The reactive astrocytes being predominant in the seizure group. The present study therefore provides empirical data on GFAP expression in the hippocampus of seizure animal model treated with aqueous extract of luffa leaves.

MTU03-17

Bakuchiol suppresses inflammatory responses via downregulation of the p38 MAPK/ERK signaling pathway in BV-2 microglia

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Purpose: The purpose of the present study was to evaluate the effects of bakuchiol on the inflammation response along with a

molecular mechanism of the inflammatory effects in lipopolysaccharide (LPS)-stimulated BV-2 mouse microglia cell line.
Methods: The production of prostaglandin E_2 (PGE_2), tumor necrosis factor-α (TNF-α), and interlukin-6 (IL-6) were measured by enzyme linked immunesorbent assay. The mRNA expression of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), TNF-α, and IL-6 were measured using reverse transcription–polymerase chain reaction analysis. Mitogen-activated protein kinases (MAPK) phosphorylation were determined by western blot analysis.
Results: Bakuchiol significantly suppressed the production of PGE_2 and IL-6 in LPS-stimulated BV-2 cells without causing cytotoxicity. In parallel, bakuchiol significantly inhibited LPS-stimulated expression of iNOS, COX-2, and IL-6 in BV-2 cells. However, bakuchiol had no effect LPS-stimulated production and mRNA expression of TNF-α. This also had no effect on LPS-stimulated c-Jun NH2-terminal kinase phosphorylation, whereas p38 and extracellular signal-regulated kinase (ERK) phosphorylation was inhibited by bakuchiol.
Conclusions: These results indicate that anti-neuroinflammatory effects of bakuchiol in the activated microglia is mainly regulated by the inhibition of p38 MAPK and ERK pathways. We suggest that bakuchiol could be beneficial for various neuroinflammatory diseases.

MTU03-18

TREM2 regulation of microglial phagocytosis is age-, activation- and target-dependent

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Microglia are the resident macrophages of the central nervous system (CNS) and act as the primary phagocyte in the CNS during normal development, tissue homeostasis and disease. In the CNS, Triggering Receptor Expressed on Myeloid cells-2 (TREM2) is expressed only by microglia. Lack of a functional TREM2 leads to cognitive dementia by the third decade of life, while a single amino acid mutation in TREM2 correlates with a 3-fold increased risk of Alzheimer's disease. We and others have previously demonstrated that TREM2 deficiency decreases phagocytosis using microglia cell lines and primary neonatal glial cultures. Because cultured microglia display significantly different phenotypes from microglia differentiated in vivo, we sought to confirm that TREM2 regulated phagocytosis in microglia differentiated within the intact mouse brain. While many reports of cultured microglia display low percentages of phagocytosing cells, we found that nearly all microglia assayed immediately after isolation from brain tissue displayed phagocytic activity. In general, phagocytic activity decreased with age. At all ages examined, wild-type activated microglia show greater phagocytosis of both targets than those isolated from untreated mice. By contrast, TREM2 deficiency decreased phagocytosis only of in vivo activated microglia and only at p15. However, the effect was target dependent. TREM2 deficiency decreased the percentage of microglia phagocytosing Staph aureus but not the amount phagocytosed per cell. By contrast, TREM2 deficiency did not alter the percentage of microglia phagocytosing synaptosomes but did decrease the amount phagocytosed per cell. Using flow cytometry, we quantified the surface expression of receptors for Staph aureus (TLR2) and for cellular targets (Tyro3, Axl and Mer). IPLPS regulated expression of these molecules but TREM2 deficiency did not. In total, our data suggest that TREM2 deficiency has little effect on homeostatic phagocytosis but has large effects on injury or inflammation-associated phagocytosis. Thus, we speculate that TREM2 mutations may increase the risk of Alzheimer's disease due to the cumulative effect of altered responses to lifelong environmental insults.

MTU03-20

Cypermethrin disrupts HB-EGF-EGFR signaling leading to neuroinflammation and memory loss in young rats: role of exogeneous HB-EGF

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Introduction: Cypermethrin is a type-II synthetic pyrethroid, used as an insecticide in commercial agricultural and domestic purposes. Cypermethrin is reported to affect the development of central nervous system (CNS). However relatively less is known about its mechanism of action on neuron survival. Here, we hypothesized that cypermethrin promotes neuronal apoptosis in young rat through inflammation where disrupted HB-EGF-EGFR signaling plays a significant role.
Method: We treated 24-day old rats with cypermethrin (10 mg/Kg) for 3 weeks, and examined neuronal apoptosis and modulation of different inflammatory proteins, and HB-EGF-EGFR signaling molecules by western blotting and immuno-histochemistry in the rat brain.
Results: Cypetrmethrin treatment increases neuronal apoptosis in the young rats. We then investigated the mechanism responsible for apoptosis and detected an elevated levels of inflammatory proteins. Increase in interleukin-1 (IL-1), its receptor and an increase in the NFκb promoted neuronal apoptosis. Exploring the mechanism revealed an attenuated signaling of growth factor HB-EGF. We observed a decrease in HB-EGF, EGFR and p-EGFR levels, indicating a compromised cell-survival pathway. This signaling pathway could be restored by exogenous supply of recombinant HB-EF, highlighting the significance of HB-EGF in cell survival. We also observed that recombinant HB-EGF cause attenuation of the Cypermethrin mediated inflammation and neuronal apoptosis. Furthermore, cypermethrin induced learning-memory impairments in the young rats, which could be prevented by recombinant HB-EGF administration.
Conclusion: Together, these data demonstrate that cypermethrin disrupts HB-EGF-EGFR signaling and increases inflammation-dependent neuronal apoptosisc which culminates into cognitive loss. The current study therefore underscores the therapeutic role exogenous recombinant HB-EGF.

MTU03-21

Parasympathetic cholinergic and peptidergic mechanisms of trigeminal pain

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Parasympathetic neurochemical mechanisms of primary headaches such as cluster headache and migraine remain little understood. In the current study, we explored the neurochemical mechanisms of this nociceptive signaling, which likely originates from interacting trigeminal and parasympathetic nerves densely innervating meninges. For his aim, we used two rat models: dissected hemiskulls with preserved innervation and isolated trigeminal neurons. We found that the main parasympathetic neurotransmitter acetylcholine (ACh), as well as its stable analogue carbachol, largely increased the nociceptive activity of meningeal trigeminal nerves. Spiking activity was also induced by nicotine indicating the primary role of nicotinic receptors in excitation of primary afferents. Similar pro-nociceptive effect was observed with the peptidergic co-transmitter of parasympathetic nerves, the neuropeptide PACAP. In contrast to neuronal mechanisms, carbachol, but not nicotine was able to degranulate meningeal mast cells, which are likely implicated in headache by releasing multiple cytokines and monoamines such as serotonin and histamine. In isolated trigeminal ganglion cultures, nicotine activated a fraction of nociceptive neuronal cells. Taken together, our data suggest that cholinergic and peptidergic mechanisms similarly contribute to induction of peripheral trigeminal pain underlying headache in migraine and likely also in other types of primary headaches.

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MTU03-22

Activated perk pathway in the brainstem was induced by masseter inflammation

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Object: To evaluate the inflammatory hyperalgesia induced by noxious stimulation of the masticatory muscle, we performed an immunohistochemical study on the expressions of phosphorylated-extracellular signal-regulated kinase (pERK) and distribution of activated microglia in the brainstem trigeminal subnucleus caudalis (Vc).

Methods: The left masseter muscle (LMM) of Sprague Dawley rats (male, 250 g) was prepared in the following methods: (i) L group; the LMM was injected with lipopolysaccharide (LPS, 2 μg/kg, 100 μL) on the 1st day of the experiment. On day 2, the same site was injected with LPS (100 μL, 5 times per 90 min). (ii) S group; the LMM was injected with LPS (2 μg/kg, 100 μL) on the 1st day of the experiment. On day 2, the same site was injected with sodium chloride solution (100 μL, 5 times per 90 min). (iii) HS group; the LMM was injected with LPS (2 μg/kg, 100 μL) on the 1st day of the experiment. On day 2, the same site was injected with 6% sodium chloride solution (100 μL, 5 times per 90 min). The rats were allowed to survive for 14 days or 25 days after the last injection. The brainstems were dissected and cut with a cryostat (at 30 μm thickness). These specimens were investigated with anti-pERK or anti-GFAP (glial fibrillary acidic protein: a marker for astrocyte) using the enzyme-labeled antibody method. The specimens were observed, recorded and analyzed using a light microscope mounted with an 3CCD digital camera system connected with a FLvFs software (Flovel Image Filling System, Tokyo, Japan).

Results: In the HS group, analysis of the IHC histology indicate that pERK-immunoreactive (IR) and GFAP-IR cells were particularly localized in the Vc until 14 days after stimulation. On the other hand, 14 days after nociception, the cells are little found in the Vc in the L and S groups.

Conclusion: The prolonged MAPK activity is related to the central sensitization and chronic pain.

MTU03-23

Evaluation of dendrimer-4 phenylbutyrate in X-linked adrenoleukodystrophy patient derived cells

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X-linked adrenoleukodystrophy (X-ALD) is a neurodegenerative disorder due to defects in the peroxisomal membrane transporter protein, ABCD1, with variable phenotypes ranging from a rapidly progressive, inflammatory cerebral demyelination (cerebral ALD) in young boys and adult men to the chronic slowly progressive adult onset adrenomyeloneuropathy affecting men and women. Hallmark pathophysiology includes accumulation of very long chain fatty acids (VLCFA), increased oxidative stress, and progressive axonopathy, with little to no genotype-phenotype correlation. Allogeneic hematopoietic cell transplantation is effective in early stages of cerebral ALD only, and no other effective interventions exist. Nanoparticle dendrimer-drug conjugates enable targeted and intracellular slow release of drugs requiring fewer treatments at lower drug concentrations. 4-Phenylbutyrate (4PBA) has been shown to increase expression of ABCD2 and proliferation of peroxisomes in models of X-ALD; however, the short half-life precludes its utility in the clinic. We have demonstrated uptake of dendrimer-drug conjugates in spinal cord neurons of the ABCD1 knockout mouse and within patient-derived primary macrophages and fibroblasts. Here, we demonstrate efficacy in ALD and AMN patient derived cells treated with PAMAM dendrimer conjugated to 4PBA (D-4PBA) as treatment significantly altered biochemical and inflammatory abnormalities. Reduced VLCFA (C26:0 and C26/C22) was detected in ALD and AMN patient derived fibroblasts after a 16-day exposure to 30 μM (AMN $p = 0.017$) or 100 μM D-4PBA (ALD $p = 0.026$; AMN $p = 0.0006$). In ALD patient monocyte-derived macrophages, a 6 h stimulation of 30 μM VLCFA leads to significant release of TNF ($p = 0.009$, compared to unstimulated cells) and pretreatment with D-4PBA prevents these increases (30 μM D-4PBA, $p = 0.0018$; 100 μM D-4PBA, $p = 0.002$; 300 μM D-4PBA, $p = 0.002$). Together, these data support feasibility and efficacy of low dose and versatile nanoparticle therapy to reduce ALD-related disease burden in patient derived cells and sets the stage for new therapeutic opportunities for complex diseases such as X-ALD.

MTU03-24

Behavioral changes, oxidative stress, brain metal and neuro-inflammatory profiles after chronic vanadium administration

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Vanadium is a potentially toxic environmental pollutant. Most studies on vanadium neurotoxicity have been after acute exposure but in reality some populations are exposed for a lifetime. BALB/c mice were divided into vanadium treated, matched controls, and animals exposed to vanadium for 3 months and thereafter vanadium withdrawn. Animals were tested using Morris water maze at 3, 6, 9, and 12 months of age. Mice were also subjected to biochemical, metal profiling and immunohistochemistry. The results showed that mice had significant loss in memory abilities from 3 to 12 months of vanadium exposure. Animals recovered significantly only 9 months after vanadium withdrawal. Vanadium exposure caused increases in levels of oxidative stress markers with a decrease in the activities of intrinsic oxidative defense markers from 6 months of vanadium exposure in the brain. Withdrawal after 3 months of vanadium exposure reversed oxidative stress from 9 to 15 months. Metal profiling showed progressive increase in vanadium uptake with regional variabilities in latter age. The withdrawal brains still show presence of vanadium metal in the brain though less than controls. There were disruption of laying pattern, and cell loss in the pre frontal cortex, Hippocampal CA1 pyramidal cells, and Purkinje cells of the cerebellum in vanadium exposed brain. With exposure into latter age, the evident neuropathology was microgliosis rather than progressive astrogliosis. In conclusion, administration of vanadium over a life time in mice resulted in behavioral deficits, derangements in brain antioxidant defense system and brain cell architecture, neuroinflammation and brain metal accumulation. While memory scores was recovered over time, the metal load and pathological effects were not completely eliminated from the brain even after a long time withdrawal from vanadium metal.

MTU03-25

Pharmacological intervention to study the effect of antinociceptive curcumin analogue in a rat model of migraine

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Objective: Migraine is a multifactorial primary headache disorder is also a disabling neurological symptoms. It is characterized by unilateral pulsatile intense headache. The pathophysiology known behind migraine is activation of the trigeminovascular system with the release of neuropeptides, results into dilation of intracranial and extracranial blood vessel. Neurogenic inflammation occurs mainly at the vascular levels, where in it comprises CGRP-mediated arteriole vasodilatation. In the present work we portrayed MG24, a novel semicarbazone as CGRP protein inhibitor. The main objective of the proposed work is to carry out in vivo evaluation of the newly synthesized compound to screen their antimigraine activity, it's effect on CGRP and endogenous inflammatory mediators levels of tumour necrosis factor –α (TNF-α), Interleukin-1beta (IL-1β) in animal model of migraine.

Methods: Since the CGRP receptor antagonists have recently been shown to be effective in migraine therapy, the pharmacology of the control mechanisms for dural CGRP release are of direct relevance to the development of newer migraine therapies. Migraine was induced by injecting Complete Freund's Adjuvant (CFA), a chemical stimulant through intracisternal route (IC) into cisterna magna of rats and effect of compound on expression levels of CGRP neuropeptide, a biomarker of trigeminal nerve activation was evaluated. To examine, blood samples were collected from retro-orbital sinus at 1, 4 and 24 h time interval. The gene expression levels of CGRP mRNA was investigated by real time quantitative reverse transcription polymerase chain reaction (RT-qPCR).

Results: CFA administration caused a significant increase in CGRP levels after 1 h when compared with baseline. The intraperitoneal treatment MG24 1 h post CFA administration lead to reduction in levels of CGRP and other endogeneous inflammatory mediators gene expression significantly.

Conclusion: The inhibition of CGRP, TNF-α and IL-1β mRNA levels by this compound offers possible mechanism in part that can account for the preventative antimigraine activity.

MTU03-26

HY2093 ameliorates brain inflammation and improves memory impairments in Alzheimer's disease mouse model

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Alzheimer's disease (AD) is the most common cause of dementia. Progressive deposition of amyloid beta (Aß) in the brain is the major pathognomonic feature. Moreover, neuroinflammation and neuronal apoptosis incur defects in cognition and memory. We have previously suggested that endogenous surfactant molecules might have co-evolved to regulate immune responses through the activation of various anti-inflammatory pathways. In this study, we showed that HY2093 could improve memory deficits in AD mice model (5XFAD). The mice were given 1 mg/kg HY2093 i.p. twice a week for 2.5 months. As a control group, the AD mice were given PBS. HY2093 significantly reduced the time to find platform, increased platform crosses and quadrant occupancy in water maze test, suggesting improvement in learning and memory of 5XFAD mice after treatment with HY2093 compared with control group. Furthermore HY2093 treatment significantly decreased the amyloid plaque formation, the number of astrocyte, microglia, and inflammation factors in the frontal cortex. In the frontal cortex, iNOS expression of astrocyte was drastically decreased by HY2093 treatment. TUNEL (+) apoptotic cells in the frontal cortex were significantly lower in HY2093 group of mice. The number of

astrocyte was decreased by HY2093 treatment in hippocampus also. Interestingly, CD11b+Gr1-F4/80 + myeloid cells were significantly increased in the spleen and in brain of 5XFAD mice that were treated with HY2093. Blood CCL3 was significantly lower in HY2093 group of mice. These findings suggest that HY2093 might be a new molecular entity that can control pathogenesis of Alzheimer disease.

MTU03-27

Lambda-cyhalothrin synergies the stress induced neuroinflammatory cytokines in rat brain through mitochondrial biogenesis

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Recently, we have found that pre-exposure to immobilization stress (IMS), a psychological stressors and forced swim stress (FSS), a physical stressors exacerbated lambda-cyhalothrin (LCT) induced brain cholinergic dysfunctions in rats. In continuation to this, studies have been carried out to understand the impact of IMS and FSS on LCT induced neuroinflammation associated with mitochondrial impairments. No significant change in the levels of brain proinflammatory (TNF-α, IL1β, IL6) and anti-inflammatory (IL10) cytokines was observed in frontal cortex and hippocampus in rats subjected to IMS (one session, placed in plastic restrainer, 15 min/day) or FSS (one session, 3 min/day) for 28 days or exposed to LCT (3.0 mg/kg body weight, p.o.) for 3 days (on days 26, 27 and 28) alone in comparison to controls. Marginal changes in mitochondrial activity, ROS generation and membrane potential both in frontal cortex and hippocampus were evident in rats subjected to IMS or FSS or those exposed to LCT alone as compared to controls. Pre-exposure to IMS or FSS for 28 days followed by LCT treatment for 3 days in rats resulted to alter the levels of brain proinflammatory and anti-inflammatory cytokines through affect the mitochondrial bioenergetics as compared to rats exposed to IMS or FSS or LCT alone. Further, pre-exposure to IMS or FSS caused a marked enhanced ROS generation and decrease in mitochondrial membrane potential in frontal cortex and hippocampus on LCT treatment as compared to rats exposed to IMS or FSS or LCT alone. These changes affect the learning and memory activities on pre exposure to IMS or FSS for 28 days followed by LCT treatment rats. The results clearly exhibit that both psychological and physical stressors contribute in the LCT induced mitochondrial impairments associated with neuroinflammatory pathway. However, alterations in behavioural and neurochemical end points were more intense in LCT treated rats pre-exposed to IMS as compared to those pre-exposed to FSS.

MTU03-28

HSP60 plays a regulatory role in il-1β-induced microglial inflammation via TLR4-p38 MAPK axis

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Neuroinflammation being the innate immune machinery of the Central Nervous System (CNS) helps to combat any neuronal insult and neurodegeneration. But as they say "excess of everything is bad", an exaggerated neuroinfalmmatory response may be detrimental for the neuronal health itself and an excessive inflammatory response associated with various neurodegenerative diseases suggests the same. The role of microglia, the resident immune cells of CNS, in neuroinflammation is evident from a number of studies. It gets activated in response to harmful stimuli and secretes various pro- and anti-inflammatory cyto-chemokines, among which IL-1β is known as "the master regulator of inflammation", as it can induce a vicious cycle of inflammation. The role of IL-1β in various neurodegenerative diseases has been extensively studied over the years but the overall molecular mechanism underlying its action are yet not well understood. Our study, therefore, focuses on a holistic approach to reveal key molecules involved in IL-1β induced inflammation in microglia. To achieve our aim, we have performed proteomic profiling of the microglial cells in response to IL-1β and identified 18 types of proteins to be differentially expressed under its influence, many of them belonging to the cellular stress pathways. Out of these proteins, we set out to analyze the role of HSP60, a mitochondrial chaperone, which is reported to be involved in neuron-glia crosstalk during neurodegeneration and appeared to be a key hub molecule by *in silico* analysis of the identified proteins.

The results show that, IL-1β induces the expression as well as secretion of HSP60 in extracellular milieu, whiche then binds with TLR4 of microglia to exert its effects. We further established that HSP60 increases the phosphorylation of ERK, JNK, and p38 MAPKs in microglia during inflammation but specific inhibition of p38 only resulted in decreased inflammation by HSP60. We thus propose that, HSP60 plays a regulatory role in IL-1β induced inflammation in microglia by activating TLR4-p38 MAPK axis, which can be specifically targeted to develop novel approaches for therapeutic applications.

MTU03-29

Nogo receptor complex (lingo-1, p75, troy) expression pattern in inflammatory foci of experimental autoimmune demyelination

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Nogo-A and its receptor complex (NgR complex) have already been implicated to inhibitory, axonal guiding and other central nervous system (CNS) modulatory aspects of the injured and demyelinating tissue. The purpose of this study was to describe the spatiotemporal expression of NgR complex molecules LINGO-1, p75 and TROY within the inflammatory sites of the experimental model of Multiple Sclerosis (MS) in mice. Thirty C57BL/6 mice were subcutaneously injected with the myelin oligodendrocyte glycoprotein (MOG) 35–55 peptide and developed chronic experimental autoimmune encephalomyelitis (EAE). The study included acute (days 18–22) and chronic (day 50) time points that were compared to controls respectively. All animals were examined daily using a 6-grade scale. Localization and neuropathological study of NgR complex was performed with double immunofluorescence (dIF) on 6 μm coronal paraffin sections while molecular analysis was performed with real-time PCR in spinal cord extracts. MOG-inoculated animals developed a typical chronic-MOG EAE pattern with mean maximal score (MMS) = 3.76 ± 0.28. The levels of the NgR complex were found to fluctuate, depending on the stage studied; LINGO-1 was increased in perivascular inflammatory foci (467.8 ± 48.18 cells/mm^2) of acute phase while an additional increase was detected in axonal structures (Integrated density,

249.156 ± 26.177) of chronic phase. Expression of p75 was increased only in residual inflammatory foci of chronic phase (IntDen, 121.521 ± 15.709) while TROY was restricted within inflammatory cells at the lesion sites of acute phase (106.7 ± 9.57 cells/mm^2). Such dynamic expression of the Nogo receptor complex, it may support an alternative role that could include the confinement of the inflammatory reaction or the rampant sprouting of axons in chronic EAE lesions.

MTU03-30

The neuroprotective properties of vitamin D, in a Parkinson's disease model, are related to its anti-inflammatory actions

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The decrease in serum levels of Vitamin D seems to be related to inflammatory diseases and this drug may exhibit neuroprotective actions. Parkinson's disease (PD) is characterized by loss of dopaminergic neurons in the *substantia nigra* and neuroinflammation is a hallmark of PD pathophysiology. The objectives were to evaluate the neuroprotective properties of vitamin D on parkinsonian rats. Male Wistar rats were untreated or treated (1 μg/kg) with vitamin D, previously or after the unilateral striatal injection of 6-OHDA. The sham-operated group was used as control. After treatments, the animals were subjected to behavioral tests and euthanized for striatal DA and DOPAC measurements and TH and DAT immunohistochemical assays. The data were analyzed by ANOVA and Tukey as the *post hoc* test. The results showed 223 apomorphine-induced rotations/h, in the untreated group, and only 13 and 77 rotations/h, respectively, after pre- and post-lesion treatments with vitamin D. No behavioral changes were noticed in the SO group. In the forced swimming test, while the untreated group showed a depressive-like behavior (increasing by 2-fold the immobility time), significant decreases were seen after vitamin D pre- and post-lesion treatments, relatively to the SO group. The immunohistochemical data for TH and DAT demonstrated decreases higher than 90% in immunostainings in the untreated 6-OHDA group, as related to the SO group. Interestingly, while a great recovery was observed in TH immunostainings after both vitamin D pre- and post-lesion treatments, this was not seen for DAT immunoreactivity, where only the pre-lesion treatment showed a good recovery. In conclusion, we demonstrated that vitamin D presents neuroprotective effects in this PD model in rats and, at least in part, these effects are related to the antioxidant and anti-inflammatory actions of this drug, as already demonstrated by us.

MTU03-31

Arachidonic acid induces are/NRF2-dependent heme oxygenase-1 transcription in rat brain astrocytes

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Arachidonic acid (AA) is a major product of phospholipid hydrolyzed by phospholipase A_2 during neurodegenerative diseases. AA exerts as a second messenger to regulate various signaling components which may be involved in different pathophysiological processes. Astrocytes are the main type of CNS resident cells which maintain and support the physiological function of brain. AA has been shown to induce ROS generation through activation of NADPH oxidases (NOX) which may play a key role in the expression of heme oxygenase-1 (HO-1). Therefore, this study was designed to investigate the mechanisms underlying AA induced HO-1 expression in rat brain astrocytes (RBA-1). We found that AA induced HO-1 protein and mRNA expression and promoter activity in RBA-1, which was mediated through the synthesis of 15d-PGJ_2 activated PPARγ receptors. This note was confirmed by transfection with PPARγ siRNA which attenuated the AA-mediated responses. AA-induced HO-1 expression was mediated through NOX/ROS generation, which was inhibited by NOX inhibitors (DPI and apocynin) and ROS scavengers (NAC). Moreover, AA-induced HO-1 expression was mediated through phosphorylation of Src, Pyk2, PDGFR, PI3K/Akt, and ERK1/2 which were inhibited by the pharmacological inhibitors including PP1, PF431396, rotterlin, AG1296, LY294002 and U0126 or by transfection with respective siRNAs. AA-enhanced Nrf2 expression and HO-1 promoter activity was inhibited by transfection with Nrf2 siRNA or by these pharmacological inhibitors. Furthermore, ChIP assay confirmed that Nrf2 and PPARγ were associated with the proximal ARE binding site on HO-1 promoter, suggesting that Nrf2/PPARγ are key transcription factors modulating HO-1 expression. AA-induced ARE promoter activity was also reduced by these pharmacological inhibitors. These findings suggested that AA increases formation of Nrf2 and PPARγ complex and binding with ARE1 binding site through Src, Pyk2, PI3K/Akt and ERK1/2, which further induced HO-1 expression in RBA-1 cells.

MTU03-32

Amyloid precursor protein modulates microglia and macrophage phenotype

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Mutations in the gene coding for amyloid precursor protein (APP) are responsible for autosomal dominant forms of Alzheimer's disease and proteolytic processing of the protein leads to a number of metabolites including the amyloid beta (Aβ) peptide. In addition to the well characterized contribution of APP and Aβ to plaque deposition in AD brains, prior work suggests that APP can function as a proinflammatory receptor on immune cells, such as macrophages and microglia. We hypothesized that APP may modulate the phenotype of these cells in diverse conditions including both obesity and Alzheimer's disease. By comparing C57BL/6 wild type and APP knockout mice we observed that amyloid precursor protein is involved in regulating the phenotype of both adipocytes and peripheral macrophages and is required for high fat diet-dependent weight gain in mice. Moreover, we determined that oligomeric but not fibrillar Aβ peptide binds directly to APP and is involved in stimulating microglial activation both in vitro and in vivo. These data suggest that APP and/or its metabolites modulate the phenotypes of peripheral immune system macrophages as well as brain resident microglia. This biology may be relevant to not only to the pathophysiology of Alzheimer's disease but also diet-associated obesity.

MTU04 Molecular Mechanism of Parkinson's Disease

MTU04-01

Analysis of the functional effects mediated by dopamine oxidation products at the mitochondrial level

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The neurotransmitter dopamine (DA) plays a critical role in many mental and physical functions, such as learning, motivation, movement control. Nevertheless, when DA is not properly stored, its oxidation could be source of neurodegeneration. In fact, DA oxidation causes the generation of reactive oxygen species (ROS) and dopamine quinones (DAQs). Since the molecular effects of DAQs are still not elucidated and it has been shown the existence of an interplay between oxidative stress and mitochondrial dysfunction, we investigated the effects of DAQs inside mitochondria.

Methods: Experiments were performed in rat brain mitochondria and in SH-SY5Y neuroblastoma cells, both exposed to DAQs. First, mitochondria were incubated with ^{14}C-DAQs to verify the entrance of these compounds inside the organelles and then, ATP synthesis and mitochondria morphology assays were performed. Finally, cell viability, calcium retention capacity (CRC), mitochondrial swelling and mitochondria membrane potential (MMP) were assessed to shed some light on the mechanisms of mitochondria-related DAQs toxicity.

Results: Our data shows that DAQs enter into isolated mitochondria, reduce ATP production, induce mitochondrial swelling and decrease CRC. In addition, we demonstrated that all above-mentioned mitochondrial dysfunctional effects are caused by the opening of the mitochondrial permeability transition pore (mPTP) *in vitro*. In cells, DAQs induce mitochondrial morphology changes and a decrease in MMP and the latter depends on a variation in mPTP.

Conclusion: Our results suggest that DAQs could induce cell death through the opening of the mPTP; therefore, inhibitors of mPTP might be a potential strategy to hamper dopaminergic neurodegeneration.

MTU04-02

The brain-specific angiogenesis inhibitor 1 has neuroprotective effect against mpp$^+$-mediated neuronal cell death

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Brain-specific angiogenesis inhibitor 1 (BAI 1) is a member of the cell-adhesion G protein-coupled receptor family that has been studied primarily for its anti-angiogenesis and anti-tumorigenesis. However, function of BAI 1 in Parkinson's disease is still unknown. Parkinson's disease is primarily resulted from the death of dopaminergic neurons. The purpose of this study was to explore the effect of BAI 1 in a model of Parkinson's disease. In order to identify the patterns of BAI 1 expression in certain cell types, we stained the substantia nigra and striatum tissues of MPTP-injected mouse briain. BAI 1 protein specifically expressed in neuronal cells including dopaminergic neurons but not in microglia and astrocytes. In addition, the level of BAI 1 expression was reduced by 1-methyl-4-phenylpyridinium (MPP$^+$) in neuronal cells. Because the activation of AMPK can reduce MPP$^+$-mediated neuronal cell death, we examined the correlation between AMPK activity and BAI 1 expression. We found that AICAR, a specific activator of AMPK, increased the expression of BAI 1 protein level and overexpression of BAI 1 protected neuronal cells against MPP$^+$-induced neurotoxicity. Collectively, our experiments suggested that BAI 1 may act as neuronal cell survival factor in Parkinson's disease.

MTU04-03

Parkinson's disease-linked *LRRK2* covers a relevant role in astroglial physiology

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Mutations in *LRRK2* are associated with familiar Parkinson's disease (PD). PD is a neurodegenerative disorder characterized by (i) neuronal death, (ii) the presence of proteinaceous accumulations in the surviving neurons and astrocytes and (iii) neuroinflammation. Accumulating evidence implicate LRRK2 in regulation of secretory vesicle trafficking and in lysosomal pathways. Since LRRK2 is ubiquitously expressed in the CNS cells, one possibility could be that mutated LRRK2 in astro- and microglia impacts neuronal functions, thus triggering neurodegeneration. Here, we show for the first time that Lrrk2 is expressed in mouse astrocytes *in vivo*. By combining confocal and electron microscopy techniques, we observe impairment in protein turnover (e.g. glutamate transporter, GLT-1) as well as abnormal lysosome accumulation in Lrrk2$^{-/-}$ striatal astrocytes. Interestingly, by co-culturing wild type primary cortical neurons with *Lrrk2*$^{+/+}$ *or Lrrk2*$^{-/-}$ mouse primary astrocytes, we demonstrate that LRRK2 depletion in astrocytes significantly abates the ability of these cells to support neuronal development *in vitro*. Impairment in the lysosomal pathway may impact calcium homeostasis, a key aspect of astrocyte functionality. To assess whether LRRK2 plays a role in the regulation of calcium handling in astrocytes, we injected AAVVs overexpressing genetically encoded calcium indicators under the GFAP promoter in *Lrrk2*$^{+/+}$ *or Lrrk2*$^{-/-}$ mouse brain cortex and performed calcium imaging in brain slices. Our results revealed that astrocyte calcium response is significantly altered in *Lrrk2*$^{-/-}$ mice. Specifically, ATP evoked calcium signals in *Lrrk2*$^{-/-}$ display a shorter latent phase, reduced oscillations and enhanced extension compared to controls. Concluding, our findings reveal a novel role of LRRK2 in the regulation of glial functions *in vivo* and *in vitro*, supporting future research aimed at understanding the mechanisms behind mutant LRRK2-linked astrocyte dysfunction in PD neurodegeneration.

MTU04-04

CSF catecholamine and kynurenine metabolites in Parkinson's disease and L-DOPA-induced dyskinesia

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Objective: Identifying potential changes in catecholamine and kynurenine (KYN) metabolism related to levodopa (L-DOPA)-induced dyskinesia (LID) in Parkinson's disease (PD).
Method: Cerebrospinal fluid (CSF) and plasma from 26 PD patients and 16 controls were analyzed using HPLC for monoamine analysis and HPLC with mass spectrometry (LC-MS) for KYN metabolite analysis. Clinical rating of disease severity and dyskinesia was performed for each PD patient. Patients were divided into groups: non-L-DOPA-treated (PD-N), L-DOPA-treated non-dyskinetic (PD-L), and L-DOPA-treated dyskinetic (PD-LID).
Results: CSF of PD-LID had higher dopamine (DA) levels compared to age-matched controls, a higher DA/L-DOPA ratio, as well as a lower DOPAC/DA ratio compared to PD-L. In plasma changes in kynurenine metabolism differentiated PD-LID patients from PD-N, PD-L and controls. PD-LID had an increased 3-hydroxykynurenine (3-HK)/kynurenic acid (KYNA) ratio and increased 3-HK/KYN ratio compared to PD-N and controls as well as significantly lower KYNA levels compared to PD-L.
Conclusion: Monitoring changes in dopamine and kynurenine metabolism could potentially be used to identify PD patients at risk of developing LID.

MTU04-05

RAB7 effector FYCO1 induces clearance of A53T-alpha-synuclein aggregates

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Introduction: Parkinson's disease (PD) is characterized by cytoplasmic aggregates of alpha-synuclein. We have previously shown that overexpression of the small GTPase Rab7 induces clearance of alpha-synuclein aggregates in cell and fly models of PD. Rab7 is known to regulate transport of autophagosomes and their fusion with lysosomes to degrade cellular content including protein aggregates. In order to understand the molecular events that mediate the beneficial effects of Rab7 on alpha-synuclein aggregates, we tested one specific effector, that mediates the transport of Rab7-positive vesicles towards the periphery, FYVE and Coiled-Coil Domain Containing 1 (FYCO1).
Methods: We expressed the A53Tmutant of alpha-synuclein in HEK293 cells and determined the effects of coexpressing Rab7 and FYCO1 on the occurrence of alpha-synuclein aggregates, on alpha-synuclein amounts and on toxicity using fluorescence microscopy, time-lapse imaging and immunoblots. Additionally, we carried out electron microscopy and tested the effect of FYCO1 in a fly model of PD.
Results: We find that FYCO1 is enriched around alpha-synuclein-containing vesicles. Moreover FYCO1 reduces alpha-synuclein amount and toxicity in a Rab7-dependent manner. FYCO1 induces the clearance of alpha-synuclein as observed by time-lapse-imaging. FYCO1 decreased the ratio of green/red fluorescence with alpha-synuclein tagged by mRFP-GFP, indicating that more alpha-synuclein is located in acidic compartments, presumably autolysosomes. Using electron microscopy we observed alterations in Golgi and rough endoplasmic reticulum when FYCO1 was coexpressed with A53T-alpha-synuclein. Moreover, we found evidence of exocytosis of electron-dense material, indicating that FYCO1 could induce secretion of alpha-synuclein. In the fly model of PD, neuronal expression of FYCO1 rescued the locomotor deficit induced by A53T-alpha-synuclein.
Conclusion: We conclude, that the Rab7 effector FYCO1 shows similar effects as Rab7 in inducing aggregate clearance. Our electron microscopic observations indicate that FYCO1 may induce exocytosis of aggregated alpha-synuclein, consistent with the recent description of FYCO1-mediated exocytosis of endosomes.

MTU04-06

The small heat shock proteins interact with aggregating α-synuclein preventing cytotoxicity

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Parkinson's disease (PD) is the second most prevalent age-related neurodegenerative disorder. The pathogenesis of PD, and other neurodegenerative diseases, has been inextricably linked with the

amyloid fibrillar aggregation and deposition of α-synuclein. The cell has a range of defense mechanisms in place to prevent aggregation and maintain protein homeostasis (proteostasis). An important element of this proteostasis network are the molecular chaperone proteins. However, the persistence of diseases associated with α-synuclein aggregation indicates that their protective capacity can be 'overwhelmed' in the context of these diseases. Our work seeks to investigate the role of the small heat shock molecular chaperone proteins (sHsps) in protecting against α-synuclein aggregation. Specifically, we have examined interactions between α-synuclein and sHsps at various stages along α-synuclein's aggregation pathway using a range of bulk and single molecule techniques. Our results demonstrate that sHsps interact transiently with aggregation-prone monomeric α-synuclein to prevent its aggregation *in vitro*. However, the efficiency by which sHsps prevent a-synuclein aggregation is highly dependent on the rate at which it aggregates. In addition, we have characterized the ability of the sHsps to interact with mature fibrillar aggregates formed by α-synuclein and established a physiologically relevant role for this interaction in preventing the cytotoxicity of the aggregates. By pursuing the mechanistic details of the manner by which sHsps interact with α-synuclein, we aim to uncover potential mechanism(s) by which sHsp chaperone activity may be targeted to attenuate diseases associated with α-synuclein aggregation.

MTU04-07

Effect of oligomerization of the Parkinson's disease related protein α-synuclein on its curvature-membrane sensitivity

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α-synulcein (AS) is a presynaptic protein extremely abundant in dopaminergic neurons where it participates in synaptic transmission acting as a critical regulator of vesicle dynamics. The abnormal amyloid aggregation of AS is related to Parkinson's disease, a neurodegenerative movement disorder associated with axon degeneration of dopaminergic nigral neurons. Prefibrillar soluble oligomers are pointed as neurotoxic species, since they might damage synapses and dendrites by both altering the physiological function of AS and acting as active pathogenic species. In this scenario, AS-membrane interactions play a key role in modulating AS physiopathology. The protein has a greater affinity for highly curved vesicles, such as that of synaptic vesicles. Therefore, we aimed at determining the loss-of-function that might be associated to the conversion of AS from its monomeric functional state to its pathological oligomeric form by evaluating the impact of AS oligomerization on its membrane-curvature sensitivity. We used Fluorescence Correlation Spectroscopy to obtain quantitative information on the interaction between monomeric and oligomeric AS and vesicles varying in sizes. Astonishingly, oligomeric AS also exhibits a higher affinity for small unilamellar vesicles than for large unilamellar vesicles. Our findings provide further evidence for the gain-of-function toxicity attributed to amyloid oligomeric species in Parkinson's disease and other synucleinopathies.

MTU04-08

The subcellular localization of human tyrosine hydroxylase isoforms

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Tyrosine hydroxylase (TH) is the rate-limiting enzyme in the biosynthesis of the catecholamines. Humans are unique in that they have four different isoforms. We hypothesised that they may show differential subcellular localisation. To examine this the neuroblastoma SH-SY5Y cell line was transfected with the human TH isoform 1 (hTH1) or isoform 4 (hTH4). Subcellular distribution was determined under basal and muscarine stimulated conditions. In basal conditions TH was found primarily in the cytosol (hTH1 81 ± 1.3% (mean±SEM) and hTH4 78 ± 1.5%) and in the membrane-associated fraction (hTH1 19 ± 1.2% and hTH4 21 ± 1.7%), with low levels in the nuclear fraction (hTH1 1 ± 0.3% and hTH4 0.7 ± 0.2%). There was no significant difference in the distribution of the two isoforms in these fractions with respect to total TH protein or for pSer19 TH. In contrast, in the membrane-associated fraction the level of pSer40 hTH4 (35 ± 0.8%) was around two fold higher than pSer40 hTH1 (19 ± 0.7%) ($p < 0.001$). After muscarine stimulation, the level of total hTH4 protein in the cytosolic fraction (68 ± 1%) was significantly decreased compared to hTH1 (81 ± 1.5%) and the level of hTH4 in the nuclear fraction was 10 ± 0.5%, whereas hTH1 was not detectable. The level of pSer19 hTH4 (7 ± 0.8%) was significantly lower than pSer19 hTH1 (21 ± 1.2%) ($p < 0.01$) in the membrane fraction after muscarine stimulation, whereas there were no differences in the distribution of the two isoforms in relation to pSer40 TH. This provides the first evidence of differential distribution of TH isoforms in subcellular fractions and that cellular stimuli can alter the subcellular distribution of TH.

MTU04-09

CSF biomarkers for Parkinson's disease: loss of glucocerebrosidase activity and alterations in modified forms of alpha-synuclein

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Biofluid markers for the diagnosis, disease activity, and progression of Parkinson's disease (PD) are much in demand. Promising

candidates are total or modified forms of alpha-synuclein and lysosomal enzymes, including β-glucocerebrosidase (GCase, EC = 3.2.1.45) activity, which can be detected in cerebrospinal fluid (CSF). A bidirectional relationship has been proposed for lysosomal dysfunction and aggregation of alpha-synuclein in PD (Parnetti et al., 2014).

CSF was collected from 22 PD patients and 15 controls. Clinical rating of disease severity (UPDRS) and dyskinesia (UDysRS) was performed for each PD patient. GCase activity was assesed by a fluorimetric assay using 25 μL of CSF and 50 μL of a reaction-buffer containing the fluorigenic substrate 4-Methylumbelliferyl bet-D-glucopyranoside. Samples were incubated at 37°C for 24 h and the the fluorescent product 4-Methylumbelliferone was measured on a plate reader. Total alpha-synuclein was assessed by ELISA as described (Heegaard et al., 2014). Oligomeric and phosphorylated species of alpha-synuclein were assessed by multiplex Western Blot analyses (Brudek et al., 2016).

In this PD cohort, we found significantly reduced GCase activity in CSF (22% reduced, $p < 0.01$), but no alterations in total alpha-synuclein levels. Western Blot analysis of PD and control CSF samples revealed significantly reduced monomeric, ser-87-phosphorylated and nitrated (Tyr125, Tyr133) alpha-synuclein in PD, but no changes in oligomeric or ser-129-phosphorylated alpha-synuclein levels.

In conclusion, reduced GCase activity and specific alpha-synuclein species in CSF may serve as biomarkers for PD.

MTU04-10

Interplay between α-synuclein and lipids in Parkinson's disease

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Abnormal α-synuclein (α-syn) aggregation in Lewy bodies is the pathological hallmark of Parkinson's disease (PD) and other synucleinopathies such as infantile neuroaxonal dystrophy (INAD), and idiopathic neurodegeneration associated with brain iron accumulation (NBIA)[1]. Despite its high propensity to aggregate under pathological conditions and when isolated *in vitro*, native α-syn is a highly abundant soluble neuronal protein in the CNS (~1% of the total proteins) and resists aggregation in normal intracellular environments[2]. However, little is known how α-syn maintains its native structure. Here we systemically investigated lipid-binding partners of α-syn using untargeted global lipidomic profiling. We found that different α-syn species (e.g. monomer, oligomer and fibril) have distinct binding preferences to lipid molecules. We identified a class of lipid molecules which specifically bind with the N-terminal of α-syn monomer, induce a compact α-helical conformation and stabilize α-syn monomer from aggregation. Importantly, this lipid mediates physiological function of α-syn in synaptic vesicle trafficking. PD familial A30P α-syn mutant shows reduced binding affinity with the lipids. Furthermore, decreased production of this class of lipids dramatically promotes α-syn aggregation in cells. Our study suggests that dysfunctions in the lipid homeostasis might be critical in the development of Lewy body diseases.

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MTU04-11

HSPA8 expression in nigral and ventral tegmental area dopaminergic neurons of control and Parkinsonian brain

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While dopaminergic neurons of the substantia nigra (SN) region of the midbrain is lost in Parkinson's disease (PD), the adjoining dopaminergic neurons of the ventral tegmental area (VTA) are relatively spared. 2-Dimensional gel electrophoresis, followed with MALDI-TOF-TOF analysis identified several proteins differentially expressed between these two regions of normal mice and one such protein, heat shock protein A8 (HSPA8), is characterised in the present study. A member of the HSC70 class protein, HSPA8 was found to have higher expression at both protein and transcript level in SN compared to VTA and this discrepancy in the protein expression was found to be specific to tyrosine hydroxylase positive dopaminergic neurons of these two regions. The parkinsonian neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mice, sacrificed after 3 days and 7 days following the last dose of the neurotoxin, were used in the study. MPTP treatment caused more than 60% loss of dopaminergic neurons in SN after 7 days, but at this juncture the loss of neurons in VTA was insignificant. Dopaminergic neuronal death in SN was concomitant with gradual decrease in HSPA8 level, whereas its expression was almost 2-fold up-regulated in VTA dopaminergic neurons following 3 days of MPTP treatment. Investigation on human post-mortem brains recapitulates similar results, where HSPA8 is significantly depleted in SN but its expression was almost 1.5-fold enhanced in VTA of PD brains. HSPA8 expression was unaltered in PD cybrids made from platelets of parkinsonian patients compared to the control cybrids. The finding signifies that HSPA8 up-regulation might be one of the strategies adopted by VTA dopaminergic neurons to combat the cellular stress associated with PD, whereas SN dopaminergic neurons become more vulnerable with the loss of this protein.

MTU04-12

Iron regulatory protein 1 (IRP1) is a required mediator in cell death induced by mitochondrial complex i inhibition

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Mitochondrial dysfunction and oxidative damage, often accompanied by elevated intracellular iron levels, plays an important role in the development of a number of neurodegenerative pathologies that include Parkinson's disease. The capacity of redox-active iron in generating free radicals underlies the "metal-based neurodegeneration hypothesis" in which ROS generated by redox-active metals (Fe, Cu) cause peroxidation of membrane phospholipids which leads to the formation of reactive aldehydes that react with proteins, producing misfolded aggregates that overwhelm the ubiquitin/proteasome protein degradation system. Here we have evaluated the role of Iron Regulatory Protein 1 (IRP1) in the death of SH-SY5Y dopaminergic neuroblastoma cells subjected to mitochondria complex I inhibition. We found that complex I inhibition was associated

with increased levels of transferrin receptor 1 (TfR1) and iron uptake transporter divalent metal transporter 1 (DMT1), and decreased levels of iron efflux transporter Ferroportin 1 (FPN1), together with increased 55Fe uptake activity and an increased cytoplasmic labile iron pool. Complex I inhibition also resulted in increased oxidative modifications and increased cysteine oxidation that were inhibited by the iron chelators desferoxamine, M30 and Q1. Silencing of IRP1 abolished the rotenone-induced increase in 55Fe uptake activity and it protected cells from death induced by complex I inhibition. IRP1 knockdown cells also presented an increased resistance to cysteine oxidation and decreased oxidative modifications. These results support the concept that IRP1 is an oxidative stress biosensor that when deregulated by mitochondrial dysfunction mediates iron accumulation and cell death.

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MTU04-13

Resveratrol restores CYP2D6 enzyme activity and upregulates NRF2-keap1 pathway in maneb- and paraquat- treated SH-SY5Y cell line

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Combined exposure to maneb and paraquat is known to induce Parkinsonism by the inhibition of complex III and I respectively, thereby increasing oxidative stress. Cytochrome P 450 2D6 (CYP2D6), a highly polymorphic enzyme, is involved in the metabolism of Parkinsonian toxins other than being involved in the metabolism of dopamine. Reduced CYP2D6 enzyme activity has been shown to increase Parkinson's disease (PD) risk. However, the role of pesticides exposure on CYP2D6 activity has not been observed. The present study investigated the role of combined exposure to maneb (2 μM) and paraquat (100 μM) on CYP2D6 activity in differentiated SH-SY5Y cells. Combined exposure to MB and PQ for 48 h in cells lead to a significant reduction in tyrosine hydroxylase (TH) expression- a hallmark of PD and CYP2D6 activity, while pretreatment of resveratrol (10 μM) significantly restored TH expression and CYP2D6 activity. Nuclear expression of Nrf2 and its downstream mediators were upregulated in resveratrol and MB and PQ treated cells. Resveratrol pretreatment in MB- and PQ- treated cells further increased the expression of Nrf2 and its downstream mediators. Similar experiments were also performed in presence of Quinidine, a CYP2D6 inhibitor. Quinidine (1 μM) lead to a significant reduction in CYP2D6 activity while no change was observed in expression of TH. However, in presence of combined MB and PQ, reduction in TH expression along with nuclear Nrf2 expression and its downstream mediators was much more pronounced as compared to either or alone. Pretreatment of resveratrol significantly restored Nrf2, its downstream mediators and level of CYP2D6 activity. The results thus indicate that resveratrol protects against maneb- and paraquat- induced Parkinsonism through restoration of CYP2D6 activity and upregulation of Nrf2-Keap1 pathway in SH-SY5Y cell line.

MTU04-14

Histone deacetylase inhibitor, SAHA, ameliorated the toxic effects of high fat diet induced insulin resistance in Hemiparkinson's

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Objectives: Insulin resistance has been reported as a possible risk factor for Parkinson's disease (PD). Preclinical and clinical studies have suggested reduced insulin receptor expression and insulin resistance in PD brains. Recently, insulin resistance was found to be present in 62% of PD patients with dementia. In our previous study we found reduction in histone H3 acetylation could be a possible risk factor for insulin resistance induced PD pathology. Therefore, the present study was designed to explore the therapeutic potential of histone deacetylases inhibitor, Suberoylanilide hydroxamic acid (SAHA), in insulin resistance induced PD pathology.

Methods: High fat diet (HFD) feeding was used for induction of insulin resistance in animals. Male Wistar rats were subjected to a normal pellet diet or HFD for 8 weeks before they were infused with low dose of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle. The animals were then divided different groups. The treatment group received SAHA (25 and 50 mg/kg i.p.)/day for 14 days. Battery of behavioral parameters was performed to check the locomotor and gait abnormalities in rats. To delineate the molecular mechanisms, we study the changes in histone acetylation in striatum region.

Results: The animals subjected to HFD feeding followed by 6-OHDA infusion showed impaired locomotion and gait abnormalities. These rats also showed significant elevation in oxidative stress markers and neuronal damage along with significant reduction in histone H3 acetylation in striatal region. In contrast, rats treated with SAHA showed significant amelioration of locomotor and gait abnormalities along with reduced oxidative stress and neuronal damage. Treatment with HDAC inhibitor, SAHA results in significant elevation of histone H3 acetylation.

Conclusions: This is the first study exploring the role of histone acetylation/deacetylation in insulin resistance induced PD pathology. This study suggests the therapeutic potential of HDAC inhibitor, SAHA in ameliorating insulin resistance induced PD pathology. Future studies in this direction might possibly confirms the role of histone acetylation in PD associated with insulin resistance.

MTU04-15

Effects of ASIC1a on regulating α-synuclein degradation via autophagic pathway in the pathogenesis of Parkinson's disease

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Acid-sensing ion channels (ASICs) are ligand-gated cation channels that respond to acidic stimuli, and ASICs are always activated when tissue acidosis occurs. In our previous study, we found that ASICs and ASIC1a inhibitors could protect cells against injury by autophagic clearance in vitro of Parkinson's disease (PD) cell model. However, the role and underlying mechanisms of ASIC1a in PD have not been fully elucidated. Hence, this study is to examine the potential role of ASIC1a in reducing α-synuclein

aggregation via autophagic clearance and its underlying mechanisms. In this study we constructed MPTP-induced PD mice model, and used gene knockout animal, immunofluorescence, Western Blot and transmission electron microscopy. We found that the expression of autophagy maker LC3-II was upregulated in ASIC1a knockout mice, and p62 and intracellar α-synuclein level was reduced, protecting cells against injury in vivo. Primary neurons were extracted and cultured from WT and knockout mice then treated by MPP^+. The results showed that ASIC1a knockout or ASIC1a blockers PcTx1, increased LC3-II then reduced α-synuclein level, exerted neuroprotective effects. In summary, these findings demonstrated that inhibition of ASICs reduced α-synuclein aggregation by enhancing its autophagic degradation and thus neurons. These findings will make contributions to disclose the possible pathogenic factor in PD and also provide novel theoretical and experimental evidence in searching for promising targets for PD therapy.

MTU04-16

Novel superoxide dismutase-1 proteinopathy is associated with Lewy pathology and neuronal loss in Parkinson's disease

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Neuronal loss in numerous neurodegenerative disorders has been linked to protein aggregation and oxidative stress. Using immunohistochemistry, we describe superoxide dismutase-1 (SOD1) proteinopathy, distinct from synucleinopathy, in the Parkinson's disease brain. Significant expression of this pathology matched the regional patterns of neuronal loss and increased Lewy neurite pathology. This is of interest given that SOD1 aggregation is suggested to be seeded by fibrillar α-synuclein. Our data highlights an association between SOD1 proteinopathy and neuronal loss in Parkinson's disease and suggests SOD1 and α-synuclein deposition may be linked in Parkinson's disease. The similarity of these novel aggregates to neurotoxic SOD1 deposits in familial amyotrophic lateral sclerosis (ALS) suggests common mechanisms leading to toxic SOD1 aggregation in these disorders. We demonstrated that SOD1 in the Parkinson's disease brain exhibits evidence of misfolding and metal deficiency, a known and potentially tractable pathway for aggregation of this protein in ALS. An understanding of the mechanisms leading to the deposition of SOD1 proteinopathy in Parkinson's disease may reveal new targets for neuroprotective therapies.

MTU04-17

Molecular mechanisms of neuroplasticity and decompensation in the nigrostriatal system at modeling Parkinson's disease

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Parkinson's disease (PD) is characterized by the appearance of motor symptoms many years after the onset of neurodegeneration, which explains low efficiency of therapy. Therefore, one of the priorities in neurology is to develop an early diagnosis and preventive treatment of PD, based on knowledge of molecular mechanisms of neuroplasticity and decompensation in the nigrostriatal system. However, due to inability to diagnose PD at preclinical stage, research must be performed in models by comparing the nigrostriatal system in the models of asymptomatic and early symptomatic stages of PD. We showed that despite the progressive loss of neurons in substantia nigra at both studied stage, almost no change were observed in the main functional characteristics, including dopamine (DA) uptake and release, DAT and VMAT2 expression and activity of MAO-A and MAO-B. In the striatum of presymptomatic mice, some parameters (DA release and uptake, MAO-A activity) remained compensatory unchanged or decreased (MAO-B gene expression and activity), while others - a reduction in DA levels in tissue and extracellular space and in VMAT2 and DAT expression, manifest functional failure. In symptomatic mice, only a few parameters (spontaneous DA release and uptake, MAO-B gene expression and activity), remained at the same level as presymptomatic stage, while most parameters (DA level in tissue and extracellular space, DA stimulated release, VMAT2 and DAT content), decreased, showing decompensation, which was enhanced by increasing MAO-A activity. Moreover, we first proved that striatum of mice comprises the monoenzymatic TH- and AADC-neurons, which synthesize DA in cooperation. Proportion of cooperative synthesis in total DA production increases as degradation of dopaminergic system proceeds. These data show that cooperative synthesis of DA is an up-regulated compensatory reaction, which is among principal mechanisms of neuroplasticity in Parkinsonism. Thus, we provide a comprehensive assessment of molecular mechanisms of neuroplasticity and decompensation in MPTP models of preclinical and clinical stages of PD being a powerful tool for translational medicine.

MTU04-18

Establishing a three-dimensional human neural cell culture model of Parkinson's disease

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Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease, affecting ~1.5% of the global population over the age of 65, with increasing prevalence with advancing age. Extensive loss of dopaminergic neurons and aggregation of the protein α-synuclein (α-syn) into large insoluble multimeric structures of Lewy bodies (LBs) represents the major neuropathology hallmarks of the disease. The influence of LB

formation in the development of PD is unknown and very little is understood regarding the mechanism of LB formation and effect of LBs on neurons that bear them. A large body of evidence from *in vitro* and *in vivo* studies have suggests a 'prion-like' hypothesis of progression in PD, implicating α-syn as a 'prion-like' protein, able to induce α-syn aggregation and cell-to-cell propagation. However, both these mechanisms remain poorly understood, mainly due to the lack of animal and cellular models that recapitulate specific neuropathy and/or behavioural features known to occur in PD. Rodent models exhibit formation of α-syn inclusions that present as diffuse aggregates present throughout the cells but do not fully recapitulate LB pathology. Here, we demonstrate addition of exogenous α-syn species are able to induce LB-pathology in a differentiated human neuroblastoma (SH-SY5Y) three-dimensional (3D) culture system as determined by immuno-positive α-syn and ubiquitin inclusions. Importantly, 3D-differentiated SH-SY5Y cells express markers of dopaminergic neurons (*DRD2,* dopamine receptor 2; *DAT*, dopamine transporter) and endogenous levels of α-syn, demonstrating a 'prion-like' seeding mechanism of LB formation that does not require the overexpression of α-syn as in previous models.

MTU04-19

The potential mechanism of salsolinol synthase induces apoptosis in PC12 cells

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Background and aims: Salsolinol (Sal) was the precursor of N-methyl-Salsolinol (NM-Sal), which can be metabolized to cytotoxic MPP^+-like neurotoxin and finally leads to the characteristic symptoms of Parkinson's disease (PD). Sal synthase is a novel enzyme which catalyzes the reaction of dopamine and acetaldehyde to produce Sal. This enzyme was the key protein in the formation of endogenous neurotoxins. Our previous work had confirmed the existence and characterization of Sal synthase and obtained its amino acid sequence. Nevertheless, the physiological function of Sal synthase has not been investigated thoroughly, especially its mechanism in the progress of neurons degeneration and the function in PD. In this study, we aimed to clarify the role of Sal synthase and the relationship between Sal synthase and PD pathogenesis. This investigation may provide a new target for the diagnosis and treatment of PD.

Method: The cDNA sequence of Sal synthase was inquired by NCBI Blast database. Then the recombinant plasmid pEGFP N2-Sal and pcDNA3.1+-Sal were obtained and transiently transfected PC12 cells, and the empty plasmid was as control group. After transfection for 48 h, cells were dually stained with Annexin V/PI to determine the population of cells in apoptosis stages and loaded JC-I probe to detect the change of the mitochondrial membrane potential. Confocal microscopy was used to detect the location of Sal synthase. Western blot was used to analyze the level of associated proteins. The content of Sal and NM-Sal was detected by HPLC-MS/MS.

Results and conclusion: The location analysis of Sal synthase showed that it was one kind of cytoplasmic protein. Compared with control, the overexpression of Sal synthase in PC12 cells could elevate the level of neurotoxins (Sal, NM-Sal) and pro-apoptotic protein (Bad), and furthermore increase mitochondrial membrane potential and promote apoptosis ($p < 0.05$, $p < 0.001$). The potential mechanism was proposed that Sal synthase could enhance the accumulation of neurotoxicity, then lead to mitochondrial dysfunction and finally induce the apoptosis of dopaminergic neurons, which would cause PD.

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MTU05 Neurological Dysfunction

MTU05-01

Short-term docetaxel-induced cognitive impairment in rats with tumour

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Purpose: Docetaxel (DTX) is an anti-cancer drug which is used widely to treat different types of cancer, but it may cause several unwanted adverse effects. One of the most debilitating side effects is cognitive impairment. The purpose of this study is to examine the short-term cognitive impairment of DTX on rats with tumour by using Morris Water Maze (MWM).

Methods: Female Sprague Dawley rats were injected with LA7 cells into the mammary gland pad to produce the tumour. The rats were then divided into 3 groups; normal control without tumour (NC, $n = 11$), rats with tumour treated with DTX (single dose 5 mg/kg i.p.) (DTX, $n = 11$) and cancer control without DTX (CC, $n = 11$). A 2 day MWM protocol was used to assess the cognitive impairment. Hippocampus (responsible for short-memory) was examined for pro-inflammatory cytokine interleukin IL-1β, and oxidative stress markers thiobarbituric acid-reactive substances (TBAR) and reactive oxygen species (ROS).

Results: Only 24 h after DTX was injected, the escape latency to find the platform was increased significantly to 58.1 s compared to 8.6 s in NC and 24.7 s in CC, also the latency difference (first hidden platform - last visible platform) was increased significantly due to DTX to 59.7 s compared to 5.5 s in NC and 31.0 s in CC. Interleukin IL-1β concentration was increased significantly in DTX to 171.56 pg/mL compared to 23.30 pg/mL in NC and 11.67 pg/mL in CC. TBARS level was increased significantly in DTX to 2.7 folds compared to NC, while CC increased it to 1.56 folds, also ROS level was increased significantly in DTX to 2.45 folds while CC raise it to 1.40 folds compared to NC.

Conclusion: DTX significantly induce short-term cognitive impairment in rats with tumour.

MTU05-02

Inhibiting soluble TNF-α signaling mitigates autonomic dysreflexia after complete high thoracic spinal cord injury

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Two leading causes of mortality and morbidity in patients with SCI is cardiovascular disease and increased susceptibility to infection. A major cause for these is SCI-induced autonomic dysfunction that results in autonomic dysreflexia (AD), a serious and potentially life-threatening syndrome that develops in people with SCI above thoracic spinal level 6 (T6). AD is characterized by episodes of extreme, sudden bouts of hypertension often accompanied by bradycardia that are triggered by a noxious stimulus below the level of injury, such as expansion of the bladder or constipation. We hypothesized that inflammation mediated by the soluble form of the pro-inflammatory cytokine sTNF-α in tissue below the injury plays a key role in plasticity associated with the development of AD. To test this, we completely transected the spinal cord in adult rats at T3, an injury model that reliably results in AD. Some animals continuously received XPro1595, a biologic that inhibits soluble TNF-α signaling, intrathecally while others received saline. All rats had radiotelemeters implanted into the descending aorta to measure blood pressure (BP) and heart rate (HR). At 2, 3 and 4 weeks post-injury, we continuously recorded hemodynamics over a 24-hour period. Preliminary data suggest that, compared to saline-treated animals ($N = 9$), the XPro1595-treated animals ($N = 10$) had significantly fewer spontaneously elicited AD events and a less dramatic increase in BP per event. Compared to saline-administered animals, XPro1595-treated animals exhibited smaller spikes in BP during CRD and shorter times to return to basal BP after CRD, implying diminished AD severity. We also found XPro1595 treatment significantly diminished SCI-induced vessel hyper-responsiveness to the vasopressor phenylephrine. Lastly, we found that XPro1595-treated animals have significantly less microglial reactivity and sprouting of nociceptive primary afferents in lumbar cord than saline animals, providing some mechanistic insight. Collectively, these data indicate that sTNF-α plays a critical role in the development of AD after SCI.

MTU05-03

Changes in behavioral and neurochemical aspects induced by manganese (ii) chloride exposure in larvae and adult zebrafish

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Manganese (Mn) is an essential metal for organisms, but high levels can cause serious neurological damage. The aim of this study was to evaluate the effects of $MnCl_2$ exposure on cognition and exploratory behavior in adult and larval zebrafish and correlate these findings with brain accumulation of Mn, overall brain tyrosine hydroxylase (TH) levels, dopamine (DA) levels, 3,4-dihydroxyphenylacetic acid (DOPAC) levels and cell death markers in the nervous system. Adults exposed to $MnCl_2$ for 4 days (0.5, 1.0 and 1.5 mM) and larvae exposed for 5 days (0.1, 0.25 and 0.5 mM) displayed decreased exploratory behaviors, such as distance traveled and absolute body turn angle, in addition to reduced movement time and an increased number of immobile episodes in larvae. Adults exposed to $MnCl_2$ for 4 days showed impaired aversive long-term memory in the inhibitory avoidance task. The overall brain TH levels were elevated in adults and larvae evaluated at 5 and 7 days post-fertilization (dpf). Interestingly, the protein level of this enzyme was decreased in larval animals at 10 dpf. Furthermore, DOPAC levels were increased in adult animals exposed to $MnCl_2$. Protein analysis showed increased apoptotic markers (p53, caspase-8, Bax-α) in both larvae and adult nervous system. The results demonstrated that prolonged exposure to $MnCl_2$ leads to locomotor deficits that may be associated with damage caused by this metal in the CNS, particularly in the dopaminergic system.

MTU05-04

Mitochondria pathway mediated neuroprotection by melatonin in valproic acid induced toxicity

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Branched chain fatty acids (BCFAs) are saturated long chain molecules with methyl groups and major lipid constituents. The influence of BCFAs accumulation on different brain cell types in the pathogenesis of neurodegeneration is an unresolved issue. Valproic acid (VPA) is a BCFA and its utility as an anticonvulsant has been supported by clinicians which was subsequently challenged due to its side-effects and induced neurotoxicity. The objective of this study was to understand the cellular mechanisms of oxidative stress mediated neuronal cell death induced by VPA and neuroprotective role of exogenous melatonin (Mel) on VPA induced cell death by using cerebral cortex and cerebellum regions of rat brain as an *in vivo* model. In addition, pre-administration of Mel (10 mg/kg body weight, *i.p.*) with VPA (200 mg/kg body weight, *i.p.*) treatment for 15 days rescued behavioral performance of rats altered by VPA administration, mitigated the level of dopamine neurotransmitter and inhibited oxidative stress by restoring some biomarkers such as acetylcholinesterase, Na^+, K^+-ATPase and monoamine oxidase, lipid peroxidation, protein carbonylation and reduced glutathione in cerebral cortex and cerebellum regions of rat brain induced by the treatment of VPA. In contrast, Mel effectively exerted an anti-apoptotic and anti-inflammatory action by regulating Bax, Bcl-2, caspase-3, Poly (ADP Ribose) polymerase -1 and nuclear factor-kappa B in cerebral cortex and cerebellum. The results of the present investigation emphasize novel insights of Mel as a supplement for the prevention and treatment of neuronal dysfunction induced by VPA.

MTU05-05

Direct modulatory effects of antiepileptic drugs on glycine receptors

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Despite the commonly held notion that glycinergic neurotransmission only occurs in lower areas of the central nervous system (CNS), mounting evidence also supports a functional role for glycine receptors (GlyR) in higher regions of the CNS. This is particularly true in the hippocampus, where electrophysiological studies have established the presence of extrasynaptic GlyR which contribute to the regulation of neuronal excitability. With consideration given to the importance hippocampal networks play in the generation of seizures, the inhibitory tone provided by GlyR has been proposed to play a homeostatic, antiepileptic role. In this study, we explored the possibility that conventional antiepileptic drugs partially exert their therapeutic effects via modulation of GlyR. Two electrode voltage clamp electrophysiology of $\alpha_{1\text{-}3}$ GlyR expressed in *Xenopus laevis* oocytes was employed in screening 24 antiepileptic drugs. The agents zonisamide, stiripentol and ganaxolone all potentiated EC_5 glycine responses within their therapeutic range, warranting full characterisation on $\alpha_{1\text{-}3}$ and $\alpha_1\beta$ GlyR. The results from this study suggests that the targeting of cerebral GlyR may represent a new strategy in the treatment of epilepsy.

MTU05-06

Individual and dual combined effects of flavonoid compounds on the inhibition of the P-glycoprotein drug efflux transporter

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The recognition that P-glycoprotein (P-gp)–mediated multidrug resistance is clinically important in several central nervous system disorders has promoted concerted efforts to search for therapeutically useful P-gp inhibitors, in order to overcome the influence of this functional barrier and increase drug availability into the brain. In the present work, the aim was to identify P-gp inhibitors among several flavonoid compounds and to investigate the inhibitory potential of dual combinations of the most promising flavonoids. Rhodamine 123 (a P-gp fluorescent probe substrate) intracellular accumulation assays were performed using the Madin-Darby canine kidney cell line expressing the human multidrug resistance-1 (*MDR1*) gene encoding P-gp (MDCK-MDR1), obtained from The Netherlands Cancer Institute (NKI-AVL; Amsterdam, Netherlands). Individual flavonoids were studied at 50, 100 and 200 μM. Overall, the results showed that baicalein, (-)-epigallocatechin gallate, kaempferol, quercetin and silymarin, at 100 and 200 μM, produced a significant increase (up to 18-fold) in the intracellular accumulation of rhodamine 123 in MDCK-MDR1 cells ($p < 0.05$), potentially through inhibiting the P-gp-mediated activity. Additionally, most of the dual flavonoid combinations increased the rhodamine 123 intracellular uptake in a greater extent than the individual flavonoids at similar concentrations. These results suggest the interest of some flavonoids [baicalein, (-)-epigallocatechin gallate, kaempferol, quercetin and silymarin], and particularly, of their dual combinations on the inhibition of the P-gp activity. Thus, baicalein, (-)-epigallocatechin gallate, kaempferol, quercetin and silymarin may be promising agents in circumventing the P-gp-mediated pharmacoresistance recognised as a major problem in several diseases of the central nervous system.

MTU05-07

Comorbid pain and depression is mediated by upregulated metabotropic glutamate receptor 5 in the prelimbic cortex

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Patients with chronic pain easily accompany the negative mood symptoms such as depression and anxiety, and these affective and emotional disturbances in return reinforce the aversive perception. However, the underlying mechanisms are largely unknown. Here we propose that the alteration of metabotropic glutamate receptor 5 (mGluR5) in the brain underlies such an aberrant amplification of tonic-aversive states. We assessed the mGluR5 level in the brain of the chronic neuropathic pain model rats and control rats using positron emission tomography (PET) technique with an mGluR5-selective radiotracer [11C] ABP688 and sought to identify the brain regions of which the mGluR5 level is relevant to the negative symptoms. We found various pain-related and mood-related brain regions show altered mGluR5 level in chronic neuropathic pain state. Among the regions, a prominent increase of mGluR5 was shown in the prelimbic region of the medial prefrontal cortex of chronic neuropathic pain animals. A pharmacological blockade of upregulated mGluR5 in the prelimbic cortex (PrL) ameliorated the negative symptoms including tactile hypersensitivity, depressive-like behavior, and anxiety-like behavior, which relieved the subjects from the unpleasant state of chronic neuropathic pain condition. Conversely, lentiviral overexpression of the mGluR5 in the PrL of naïve rats successfully induced comorbid pain and negative moods. Our data provide deeper insight into the shared mechanism of pain perception and negative emotions, identifying a therapeutic target for the treatment of chronic pain and mood disorders.

MTU05-08

Pathophysiological mechanism of Munc18-1 mutations in early infantile epilepsies

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Objective: While Munc18-1 is essential for presynaptic vesicle fusion in developed neurons, this molecule is likely to be involved in brain development since gene abnormalities in *MUNC18-1* (*STXBP1*) cause early infantile epileptic encephalopathy with suppression-burst (Ohtahara syndrome), neonatal epileptic encephalopathy and other neurodevelopmental disorders. We analyzed physiological and pathophysiological relevance of Munc18-1 during the cortical development.

Methods: With acute knockdown and expression with the *in utero* electroporation technique, we performed *in vivo* and *in vitro* investigation, including confocal laser microscope-associated live-imaging, to clarify the role of Munc18-1 and its epilepsy-causing mutants in the mouse corticogenesis.

Results: Munc18-1-knockdown caused abnormal migration of cortical neurons during corticogenesis. The phenotype was rescued by an RNAi-resistant Munc18-1. Protein kinase C, but not Cyclin-dependent kinase 5, was likely to be implicated in the migration. Notably, Munc18-1-binding partner, Syntaxin1A but not B, rescued the knockdown phenotype. Time-lapse imaging revealed that the radial migration step was hampered in the cortical plate. Although functional synapses are not formed in the neocortex during the embryonic stage, these results suggest that Munc18-1 has a specific role in Syntaxin1A regulation, which is modulated by Protein kinase C, in the radial migration during the corticogenesis. In addition, disruption of N-Cadherin localization by hampered vesicle trafficking appeared to be involved in the migration defects.

Interpretation: Functional abnormalities of MUNC18-1 may induce aberrant cortical neuron migration leading to functional defects of the cerebral cortex, and consequently contribute to the pathophysiologies of epilepsies and other disorders with *MUNC18-1* abnormalities.

MTU05-09

Retrosplenial cortex modulates neuropathic pain via NMDA-glutamate receptors

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Retrosplenial cortex (RSC) activates descending mechanisms of pain control and plays a relevant role in the modulation of phasic and persistent nociception. However, the role of RSC in the modulation of chronic neuropathic pain is unknown. Thus, we evaluated the role of RSC in a model of neuropathic pain in rats and explored the involvement of NMDA-glutamate receptors in this process.

To induce neuropathic pain, male Wistar rats were submitted to a complete spinal nerve ligation (SNL), while serine racemase (SRR) mutant mice were submitted to chronic nerve constriction. Changes in mechanical allodynia were evaluated using the Von Frey test in 2, 7, 14 and 21 days after surgery.

Lidocaine administration in RSC increased tactile hipersensivity 2 and 7 days after SNL. On the other hand, optogenetic stimulation of the RSC decreased the tactile hypersensitivity observed in 2 and 7 days after SNL, but did not have effect after 14 and 21 days after SNL. The NMDA receptors stimulation with glutamate or the NMDA receptor co-agonist D-serine in the RSC also decreased tactile hypersensitivity observed 2 days after SNL. In contrast, the concomitant injection of glutamate and D-serine reduced tactile hipersensivity not only 2 days after SNL, but also 7 and 14 days after SNL. To further study the role of D-serine on these processes we investigated the effect of chronic constriction injury in SRR mutant mice. Chronic lidocaine inactivation of RSC increased tactile hypersensitivity in *wild type* (WT) and SRR mutant mice and this effect was more robust and prolonged in the SRR mutant group. Finally, D-serine administration in the RSC significantly reduced tactile hipersensivity in WT and SRR mutant mice.

Our findings indicates that RSC modulated the severity of neuropathic pain, and this effect is mediated by the activation of glutamate receptors, including the activation of the co-agonist site of NMDA-glutamate receptors.

MTU05-10

The progression of kaolin-induced hydrocephalus: light and electron microscopic features in rats

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Hydrocephalus is a common neurological disorder caused by an abnormal accumulation of cerebrospinal fluid (CSF) within the brain which results in injury to the surrounding brain tissue with neurological deficits. A major factor not accounted for in most studies is the progressive change over time. In this study, we examined, the changes that occur with time in neurons, glia, extracellular space in the brain parenchyma and ependymal lining of the ventricles in neonatal rats with kaolin-induced hydrocephalus.

We induced hydrocephalus in 12 three week-old Wistar rat pups by intracisternal injection of 0.05 mL of kaolin solution (250 mg/mL in sterile water) while 12 controls had sham injection. The hydrocephalic rats were divided into 3 groups consisting of 4 rats each which were sacrificed at 1, 4and 8 weeks post-induction of hydrocephalus along with their age-matched controls. Following sacrifice, half of the brain samples were stained with haematoxylin and eosin, cell counts were determined and data analysed using ANOVA at $\alpha 0.05$. The other half were processed for Transmission and Scanning Electron Microscopy (TEM and SEM) and the images analysed descriptively.

The laminar organisation of the cerebral cortex was disrupted in all hydrocephalic rats, but neuronal density was significantly increased at 8 weeks (127.80 ± 8.68 / HPF vs 85.50 ± 5.42 / HPF in controls). An initial denudation observed in the ependymal cell cilia of the ventricular wall was followed by gradual restoration of cilia size and population over time. Ultrastructural changes in the brain parenchyma including enlargement of extracellular space, disruption of intracellular architecture, neuronal degeneration and hydropic changes in cell organelles like the mitochondria were observed with increasing severity as the duration of hydrocephalus increased.

Hydrocephalus produces significant structural injury within the brain parenchyma which increases with duration and severity, but there is also evidence of partial structural recovery on the ventricular wall over time.

MTU05-11

Arsenic induces apoptosis in hippocampal neurons and cognitive impairment in rats via BMP2 dependent BDNF/PTRKB signaling pathway

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Arsenic stimulates apoptosis in the brain cells and induces cognitive deficits. However, mechanism promoting arsenic-mediated neuronal apoptosis and cognitive impairment is less investigated. Bone morphogenetic proteins (BMP) are expressed in the hippocampus, that controls cognitive performances, and we hypothesized that a deregulated BMP signaling may affect the hippocampal neuronal apoptosis and cognitive functions. We first validated an arsenic-mediated dose-dependent loss in the hippocampal neurons, through Western blotting (WB) and Nissl's staining. Increased Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-reactivity in the neuronal nuclei (NeuN) cells and an enhanced cleaved Poly ADP-ribose polymerase (c-PARP) and cleaved caspase-3, detected through Immunoflourescence (IF) and WB, verified As-induced neuronal apoptosis. Investigating the mechanism through *in vivo* and *in vitro* studies revealed that arsenic promoted Bone Morphogenetic protein-2 (BMP2) expression, and a downstream BMP Receptor2 (BMPR2) level and p-SMAD1/5 signaling in the hippocampal neurons. Interestingly, a BMP antagonist, noggin, reduced the arsenic–induced TUNEL reactivity and neuronal loss, proving participation of increased BMP2 in neuronal apoptosis. We further found that the increased BMP2 signaling suppressed Brain-Derived Neurotrophic Factor (BDNF) expression levels and BDNF/TrkB signaling in the arsenic-treated hippocampal neurons. This decreased BDNF/TrkB pathway appeared essential for neuronal apoptosis, as evident from a TrkB inhibitor (K252a)-mediated abrogation of noggin-induced protection to the hippocampal neurons. Ultimately, we verified cognitive impairments in the arsenic-treated rats through Passive avoidance test and Y-Maze test, and proved a restoration following noggin treatment. Overall present study proves that arsenic induces apoptosis in the hippocampal neurons through a BMP2/p-Smad1/5-dependent BDNF/TrkB pathway, affecting normal cognitive performances.

MTU05-12

Response of dorsal root ganglion neurons innervating intervertebral disc to TRPV1 agonist capsaicin

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Intervertebral disc (IVD) can be a major source of low back pain (LBP). Some studies reported that degenerated IVD release cytokines, beta-nerve growth factor (β-NGF), and brain-derived neurotrophic factor (BDNF) and in dorsal root ganglion (DRG) these neurotrophins induce the upregulation of transient receptor potential cation channel subfamily V member 1 (TRPV1). However, it is not clear whether TRPV1 can participate in sensory or nociceptive processing associated with IVD. The purpose of this study was to characterize the inward current and calcium influx activated by TRPV1 agonist capsaicin in DRG neurons innervating lumbar IVD.

We used male SD rats (300 ~ 350 g, South Korea) and injected DiI (3 μL; a lipophilic and fluorescent dye) into L4-5 IVD under anesthesia. Two weeks later, neural cells were extracted from T13-L4 DRG and the dissociated cells were plated onto circular glass coverslips coated with poly D-lysine. Intracellular calcium imaging and whole-cell patch clamp technique were used to check capsaicin response in DiI-labeled neurons.

Intracellular calcium imaging revealed that 37 (71%) of 52 labeled neurons responded to 1 μM capsaicin. Capsaicin-induced peak inward current density (pA/pF) of labeled neurons was measured in dose-dependent manner (responder/tested cells; n = 2/10, 0.03 μM; n = 8/12, 0.1 μM; n = 7/12, 0.3 μM; n = 8/

11, 1 μM; n = 7/11, 3 μM; n = 8/12, 10 μM), and a calculated EC50 of dose-response curve (fitted to four-parameter logistic equation) was 0.82 μM.

The present study implicate that the nociceptive information from IVDs can be multisegmentally transmitted to lumbar DRG neurons and TRPV1 in these DRGs can have a critical role in discogenic low back pain.

MTU05-13

Ubiquitination and stability of choline acetyltransferase mutants are regulated by molecular chaperones HSC/HSP70 and HSP90

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Choline acetyltransferase (ChAT) synthesizes the neurotransmitter acetylcholine required for cholinergic neurotransmission and some mutations in ChAT have been linked to congenital myasthenic syndrome (CMS), a rare neuromuscular disorder. The CMS-related ChAT mutation V18M is located within a highly-conserved proline-rich motif [residues 14-PKLPVPP-20] that shares homology with SH3-binding motifs and reduces ChAT enzyme activity and steady-state protein levels. We showed previously that mutation of this proline-rich motif enhances ChAT ubiquitination and reduces cellular protein levels of P17A/P19A and V18M-ChAT, though the mechanism is unclear. Using a proximity-dependent biotin identification (BioID) assay followed by mass spectrometry, we identified HSC/HSP70 and HSP90, members of the heat shock protein (HSP) family of molecular chaperones, as novel ChAT protein interactors; these interactions are enriched in HEK-293 cells expressing P17A/P19A-ChAT. By anti-ChAT co-immunoprecipitation (co-IP) we confirm these interactions in both HEK-293 and cholinergic SN56 cells and show that they are enhanced for both P17A/P19A and V18M-ChAT. HSC/HSP70 inhibition by 2-phenylethynesulfonamide (PES) results in accumulation of Triton X-100-insoluble wild-type (WT) ChAT and aggregation of mutant P17A/P19A, V18M, and CMS-related A513T-ChAT. Additionally, HSC/HSP70 inhibition by VER-155008 enhances ubiquitination and promotes proteasomal degradation of WT and mutant ChAT, whereas HSP90 inhibition by 17-AAG specifically promotes proteasomal degradation of mutant ChAT. Lastly, we show that ChAT interacts with the E3 ubiquitin ligase C-terminus of HSC70-interacting protein (CHIP) using both anti-ChAT co-IP and *in situ* proximity-ligation assays, though siRNA-mediated knock-down of endogenous CHIP had no effect on steady-state protein levels of WT or mutant ChAT. Collectively, these results identify a novel role for the heat shock family of molecular chaperones in the regulation of ChAT protein solubility and stability, and support further research into the regulation of ChAT ubiquitination by HSPs and the role that ubiquitination may play in relation to ChAT function during cellular stress and disease.

MTU05-14

The sigma-1 receptor binds hexanucleotide repeat expansions of C9orf72: implication in ALS and FTD

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The GGGGCC (G4C2) hexanucleotide expansions in the non-coding region of C9orf72 gene have been reported in cases of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Recent studies from large-scale genetic screen in Drosophila showed that G4C2 repeat expansion compromises the nucleocytoplasmic transport at the nuclear pore complex (NPC) that represents a newly discovered mechanism of neurodegeneration. Sigma-1 receptors (Sig-1Rs) are ligand-regulated molecular chaperones and are known to be a pluripotent modulator in living systems. Previous studies showed that a missense mutation at Sig-1R amino acid residue 102 (E102Q) was seen in familial ALS patients. However, whether Sig-1Rs are involved in G4C2 repeats-mediated ALS/FTD is unknown. Here we found that Sig-1Rs exist also at the NPC. Further, by using the biotin pull-down assay we found that biotin-labeled (G4C2)10 RNA repeats binds and interacts with Sig-1R-YFP in Neuro2A cells. The endogenous Sig-1R in rat liver microsomes was also found to bind the (G4C2)10 RNA repeats. To further dissect the interaction domains between Sig-1R and G4C2 RNA repeats in vitro, we generated recombinant glutathione S-transferase (GST)-tagged proteins including full-length and 3 truncated fragments of mouse Sig-1R. The pull-down assay revealed that full-length of recombinant Sig-1R (amino acids 1–223) physically interacts with G4C2 RNA repeats, but not GST alone. The majority of the Sig-1R-(G4C2)10 RNA interaction occurs on the N-terminal (amino acids 1–79) and C-terminal fragments (amino acids 174–223) of the recombinant Sig-1R. We propose here a novel regulatory mechanism that Sig-1R may participate in the regulation of hexanucleotide repeat expansions in C9orf72 by serving as a molecular 'sponge' to reduce the toxicity of the RNA repeats. The dysfunction of Sig-1R may thus relate to the disease state of ALS and FTD. (This work was supported by IRP/NIDA/NIH/DHHS)

MTU05-15

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

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Aim: 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 phospho- and ubiquitinated-proteins.

Methods: C57BL/6 mice were sacrificed at 3 or 21 months of age, and their coritices 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.

Results: There were 328 phospho-proteins to be identified in mice cortex, and 15 phospho-proteins were significantly changed between 3 vs 21 months mice cortex. Six proteins were increased in 21 months mice cortex. Nine proteins were decreased in 21 months mice cortex. Moreover, there were ~200 ubiquitinated-proteins to be identified in mice cortex, and 7 ubiquitinated -proteins were changed between 3 vs 21 months mice cortex.

Conclusion: These findings indicate that the changed post-transcriptional modifications may play an important role in developing neurodegenerative disorders and dementia.

MTU05-16

In vivo regulation of chondroitin sulfate gene to recovery from spinal cord injury and brain infarction

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Injured adult neurons in the mammalian central nervous system (CNS) rarely regenerate, because some of the intracellular and cell-surface environmental factors inhibit axon regrowth. Chondroitin sulfate (CS) is the most abundant and potent exogenous inhibitor of axonal regeneration. CS degradation induces some of the axonal regrowth following spinal cord injury by treatment of chondroitinase ABC (ChABC). We generated null (KO) mice of CSGalNAcT1, a key enzyme in CS biosynthesis. We show that KO mice recovered much faster and more completely from induced SCI than do wild-type mice and even ChABC treatment mice (Takeuchi et al., *Nature Commun.*). Cerebral Infarct volumes of CSGalNAcT1 KO mice were smaller than those of WT in mice subjected to cerebral artery ligation. Our results show that reduction of CS synthesis by the controlling the CSGalNAcT1-expression is a best strategy for spinal cord injury and stroke treatment. We try to establish the accurate inhibition systems of CS-expressions in vivo from the drug screening system (small molecule compound) and siRNA and antisense oligo study, to regulate of the CSGalNAcT1-expression in the injury area. We selected the many kinds of drugs to regulate the CS-expressions and siRNAs to inhibit the up-regulations of those genes. The sponge forms biomaterials impregnated with a mixture containing small molecule compounds or siRNAs was placed on the lesion area in mice suffered neural injuries. The recovery of these mice which treated with drug delivery systems reached the levels of satisfactory amelioration comparable to those of KO mice. Taken together, our results indicated that our drug and delivery system is a promising therapeutic target for treatment of the spinal cord injury and brain infarction, and many treatments of the neural damage.

MTU05-17

Protective role of PTEN in cardiovascular regulation during experimental brain stem death

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We established previously that activation of PI3K/Akt signaling, leading to upregulation of nitric oxide synthase II (NOS II)/peroxynitrite cascade in the rostral ventrolateral medulla (RVLM), the origin of a 'life-and-death' signal detected from arterial pressure that reflects failure of central cardiovascular regulation that precedes brain stem death, underpins the cardiovascular depression induced by the organophosphate pesticide mevinphos. The tumor suppressor phosphatase and tensin homolog (PTEN) is a lipid phosphatase and a major negative regulator of PI3K/Akt signaling. This study investigated the role of PTEN in cardiovascular regulation during experimental brain stem death. In adult male Sprague-Dawley rats maintained under propofol anesthesia (25 mg/kg/h, i.v.), microinjection of Mev (10 nmol) into the RVLM induced an increase (pro-life phase), followed by a decrease (pro-death phase) in the 'life-and-death' signal and elicited cardiovascular depression (pro-death phase) during experimental brain stem death. Real-time PCR or Western blot analysis showed that the mRNA or protein level of PTEN was insignificant changes in RVLM during experimental brain stem death. However, progressive augmentation in PI3K, Akt or PTEN activity and decrease in oxidized form of PTEN that paralleled the increase in NOS II or peroxynitrite level in RVLM. Pretreatment by microinjection into the bilateral RVLM of anti-PTEN antiserum 1 h before or PTEN siRNA 48 h before administration of Mev significantly diminished the augmented in 'life-and-death' signal during pro-life phase and enhanced the progressive hypotension during pro-death phase, and potentiated the increase in Akt activity, NOS II or peroxynitrite level. We conclude that PTEN in RVLM sustains cardiovascular regulatory functions during experimental brain stem death via downregulation of NOS II/peroxynitrite signaling pathway as a negative regulator of PI3K/Akt cascade. In addition, the activation of PTEN was regulated by post-translational mechanism in this process.

MTU05-18

The ERR gene expression on spinal cord after brachial plexus root avulsion

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Estrogen-Related Receptor γ (ERR γ) is a member of a small group of orphan nuclear receptor transcription factors that been implicated in diverse physiological and pathological processes. In the central nervous system of mice, the transcription factor ERR γ is highly expressed during neuronal differentiation and in mature gamma but not alpha motor neurons. Studies in mice provided evidence that transcriptional programs define functionally distinct motor neuron subpopulations, even within anatomically defined motor pools. Therefore, ERR γ is a potential therapeutic target and also a subject of further signalling inquiry due to co-localisation with other transcription factors. In our rat model of brachial plexus injury, the pattern of expression of ERR γ and its co-localisation with other transcription factors in the rat spinal cord is unknown hence this pilot study. The expression profile of ERR γ and its co-localisation with NeuN or ATF-3 in motor neurons of brachial plexus avulsed rats was assessed using Western Blotting, Immunohistochemistry and Immunofluorescence. The results of western blotting showed that the level of ERR γ protein at 3, 7 and 14 days post avulsion was significantly lower in the ipsilateral half than that in the contralateral half of spinal cord (All $p < 0.05$). ERR γ positive motor neurons were also notably lower in number in the ipsilateral side compared to that in the contralateral side at 3, 7 and 14 days post avulsion; implying they were progressively being lost.

ATF-3-positive motor neurons were the same as Fluorogold or ERR γ-positive motor neurons at day three. Almost all large (alpha) and small (gamma) ERR γ-positive motor neurons were also NeuN-positive. However, a few of these were ERR γ^{on}/NeuN$^{off.}$ These results provide proof that ERR γ is a non-specific marker of gamma motor neurons in rats; as such this specific transcriptional program cannot be used to define functionally distinct motor neuron subpopulations in rats as was the situation in mice.

MTU07 Synaptic Transmission

MTU07-01

Differential distribution of kainate receptor auxiliary subunits Neto1 and Neto2 in the developing central nervous system

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Neuropilin and tolloid like proteins (Neto1 and Neto2) were identified as auxiliary subunits of endogenous kainate receptors (KARs) in the central nervous system (CNS). They affect almost all aspects of KAR signalling, including channel gating, pharmacology and trafficking. The regional distribution and spatio-temporal correlation between pore-forming (GluK1-5) and auxiliary KAR subunits is fundamental to understand the molecular composition and functional properties of these receptors in the developing and adult CNS. Here, we show the regional expression profiles of Neto1 and Neto2 proteins at different stages of rat brain development from embryonic day E14 to postnatal day P90 using an *in situ* blotting technique. Our results established a complementary and often overlapping expression profiles of Neto1, Neto2 and pore-forming KAR subunits GluK2/3 and GluK5 in developing and mature CNS. In the hippocampus, Neto1 is mainly expressed in the stratum lucidum of the CA3 region whereas Neto2 was identified mainly in the hilus of the dentate gyrus region of the hippocampus. The immunoreactivity of Neto1 in the cerebellum was very weak and correlated to GluK5 subunit protein expression pattern. On the other hand, Neto2 was strongly expressed in the cerebellar granular cell layer in the same way as GluK2/3 KAR subunit proteins. Both Neto1 and Neto2 showed a higher expression level in the inner cortical layers than the outer layers, a finding that well matched with pore-forming KAR subunits expression profiles. Our experiments established different spatio-temporal changes for individual KAR proteins during development. For example, we have detected a gradual increase in Neto1 expression between P0 and P90 in the stratum lacunosum moleculare area of the hippocampus, while Neto2 expression increased until P14 followed by a gradual decrease until P90 in the same region, which indicates a differential change in subunit ratios. Collectively these results suggest region-specific changes in subunit compositions and functional properties of KARs throughout development. This work was supported by the Hashemite University, Jordan and BBSRC, UK (grant BB/J015938/1).

MTU07-02

A novel role for very long chain fatty acids in brain function

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Purpose: ELOngation of Very Long chain fatty acids-4 (ELOVL4) is an elongase responsible for biosynthesis of very long chain (VLC; ≥ C28) fatty acids, making VLC polyunsaturated fatty acids (VLC-PUFA) in retina and testes, and VLC saturated fatty acids (VLC-SFA) in skin and brain. Homozygous inheritance of the Stargardt (STGD3) mutation in ELOVL4 causes a CNS phenotype in humans, including seizures, intellectual disability, spastic quadriplegia, and death. We hypothesize that ELOVL4-synthesized VLC-SFA play an essential role in neural cell structure and function.
Methods: We generated a successful animal model for STGD3/STGD3 inheritance (−/−). ELOVL4 localization within the CNS was determined in wild type mice (+/+) using immunofluorescence (IF) microscopy. Hippocampal lipids were analyzed by mass spectrometry. Synaptic membrane fractionation was performed on baboon hippocampus for lipid analysis. Hippocampal slices from (+/+) and (−/−) mice were subjected to spontaneous multi-electrode array (MEA) recordings. Primary neuronal cultures from hippocampus of (+/+) and (−/−) mice were subjected to FM1-43 assessment of synaptic vesicle exocytosis rates.
Results: STGD3/STGD3 mice developed seizures at P19 followed by death at P21. Hippocampal lipidomic analysis of (+/+) mice confirmed the presence of 28:0/30:0 in sphingolipids. Membrane fractionation of baboon hippocampus revealed enrichment of 28:0/30:0, but not VLC-PUFA, in synaptic vesicle membranes. MEA recordings showed a significant increase in the amplitude and decrease in the inter-spike interval of action potentials in (−/−) vs (+/+) hippocampal slices. FM1-43 studies showed a significant increase in synaptic vesicle exocytosis rates in (−/−) vs (+/+) primary hippocampal neurons.
Conclusions: This is the first study to demonstrate that mutations in *Elovl4* cause a CNS phenotype in an animal model. These studies suggest a neuron-specific role for VLC-SFA in the regulation of pre-synaptic synaptic function by impacting the rate of synaptic vesicle release.

MTU07-03

In vivo evaluation of interaction between GTP cyclohydrolase 1 and its regulatory protein GFRP in the rat brain stem

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6R-L-erythro-5,6,7,8-tetrahydrobiopterin (BH4) is an essential co-factor required for the enzymatic activity of the nitric oxide synthases and the BH4-dependent amino acid hydroxylases: phenylalanine hydroxylase, tyrosine hydroxylase, and tryptophan hydroxylase. BH4 is therefore important for the synthesis of nitric oxide and monoamine neurotransmitters such as 5-hydroxytryptophan (serotonin), dopamine, adrenaline and noradrenaline.

The enzyme GTP cyclohydrolase 1 (GCH1) (EC: 3.5.4.16) is the first and rate-limiting enzyme in the metabolic pathway for *de novo* biosynthesis of BH4. Several factors are involved in the regulation of GCH1 activity. Among them, the GCH1 feedback regulatory protein (GFRP) is known to mediate the feedback inhibition of GCH1 activity by BH4 at least *in vitro*. *In vivo*, little is known about regulation of GCH1 activity by GFRP.

We investigated the distribution and colocalization of GCH1 and its regulatory protein GFRP on paraformaldehyde-fixed sections of rat substantia nigra and locus coeruleus using home-made antisera. Interaction between GFRP and GCH1 was examined using blue-native-PAGE, and BH4 levels were monitored using ECD-HLPC in the presence of a GCH1 inhibitor. Immunolocalization revealed heterogeneous expression of GFRP and GCH1 proteins among the brain cell groups synthesizing monoamines or nitric oxide. This preliminary biochemical investigation indicated that GFRP/GCH1 interactions seemed very scarce in these brainstem samples, implying little or no GFRP regulation of BH4 production by the monoaminergic neurons. These results suggest that, *in vivo* in the brainstem, feedback inhibition of GCH1 activity mediated by GFRP is unlikely to occur, at least in normal physiological condition.

MTU07-04

A new photochromic modulator of inhibitory CYS-loop receptor channels

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Photoswitchable molecules provide a unique tool for ion channel's functioning control. The technique is based on the ability of certain molecules (azobenzenes, spiropiranes, diarylethenes) to change their conformation upon specific wavelength illumination. We report a soluble photochromic ligand, UR-DW285, composed of a diazepam moiety and an azobenzene-photoisomerizeable group. Using the patch-clamp recording of currents mediated by ionic channels expressed in *CHO* cells we have analyzed its action on GABA receptors (GABARs) and 5 subtypes of homomeric glycine receptors (GlyRs) formed by human alpha1, 3, mouse alpha2 and zebrafish alpha1, 2 subunits. At a visible light, UR-DW285 (50 μM) inhibited GABAR-mediated currents by ~60%. UV illumination abolished the inhibition, suggesting that UR-DW285 depresses GABARs only in *trans*-state. UR-DW285 also potently modulated GlyRs, with distinct effects for different subunits. In trans-state UR-DW285 inhibited zebrafish alpha1 GlyRs-mediated currents (34 ± 2%) and UV reinforced the inhibition (47 ± 2%). At saturating glycine (> 300 μM), the effect of DW285 was negligible, suggesting a competitive-like mechanism of action. In contrast, on mammalian alpha2 and alpha3 GlyRs, UR-DW285 was not active at UV illumination, while at the visible light it caused strong inhibition (IC_{50} ~ 20 μM), which was preserved at saturating glycine, suggesting an additional, non-competitive pore-site of UR-DW285 action. This was supported by experiments with alpha1 GlyRs containing a mutation in the pore-lining TM2 helix (G254A) and by molecular modeling. The mutant behaved similarly to alpha2 GlyRs. However, in behavioral experiments performed with whole-body illumination of zebrafish, no significant effects of UR-DW285 were observed, possibly due to opposite UV effects imparted by UR-DW285 to the main inhibitory ligand-gated receptors - GABARs and alpha1 GlyRs. This discrepancy is currently under study.

MTU07-05

Adenosine A2A receptors stabilizes inhibitory synapses during development

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In the adult brain, adenosine, controls neurotransmitter release mainly through inhibitory A1 and facilitator A2A receptors. However, its role in development remains to be elucidated. Here, we addressed the role of A2AR-mediated signalling during GABAergic synaptogenesis in the hippocampus.

Using rat primary hippocampal cultures, we found an increase of A2AR expression during the period of synaptogenesis. This developmental expression of A2AR was correlated with a role of A2AR in the stabilization of nascent GABA synapses, a regulation restricted to the period of synaptogenesis. Downregulating A2AR expression with a shRNA approach in isolated postsynaptic cells led to a loss of synapses equivalent to that seen upon A2AR activity blockade, reporting the A2AR-mediated synapse stabilization is a cell autonomous process that requires A2AR activation in the postsynaptic cell.

Adenosine can be secreted by both glia and neurons; however we found that activity-dependent release of neuronal adenosine is sufficient to stabilize newly formed GABA synapses. Using live cell imaging, we showed adenosine signalling stabilizes active nascent inhibitory synapses. We then characterized the molecular mechanism downstream postsynaptic A2AR. We report the contribution of the Adenylyl cyclase/cAMP/Protein Kinase A (PKA) signalling cascade and we identified a key target of PKA in this regulation, the postsynaptic scaffolding molecule gephyrin. Finally, we showed the A2AR-mediated stabilization of the post- and pre- synapse required the trans-synaptic Slitrk3-PTPδ complex.

These data allowed us to propose that adenosine signalling acts as a sensor of active presynaptic terminals to stabilize newly formed GABAergic synapses during synaptogenesis.

MTU07-06

In vivo photomodulation of GABA and glycine receptor channels

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The effects of the photochromic compound, UR-DW290, composed of diazepam and azobenzene groups, were studied in zebrafish behaviour and function of ionic currents mediated by heterologously expressed GABARs and GlyRs using whole-cell patch-clamp recordings. Under visible light, currents mediated by GABA receptors ($\alpha1/\beta2/\gamma2$ subunits) were weakly inhibited by 50 μM UR-DW290, while under UV illumination the inhibition was entirely absent. GlyRs were more effectively and subunit-specifically inhibited by UR-DW290. On mammalian or zebrafish α2 GlyRs, at visible light, UR-DW290 provoked inhibition of currents induced by 20 μM glycine on 38 ± 7% and 32% ± 3, respectively. Upon UV illumination suppression was stronger: 73 ± 6% and 64% ± 2 for mammalian or zebrafish α2 GlyRs, respectively. Action on α1 GlyRs exhibited similar tendency, although with less efficiency. With increase of glycine concentration inhibitory power of UR-DW290 decreased, suggesting a competitive mechanism of the antagonist action. The behavioural analysis of zebrafish larvae (7 and 8 days post fertilization) showed an excitatory effect of UR-DW290. Animals treated with UR-DW290 (100 μM) experienced higher activity behaviour during the relaxation period (20 min in absence of light stimuli) in comparison to controls. Notwithstanding their permanent excitation state, under 365 nm light animals treated with UR-DW290 evoked an overreaction to light changes which was reduced to control animal levels applying blue light (455 nm). We found significant differences for different behavioural parameters between UR-DW290 treated groups and controls including total animal activity and swimming distance, startle responses and habituation time. We observed how in the presence of UR-DW290 animal responses to light stimuli can be tuned in a dose dependent way and how swimming behaviour, such as exploratory capacities, can be triggered.

MTU07-07

Impaired retrieval of synaptobrevin to synaptic vesicles causes a progressive reduction in exocytosis

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Synaptophysin is an integral synaptic vesicle (SV) protein which is responsible for facilitating the retrieval of the essential v-SNARE synaptobrevin II (sybII) back to SVs during endocytosis. Here, we use synaptophysin knockout hippocampal neurons to investigate the consequence of impaired sybII retrieval on the efficiency of exocytosis. Synaptophysin knockout (KO) hippocampal neurons were transfected with vGLUT-pHluorin and mCerulean empty vector (KO) or synaptophysin-mCerulean (rescue), and were subjected to 4 repeated trains of 300 action potentials (10 Hz), and changes in pHluorin fluorescence monitored. We find that upon repeated stimulation there is a progressive reduction in evoked exocytosis in synaptophysin KO neurons ($p < 0.05$), whilst the efficiency of exocytosis is maintained in neurons rescued with synaptophysin. This is due to a reduction in sybII retrieval back to synaptic vesicles, and importantly, can be rescued by increasing the basal load of sybII on SVs by transfecting neurons with exogenous sybII. These findings demonstrate that perturbed sybII retrieval has knock-on consequences for exocytic efficiency, and suggests that the fidelity of neurotransmission may be compromised in systems with defective sybII trafficking. This may provide the molecular basis for the cognitive impairments that are seen in both synaptophysin knockout mice, and in individuals with X-linked intellectual disability who harbour mutations in synaptophysin.

MTU07-08

Calcium channel surface dynamic influences synaptic transmission

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The localization of voltage gated calcium channels (VGCCs) within the presynaptic active zone is critical for synaptic transmission. Using single particle tracking photoactivation localization microscopy (sptPALM) we localize VGCCs within active synapses and determine their surface dynamics in the membrane. Molecular interactions between active zone proteins, the C-terminus of VGCCs and vesicles have been shown to essentially regulate synaptic transmission. Whether interactions between VGCCs and scaffold proteins guide channel localization and mobility has been tested by using $Ca_V2.1$ C-terminal splice variants. Here, alternative splicing of exon 47 results in the expression of a shorter C-terminus (Δ47) lacking a variety of described protein-protein interactions.

Both splice variants, $Ca_V2.1_{\Delta47}$ and $Ca_V2.1_{+47}$ accumulated into the presynaptic terminals and co-localize with presynaptic proteins as Bassoon, RIM and Munc13 and the vesicular protein synapsin. The shorter $Ca_V2.1_{\Delta47}$ was significantly more mobile compared to $Ca_V2.1_{+47}$ but showed similar confinement and dwell time within the synapse. Evoked presynaptic calcium signals as well as postsynaptic currents were similar in $Ca_V2.1_{+47}$ or $Ca_V2.1_{\Delta47}$ dominated synapses.

Transient light induced immobilization of the presynaptic $Ca_v2.1$ via cryptochromes lead to a recruitment of VGCCs to the synapse, alterations in the presynaptic calcium response and enhancement of vesicular release probability. Thus, despite their confinement in the active zone, VGCCs are highly mobile, changing their position in

respect to ready releasable vesicles and consequently dominating the mode of vesicular release. Mobile VGCCs primarily promote single vesicular release, whereas clustering of the channels induces multi-vesicular release. Here, we postulate that fast reorganization of the relative positioning of calcium channels towards synaptic vesicles is a dominant component of short term plasticity and hence most relevant for sensory input processing and neuronal communication in local networks.

MTU07-09

New photoswitchable neuromuscular blockers: design, synthesis, and physicochemical/biological evaluation

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Nicotinic acetylcholine receptors (nAChRs) are widely distributed in both central and peripheral nervous system (CNS and PNS, respectively) and belong to the ligand-gated ion-channel superfamily. They are composed by combinations of α, β, g, δ, and ε subunits, which define different tissue-specific nAChR subtypes. nAChRs can be broadly classified into 2 classes: neuronal nAChRs (i.e. α7 or α4β2) and skeletal muscle nAChRs (mainly composed by αγαδβ subunits). In the PNS, muscle-type nAChRs mediate synaptic transmission at the neuromuscular junction.

The alkaloid pancuronium is used in general anesthesia as muscle relaxant. In spite of being common used in the clinic, pancuronium cause a number of adverse side effects in patients, including increased heart rate, blood pressure and cardiac output, respiratory depression and apnea. The cause of these side effects remains unknown, although a recent study suggests a mechanism involving targeting of nAChR in the CNS.

Recently, we have started the design, synthesis, and biological evaluation of new selective photoswitchable ligands of muscular nAChR with lower affinity for neuronal receptors, with the objective of inducing light-controlled skeletal muscle paralysis hence reducing CNS-related adverse effects. In this work, we have obtained new compounds that combine structural requirements of pancuronium with a photoisomerizable azobenzene scaffold.

The new photoswitchable diazene-based compounds display good water solubility, can be easily isomerized between the *E*- and *Z*- conformations by irradiation at 254 and 365 nm and are potent nicotinic ligands with a clear selectivity (up to 60-fold) for the muscular nAChRs (K_i = 35–42 nM), compared to neuronal α7 nAChRs (K_i = 910–2500 nM) and α4β2 nAChRs (K_i > 10 μM). Moreover, all compounds are predicted to be not able to enter in the CNS, thus avoiding potential undesired central side-effects.

MTU07-10

Sleep deprivation (SD) impair learning and memory by altering synaptic transmission: a proteomic approach

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Sleep quality have shown a direct correlation with good health. Sleep Deprivation (SD) may increase the risk for neurological disorders like stroke and Alzheimer's disease. SD can influence cognition, memory, and sleep/wake homeostasis and can cause impairments in many physiological processes. As, the Hippocampus plays a pivot role in learning and memory, therefore the present study was undertaken to examine proteomic changes occurring in hippocampus following chronic partial SD. Male Sprague Dawley rats were exposed to 72 h simultaneous SD in a novel SD cage. After exposure hippocampi was isolated and further processed for proteome profiling through LC-MS/MS. Comparisons of the proteome profiles of hippocampus revealed that chronic SD exposure cause alteration in (≥1.5-fold) in 71 proteins; these changes in protein expression were validated by western blot or immunohistochemistry. String and IPA analyses of identified proteins suggested that SD may influence proteins which belong to a diverse variety of functional classes including cell death, proteins involved in synaptic transmission, gliotransmission oxidative stress metabolism, growth factors and proteins associated with signalling. SD decreases expression of synaptic proteins i.e. synaptophysin, PSD-95and synapsin which further validated by western blotting. Golgi staining revealed deceases in dendritic arborisation and spine density on SD exposure. Present study also reveals SD mediated impairment in BDNF/TrkB signaling which may accounts for SD induced memory impairment.

MTU07-11

Physiological roles of glutamate secreted from VGLUT3-expressing neurons

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Vesicular glutamate transporter 3 (VGLUT3) stores glutamate (Glu) in vesicles of neurons that commonly secrete other neurotransmitters, such as striatal cholinergic interneurons (CINs) and serotonergic neurons. In CINs, VGLUT3 expression allows for Glu release, but can also facilitate vesicular storage of acetylcholine (ACh). Whether Glu released by VGLUT3-expressing neurons has significant physiological functions beyond supporting ACh neurotransmission is still poorly understood. We hypothesized that direct activation of VGLUT3 neurons can modulate behaviour. To investigate this possibility, we used a VGLUT3Cre driver to chemogenetically activate VGLUT3-positive neurons in the mouse brain. Two mouse lines were generated: one in which an excitatory Designer Receptor Exclusively Activated by Designer Drugs (qM3-

DREADD) is expressed in VGLUT3-positive neurons (VGLUT3Cre-DREADD) and a second line in which we knocked out release of ACh from these neurons (VGLUT3Cre-VAChTfx/fx-DREADD) in addition to expressing DREADD. This allowed us to activate neurotransmitter secretion and start to isolate Glu released from VGLUT3-positive neurons. Upon clozapine-N-oxide (CNO) injection, we found that activation of VGLUT3Cre-DREADD neurons caused decreased exploratory activity. However, the hypoactivity was not related to motor deficits or alterations in mood and anxiety. Moreover, elimination of ACh release in VGLUT3Cre-VAChTfx/fx-DREADD mice produced the same behavioural phenotypes, indicating ACh release may not impact the hypoactive phenotype. Thus, these results suggest that activation of VGLUT3-positive neurons produces an overall suppression of movement. Future experiments will investigate the brain regions involved in this phenotype and the contributions of other neurotransmitters. Ultimately, these experiments will broaden our understanding of glutamatergic transmission, specifically clarifying if Glu secretion from VGLUT3 neurons has specific physiological functions independent of their co-transmitter.

MTU07-12

Novel pharmacological modulators of glycine receptors function

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In our studies, we are searching for new pharmacological ways of glycine receptors (GlyRs) modulation using the expression of specific subunits, mutagenesis, electrophysiology and molecular modeling. Here will be presented 2 new compounds and photoswitchable drug for GlyRs activity regulation. Using electrophysiological recordings from cells heterologically expressing receptors of known composition, we discovered that ginkgolic acid (GA) is a subunit-specific potentiator of GlyRs. In nanomolar concentration, it strongly augmented the glycine-induced currents without effect on alpha2 or alpha3 GlyRs. Mutagenesis analysis suggests that residues T59A/A261G/A303S are involved in this potentiation. We also discovered that anti-inflammatory drug, niflumic acid (NFA), blocks the ion-conducting pore of GlyRs with higher affinity to alpha2 and alpha3 subunits in comparison with alpha1. By mutagenesis analysis and molecular modeling, the bonding sites of NFA action in the pore of GlyR were determined. Recently, we have developed a first subunit-specific azobenzene-based photoswitchable modulator of GlyRs (UR-DW285). At UV illumination, UR-DW285 was not active on alpha2 and alpha3 GlyRs while at the visible light it caused strong inhibition ($IC_{50} \sim 20$ μM). In contrast, on alpha1 GlyRs, UV illumination reinforced inhibition of currents and UR-DW285 worked as the competitive antagonist, suggesting 2 different binding sites for the drug. Mutation G254A in the pore-forming TM2 domain of alpha1 GlyRs prevented the action of UR-DW285 at UV illumination, indicating one of the sites of UR-DW285 action. These observations established novel modulators of GlyRs and might be used for specific control of glycinergic activity in experimental conditions and for the development of clinically relevant modulators.

MTU07-13

Pharmacology and crystal structure of novel 2,3-quinoxalinediones at kainate receptors

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Ionotropic glutamate receptors (iGluRs) are the primary mediators of fast excitatory neurotransmission in the mammalian CNS where they are involved in learning and memory formation. iGluRs are important for normal brain function and thus disturbances in the iGluR system are associated with the pathophysiology of CNS diseases such as epilepsy, schizophrenia and depression. Selective tool compounds are therefore needed to address the functional roles of different types of iGluRs. A few selective compounds that can discriminate between AMPA and kainate (KA) receptors are available. However, within the KA receptor family (GluK1-5) only compounds with selectivity towards GluK1 exist [1]. Thus, there is an unmet need for tool compounds with selectivity towards the remaining KA receptor subunits.

Here we report the pharmacology of a series of novel N1-substituted 2,3-quinoxalinediones, as well as the crystal structure of one compound (JP-10-7A) in the GluK1 ligand binding domain (GluK1-LBD) at 1.85 Å resolution. Radioligand binding experiments indicated that most of the compounds had similar binding affinities at GluK1 and GluK3, but a few had higher affinity at GluK3 and were thus GluK3-preferring.

The GluK1 binding mode of the JP-10-7A 2,3-quinoxalinedione scaffold is similar to that of another published 2,3-quinoxalinedione ligand, (*S*)-2-amino-4-(2,3-dioxo-1,2,3,4-tetrahydroquinoxalin-6-yl)-butanoic acid (PDB-entry 4QF9), with the substituent in the N1-position pointing out of the binding pocket. Whereas agonists induce a closure of domain D2 towards D1, antagonists stabilize an open conformation of the GluK1-LBD. Domain opening of GluK1-LBD with JP-10-7A bound (compared to glutamate bound GluK1-LBD PDB-entry 2F36, molA) is ~30°, which is consistent with an antagonist binding mode. Functional electrophysiological (TEVC) experiments indeed showed these compounds to be antagonists at cloned, homomeric KA receptors. The structure and pharmacology will be valuable for design of new and more GluK3-selective quinoxalinedione analogues.

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MTU07-14

Influence of astrocytic glutamine transporter SN1 deficiency on electrophysiological correlates of glutamatergic transmission

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The N-system glutamine transporter SN1 preferentially transfers glutamine out of astrocytes (Chaudhry et al., Cell 1999), and its depletion causes accumulation of ammonia-derived glutamine in astrocytes in vitro (Zielińska et al., 2015). Since glutamine delivery to neurons is a prerequisite of active glutamatergic transmission (Billups et al., 2013), we hypothesized that SN1 deficiency will impair its electrophysiological manifestations.

We used C57Bl6 mice in which knockdown SN1 protein in prefrontal cortex was induced by *vivo-morpholino* (VM) technique. In all groups SN1 protein expression analysis, HNMR measurements and electrophysiological studies of the pertinent brain regions were conducted.

In the prefrontal cortex of mice with local knockdown we observed decreased expression of SN1 protein level by ~55%. HNMR analysis of a pertinent brain region revealed ~12% decrease in glutamate level (a sum of glutamine plus glutamate was decreased by ~15%) and a lack of changes in total glutamine level. KO-SN1 mice showed reduced amplitudes of field potentials evoked in layer V horizontal connections (V_{max} was reduced by ~50%) while amplitudes of responses evoked in vertical layer V – layer II/III connections were unchanged. The resting membrane potential of KO-SN1 pyramidal neurons from layer II/III was less negative than control neurons (unchanged in layer V). A tendency toward increase in the mean frequency of sEPSCs in pyramidal neurons originating from the layer II/III of KO-SN1 animals was observed, opposite to the changes in layer V. The causes of insensititvity of cerebral cortical layers other than layer V to SN1 knockout remains to be elucidated.

This result supports the hypothesis that SN1 transporter deficiency reduces the neurotransmitter glutamate content and glutamatergic tone in a defined layer of the cerebral cortex, and implicates glutamine retention in astrocytes as a most likely causative factor.

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MTU07-15

Caffeine exposure during the period of synaptogenesis alters hippocampal circuitry and related functions

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In the adult brain, adenosine, a degradation product of ATP, controls neurotransmitter release and synaptic plasticity through G protein coupled A1 and A2A receptors. The activity of these receptors is essential in normal behaviour, including learning and memory, sleep and arousal, locomotor activity and exploration, feeding behaviour and mood and motivation. However, the role of these receptors in development is not well known.

Our lab has recently identified a novel mechanism by which the adenosine signaling pathway acts as a detector and stabilizer of active nascent inhibitory GABAergic synapses in the hippocampus. Based on our recent findings, we propose that the deleterious consequences of *in utero* and post-natal brain exposure to caffeine (Sci Transl Med. 2013; 5(197), an antagonist of A1 and A2A receptors, are primarily due to A2A receptor-dependent alterations in synaptogenesis. We are now testing this hypothesis *in vivo* by assessing synaptic protein expression, synapse density as well as neuronal and network activity in the hippocampus. These experiments are performed in both juvenile and adult mice injected daily during the period of hippocampal synaptogenesis with caffeine or the selective A2A receptor antagonist SCH58261. We are also exploring the impact of these treatments on hippocampal-dependent memory and susceptibility to seizures. Preliminary data show altered hippocampal synaptogenesis, increased network activity, cognitive deficits and sensitivity to pharmacologically induced epilepsy in treated animals. Our findings provide a better understanding of the pathological mechanisms engaged upon early-life exposure to caffeine.

MTU07-16

Optical control of muscarinic acetylcholine receptors using photoswitchable bitopic ligands

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Muscarinic acetylcholine receptors (mAChRs) are class A GPCRs characterized by a widespread tissue distribution and involved in the control of numerous central and peripheral physiological responses. The high sequence homology of the different subtypes (M1–M5) in the transmembrane region hampers the development of subtype selective orthosteric agonists. On the other hand, the allosteric site, located in the extracellular loop, is less conserved, thus muscarinic allosteric agents are commonly endowed with a more pronounced subtype-selectivity. Recently, a new strategy was developed towards the selective modulation of mAChRs, i.e. the development of dualsteric ligands, which are molecules that can bind simultaneously to both the orthosteric and the allosteric sites of such receptors. The most interesting bitopic ligands emerging from this investigation were hybrid derivatives incorporating (i) iperoxo, an oxotremorine-related unselective orthosteric superagonist, (ii) a polymethylene spacer, and (iii) a moiety targeting the allosteric site.

Inspired by this strategy, in the course of our ongoing development of photoswitchable ligands for the optical control of (neuro) biological functions, we designed and synthesized a new set of light-regulated muscarinic bitopic ligands by replacing the polymethylene spacer chain with an azobenzene linker to serve as molecular photoswitch. This modification enabled the remote control of the

mutual position between the 2 pharmacophoric moieties with light, thus potentially modulating affinity and efficacy of our compounds as a function of their photoisomerization state. One of our ligands (P-Azo-Iper) turned out to be a potent activator of M2 receptors under UV illumination (*cis* isomer), but inactive after relaxation in the dark or under illumination with blue light or white light (*trans* isomer). All compounds were investigated in binding and enzymatic experiments. Their cellular responses were evaluated *in vitro* and *in vivo* in the Xenopus tropicalis heart.

MTU07-17

Glycine receptor subunits expressed in retina during rat development

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Considerable evidence indicates that glycine functions as inhibitory neurotransmitter in the vertebrate retina. Glycine exerts its action through the glycine receptor (GlyR), which consists of 2 α (α 1–4) and 3 β subunits. Immunohistochemical studies demonstrated the expression of the 4 α subunits in the adult retina; however, their proportion in adult and immature retina is unknown. In an attempt to know the relative quantities of these subunits and its possible significance, we studied the mRNA and protein expression of these subunits in the retina during the postnatal Long Evans rats development, by qPCR and Western Blot. Animals were handled according to the Mexican Institutes of Health Research rules. The oligonucleotide primers employed were those described by Aroeira et al 2011; for the α4 subunit we used exon 7–8 (Integrated DNA Technologies). The 18S gene was used to construct a concentration curve for GlyR subunits quantification (T4 Oligo, Mexico). For Western Blot we used commercially available antibodies for all the α subunits, and quantified them using actin as loading control. We found that mRNA expression of α1, α3, α4 and β GlyR subunits increased gradually during development; these results match with the protein expression pattern found for these subunits. The α2 GlyR subunit showed the highest expression values for both mRNA and protein, at all stages studied; being the α1 and α2 the predominant subunits in the adult retina.

We concluded that the expression of GlyR subunits correlated with retina cells differentiation, supporting also the role of GlyR in retina development. The highest proportion of the α2 subunit in the adult retina suggested the presence of monomeric and /or heteromeric α2 GlyR in the adult and immature retina, emphasizing its role in retina function.

MTU07-18

NG2 GLIA-specific gene knockout as a tool to understand the impact of neuron-GLIA synaptic signaling

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NG2 glia in grey matter receives direct synaptic input from glutamatergic and GABAergic neurons. However, the functional consequence of this input is not yet understood. During development, NG2 glia upregulates Kir4.1 channels, leading to low membrane resistance and a resting potential close to the K^+ equilibrium potential. To test if Kir currents regulate the efficiency of synaptic activation of NG2 glia, we generated NG2-CreERT2 knock-in mice where conditional knockout of the Kir4.1 gene upon tamoxifen administration is induced.

In tamoxifen-treated mice, semi-quantitative RT-PCR of FAC sorted NG2 glial cells proved a downregulation of Kir4.1 mRNA to 15% in the hippocampus and 50% in the cerebellum. NG2 glia devoid of Kir currents displayed more positive resting potentials as compared to Kir-expressing cells and a significantly increased membrane resistance. Monitoring NG2 glia responses upon Schaffer collateral stimulation revealed similar EPSC amplitudes in Kir-deficient NG2 glia compared to control cells. Interestingly, short-term plasticity of neuron-NG2 glia synapses was affected as the presynaptic transmitter release probability at neuron-recombined NG2 glia synapses was enhanced. To investigate the impact of Kir4.1 deletion in NG2 glia on neural signaling, field potentials were recorded in the hippocampus after stimulation of Schaffer collaterals. Long term potentiation, induced by theta-burst stimulation, was significantly impaired in the hippocampal CA1 region of mice with NG2 glia-targeted Kir4.1-deficiency. In the hippocampus and cerebellum, NG2 glia-targeted deletion of the Kir4.1 gene entailed an increase of MBP and MAG mRNA in recombined cells and an upregulation of MBP protein 8 weeks after tamoxifen injection. These findings show that Kir4.1 channels in NG2 glial cells regulate their excitability, influence myelination and are important for proper hippocampal synaptic plasticity.

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MTU07-19

Altered neuronal excitability in system XC- null mice in vivo uncovered by chemoconvulsant challenge

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System x_c^- (Sx_c^-), a cellular antiporter that links the import of L-cystine with the export of L-glutamate, is an important contributor to ambient extracellular glutamate levels. Changes in the concentration of extracellular glutamate are known to alter synaptic strength and neuronal excitability. Thus, whether mice null for *SLC7a11* — the gene that encodes the substrate specific light-chain (xCT) for Sx_c^- — demonstrate alterations in neuronal excitability was assessed herein using the chemoconvulsant kainic acid (KA). Male and female wild-type and *SLC7a11* null littermates were administered KA either acutely (single dose of 12–15 mg/kg) or sub-acutely (6 doses administered over 150 min [22.5 mg/kg total]) after which behavioral seizure activity as an indirect measure of excitability was scored by an investigator blinded to genotype. Following a single

injection of KA, 95% (19/20) of *SLC7a11* null mice entered a hyperexcitable state characterized by clonic seizure activity (i.e. rearing with forelimb clonus) as compared to only 24% (4/17) of wild-type mice ($p < 0.0001$). Strikingly, within 90 min of the sub-acute KA dosing paradigm, 85% of wild-type mice (23/27) entered status epilepticus whereas only 28% (7/25) of *SLC7a11* null mice did ($p < 0.0001$). Most of the *SLC7a11* null mice (84%) became hypomobile. Overall, our data demonstrate that neuronal excitability in *SLC7a11* null mice provoked by chemoconvulsant challenge deviates from that of wild-type littermates in a complex manner that differs in sign depending on the KA dosing paradigm employed.

MTU07-20

Alfaxalone alters inhibitory but not excitatory synaptic transmission or action potential firing in rat hypoglossal motor neurons

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Owing to its large safety margin, low cardiorespiratory depression and good pharmacokinetic and pharmacodynamic profile, alfaxalone is widely used for veterinary anaesthesia in large animals (horses, pigs and dogs). However, small rodents like rats and mice often show neuromotor excitation during alfaxalone anaesthesia. Prior work suggests that alfaxalone suppresses inhibitory synaptic transmission to hypoglossal (XII) motor neurons (MNs). However, the effects of alfaxalone on miniature inhibitory transmission, on spontaneous excitatory transmission, and on action potential (AP) firing have not been studied. Whole-cell patch-clamp recordings were made from XII MNs in 300 μm-thick transverse brainstem slices from 7–14 days-old Wistar rats after sodium pentobarbitone anaesthesia ($n = 46$ from 26 rats). Spontaneous and evoked excitatory postsynaptic currents (EPSCs), miniature inhibitory glycinergic transmission ($_{mini}$IPSCs) and AP firing were recorded at a holding potential of −60 mV using CsCl or K^+ methyl sulfate-based internal solutions, respectively. Our results show that alfaxalone significantly reduces $_{mini}$IPSCs frequency (63.8%) and amplitude (70.5%) to XII MNs, consistent with a reduction in inhibitory transmission to MNs, leading to neuromotor excitation. This effect on inhibitory transmission was particularly notable, as alfaxalone, even at higher concentrations (10 nM–3 μM), failed to significantly alter either spontaneous or evoked EPSC frequency, amplitude, half-width, rise-time and baseline holding current. Similarly, repetitive action potential firing by XII MNs was not altered by alfaxalone. Our results show that neuro-muscular excitation during alfaxalone anaesthesia are most likely to be mediated by decreases in inhibitory synaptic input, without alteration in spontaneous or evoked excitatory synaptic transmission, and that alfaxalone does not excite XII MNs sufficiently to cause any changes in action potential firing.

MTU07-21

Probing subunit interfaces using genetically-encoded photocrosslinkers in glutamate receptors

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NMDA receptors (NMDARs) are ionotropic receptors activated by glutamate, the major excitatory neurotransmitter in the brain. NMDARs are fundamental to brain development and function by their unique capability to induce long-term synaptic plasticity that underlies higher cognitive functions such as learning and memory. Functional NMDARs require at least two different subunits to assemble as a heterotetrameric complex typically composed of two GluN1 and two GluN2 subunits, of which there are 4 subtypes (GluN2A-D). The GluN1 and GluN2 N-terminal domains (NTDs) which are distinct from the agonist-binding domains and lay most distal to the pore region, are a major locus for allosteric regulation of NMDARs. Recent cryo-EM and high-resolution crystal structures of GluN1/GluN2B NMDARs revealed the dimeric arrangement of the NTDs and the importance of subunit interfaces in conformational rearrangements during receptor gating. However, little is known about the structural basis for conversion between inactive, active and allosterically-regulated states of the receptor. Here we combined site-specific genetic incorporation of a photo-cross-linking unnatural amino acid (Uaa), *p*-azido-L-phenylalanine (AzF), and electrophysiology to report the dynamics of NTD interfaces in intact receptors. We identified GluN1-AzF NTD mutants whose function can be robustly and specifically increased by UV stimulation. We then characterize the influence of agonists and allosteric modulators (ifenprodil, zinc, spermine) on this photosensitivity. We propose that GluN1 and GluN2A/GluN2B NTDs can dimerize through close apposition of two alpha helices from lower-lobe. The closure of the dimer interface is required for activation, because locking the interface with 2 lower-lobes closely apposed increases receptor activity, whereas allosteric inhibition stabilizes an open interface conformation. These provide important dynamic information on structural basis of gating and modulation mechanism, and will guide screening of therapertic compounds for brain diseases associated with malfunctioning of NMDARs.

MTU08 Signal Transduction

MTU08-01

Hippocampal activity after local administration of amphetamine into the medial mammillary nucleus in urethane-anesthetized rats

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Although the importance of the mammillary body for memory and learning processes is well known, its exact role has remained vague. The fact, that many neurons in one nucleus of the mammillary body in rats, i.e. the medial mammillary nucleus (MM), fire according with hippocampal theta rhythm, makes this structure crucial for a theta rhythm signaling in so-called extended hippocampal system. In the present study, we investigated the effect of pharmacological activation (local amphetamine infusion) of the MM on theta rhythm activity and immediate-early gene (*c-fos*) distribution in the hippocampus in urethane-anesthetized rats. We found that intra-MM amphetamine microinjections have mild influence on sensory-elicited theta rhythm in the hippocampus. Amphetamine infusion decreased the EEG signal power of very low theta frequency band, i.e. 3–4 Hz, down to 76% in comparison to pre-injection conditions, whereas in theta frequency bands from 4 to 9 Hz, the infusion increased the hippocampal EEG power, up to 265% in 4–5 Hz band and 174% in 8–9 Hz band. An immunohistochemical analysis has shown a lack of changes in regard to Fos distribution in the hippocampus after amphetamine infusion to the MM. These results indicate that pharmacological activation of the MM may influence electrophysiological activity of the hippocampus in urethane-anesthetized rats. This research was supported by the National Science Centre (DEC-2014/12/S/NZ3/00621).

MTU08-02

Aging in a dish - mechanical signaling in juvenile and aged neuronal cultures

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From development throughout maturation neurons interact with their environment by sensing chemical signals in order to regulate integration into the neuronal network. Recently, evidences increase that neuronal cells also respond to mechanical cues. Neuronal tissue and even different cell types are mechanically inhomogeneous and so cells are exposed to a variety of mechanical stimuli shown to influence cell properties like cell differentiation, maturation and survival.

In consequence, abnormalities in mechanical properties within a certain micro compartment of the brain can interfere with its physiological function. The brain stiffens with age and also protein aggregates like Abeta-42 or alpha-synuclein increase the elastic modulus drastically, leading to deficits in mechanotransduction.

So far, studies on neuronal responses to substrate rigidity have mainly focused on axonal outgrowth and regeneration. Further it is of outstanding interest to understand the properties of mechanotransduction during neuronal development and aging.

To examine the potential relationship between mechanical cues and neuronal development we use polyacrylamide (PAA) gels. These PAA gels allow the production of cell culture substrates with defined mechanical properties resembling substrate rigidity of the living brain: a young brain with elastic modulus of around 100 Pa, or a mature brain with an elastic modulus beyond 1000 Pa.

First results showed that dendritic arborisations increased up to 5-fold when neuronal cultures are grown on a soft PAA gel, mimicking young brain tissue, compared to a hard substrate (10 kPa). Looking deeper into synaptic development, we observed a shift in the onset of synaptic development in dependence on substrate rigidity. Functionally, we showed a clear increase in *de novo* protein synthesis for neurons cultured on soft substrates during development, maturation and aging.

These results point towards mechanic control of synaptogenesis and synaptic vesicle recycling. Further, the mechanical properties of the surrounding environment influence complex cellular processes like d protein translation control throughout neuronal lifetime.

MTU08-03

Serotonin receptor 5-HT7 mediated activation of CDK5

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Cyclin-dependent kinase 5 (Cdk5) is involved in the regulation of various aspects of brain development and function. In contrast to other members of Cdk family, which are activated by cyclin binding, enzymatic activity of Cdk5 is controlled by the activator proteins p35/p39. One of Cdk5 substrates is the microtubule-associated protein tau. Physiologically, tau phosphorylation regulates its binding affinity to microtubules. However, under pathological conditions, tau hyperphosphorylation by Cdk5 and other tau kinases results in destabilisation of the microtubule network and the formation of neurofibrillary tangles.

The serotonin receptor 5-HT7 is a G-protein coupled receptor implicated in learning and memory. On cellular level it regulates neuronal morphology, is involved in axonal and dendritic outgrowth and influences synaptogenesis and spinogenesis. Via G_s protein coupling the receptor activates adenylylcyclase leading to an increase of cellular cAMP levels resulting in multiple signalling cascades including Akt and Erk activation. Additionally, the receptor couples with G_{12} protein and activates small GTPases RhoA and Cdc42, which are important regulators of neuronal morphology.

Here we demonstrate that the 5-HT7 receptor increases tau phosphorylation via activation of Cdk5. Moreover, using co-immunoprecipitation as well as lux-FRET analysis we demonstrated direct interaction of the 5-HT7 receptor and Cdk5 at the plasma

membrane both in neuroblastoma cells and in mouse cortex. To reveal the underlying molecular mechanism of 5-HT7 receptor-mediated Cdk5 activation we use molecular modelling and site directed mutagenesis. Based on the interaction model we created several mutants to identify the interaction domain(s) and specify the interaction interface. We used these mutants to examine their impact on the direct interaction of both proteins as well as on Cdk5 activation and localization.

MTU08-04

Intrinsic control of AKT signaling in CNS axon growth and regeneration by regulation of novel substrates,

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Developmental axon growth or axon regeneration requires active molecular machinery that regulates specific transcription factors, growth cone components, and mediators of signal transduction. Akt is a crucial growth promoting enzyme which is implicated in axon elongation. However, molecular mechanism of Akt signaling in CNS axon growth remains to be determined. Here we demonstrate that Akt contributes growth cone formation and thus promotes axon growth employing Id2 and Radixin as novel substrates by specific phosphorylation in the developing neurons. Akt mediated phosphorylation of Id2 augments its protein stability and steers localization of Id2 at growth cone. Interruption of Id2 expression or phosphorylation abolished the interaction of Id2 with Radixin, which is important for the construction of normal structure and functional organization of the growth cone, and resulted in impaired growth cone formation along with reduced axonal outgrowth. Reconstitution of Akt/Id2/Radixin signaling after injury in hippocampus slice culture redeem growth promoting ability, revealing obvious axon regeneration whereas phosphor-ablated mutant of either Id2 or Radixin does not demonstrate axon regrowth. Thus, Akt signaling plays a key role in the regulation of axonal growth and regeneration by controlling the organization of the growth cone.

MTU08-05

Spider acetylcholine binding proteins: an ideal model to study the interaction between insect nAChRs and neonicotinoids

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Acetylcholine binding proteins (AChBPs), homologous to extracellular domains of nicotinic acetylcholine receptors (nAChRs), provide an appropriate model for the studies on nAChRs, especially for invertebrates due to difficulties in heterologous expression of their nAChRs. Until now, AChBPs were only characterized in aquatic mollusks, which showed low sensitivities to neonicotinoids, insecticides targeting on insect nAChRs. Fortunately, AChBP subunit was also found in spiders based on the sequence and tissue expression analysis. Here we reported five AChBP subunits in *Pardosa pseudoannulata*, a predator enemy against rice insect pests. Spider AChBP subunits show higher sequence similarities to nAChR subunits from both insects and mammals, when compared to mollusk AChBP subunits. From *P. pseudoannulata* AChBP1 subunit, the polymer AChBP (Pp-AChBP) was heterologously recombined in Sf9 cells, and Ls-AChBP from *Lymnaea stagnalis* was also constructed for the comparison. For both AChBPs, there existed one ligand site per subunit in each interface between two adjacent subunits. Neonicotinoids bound on Pp-AChBP with much higher affinities (7.9–18.4 times based on K_d or K_i values) than that on Ls-AChBP, although epibatidine and α-Bgt showed higher affinities on Ls-AChBP contrarily. The results indicated that the spider AChBP might be a more suitable model to study the interaction of insect nAChRs and neonicotinoids. The discussion on physiological roles and adverse effects of AChBPs in spiders was included, due to the high affinity binding of neonicotinoid on spider AChBPs.

MTU08-06

Dopamine-induced phosphorylation of NPAS4 through MAPK regulates reward-related learning and memory

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Dopamine (DA) type 1 receptor (D1R) signaling activates cAMP/PKA and then activates MAPK through Rap1 in striatal medium spiny neurons (MSNs) and plays a pivotal role in regulating neuronal excitability and reward-related behaviors (Nagai et al., Neuron., 2016). However, how D1R signaling regulates reward-related learning and memory through the gene expression is not fully understood. To isolate and concentrate the transcriptional factors (TFs) regulated by D1R signaling in mouse striatum, we performed proteomic analyses using affinity beads coated with CREB-binding protein (CBP), which acts as co-activator of numerous TFs and is involved in reward-related learning and memory. We identified Neuronal Per Arnt Sim domain protein 4 (NPAS4), as a novel CBP-interacting protein in striatum. NPAS4 was phosphorylated at Thr-427 by MAPK downstream of the D1R and the phosphorylation of NPAS4 increased the interaction of NPAS4 with CBP. The phosphomimic mutant of NPAS4 enhanced the BDNF exon I and IV promoter activity. Furthermore, the deletion of NPAS4 in accumbal D1R-expressing MSNs impaired the cocaine-induced place preference. The deficit in cocaine-induced place preference in NPAS4 deletion was restored by co-transfection with NPAS4 but not NPAS4 mutant. These results suggest that MAPK phosphorylates NPAS4 downstream of D1R and increases its binding with CBP, thereby regulating BDNF expression and reward-related learning and memory.

MTU08-07

N-terminus phosphorylation in the dopamine transporter mediates gβγ-stimulated dopamine efflux

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Dopaminergic neurotransmission is altered in complex psychiatric conditions such as depression, attention-deficit hyperactivity disorder and drug addiction. The dopamine transporter (DAT) clears extracellular dopamine through a sodium-coupled transport mechanism. The DAT function is regulated by many intracellular mechanisms including phosphorylation, ubiquitination, and protein-protein interactions. We recently reported a novel mechanism of regulation of DAT by heterotrimeric G-proteins. We found that Gβγ subunits bind directly to the C-terminus of DAT, and upon G-protein activation, the release of Gβγ results in a decrease in DA uptake. In a new set of studies, it was found that the decrease in DA-uptake is a result of the promotion of DA-efflux, a mechanism that has been described in the actions of amphetamines. DA-efflux is dependent on calcium, sodium, and membrane potential. It also involves phosphorylation of the N-terminus by Serine/Threonine kinases such as protein kinase C (PKC) and calmodulin kinase II (CamKII). Using radio-labeled-DA to measure DAT function, we are now exploring whether the DA efflux promoted by the activation of Gβγ subunits also requires phosphorylation of the N-terminus. We used two DAT mutants, hDAT-S/A and hDAT-S/D. These mutants have five N-terminal serines (S2, S4, S7, S12, S13) substituted to alanine (S/A) or pseudo-mimicking phosphorylation with a substitution to aspartate (S/D). The induction of [^{3}H]-DA efflux by mSIRK, a Gβγ biding/activating peptide, was abolish in the mutant carrier hDAT-S/A, suggesting that those serine residues are important for the Gβγ-stimulated efflux. We also used pharmacological tools to inhibit kinases and phosphatases in order to explore how the general phosphorylation state of DAT is important in the regulation of DAT. Our data suggests the effect on uptake and efflux mediated by Gβγ activation is dependent on the availability of phosphorylation sites in the DAT-N-terminus. These results are leading our investigation to determine if the substitutions of putative phosphorylation sites modify the binding of Gβγ to the C-terminus or if phosphorylation is required for the conformational state of the transporter to shift into efflux mode.

MTU08-08

A new way to create synapses: neuroplastin-TRAF6-dependent signaling induces excitatory synapse formation

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The cell adhesion molecules Neuroplastins 55/65 (Np55, Np65) are present in synapses. Polymorphisms in the Np gene promotor are linked to cortical thickness, intellectual ability and schizophrenia. In mice, ablation of Np expression triggers deficits in cortex- and hippocampus-dependent learning, retrograde amnesia for associative memories as well as reduces synapse plasticity (Bhattacharya et al., 2017). Furthermore, Nps regulate acutely the number and structural stability of hippocampal excitatory synapses (Herrera-Molina et al., 2014). Because Np cytoplasmic domain contains a tumor necrosis factor receptor-associated factor 6 (TRAF6) binding motif, we focus on the role of Np-TRAF6 interaction in synapse formation/stabilization. Np-deficient hippocampal neurons form less and shorter dendritic protrusions during synaptogenesis. Rescue of dendritic protrusion formation by Np expression did not occur in mutant neurons co-transfected with TRAF6 siRNA. Different Np mutants in the TRAF6 binding site failed to promote dendritic protrusion formation in wild type and Np-deficient neurons. Np-TRAF6 direct interaction was confirmed using molecule docking modelling *in silico*, surface plasmon resonance, pulldown and immunoprecipitation assays and a series of negative dominant and mutant constructs. In HEK cells overexpressing different Nps tagged with fluorescent proteins, we observed multimerization of Nps (FRET experiments), recruitment of cytosolic TRAF6 and robust Np-TRAF6 co-localization (confocal imaging) in newly formed actin-based filopodia. All these effects were reduced by TRAF6 siRNA transfection. Examination of downstream signaling cascades lead us to identify a role for NF-κB and PI3K/Akt/WASP pathways in Np-TRAF6-induced formation of dendritic protrusions and filopodia in neurons and HEK cells respectively. Our data support the existence of a new synaptogenic interaction between Np and TRAF6 resulting in mechanisms to initiate signaling cascades able to regulate gene transcription and actin cytoskeleton organization during early synapse formation/stabilization.

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MTU08-09

In vivo regulation of glycogen synthase kinase 3β activity in neurons and brains

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Glycogen synthase kinase 3β (GSK3β) is a multifunctional protein kinase involved in many cellular activities including development, differentiation and diseases. GSK3β is thought to be constitutively activated by autophosphorylation at Tyr216 and inactivated by phosphorylation at Ser9. The GSK3β activity has previously been evaluated by inhibitory Ser9 phosphorylation, but it does not necessarily indicate the kinase activity itself. Here, we applied the Phos-tag SDS-PAGE technique to the analysis of GSK3β phosphoisotypes in cells and brains. There were three phosphoisotypes of GSK3β; double phosphorylation at Ser9 and Tyr216, single phosphorylation at Tyr216 and the nonphosphorylated isotype. Active GSK3β with phosphorylation at Tyr216 represented half or more of the total GSK3β in cultured cells. Although levels of phospho-Ser9 were increased by insulin treatment, Ser9 phosphorylation occurred only in a minor fraction of GSK3β. In mouse brains, GSK3β was principally in the active form with little Ser9 phosphorylation, and the phosphoisotypes of GSK3β changed depending on the regions of the brain, age, sex and

disease conditions. These results indicate that the Phos-tag SDS-PAGE method provides a simple and appropriate measurement of active GSK3β *in vivo*, and the activity is regulated by the mechanism other than phosphorylation on Ser9.

MTU08-10

Membrane cholesterol prevents persistent activation of muscarinic receptors by wash-resistant xanomeline

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Alterations in signalling via muscarinic receptors play an important role in a variety of neurological, psychiatric and internal diseases, e.g. Alzheimer's disease, schizophrenia, asthma and syndrome of overactive bladder. Muscarinic receptors contribute to the development of addiction and modulate analgesia, immunity, thermoregulation, and other processes. Muscarinic receptors are thus an important but difficult pharmacotherapeutic target. Xanomeline is muscarinic agonist that is unique prototypical M_1/M_4 functionally selective agonist. Xanomeline has the same affinity, potency and efficacy at all five subtypes of muscarinic receptors. Part of xanomeline binding is resistant to washing. Wash-resistant xanomeline activates muscarinic receptors persistently, except of M_5 subtype. Mutation of leucine 6.46 to isoleucine at M_1 or M_4 receptors abolished persistent activation by wash-resistant xanomeline. Reciprocal mutation of isoleucine 6.46 to leucine at M_5 receptor made it sensitive to activation by wash-resistant xanomeline. Lowering of membrane cholesterol made M_1 and M_4 mutants and M_5 wild type sensitive to activation by wash-resistant xanomeline. Molecular docking revealed cholesterol binding site in the groove between transmembrane helices 6 and 7. Molecular dynamics showed that interaction of cholesterol with this binding site attenuates receptor activation. We hypothesise that differences in cholesterol binding to this site between muscarinic receptor subtypes may constitute basis for xanomeline apparent functional selectivity and may have notable therapeutic implications. Differences in receptor-membrane interactions, rather than in agonist-receptor interactions, represents a novel possibility to achieve pharmacological selectivity. Our findings may be applicable to other G protein coupled receptors.

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MTU08-11

Post-translational modification of apelin receptor is likely to change the pharmacological function in the central nervous system

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Apelin receptor, Aplnr, is a member of G protein coupled receptor. The amino acid sequence suggests that Aplnr has two N-glycosylation sites. Here, we have detected the Aplnr proteins which was overexpressed in HEK293 cells, or was purified from the central nervous system of the mouse. Western blot analysis revealed that, in the HEK cells that was overexpressed of Aplnr, there are two bands on the membrane around the molecular weight of Aplnr, that have already been reported. Among of the two, the band with slow mobility, was disappeared by the sample incubation with Peptide-N-Glycosidase F (PNGase F) for cleaving N-glycosylation sites. On the other hand, when attempting to detect Aplnr using the mouse spinal cord, multiple bands at approximately 20 kDa greater than the expected molecular weights were detected. Among of them, the band with a slow mobility, was disappeared by PNGase F in the same manner as described above. Therefore, it was suggested that Aplnr in the central nervous system expresses with several post-translational modification, including N-glycosylation, and it functions differently to peripheral organs.

MTU08-12

PAR1 activation induces calcium-dependent glutamate release from RPE cells

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The retinal pigment epithelium (RPE) is a highly specialized cell monolayer located between neural retina and the choroid. Although differentiated RPE cells remain quiescent, their proliferation is activated under pathological conditions involving the alteration of the blood-retina barrier (BRB). Among these pathologies, proliferative vitreoretinopathy (PVR) is characterized by the uncontrolled proliferation of RPE cells, leading to the formation of contractile membranes on both surfaces of the retina. The contraction of these membranes results in retinal detachment and ultimately leads to blindness. Under these conditions, RPE cells are exposed to serum components, thrombin among them. Thrombin is a multifunctional serine protease which participates in a wide range of cellular processes such as proliferation, differentiation and survival in a variety of cell types, including RPE cells. Thrombin effects are exerted through the proteolytic activation of protease-activated receptors (PARs 1, 3 and 4), particularly by PAR-1. Clinical studies have shown that glutamate (GLU) concentration as well as thrombin activity are significantly increased in the vitreous humour of patients suffering from retinal detachment. On this line, we have demonstrated that GLU induces RPE cell proliferation through the activation of signaling pathways involving type I metabotropic glutamate receptors and NMDA receptor-mediated calcium increase. In the present study we analyzed the effect of thrombin on GLU release from rat RPE cells in primary culture. Results showed that, under physiological conditions, the activation of PAR-1 by

thrombin or by PAR-1 agonist peptide stimulates GLU release from RPE cells in a specific, dose-dependent manner. This effect was prevented by the chelation of intracellular calcium, suggesting the requirement of calcium release form the endoplasmic reticulum. Together with our previous findings, these results suggest that thrombin and glutamate might exert a synergistic effect on the promotion of RPE cell proliferation in fibroproliferative diseases derived from the disruption of the BRB, such as PVR.

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MTU08-13

Modification of perisynaptic extracellular matrix upon D1-like dopamine receptor activation is PKA-dependent

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The extracellular matrix (ECM) of the central nervous system consists of chondroitin sulfate proteoglycans such as Aggrecan and Brevican bound to hyaluronic acid, link proteins and tenascins. The brain's ECM has been shown to surround pre- as well as postsynapses, to be formed and remodeled in an activity-dependent manner; and could be important for synaptic plasticity and learning.

Dopamine, a crucial neuromodulator for motivated learning, acts through either D1- or D2-like dopamine receptors in the brain. Activation of protein kinase A (PKA) via stimulated D1/D5 dopamine receptors was shown to lead to enhanced extracellular tissue-type plasminogen activator (tPA) activity probably associated with an increased release of this protease. We hypothesized that a similar mechanism may underlie ECM remodeling by proteases, such as ADAMTS 4/5.

Here, we studied the impact of dopamine in ECM modification by proteolytic cleavage and its relevance for synaptic plasticity, thus learning. Therefore, D1-like or D2-like receptors were activated by dopamine receptor agonists SKF81297 and Quinpirole, respectively. Immunocytochemical analysis revealed that perisynaptic Brevican cleavage is increased only after D1-like receptor activation at excitatory synapses. We could block this effect by the D1-like dopamine receptor antagonist SCH23390 as well as by using a PKA inhibitor (cAMPS-Rp) or an inhibitor of ADAMTS 4 (TIMP-3). Taken together, these findings underline the possibility of an interplay between the dopaminergic system and the ECM, though the exact molecular and cellular signaling mechanism remain to be clarified.

MTU08-14

Cleavage of ErbB4 after G-protein -coupled receptor stimulation in hypothalamic neurons and anterior pituitary cells

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ErbB4 belongs to the ErbB protein family of receptor tyrosine kinases, and Neuregulin 1 (NRG1) is one of the ligands of ErbB4. When NRG1 binds to ErbB4, tyrosine kinase is activated and multiple tyrosine residues are autophosphorylated, resulting in the initiation of multiple signal transduction pathways. Hypothalamic GnRH neurons have a GnRH receptor belonging to the G-protein-coupled receptors (GPCR). In the present study, we found that EGFR and ErbB4 were involved in the activation of extracellular signal-regulated protein kinase (ERK) by weak stimulation of GnRH receptor in cultured GnRH neurons (GT1-7 cells). Moreover, strong stimulation of GnRH receptor induced the cleavage of ErbB4 and accumulation of an 80-kDa fragment. After treatment of the cells with 50 nM GnRH for 5 min, about 80% of ErbB4 was cleaved. The studies with selective inhibitors indicated that G_q or G_{11} were involved in ERK activation and ErbB4 cleavage. TAPI-2, an inhibitor of tumor necrosis factor-α-converting enzyme (TACE), and siRNA for TACE inhibited the cleavage of ErbB4, suggesting that TACE was involved. After ErbB4 cleavage, the activation of ERK by NRG1 was almost completely inhibited. In addition to GT1-7 cells, we found that ErbB4 was expressed in cultured anterior pituitary gonadotroph cells, αT3-1 cells. GnRH treatment of αT3-1 cells also induced ErbB4 cleavage in a TACE-dependent manner. These results suggested that the down-regulation of ErbB4 was induced by GPCR stimulation.

MTU08-15

Dendritic spine formation is regulated by lemur kinase 1a (LMTK1A) via Rab11A -positive endosome trafficking

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Synaptic transmission is crucial for propagation of excitation between neurons. Synapse is a specialized structure composed of pre- and post-synaptic terminals. In particular, a postsynaptic region of excitatory synapses forms a mushroom-like protrusion called dendritic spine. Spine formation and stabilization are associated with neuronal maturation and activity. However, it is not known yet how the spine density is determined. While actin cytoskeleton plays an essential role in spine formation, membrane supply is also required because cell surface expands when spines are formed. In fact, it is reported Rab GTPases are involved in spine formation. Nonetheless, the detailed mechanisms how Rabs participate have not been addressed yet.

Lemur kinase 1A (LMTK1A) is a novel kinase that negatively regulates neurite outgrowth via trafficking of Rab11-positive recycling endosomes. In this study, we investigated the role of LMTK1A in dendritic spine formation. Knockdown of LMTK1 or overexpression of kinase negative LMTK1A in primary hippocampal neurons or mouse brains using *in utero* electroporation increased the spine density. LMTK1-knockout neurons showed the same phenotype. These results indicate that LMTK1A negatively

regulates the dendritic spine formation. Most of those spines were PSD-95 positive and showed the staining of anti-synaptophysin presynaptic protein in its vicinity, indicating that spines induced by downregulation of LMTK1 are mature and functional. Next we investigated the role of Rab11A in dendritic spine formation. Overexpression of constitutively active Rab11A (Q70L) increased spine density and, on the contrary, constitutively inactive Rab11A (S25N) decreased. To test the LMTK1-Rab11A-spine cascade, we performed the knockdown of LMTK1A in the presence of active or inactive Rab11A. The spine formation was mainly determined by the activity of Rab11 but not by LMTK1A, indicated that LMTK1A regulates spine formation upstream of Rab11A. Together, we show here for the first time the role of LMTK1A in spine formation. LMTK1A would prevent overgrowth of axon, dendrites and spine during neuronal development through endosomal trafficking. We are now analyzing what membrane components are supplied by the LMTK1-Rab11A system in spine formation.

MTU08-16

Vitamin C regulates NMDA receptor activity and neuronal survival in the retina

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Our previous work showed the presence of transporters regulating the uptake and release of ascorbate (AA) in the retina. Interestingly, the activation of glutamate receptors induces AA release from retinal cells in culture through a mechanism mediated by the sodium-dependent vitamin C transporter type 2 (SVCT2). In the present work we used mixed cultures of chick retinal cells in order to study the reciprocal roles of vitamin C on glutamate signaling in the retina. We show here that AA or its oxidized form, dehydroascorbate (DHA), regulates NMDA receptor activity in a biphasic way, since both forms activate receptor activity, as measured by an increase of (^{3}H) MK801 binding, but also prevent receptor activation stimulated by glutamate. The increase of (^{3}H) MK801 binding was dependent on the activation of type III metabotropic glutamate receptors as it was blocked by the selective antagonist MAP4 and mimicked by the agonist L-SOP. On the other hand, the inhibition of glutamate-stimulated (^{3}H) MK801 binding may be explained by the decrease of NMDA receptor N1 subunit expression at the surface membrane as measured by biotynilation and confocal imaging protocols. Interestingly, vitamin C is also able to increase AKT and CREB phosphorylation in a NMDA-dependent manner, to decrease (^{3}H) D-aspartate uptake and to promote the accumulation of glutamate in the extracellular medium of cultured retinal cells. Moreover, a long-term incubation of retinal neuronal cultures with low concentrations of AA (10 μM) promotes the protection of retinal neurons from glutamate excitotoxicity. These results demonstrate an intense modulation of glutamate signaling by AA or DHA in the retina and therefore are consistent with a neuromodulatory role for vitamin C in this tissue.

MTU08-17

Fluorescence anisotropy based assay for the characterization of ligand binding to G protein-coupled receptors

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G-protein-coupled receptors (GPCRs) are involved in a wide variety of regulatory processes in the nervous system and abnormalities in GPCR mediated signal transduction are associated with numerous diseases. The development of novel drugs with less side effects and higher efficacy requires better understanding of the mechanisms and kinetics of receptor-ligand interactions. Implementation of fluorescence anisotropy assay for monitoring ligand binding processes to GPCRs have opened new possibilities for this kind of studies. However, there are several issues which have to be solved before reliable results can be obtained. Ratiometric nature of the assay requires that the concentrations of the reporter ligand and the receptor are in the same range. We have shown, that budded baculovirus particles, which display GPCRs on their surfaces are very suitable for this kind of studies as they ensure high expresssion levels, homogeneity and stability of receptor samples as well as good signal to noise ratio in the assay [1]. Interpretation of the kinetic results of the non-pseudo first order reactions is also more demanding and may even require global numerical analysis to achieve physically meaningful results. The ligands used have to be labelled with a suitable fluorophore, which life-time, color, bleaching stability and hydrophobic properties are suitable for the particular assay. Coupling of the fluorophore has to retain also ligand's high affinity and specificity and the obtained reporter ligand has to have suitable kinetic properties for the characterization of different unlabeled ligands. Up to now, we have achieved working systems for melanocortin 4 receptors [2, 3], neuropeptide Y_1 receptors, dopamine D_1 receptors, serotonin 5-HT_{1A} receptors [4] and muscarinic acetylcholine M_2 receptors.

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MTU08-18

Deepen human D-amino acid oxidase properties to get insight in D-serine metabolism

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In the brain, the FAD-dependent enzyme D-amino acid oxidase (DAAO, EC 1.4.3.3) plays a key role in the catabolism of D-serine, the main endogenous co-agonist of the N-methyl-D-aspartate receptors (NMDAR). Indeed, the flavoenzyme activity, through the regulation of D-serine cellular levels, may affect NMDAR-dependent physiological functions. The over- or down-stimulation of NMDARs is involved in neurodegenerative diseases and in

psychiatric disorders, and a dysregulation in the processes defining the dynamics of D-serine levels likely concur to their onset. Hence, there is an increased interest in shed light on the molecular mechanisms modulating human DAAO (hDAAO) activity.

Over the past few years, hDAAO structural/functional relationships have been inquired, but several aspects remain elusive. To fill this gap, we deepened the characterization of hDAAO properties investigating the enzyme activity and stability at different pH and temperature values, the kinetic parameters on alternative substrates, the binding of potential ligands and the effect of L-amino acids on the enzyme activity. Moreover, we evaluated the effect of ions on hDAAO structural and functional properties.

hDAAO proved to be highly stable at physiological pH and temperature. It oxidizes both D-DOPA and D-kynurenine, but with a low kinetic efficiency. ATP, NMDA, glycine and glutamate do not interact with hDAAO. Conversely, free L-amino acids can bind to hDAAO. In particular, L-serine acts as a competitive inhibitor (estimated K_i=26.2 mM); however, at physiological concentrations (1 mM) it should not affect the enzyme activity. hDAAO conformation appears moderately altered by Ca^{2+} and the concomitant presence of ATP or GTP and 10 mM Mg^{2+}, but its functionality is not affected.

These results extend our knowledge and will help the design of new effective strategies to modulate hDAAO activity.

MTU08-19

Lipid raft integrity impacts cannabinoid receptor 1 signalling

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The Cannabinoid 1 receptor, CB1R, has evolved as a major regulatory molecule for almost all known aspects of CNS development and function, with actions ranging from proper cellularity to complex behaviors such as fear, appetite, addiction. We have previously demonstrated in neurons that CB1R forms signalling complexes with proximal kinase PKCε to induce a/G_q/PLC/PKCε/Src-Fyn/Ras/Raf-dependent first peak of ERK pathway, while a second CB1R pool induces a $G_{i/o}$/Src-Fyn/FGFR1/Ras/Raf-dependent second amplification of ERK activation, all emanating from lipid rafts. To further elucidate the significance of rafts in CB1 signalling we disrupted lipid rafts and associated cytoskeleton systems and studied by confocal microscopy localization and trafficking of overexpressed CB1R in COS cells. In basal conditions, GFP-CB1R fluorescence was seen at the plasma membranes of lamellipodia and filopodia, and in juxta/paranuclear compartments - consistent with an ER/Golgi localization. The specific CB1R-agonist methanandamide, R(+)-MA (15 min), minimized CB1R plasma membrane localization and increased by 3-fold some perinuclear pools, seen as perinuclear rings, with no significant localization in lysosomes. Nocodazole-disruption of microtubules, a functional partner of lipid rafts, distorted CB1R patterns both in the surface and in small vesicles, seen along thicker microtubule bundles, including those in mitotic structures. Methyl-β-cyclodextrin, which sequesters cholesterol to disrupt rafts, minimized receptor presentation at plasma membranes and increased it in some irregular vescicular structures. Similarly, after collapsing the F-actin cytoskeleton with cytochalasin-D, CB1-GFP fluorescence appeared mostly as irregular, scattered cytoplasmic foci; R(+)-MA did not cause, however, any increases in the perinuclear pools, indicating that CB1R signalling may still occur when F-actin is disrupted, yet, the signal may be modified because of lesser intracellular trafficking of CB1R and thus 'consumption' of the signal in the cell periphery. Moreover, each of these perturbations impacted both the duration and magnitude of ERK activation. These data collectively indicate that CB1R targeting to and signalling/trafficking from the plasma membrane is regulated by lipid rafts and its functional partners, the F-actin and the microtubule cytoskeletons.

MTU08-20

β3-adrenoceptors inhibit cholinergic neurotransmission in the bladder indirectly via ado release and A1 receptor activation

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The selective β_3-adrenoceptor agonist, mirabegron, was recently introduced in clinical practice for the treatment of overactive bladder (OAB) syndromes. The direct detrusor relaxant effect of β_3-adrenoceptor agonists as the sole mechanism to improve OAB symptoms has been increasingly questioned. Activation of β_3-adrenoceptors may negatively modulate nerve-evoked acetylcholine (ACh) release, but there is no evidence for the presence of these receptors on cholinergic nerve terminals. Our hypothesis is that adenosine formed from the catabolism of cyclic AMP in the detrusor may act as a retrograde messenger via prejunctional A_1 receptors to explain inhibition of cholinergic activity by β_3-adrenoceptors. Isoprenaline (1 μM) decreased [^{3}H]ACh release from stimulated (10 Hz,200pulses) human ($-47 \pm 5\%$) and rat ($-38 \pm 1\%$) detrusor strips.Mirabegron (0.1 μM, $-53 \pm 8\%$) and CL316,243 (1 μM, $-37 \pm 7\%$) mimicked isoprenaline (1 μM) inhibition and their effects were prevented by blocking β_3-adrenoceptors with L748,337 (30 nM) and SR59230A (100 nM), respectively in human and rat detrusor. Mirabegron and isoprenaline increased extracellular adenosine in the detrusor. Blockage of A_1 receptors with DPCPX (100 nM) or the equilibrative nucleoside transporters (ENT) with dipyridamole (0.5 μM) prevented mirabegron and isoprenaline inhibitory effects. Dipyridamole prevented isoprenaline-induced adenosine outflow from the rat detrusor and this effect was mimicked by the ENT1 inhibitor,NBTI(30 μM). Cystometry recordings in anaesthetized rats demonstrated that SR59230A, DPCPX, dipyridamole and NBTI reversed the decrease of the voiding frequency caused by isoprenaline(0.1-1000 nM). Data suggest that inhibition of cholinergic neurotransmission by β_3-adrenoceptors results from adenosine release via equilibrative nucleoside transporters and prejunctional A_1 receptors stimulation in human and rat urinary bladder. Work supported by FCT(PEst-OE/SAU/UI0215/2014 and UID/BIM/4308/2016). IS is in receipt of a PhD fellowship by FCT(SFRH/BD/88855/2012).

MTU08-21

Metabotropic glutamate receptor 7 (mGluR7) trafficking and signaling by post-translational modifications

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The metabotropic glutamate receptors (mGluRs) are seven membrane-spanning proteins that are linked via G-proteins to intracellular signaling cascades. Among eight family members of mGluRs, mGluR7 is highly expressed at the presynaptic active zone where it modulates excitatory neurotransmission and synaptic plasticity by limiting further neurotransmitter release in an auto-regulatory manner. Like many GPCRs, mGluR7 undergoes constitutive and agonist-dependent endocytosis. Although it has been reported that protein turnover and endocytosis of GPCRs are dynamically regulated by the ubiquitin-proteasome system, it remains elusive that mGluR7 undergoes ubiquitination in response to synaptic activity. In this study, using biochemical approaches coupled with confocal imaging, we have explored whether mGluR7 is a target of ubiquitination. We found that mGluR7 is ubiquitinated at the cytoplasmic loop 2 region and the C-terminal tail by the treatment of agonist in HEK 293T cells and primary cortical neurons. In addition, we identify Nedd4, the HECK domain ubiquitin E3 ligase is responsible for mGluR7 ubiquitination and regulate its endocytosis and degradation. Taken together, these data support a model that the ubiquitination of mGluR7 is critical for stable surface expression and function of mGluR7 in neurons.

MTU08-22

Substrate transport and anion permeation proceed through distinct pathways in glutamate transporters

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Advances in structure-function analyses and computational biology have enabled a deeper understanding of how excitatory amino acid transporters (EAATs) mediate chloride permeation and substrate transport. However, the mechanism of structural coupling between these functions remains to be established. Using a combination of molecular modeling, substituted cysteine accessibility, electrophysiology and glutamate uptake assays, we identified a chloride-channeling conformer, *iCh*S, transiently accessible as EAAT1 reconfigures from substrate/ion-loaded into a substrate-releasing conformer. Opening of the anion permeation path in this *iCh*S is controlled by the elevator-like movement of the substrate-binding core, along with its wall that simultaneously lines the anion permeation path (*global*); and repacking of a cluster of hydrophobic residues near the extracellular vestibule (*local*). Moreover, our results demonstrate that stabilization of *iCh*S by chemical modifications favors anion channeling at the expense of substrate transport, suggesting a mutually exclusive regulation mediated by the movement of the flexible wall lining the two regions.

MTU08-23

Mechanism of dopamine signaling for membrane excitability

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Neuromodulators, including dopamine and acetylcholine, are key factors for reward-related behavior. Striatum/nuclear accumbens (NAc) is the key brain region for determining reward-related behavior by controlling dopamine D1 receptor-medium spiny neuron (D1R-MSN)-mediated direct pathway and dopamine D2 receptor-medium spiny neuron (D2R-MSN)-mediated indirect pathway. Dopamine enhances reward-related behavior by activating D1R-MSN-mediated direct pathway. Recently, we developed a novel proteomic system and clarified that dopamine activates protein kinase A (PKA)-Rap1-MAPK pathway to enhance D1R-MSN neuronal excitability, leading to facilitated reward-related behavior. However the mechanism of dopamine signaling underlying neuronal excitability remains to be understood at the molecular level. In this study, we identified a voltage-gated potassium channel KCNQ2, which is involved in neuronal excitability, as a phosphoprotein regulated by MAPK. Phosphorylation of KCNQ2 by MAPK modulated the open propability of KCNQ2/3 heterochannels in Xenopus oocyte system. Activation of D1R and PKA modulated the KCNQ-sensitive current in striatal slice. These results suggest that dopamine signaling regulate the activity of KCNQs channel, thereby controling neuronal excitability.

MTU08-24

Amphetamine induced internalization of the dopamine and glutamate transporters is mediated by the trace amine receptor 1

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Psychostimulants such as amphetamine (AMPH) are often useful therapeutic agents, but can also pose a danger as a consequence of their addictive properties. A better understanding of the biochemical cascades that mediate the physiological outcomes of this class of drugs is needed.

We previously reported that AMPH activates endocytosis of the dopamine and glutamate transporters, DAT and EAAT3. This trafficking requires activation of RhoA, a small GTPase that can be regulated by protein kinase A (PKA). PKA phosphorylation inactivates RhoA as well as internalization of the transporters. The trace amine-associated receptor 1 (TAAR1) is an intracellular GPCR known to couple through Gs and contributes to the actions of psychostimulants in dopamine neurons. These observations led us to

examine the potential role of TAAR1 in RhoA activation and inactivation.

We used CRISPR-Cas9 gene editing to disrupt endogenous TAAR1 gene expression in HEK293. In cells that lack TAAR1, AMPH did not induce DAT or EAAT3 internalization. We also could not detect Rho activation in TAAR1(-) cells despite robust AMPH-induced Rho activation in TAAR1(+) cells.

To identify the pathway of Rho activation through TAAR1, we co-expressed mini-genes that interfere with activation of various GPCR alpha-subunits and found that TAAR1 not only couples with Gs, but also couples with the G13 alpha subunit, an established activator of RhoA signaling. We designed cell-permeable peptides based on these interfering sequences to confirm these observations in adult murine midbrain tissue.

To resolve the potential of distinct subcellular compartments where these two different TAAR1-mediated events occur, we used FRET sensors to assess PKA activation or RhoA activation that were targeted to various cellular compartments. AMPH-stimulated PKA activation was broadly distributed throughout the cell; however, RhoA activation was highly concentrated in regions surrounding the ER.

These observations demonstrate that TAAR1 serves as the intracellular target for AMPH that mediates RhoA activation, cAMP signaling and subsequent regulation neurotransmitter transporter trafficking. These data suggest new pathways to target in order to better understand the mechanisms of action of AMPH.

MTU08-25

A fundamental chemical analysis of serotonin transmission in genetic and environmental autism spectrum disorder models

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Autism spectrum disorder (ASD) is a collection of developmental disorders with growing prevalence. The pathophysiology of ASD is not yet fully understood, hindering suitable prevention and treatment options. Specifically, a universal underlying chemical mechanism is lacking. We believe that serotonin dysfunction can be identified as a common neurochemical mechanistic feature of this disorder, however current techniques do not provide a complete representation of the serotonin system as they are only capable of measuring basal levels at low temporal resolution. Here, we describe the application of Fast Scan Cyclic Voltammetry, which operates on a neurotransmission temporal resolution and allows us to examine serotonin release and reuptake, to genetic and environmental ASD models. These models allow us to establish a chemical phenotype accompanying stereotypical ASD behavioral in mice. The results demonstrated a significant difference in the serotonin chemistry between ASD models and controls. Identifying this chemical phenotype will allow us to redefine the serotonin chemistry within ASD.

MTU08-26

Activation of PYK2 by PKD and CaM kinase II in cultured hypothalamic neurons

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Gonadotropin-releasing hormone (GnRH) is secreted from hypothalamic neurons (GnRH neurons). GnRH neurons have a GnRH receptor belonging to the G-protein-coupled receptors (GPCRs). In the previous study, we found that CaM kinase IIδ2 was involved in GnRH-induced ERK activation in cultured GnRH neurons (GT1-7 cells). Recently, we found that novel protein kinase C (nPKC) was also involved in ERK activation. It is well known that protein kinase D (PKD), belonging to the CaM kinase family, is activated by nPKC. It has been reported that proline-rich tyrosine kinase 2 (PYK2) was activated by activation of PKC, as well as by the increase in the intracellular Ca^{2+}. It was reported that PYK2 was involved in GnRH-induced ERK activation in GT1-7 cells. In the present study, we examined the possibility that PKD and CaM kinase IIδ2 were involved in GnRH-induced PYK2 activation. (i) Fyn existed in the activated form in the cells, and dasatinib, a Src family inhibitor, completely inhibited Fyn activation and GnRH-induced PYK2 activation. (ii) PKD1 was activated by GnRH in an nPKC-dependent manner. (iii) Knockdown of PKD1 and a PKD inhibitor inhibited GnRH-induced PYK2 activation, while they had no effects on Fyn activation. (iv) Knockdown of CaM kinase IIδ2 and KN93, an inhibitor of CaM kinases, inhibited GnRH-induced PYK2 activation. These results strongly suggested that PKD1 and CaM kinase IIδ2 activated the ERK pathway through PYK2 activation by Fyn.

MTU09 Neurogenesis and Cell Differentiation

MTU09-01

Induced haploinsufficiency of *kit* receptor tyrosine kinase impairs development of central nervous system

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Background: Kit receptor tyrosine kinase has been shown to regulate a wide range of biological functions in various cell lineages including pigment cells, hematopoietic cells, germ cells, neuronal cells of central nervous system (CNS). Kit mRNAs are expressed in cells affected by *Kit* locus mutants, however, in developing or adult brain which is one of the major tissues expressing Kit mRNA, no clear phenotype lead to the loss of specific cell types or functions was detected in the brain of *Kit* mutants.
Objective: To investigate the Kit function in neural lineage, we introduced a conditional loss of function mutation of *Kit* from a certain point of the developmental stage.
Methods: Transmembrane region of *Kit* was flanked by loxP to excise the *Kit* floxed allele by Cre recombinase. To take advantage of CNS specific induction of Cre, $Kit^{floxed/+}$ mice were crossed with *Sox1-Cre* mice expressing Cre directed by the neural lineage specific promoter sequence. The morphology, histology, gene expression, and the growth and differentiation of neural stem/precursor cells were analyzed.
Results: Expression of endogenous *Sox1* gene starts as early as E8.0 and *Kit* mRNA expression in their brain reduced into one half of the control in E10.5. The resultant $Kit^{floxed/+}$; *Sox1-Cre+* embryos showed significant reduction of the size of the forehead in E12.5, which generate a severe hypoplasia of CNS including brain, spine, and eye. This was accompanied by the increase of apoptotic cells in early embryonic brain and the gradual loss of self-renewal capacity of neural stem/precursor cells leading to the accelerated differentiation of neural stem cells to mature neural cells.
Conclusion: The phenotype appeared in $Kit^{floxed/+}$; *Sox1-Cre+* conditional Kit haploinsufficient embryos suggests that Kit expressed in developing CNS is functional signaling molecules necessary for proper brain development as the other cell lineages solely dependent for Kit in its certain developmental stage.

MTU09-02

Uncovering floor plate descendants in the ependyma of adult mouse CNS using mapping of *Nato3*-expressing cells

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During embryonic development of the central nervous system (CNS), the expression of the bHLH transcription factor *Nato3* (*Ferd3 l*) is unique and restricted to the floor plate of the neural tube. In mice lacking *Nato3* the floor plate cells of the spinal cord do not fully maturate, whereas in the midbrain floor plate, progenitors lose some neurogenic activity, giving rise to a reduced population of dopaminergic neurons. Since the floor plate is considered to be disintegrated at the time of birth, *Nato3* expression was never tested postnatally and in adult mice. Here, we utilized a *Nato3* knockout mouse model in which a *LacZ* reporter precisely replaced the coding region under the endogenous regulatory elements, such as its expression recapitulates the spatiotemporal pattern of *Nato3* expression. *Nato3* was found to be expressed in the CNS throughout life in a highly-restricted manner along the medial cavities: in subpopulations of cells in the third ventricle, the cerebral aqueduct, the fourth ventricle, the central canal of the spinal cord, and the subcommissural organ, a gland located in the midbrain. A few unifying themes are shared among all *Nato3*-positive cells: all are positioned in the midline, are of an ependymal type, and contact the cerebrospinal fluid (CSF) similarly to the embryonic position of the floor plate bordering the lumen of the neural tube. Taken together, *Nato3* defines an unrecognized subpopulation of medial cells positioned at only one side of circular ependymal structures, and it may affect their regulatory activities and neuronal stem cell function.

MTU09-03

ENU mutagenesis screening to identify mutations that control corpus callosum development

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The two hemispheres of the cerebral cortex are well interconnected by commissural axons, which allow for the synchronization and lateralization of higher brain functions. The dominant commissural fiber tract of the mammalian brain is the corpus callosum. Most commissural axons originate from pyramidal neurons located in layer II/III or to a lesser extent in layer V of the neocortex. During evolution of higher mammals and specifically humans, the proportion of upper versus deeper layer neurons has substantially increased. This lead to strongly increased cortico-cortical connectivity, which is thought to have enabled the emergence of higher cognitive abilities. Corpus callosum (CC) essential for the lateralization of highly specialized brain functions, such as speech processing. The relatively high incidence of CC agenesis in humans (1 : 4000) illustrates that callosal axon guidance and CC formation during embryonic brain development are complicated and relatively fragile processes. Abnormal CC development is associated with more than 50 neurodevelopmental syndromes and often impairs emotional, social and mental functions.

In order to identify genes that control CC formation we carried out ENU mutagenesis in mice that express LacZ gene as a tag in all callosal neurons. We identified several mutants that demonstrate CC development abnormalities. The phenotype of these mutants will be presented and discussed.

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MTU09-04

Neurons on nanotopographies

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Topography, the physical characteristics of an environment, is one of the most prominent stimuli neurons can encounter in the body. Many aspects of neurons and neuronal behavior are affected by the size, shape, and pattern of the physical features of the environment. A recent increase in the use of nanometric topographies, due to improved fabrication techniques, has resulted in new findings on neuronal behavior and development. Factors such as neuron adhesion, neurite alignment, and even the rate of neurite formation have all been highlighted through nanotopographies as complex phenomena that are driven by intricate intracellular mechanisms. The translation of physical cues is a biologically complex process thought to begin with recognition by membrane receptors as well as physical, cell-to-surface interactions, but the internal biological pathways that follow are still unclear. In this respect, nanotopography would be a more suitable platform on which to study receptor interfaces than microtopography because of the subcellular topographical features that are relevant in scale to the receptor activity. Ultimately, the characterization of this unknown network of pathways will unveil many aspects of the behavior and intracellular processes of neurons, and play an important role in the manipulation of neuronal development for applications in neural circuits, neuroregenerative medicine and prostheses, and much more.

MTU09-05

Obesity leads to impairment in neurogenesis and reduces brain size

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Overweight and obesity are public health problems that affect 30% of the world population. It is known that accumulation of body mass causes peripheral insulin resistance, diabetes, among other comorbidities. Recent studies have show that overweight also affects the nervous system, impairing cognition and increasing the propensity to develop neurodegenerative diseases, particularly Alzheimer's disease. However, the modifications in the brain as a consequence of obesity and how they affect cognition and behavior remains unclear. To investigate the impact of obesity in brain structure, we used C57/Bl6 transgenic ob/ob mice, which eats excessively due to mutations in the gene responsible for the production of leptin. Magnetic resonance imaging (MRI) was initially performed for *in vivo* brain volume analysis. Preliminary data show a 10% decrease in the total brain volume of the obese mice when compared to age-matched non –transgenic C57/BL6 control mice. We next carried out analysis to investigate neurogenesis, and further looked at immature neurons (using anti-doublecortin-DCX) and at the cell proliferation marker anti- Ki-67 in the brain. Both the lateral ventricle and the hippocampus were analyzed. We found important decreases in positive cells for doublecortin, as well as in cells expressing the cell cycle marker ki-67 in the lateral ventricle and in the hippocampus of ob/ob mice as compared to controls. Our data suggests that impaired neurogenesis in the brain may contribute to cognitive deficits that obese mice develop and to the overall impact of obesity in the brain.

MTU09-06

Study of the neurodifferentiative role of GM1 oligosaccharide chain in mouse primary cerebellar neurons

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One of the best studied ganglioside for its neurotrophic function is ganglioside GM1. GM1 neuro-properties are exerted when the GM1 membrane content increases in membrane microdomains, known as lipid rafts. This local enrichment could be responsible for (i) the GM1 content-dependent membrane reorganization, that alter the membrane properties, ensures that the physical parameters required for proper protein function is reached, (ii) the GM1 oligosaccharide-protein direct interactions, which can stereo chemically and allosterically modify protein structure and function.

Despite several experimental data have been demonstrated the GM1 involvement in neuronal differentiation, the molecular mechanism by which GM1 exerts its neurotrophic action has not yet been elucidated. In this view, we decide to investigate the importance of its oligosaccharide portion by using primary neurons from mice cerebellum.

We found that the GM1 oligosaccharide portion is able to influence the differentiation of these cells. By morphological analysis we evidenced the outstanding ability of oligosaccharide-treated cells to aggregate forming clusters. Moreover, the immunoblotting analysis highlighted the acceleration of the neuronal differentiation: there is an increase in the expression of neurodifferentiation markers such as MAP2, Synapsin and Neuroglican C, with respect to untreated cells. We also found an increase in GTPase RAC3 expression, that is involved in radial and tangential migration.

Our results suggest that the oligosaccharide portion of GM1 is responsible for the ability of GM1 to induce neuritogenesis. We surmise that the neurotrophic effect of GM1 is due to a direct interaction with extracellular domain plasma membrane proteins.

MTU09-07

Tau-dependent suppression of adult neurogenesis in the stressed hippocampus

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Stress, a well-known sculptor of brain plasticity, is shown to suppress hippocampal neurogenesis in the adult brain; yet, the underlying cellular mechanisms are poorly investigated. Previous studies have shown that chronic stress triggers hyperphosphorylation of the cytoskeletal protein Tau, a process that impairs the

cytoskeleton-regulating role(s) of this protein with impact on neuronal function. Here, we analyzed the role of Tau on stress-driven suppression of neurogenesis in the adult dentate gyrus (DG) using animals lacking Tau (Tau-KO) and wild-type (WT) littermates. Unlike WTs, Tau-KO animals exposed to chronic stress did not exhibit reduction in DG proliferating cells, neuroblasts and newborn neurons; however, newborn astrocytes were similarly decreased in both Tau-KO and WT mice. In addition, chronic stress reduced PI3K/mTOR/GSK3β/β-catenin signaling in the DG, known to regulate cell survival and proliferation, in WT, but not in Tau-KO. These data establish Tau as a critical regulator of the cellular cascades underlying stress deficits on hippocampal neurogenesis in the adult brain.

MTU09-08

SIP1 controls dendritic arbor formation in the mammalian neocortex

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The dendritic arbor is an elaborately branched and specialized characteristic neuronal structure that acts as the main site of information input to the neuron. Unsurprisingly, defects in the formation of the dendritic arbor or in its differentiation and maintenance are associated with cognitive impairment and occur in a wide variety of human neurodevelopmental disorders in particular intellectual disability-associated syndromes and neuropsychiatric disorders. Heterozygous mutations in the transcription factor Sip1 (Smad-interacting protein 1; also called ZFHX1b or ZEB2) have been found to cause Mowat-Wilson syndrome, a human condition associated with severe intellectual disability, multiple congenital abnormalities and epilepsy. We used *in utero* electroporation (IUE) to generate mosaic deletion of Sip1 gene in the developing mouse neocortex. Mosaic deletion of Sip1 in the mouse neocortex was achieved by electroporation of a construct expressing Cre recombinase under the control of an ubiquitous (CAG) promoter. During development, Sip1 is expressed in all postmitotic neurons and is absent from progenitor cells.

In these experiments we observed striking alterations in the dendritic morphology of mature neurons at P23. Apical dendrites of Sip1 deficient neurons did not maintain correct polarity. While the orientation of an apical dendrite to pial surface in the neocortex is 90°, in Sip1 deficient neurons it was randomised. On the other hand the dendritic complexity was also affected by Sip1 deletion. Our data indicate that Sip1 is required in the differentiating neurons to establish neuronal morphology.

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MTU09-09

Pyridoxine modulates neurogenesis by regulating CB1 cannabinoid receptor-interacting protein

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Pyridoxal 5′-phosphate has a major coenzyme to synthesize monoamines such as serotonin, dopamine, and serotonin. In the present study, we investigated the effects of pyridoxine on memory function using a novel object recognition test as well as the changes in protein profiles based on the proteomic approach. Eight-week-old mice received intraperitoneal injections of physiological saline (vehicle) or 350 mg/kg pyridoxine twice a day for 21 days. Changes in protein and serotonin turnover levels were analysed using two-dimensional fluorescence difference gel electrophoresis (2D-DIGE) and high-performance liquid chromatography (HPLC), respectively. Differentially expressed proteins were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Approximately 2690 protein spots were detected by 2D-DIGE, and increases > 1.5-fold were observed in several proteins in the hippocampal homogenates of vehicle-treated mice relative to those of pyridoxine-treated mice. Phosphoglycerate mutase 1 was up-regulated, while CB1 cannabinoid receptor-interacting protein 1 (CRIP1) was down-regulated, in the pyridoxine-treated group. Additionally, the 5-hydroxyindoleacetic acid/5-hydroxytryptamine ratio was significantly lower in the hippocampus of the pyridoxine-treated group than in that of the vehicle-treated group. Furthermore, discrimination indices based on the novel object recognition test were significantly higher in the pyridoxine-treated group than in the vehicle-treated group. Administration of CRIP1a siRNA significantly increases the discrimination index as well as cell proliferation and neuroblast differentiation in the dentate gyrus. These results suggest that pyridoxine promotes short-term recognition memory and increases serotonin levels in the hippocampus via CRIP1a modulation.

MTU09-10

Glycoprotein M6a clustering of lipid-rafts and associated signaling proteins for neuronal polarity

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Lipid-raft domains, where sphingolipids and cholesterol are enriched, concentrate signaling molecules. We focused on glycoprotein M6a (GPM6a), which is expressed at a high concentration in developing neurons, and is known to be one of the major palmitoylated proteins in the brain. Since palmitoylated membrane

proteins are generally known to accumulate in lipid-raft domains, this protein should be a good candidate for the study of neuronal lipid-rafts. We found that GPM6a is colocalized with D4, a cholesterol-binding protein and a molecular imaging marker for lipid-rafts. Lipid-rafts containing GPM6a were clustered; and, in the absence of GPM6a, no rafts were clustered and D4 was dispersed in the membrane. We identified the downstream signaling molecules of GPM6a as the Rufy3-Rap2-STEF/Tiam2 complex, and the components of this complex have been previously reported to be determinants for cell polarity. GPM6a signaling pathway molecules are collected and concentrated in lipid-rafts in a manner that is dependent on the palmitoylated form of GMP6a, even at stage 1 of neuron formation. The combined data indicate that palmitoylated GPM6a induces the clustering of lipid-rafts, and that a palmitoylation-dependent GPM6a-signaling protein complex is formed in the lipid-rafts at stage 1 of neuron formation, which acts as one of the earliest polarity determinants and accelerators for neuron formation[1), 2)].

References: 1) Nozumi, M. et al. Cell Rep. 18: 2203-18 ['17]; 2) Honda, A. et al.: J Neurosci 37: (*in press*) ['17].

MTU09-11

Branching patterns and volume density of hippocampal immature neurons exposed to exercise and complex enriched environments

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Adult neurogenesis in the dentate gyrus of the hippocampus was investigated in the Long-Evans (LE) rats exposed to the standard laboratory environment, running wheel exercise as a single influencing factor and a complex enriched environment. After 28 days exposure six LE rats in each group were transcardially perfused with 4% paraformaldehyde (PFA) in PBS. Brains were carefully removed, post-fixed in PFA sagittal frozen sections cut at 50 μm. The brain sections were treated with Cresyl violet for cytoarchitecture. Immunohistochemical and immunofluorescence techniques using doublecortin (DCX) identified immature neurons and their processes, synaptophysin for synapses and synaptobrevin for spinogenesis. Volume density of the dentate gyrus was measured using the VOLUMEST application on the Image J software. Results showed a significant increase in brain weight ($p \leq 0.5$) for the complex enriched compared to the running and control groups but no statistical significance in the brain/body index. The DCX immunopositive results indicated the neuron structure, dendritic branching patterns, as well as neuronal arrangements on the dorsal and ventral limbs of the dentate gyrus was variable among groups. DCX post mitotic neurons were distributed more on the ventral limb of the dentate gyrus compared to the dorsal limb in exercise and enriched groups compared to the standard group. In enriched group were post mitotic neurons with single dendrite that extended to the molecular cell layer of the dentate gyrus before dividing into secondary and tertiary dendrites. Immunofluorescence showed neuronal soma and process of the DCX positive cells. Synaptophysin and synaptobrevin presented as diffuse neuronal clusters over the subgranular zone with no significant differences. Volumetric density was least in the control and greatest in the enriched environment with no statistical significant differences observed between the groups. Enriched environment increased the potential for preferential generation and integration of new neurons in specific limb of the dentate gyrus.

MTU09-12

Promotion of mTOR signaling in neural progenitors exposed to the green tea amino acid theanine

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Chewable tablets enriched of theanine, which is an amino acid ingredient in green tea by 2–3% with a structural analogy to glutamine rather than glutamate in terms of the absence of free gamma carboxylic acid, is now on sale in Japan as a dietary supplement expected to be beneficial for the prophylaxis of cognitive impairments on the basis of our previous findings on neural progenitor cells. In this study, we evaluated the intracellular mechanisms underlying the promotion of both proliferation and subsequent neuronal differentiation in neural progenitor cells exposed to theanine. Neural progenitor cells were cultured with theanine at different concentrations, followed by subsequent determination of the endogenous levels by Western blotting of several key intracellular proteins for the mammalian target of rapamycin (mTOR) pathway responsible for the cell growth in a manner sensitive to intracellular glutamine levels. Exposure to theanine not only induced marked upregulation of transcript expression of the glutamine transporter Slc38a1 in neurospheres composed of clustered proliferating progenitors from embryonic mouse neocortex at concentrations above 10 mM, but also resulted in facilitated phosphorylation of mTOR and downstream p70S6K and S6 proteins without affecting the p70S6K protein level. Stable overexpression of Slc38a1 markedly promoted the phosphorylation of mTOR and downstream relevant proteins in proliferating embryonal carcinoma P19 cells, while theanine failed to additionally promote the facilitated phosphorylation of proteins related to the mTOR signaling in these stable Slc38a1 transfectants. In embryonic murine neural progenitor cells previously exposed to theanine, a significant increase was seen in the number of cells immunoreactive for the neuronal marker protein MAP2 along with a decreased number of GFAP-positive cells after spontaneous differentiation in the absence of theanine. These results suggest that theanine promotes proliferation and neuronal differentiation through a mechanism relevant to activation of the mTOR signaling pathway required for self-renewal toward accelerated neurogenesis in murine undifferentiated neural progenitor cells.

MTU09-13

GM1 neurotrophic properties are related to GM1 oligosaccharide - TRKA interaction

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Several data suggest a specific role of ganglioside GM1 in neuronal differentiation and development, but the molecular mechanisms of these processes are largely unknown. The involvement of GM1 ganglioside in the process of neurite production has been reported for many years. Here, we report that the only GM1 oligosaccharide, rather than the ceramide portion or the total molecule, is directly involved in these processes.

GM1, or its oligosaccharide, was added to the neuroblastoma cell culture medium and neurite outgrowth evaluation was accomplished by phase contrast microscopy; neurofilament expression and Trk pathway activation were evaluate by Western Blotting.

GM1 oligosaccharide induced neuritogenesis and stimulated Trk pathway activation. Neurite elongation was accompanied by an increase of the neurofilament protein expression. The comparison of the results obtained with GM1 suggests a direct action of GM1 oligosaccharide in the neuritogenesis processes and in Trk activation in murine neuroblastoma cells. This means that the specific role exerted by changes of the membrane ganglioside GM1 content, described in the past, is determined by a direct interaction between the GM1 oligosaccharide portion and specific proteins. This allows considering both the *trans*- and *cis* -interaction via a head-to-head and side-by-side interaction, respectively. Trk receptor is involved in the process by direct interaction or by interaction through intermediate proteins.

MTU09-14

Functional analyses of TPT1 in neural stem/progenitor cells and glioma initiating cells

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MIF (Macrophage migration inhibitory factor) was identified as a functional molecule, which supports the proliferation and/or survival of murine neural stem/progenitor cells (NSPCs) using functional cloning strategy (Ohta et al., JCS. 2012). In the functional cloning procedure, we also identified a new factor, TPT1 (Tumor Protein Translationally-Controlled 1). TPT1 is expressed in cultured mouse NSPCs and the ventricular zone of mouse brain at embryonic day 14.5. Intriguingly, MIF-treated murine NSPCs increased the Tpt1 gene expression. Overexpression of Tpt1 in mouse NSPCs increased the cell proliferation *in vitro*. In human ES-derived NSPCs (hES-NSPCs), lentivirus-mediated gene silencing of MIF decreased the gene expression of TPT1. Over-expression of TPT1 increased the cell proliferation and in contrast, lentivirus-mediated gene silencing of TPT1 decreased the cell proliferation and neurogenesis in hES-NSPCs. In addition, the TPT1 gene silenced hES-NSPCs showed the decrease of the S-phase fraction accompanied with the up-regulation of p21 gene expression, and increased the apoptotic activity. We also performed RNA sequencing and confirmed the gene expression changes of cell cycle related factors in the TPT1 gene silenced hES-NSPCs. Moreover, we tried to identify miRs regulated by TPT1 in hES-NSPCs using Taqman array gene cards, and identified miR338-3p as a TPT1 downstream target. The TPT1-miR338-3p-SMO axis was newly identified as regulating the cell proliferation of hES-NSPCs *in vitro.* Taken together, MIF-regulated TPT1 contributes to the proliferation and/or survival of NSPCs in both mouse and humans. Otherwise, we also reported the functions of MIF and CHD7 (chromodomain-helicase-DNA-binding protein 7) in glioma initiating cells (Fukaya et al., Cancer Res., 2016; Ohta et al., Mol. Brain, 2016), regulating the cell proliferation. Finally, we found that TPT1 gene expression was regulated by MIF and CHD7, respectively, and TPT1 gene silencing decreased the cell proliferation in the glioma initiating cells. Together, these results may contribute to the development of new therapeutic targeting for glioma.

MTU09-15

Endogenous galectin-1 is not required for normal axonal development in early embryo stages and posterior locomotor function

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It was recently described that Galectin-1 (Gal-1) promotes axonal growth after spinal cord injury. This effect depends on protein dimerization, since monomeric Gal-1 fails to stimulate axonal re-growth. Gal-1 is expressed *in vivo*, at concentrations that favor the monomeric species. The present study aims to investigate the role of endogenous Gal-1 in normal axonal development and locomotor behavior in mice.

In order to characterize axonal development in *lgals-1*$^{-/-}$ mouse embryos, we resorted to a combination of the 3-DISCO technique and optical cutting-edge technologies, such as 1-photon and epifluorescence microscopies under high power LED illumination followed by serial image section deconvolution and 3-D reconstruction. Using cleared whole *lgals-1*$^{-/-}$ embryos, 3-D axonal cytoarchitecture was analyzed, evaluating axonal development, number of fibers and fluorescence density, length and shape of single neurofilaments in the axonal sprouting, deep in the whole tissue. Gal-1 deficient embryos did not show morphological/anatomical alterations in any of the axonal populations and different parameters analyzed. In addition, specific guidance receptor PlexinA4 did not change its axonal localization in the absence of endogenous Galectin-1. Finally, the absence of endogenous Gal-1 did not change the normal locomotor activity in post-natal stages.

Taken together, our results show that the absence of endogenous Gal-1 does not modify normal axonal development and *in-vivo* locomotor abilities. In agreement with our previous observations, the present results further validate the use of *lgals-1*$^{-/-}$ mice as a model system to evaluate the action of this lectin on different traumatic neuropathologies such as spinal cord- or traumatic brain injury.

MTU09-16

Molecular and cellular causes of severe heterotopia: identifying new genes playing a key role in radial glial cells

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Subcortical heterotopias are malformations of cortical development associated with epilepsy and intellectual disability, and characterized by the presence of ectopic neurons in the white matter. Mouse models of this disorder are rare, although mutations were identified in the microtubule-binding protein Eml1/EML1 in the spontaneous *HeCo* (‘*heterotopic cortex*’) mouse, and in patients exhibiting giant ribbon-like heterotopia.

To explore the bases of this disorder, a cohort of patients showing an EML1-like phenotype was selected for further investigations. These patients showed giant heterotopia with polymicrogyria, or periventricular heterotopia and partial agenesis of the corpus callosum. Patient DNA samples were analyzed by exome sequencing. Candidate genes and pathways are being studied, particularly *DLGAP4,* which belongs to a membrane-associated guanylate kinase family. The role of this gene during neurodevelopment in progenitor cells has not been previously studied. Expression analyses confirmed its presence in the ventricular zone from early corticogenesis in the mouse brain. Using different cell lines, potential partners of Dlgap4 were identified. Predicting the consequences of a *de novo* human mutation using the I-Tasser method, revealed the extent of loss and modification of DLGAP4′s potential functions. To further test its role in RGCs during cortical development, *in utero* electroporation was performed in mouse embryos. Our results strongly suggest that Dlgap4 is involved in the maintenance of cell polarity at the ventricular lining and when it is mis-regulated, induces changes in ventricular lining integrity.

This work reveals unsuspected molecular mechanisms important for the function of cortical progenitors, which when perturbed produce severe EML1-like heterotopia phenotypes in mouse and human.

MTU09-17

ENU mutagenesis screening in mice identifies a mutation that causes microcephaly

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Microcephaly is a neurodevelopmental disorder that is characterized by smaller brain volume. Primary microcephaly is present at birth, whereas secondary microcephaly develops postnatally and is a progressive neurodegenerative condition. The birth incidence of primary microcephaly in humans differs from 1.3 to 150/100000, depending on the population type and consanguineous populations.

In order to identify genes causing primary microcephaly in mice we conducted chemical mutagenesis using N-ethyl-N-nitrosourea (ENU) as a mutagene. ENU was injected into 8 weeks old C3H males in order to induce mutations in the sperm. 78 males recovered fertility after 5 months and were mated to C3H females. The offspring of these mating were crossed to C57B6 mice in order to conduct screening for recessive mutations causing microcephaly. We identified a recessive mutations S1-5 that demonstrated primary microcephaly. The phenotype of this mutant will be presented.

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MTU09-18

Adhesive property of neuromesodermal progenitor-derived neural stem cells is regulated by wnt signalling

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It is becoming evident that the properties of neural stem cells (NSCs) are highly heterogeneous depending on their locations and/or the developmental origin. A subset of posterior NSCs have recently shown to be derived from tail tip neuromesodermal progenitors (NMPs) which exhibit bi-potential to produce either neural cells or mesodermal cells. Junctional neurulation is a unique developmental program where primary and secondary neurulation meets to shape a discrete region of the spinal cord. This transition zone of junctional neurulation is highly susceptible to neural tube defects. *In vivo* lineage tracing with TcreER2:Rosa-EGFP transgenic mice revealed that NMPs-NSCs are confined to the secondary neural tube at the lumbosacral level that overlaps dorsally with primary neural tube to form the junctional neural tube. Interestingly, here we discovered that NSCs in the secondary neurulation regions is significantly adhesive and exhibit collective migration properties comparing to the NSCs in the primary neurulation region. By alteration of Wnt/β-catenin signalling significantly altered primary neural tube-NSCs toward secondary neural tube-NSC-like phenotypes. These data illustrate that different adhesive properties of NSCs along with longitudinal axis of neural tubes may be implicated in the formation of junctional neurulation.

MTU09-19

Establishment of human pluripotent stem cell reporter lines expressing tdtomato in oligodendrocyte progenitors

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Several protocols to generate oligodendroglial lineage cells from human pluripotent stem cells (hPSC) have been successfully established. A major obstacle to improving the efficiency of these protocols is the inability to monitor differentiation in live cells in real time. Here we report the generation of lineage-specific hPSC reporter lines expressing tdTomato under the control of the oligodendrocyte progenitor marker PDGFRα. We show that PDGFRα-tdTomato hPSC reporter lines retain the features of undifferentiated hPSCs. We also show that the expression of reporter gene closely parallels that of endogenous PDGFRα. In future applications, we aim to use these reporters to assess changes in oligodendrocyte differentiation in models of neurological disorders. These reporter lines can also be used to refine differentiation methodologies to increase the efficiency and homogeneity of oligodendrocyte derived from hPSCs, an important requisite for therapeutic applications.

MTU09-20

Radial glial cell anomalies contributing to ectopic progenitors in Eml1 mouse mutants

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Cortical development is a finely regulated process that depends on different neuronal progenitors, whose division modes, morphology and location within the developing cortical wall are critical characteristics that determine accurate development of the neocortex.

We are studying the spontaneously arisen *HeCo* mouse which constitutes a model for subcortical band heterotopia (SBH), a severe cortical malformation characterized by the presence of mis-localized neurons in the white matter and beneath the normotopic cortex. Mutations were found in the microtubule-associated protein Eml1/EML1, in the *HeCo* mouse, as well as in patients with severe atypical heterotopia (Kielar et al., 2014). In early corticogenesis, *HeCo* mice present ectopic progenitors in regions where the heterotopia forms, although the causes leading to their delamination from the ventricular zone still need to be fully understood.

We are investigating the mechanisms leading to ectopic progenitors, by focusing on a main neural progenitor type particularly affected in this mouse mutant, the radial glial cell (RGC). We have shown *HeCo* RGCs present spindle orientation (Kielar et al., 2014) and mitotic spindle length abnormalities when compared to the WT. To study the *HeCo* ventricular surface in more detail, we are focusing on interphase RGCs. In early corticogenesis, we observe a proportion of bigger cells in the *HeCo* ventricular lining, associated with a decreased density of the total number of apical domains observed. In addition, we detected anomalies in centrosome and primary cilia, indicating that apical end-feet of *HeCo* interphase RGCs are perturbed. Experiments with patient fibroblasts support the defects in primary cilia revealed in the mutant mouse. Studying these apical components in *HeCo* mice using different microscopy techniques, as well as elucidating molecular mechanisms involving Eml1, will shed light on progenitor cell regulation and position, critical for correct cortical development.

MTU09-21

PGC-1α induces neural stem cell differentiation and reverses cognitive deficits in aβ- induced toxin model of AD

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New neurons are continuously being generated from neural stem cells (NSCs) to maintain critical functions and repair processes in the adult brain. However, in Alzheimer's disease (AD) the proliferation and differentiation of NSCs is reduced and generation of functional neurons is principally impaired resulting in progressive memory decline and subsequent loss of cognitive functions. Therefore, potential mechanisms that can prevent Aβ toxicity and support neuronal regeneration against Aβ induced neurotoxic insults are much needed. PGC-1α, a master integrator of energy metabolism is known to regulate diverse functions, however, so far its role in the regulation of NSCs self-renewal, proliferation and differentiation remain largely uncharacterized. In the present study, we first evaluated whether PGC-1α integrates with the genes and transcription factors that control self-renewal, proliferation, differentiation and survival of NSCs in the hippocampus. Second, whether promotion of PGC-1α expression in AD model can restore diminished NSC pool, damaged neural circuitry and impaired cognitive functions. To discern the role of PGC-1α in NSCs proliferation and differentiation, we performed RNA interference and AAV-mediated overexpression of PGC-1α in the hippocampus. The shRNA-mediated knockdown of PGC-1α in the hippocampus resulted in decreased pool of NSCs and BrdU-positive cell proliferation consequently leading to dysregulation of neurogenesis. On the contrary, PGC-1α over-expression both *via* viral-mediated gene transfer and pharmacologically by Nicotinamide/AICAR up-regulated the mRNA expression of neurogenic transcription factors/genes such as neuregulin, neuroD1 and suppressed the expression of STAT3. Furthermore, the co-localization of BrdU with DCX, NeuN was enhanced signifying the importance of PGC-1α in the generation of mature and 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 Aβ 1-42 induced AD model. AAV and Nicotinamide mediated upregulation of PGC-1α expression in AD model enhanced cognitive function. Overall, these results illustrate a highly specific role of PGC-1α in regulating adult hippocampal neurogenesis and selective upregulation of PGC-1α may provide an effective strategy in ameliorating memory loss associated with AD.

MTU09-22

The green tea amino acid theanine for possible improvement of cognitive declines

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Theanine is an amino acid enriched in green tea by 2–3% with a chemical structure analogous to glutamine rather than glutamic acid. We have been studying pharmacological profiles of this green tea amino acid in neural progenitor cells, which are endowed to proliferate for self-replication and to differentiate into progeny lineages such as neuronal, astroglial and oligodendroglial cells, in embryonic, developing and adult rodent brains. Progenitor cells were exposed to theanine at different concentrations for determinations of the size of neurospheres composed of clustered proliferating cells and MTT reducing activity, followed by culture in the absence of theanine for immunocytochemical detection of the cells immunoreactive for MAP2 and GFAP among cells stained with Hoechst33342. In cultured neural progenitor cells from embryonic rat and mouse neocortex, theanine invariably promoted the formation of neurospheres and MTT reduction in a concentration-dependent manner at 1 to 100 μM, followed by facilitation of spontaneous differentiation into cells immunoreactive for MAP2 with a concomitant decreased number of GFAP-positive cells. In cultured progenitor cells from the hippocampus of adult nestin-GFP mice, theanine significantly increased the size of neurospheres. In murine embryonic carcinoma P19 cells exposed to theanine, similar facilitation was seen in proliferation and subsequent spontaneous neuronal differentiation. Upregulation was induced for the glutamine transporter *Slc38a1* transcript in rat and mouse progenitors exposed to theanine for 4 days, but not for 2 days, whereas theanine failed to further promote both proliferation and neuronal differentiation abilities already facilitated in P19 cells stably overexpressing *Slc38a1*. Significant alleviation was seen in cognition impairment scores measured by double-blinded physicians in healthy age-matched elderly people given capsules of green tea enriched of theanine than those given normal green tea capsules after daily oral intake for 7–12 consecutive months. We have made a dietary supplement product enriched of theanine as a chewable tablet for online sale to expect a beneficial support for the prophylaxis of particular cognition impairments.

MTU09-23

Regulation of proliferative activity by protease-activated receptor 1 in neural stem/progenitor cells generated after neuronal deg

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It is now clear that there is a continual turnover of the mammalian hippocampal dentate gyrus (DG) neurons throughout life even in adult. Various neurological injuries are widely recognized as promoting endogenous neurogenesis in DG. Thrombin-activated/protease-activated receptor-1 (PAR-1) is known to regulate proliferation of neural cells following brain injury including intracellular hemorrhage. Our previous studies demonstrated that the systemic treatment with trimethyltin chloride (TMT) causes the granule cell loss in the mouse DG, with being regenerated in the dentate granule cell after neuronal loss. To elucidate the roles of PAR-1 in neuroregeneration after neuronal degeneration, we evaluated the expression of PAR-1 in the newly generated cells following neurodegeneration in the DG of adult mouse. *In vivo* experiments, mice were given TMT to prepare hippocampal slices for immunohistochemical analysis using antibody against PAR-1 and nestin [neural stem/progenitor cells (NPCs) marker]. Cells positive for PAR-1 and nestin markedly increased in the DG on day 3–5 after TMT treatment. *In vitro* experiments, the exposure of NPCs derived from the DG after neuronal degeneration to thrombin significantly attenuated cell proliferation by bromodeoxyuridine incorporation assay. Our results suggest that PAR-1 has a critical role in proliferative activity inNPCs generated following neuronal degeneration in the DG.

MTU10 Brain Bioenergetics

MTU10-01

Impaired mitochondrial and metabolic activity in neurons derived from human induced pluripotent stem cells of FTD patients

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Frontotemporal dementia (FTD) is the third most common form of primary degenerative dementia, accounting for up to 20% of young onset dementia. FTD is a neurodegenerative disorder characterized by cognitive impairment affecting the frontal and/or temporal lobes of the brain associated with progressive brain atrophy. Given that mitochondrial defects are commonly observed in neurodegenerative diseases, we investigated whether mitochondrial respiration and energy metabolism are affected in neurons derived from human induced pluripotent stem cells (hIPSC) obtained from FTD patients. With this aim, forebrain region-specific neurons were derived from FTD patients hiPSC lines expressing NESTIN, FOXG1, OTX2 and PAX6 mRNA. After subsequent maturation, glutamatergic cortical neurons expressing MAP2AB, TUJ1, TAU, VGLUT1, TBR1 and CTIP2 mRNA were obtained. Mitochondrial morphology and function were assessed using transmission electron microscopy and real-time monitoring of oxygen consumption via the Seahorse XFe96 Analyzer, respectively, in cultured hiPSC-derived neurons. The results were compared to CRISPR/Cas9-edited isogenic controls and neurons derived from an age-matched healthy subject. Ultrastructure analysis revealed mitochondria with poorly developed cristae as well as abnormal localization in the FTD patient-derived neurons. In line, oxygen consumption associated with mitochondrial activity was decreased in the FTD neurons. All of the observed phenotypes were reversed after targeted gene corrections in the isogenic controls. Our findings indicate that hiPSC-derived neurons from FTD patients display significant mitochondrial dysfunction that could potentially be targeted for treatment development.

MTU10-02

Augmented cerebral mitochondrial function and hippocampal ketone body metabolism in Db/db mice

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Aim: Type 2 diabetes mellitus (T2DM) is a risk factor for the development of Alzheimer's disease (AD). Hypometabolism of glucose have been observed in pre-symptomatic patients and animal models of AD, suggesting a fundamental pathological mechanism. The aim of this study is to elucidate cerebral metabolic consequences of T2DM to possibly reveal accelerating factors of T2DM on AD pathology.

Materials and methods: Db/db mice were used as a T2DM animal model at 16 weeks of age. Acutely isolated cerebral cortex and hippocampus slices of db/db mice were incubated in media containing [U-^{13}C]glucose or [U-^{13}C]β-hydroxybutyrate. Oxygen consumption and ATP synthesis rate of isolated whole-brain mitochondria were assessed by SeaHorseXFe96 and on-line luciferase based assay, respectively.

Results: Decreased ^{13}C enrichment from [U-^{13}C]glucose metabolism were observed in key metabolites in extracts of both cerebral cortical and hippocampal slices of the db/db mice. However, the glucose hypometabolism was more prominent in the cerebral cortex. Incubations with the ketone body [U-^{13}C]β-hydroxybutyrate showed increased ^{13}C labeling in citrate, glutamate and glutamine in hippocampal slices of db/db mice. These changes were absent in the cerebral cortex. Isolated whole-brain mitochondria from db/db mice, surprisingly displayed augmented oxygen consumption when stimulated with ADP, when pyruvate and malate were provided as substrates. This finding was supported by a significantly increased ATP production from isolated brain mitochondria of the db/db mice.

Conclusion: Cerebral metabolism and energetics are affected in the db/db mouse. Hypometabolism of glucose is evident in the cerebral cortex and hippocampus. However, the hippocampus of db/db mice, exhibits augmented ketone body metabolism. Mitochondria isolated from the db/db brain showed a significant increase in respiration and ATP synthesis. The results suggest that the hypometabolism of glucose is the major deleterious effect of T2DM on brain energy metabolism. However, the increased ketone body utilization and augmented mitochondrial efficiency suggests compensatory mechanisms, possibly related to the glucose hypometabolism, in the diabetic brain.

MTU10-03

Compartmentalised signalling-metabolism coupling in brain cells - putative drug targets for neurological diseases?

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Faulty cellular signalling and metabolism is a hallmark of a number of brain diseases. In the field of cell biology it is becoming increasingly apparent that signalling pathways are compartmentalised in both space and time within the cell, and that an increased understanding of these signalling events may lead to the discovery of novel drug targets.

Compartmentalised coupling of cAMP and calcium signalling to brain cell metabolism has already been demonstrated implying a relevance to brain physiology and pathology.

In this poster presentation, we attempt to make the case that an appreciation of compartmentalised cellular signalling and metabolism is warranted to advance our understanding of cellular neuroscience, and to discover novel drug targets.

MTU10-04

Comparison diffusion behavior of metabolites in brains of congenital portal systemic shunt and healthy mice *in vivo* at 14.1 t

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Diffusion-weighted ^{1}H MRS allows investigating the cellular compartmentalization of molecules in the living organs and may shed insight on alterations of cellular restrictions faced by metabolites in different cerebral abnormalities and diseases. The aim of this study was to determine whether diffusion behavior of metabolites in the congenital portal systemic shunt (PSS) mouse brain is different from ones of the healthy mouse *in vivo,* combining large diffusion weighting and ^{1}H MRS methods.

All experiments were performed on a 14.1T magnet using a home-built quadrature transceiver. Six adult PSS and six age-matched healthy (Ctrl) C57BL/6J mice have been prepared and anesthetized using isoflurane. ^{1}H-MRS data were acquired using localized diffusion-weighed STEAM-based spectroscopic pulse sequence (TE=16 ms and a mixing time of 113 ms), covering the b range from 0 to 45 ms/μm^2. Quantified data by LCModel were fitted using bi-exponential equation. Diffusion behavior of metabolites in the mouse brain was compared between PSS and Ctrl group.

The remarkable sensitivity and spectral resolution of localized short-echo ^{1}H MRS at 14 T allowed a precise measurement of the diffusion properties of metabolites in the brain of PSS and Ctrl mouse *in vivo* at very high diffusion weighting. The comparable diffusion properties of most investigated metabolites in the brain *in vivo* between PSS and Ctrl mice may indicate that unaltered barrier and cellular restriction dominate on the diffusion of metabolites in both group and therefore could support the hypothesis about the similarity of intracellular distribution space for these metabolites in PSS mice when compared to Ctrl mice. The slightly different diffusivity of Tau may be ascribed to possible cellular redistribution of Tau in PSS mice, however, it needs to be further explored.

MTU10-05

Intracisternal injection of [U-^{13}C]glucose for investigating brain metabolism in freely moving mice

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Purpose: To investigate brain metabolism using intracisternal delivery of [U-^{13}C]glucose, thus bypassing blood-brain barrier and avoiding effects of peripheral metabolism.

Methods: Mice (C57BL/6JRj, 8wo) were implanted a chronic cannula into cisterna magna. After recovery (24 h) an isosmolar 0.3M [U-^{13}C]glucose solution was infused using a microinjection pump. Animals were sacrificed by microwave irradiation. ^{13}C-labeling and metabolite amounts were determined using mass spectrometry and HPLC. Glycogen content was determined as glucose units after amyloglucosidase treatment.

Results: [U-^{13}C]Glucose injected at 2 μL/min (10 μL) resulted in fast label incorporation into brain lactate as well as glutamate and glutamine. Lactate labeling rapidly (within 10 min) decreased by about 50%, while enrichment in glutamate and glutamine kept increasing in the same time interval. Lactate was the only labeled compound recovered in cervical lymph nodes. [U-^{13}C]Glucose injected at 0.3 μL/min (4.5–18 μL) resulted in progressive rise of label incorporation into brain lactate, glutamate, glutamine, aspartate and GABA. Labeling of these compounds was significantly faster in awake than anesthetized animals. The absolute concentrations of glutamate and GABA were higher in the awake state whereas that of glutamine was lower (~20% changes). Brain glycogen was higher (+50%) during anesthesia and was negatively correlated with glutamate/GABA and positively correlated with glutamine.

Conclusions: Our results indicate that lactate is produced in excess of its utilization and rapidly leaves the brain, possibly through brain lymphatics. The rate of aerobic glycolysis is higher during wakefulness than anesthesia and so is the rate of transmitter synthesis, suggesting higher glutamatergic and GABAergic tone in awake animals. The correlations between brain glycogen content and glutamate/GABA and glutamine in different states indicate that glycogen synthesis/breakdown is modulated by brain activity and contributes as substrate to neurotransmitter synthesis, underlining its functional importance.

MTU10-06

Physiological roles of brain glycogen

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The role of brain glycogen has been traditionally associated with the preservation of neuronal function during energetically challenging states such as hypoxia, hypoglycaemia, ischemia, and seizures. Nevertheless, glycogenolysis also occurs in euglycaemia during an increase in neuronal activity, thus indicating that brain glycogen also supports neuronal function in non-pathological conditions. In order to address the physiological roles of glycogen in the brain, we generated a brain-specific glycogen synthase knockout mouse. These animals, that completely lack brain glycogen, show a significant deficit in learning capacity and in the activity-dependent changes in synaptic strength. Furthermore, they show greater susceptibility to hippocampal seizures and myoclonus following the administration of kainate and/or a brief train stimulation of Schaffer collaterals, which is in agreement with reports describing a relationship between brain glycogen and susceptibility to epilepsy. Within the brain, the presence of glycogen has been restricted mainly to astrocytes. Therefore, all physiologic roles of brain glycogen have been attributed exclusively to astrocytic glycogen. However our findings demonstrate the presence of an active glycogen metabolism also in neurons, which changes the current view of the role of glycogen in the brain. Taken together, our results reveal the relevant role played by glycogen in brain metabolism.

MTU10-07

Heterogeneity of energy metabolism of astrocytes

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Astrocytes are a cell type in the brain which plays an important role in brain energy metabolism. However, astrocytes are most likely a heterogeneous population of cells as the environment and therefore the requirements for these cells are very different in different areas of the brain and – most likely – also at different positions within the same brain region. Therefore, we hypothesized that both basal and stimulated energy metabolism as well as functional properties of astrocytes might be substantially different in different brain regions reflecting these diverse environments and requirements. To address these questions we took advantage of genetically encoded, fluorescent sensors for metabolites and studied in cultured cells and in acutely isolated brain slices the dynamics of key metabolites including lactate, ATP, and the NAD^+/NADH-redox state. We identified distinct differences in basal energy metabolism as well as in the main regulatory mechanisms within populations of astrocytes but also between astrocytes located in different brain regions. These results support the hypothesis that metabolism of astrocytes is subject to cellular heterogeneity, which might also contribute to brain region specific vulnerability in disease states.

MTU10-08

Inhibition of soluble adenylyl cyclase (SAC) causes a robust increase in glycolysis in cultured astrocytes

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One of the rate limiting steps in glycolysis is catalyzed by the enzyme phosphofructokinase-1, which is allosterically activated by fructose 2,6-biphosphate (F2,6BP). The bifunctional enzyme phosphofructokinase 2/fructose-2,6-bisphosphatase (PFK2/FBPase-2) is responsible for the production and degradation of F2,6BP and thus involved in regulating glycolysis. The activity of PFK2/FBPase-2 is regulated by phosphorylation by cAMP dependent Protein Kinase A (PKA) which couples cAMP signaling and thereby the adenylyl cyclases to glycolytic activity. At present time, there is no knowledge on which role sAC has in regulating PFK2/FBPase-2 and thereby glycolysis.

This work characterizes the effect of inhibition of sAC on glycolytic activity and whether this change is mediated via a change in F2,6BP levels. Working with cultured astrocytes, C6 glioma cells and HEK cells we show that that inhibition of sAC causes a robust increase in glycolytic activity and glucose uptake. The ratio between PFK2 and FBPase-2 activity is investigated to determine if the increase in glycolytic activity is caused by an increase in the level of F2,6BP. The findings provide novel insight into the cAMP signaling mechanisms mediated by sAC and how these are involved in metabolic regulation.

A greater understanding of the pathways between cAMP formation and the endpoint, increased glycolytic activity in this case, can potentially provide new drug targets. In neurogenerative diseases such as Alzheimer's disease a decreased cerebral glycolytic activity has been observed in both patients and animal models of the disease. Thus, sAC could potentially become a drug target for treating Alzheimer's disease.

MTU10-09

Homocysteine affects the glucose and glutamate metabolism of human glioblastoma cells

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Homocysteine is an intermediate of S-adenosylmethionine cycle, which increased level in blood positively correlates with occurrence of several diseases including neurodegeneration. In addition to its neurotoxic effect, homocysteine is also capable to initiate death of human astrocytes. Since glia-neuronal cooperation is considered to be essential for sustaining neuronal energy metabolism and glutamate-glutamine cycle, we investigated the putative effect of homocysteine on the human glial cells to metabolize glucose and glutamate. The human glioblastoma cells, T98G, were used as a study model. The cells were incubated in medium supplemented with D,L-homocysteine in levels up to 0.1 mM for 4 or 28 h. After desired time of incubation, the concentrations of glucose, lactate and glutamate in the media were analyzed enzymatically. Short term incubation of human glioblastoma cells in the presence of homocysteine had no effect on their glucose, lactate and glutamate metabolism. Prolonged incubation for 28 h with D,L-homocysteine, even at the level of homocysteine 0.05 mM, has significantly stimulated the glucose and glutamate uptake. The rate of lactate released remained unchanged at all tested conditions. Our results show that prolonged presence of homocysteine at the levels reachable during hyperhomocysteinemia in human may affect the glial metabolism of glucose and glutamate. Since glucose is a major substrate for energy metabolism in brain parenchyma, increased glial uptake of glucose during hyperhomocysteinemia that is not accompanied by lactate release may lead to decreased availability of the fuel molecules for sustaining the energy needs of neurons. Furthermore, stimulated uptake of the glutamate into the glial cells could disturb the glutamatergic neurotransmission. Taking together, homocysteine could influence not only the physiological functions of neurons but also glial cells and such combine effect may play a role in etiopathogenesis of neurodegeneration associated with hyperhomocysteinemia.

MTU10-10

Beta-hydroxybutyrate metabolism and metabolic compartmentation in brain

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There is renewed interest in the use of the ketone body β-hydroxybutyrate (βOHB) to treat neurological disorders but its metabolism in brain still requires thorough characterisation. Here,

we studied guinea pig cortical brain slices using increasing concentrations of [U-^{13}C]D-βOBH ([U-^{13}C]D-βOHB) in conjunction with [1-^{13}C]D-glucose under conditions of normo- and hypoglycaemia, as well as under high potassium (40 mmol/L K^+) depolarization in normo- and hypoglycaemic conditions. [U-^{13}C]D-βOHB at lower concentrations (0.25 and 1.25 mmol/L) was mostly metabolized in neurons and stimulated mitochondrial metabolism. In astrocytes it was incorporated into glutamine but did not stimulate metabolism in the cytosol. At higher concentrations (2.5 mmol/L) βOHB inhibited metabolism of [1-^{13}C]D-glucose and reduced total label incorporation and total metabolite pools. [U-^{13}C]D-βOHB could not substitute for glucose when glucose levels were reduced. Incorporation of label from [U-^{13}C]D-βOHB was decreased under depolarising conditions, showing that glucose was the preferred fuel under these circumstances. Unlike the case with labelled glucose, lactate and pyruvate, label from [U-^{13}C]D-βOHB was not used to make labelled acetate, suggesting that mitochondrial citrate made from [U-^{13}C]D-βOHB was not exported to the cytosol. Finally, inhibition of glutamine synthesis with MSO had no significant effect on incorporation of label from [U-^{13}C]D-βOHB into GABA C2,1 indicating that the majority of this GABA was synthesized *in situ* from [U-^{13}C]D-βOHB rather than from Gln C4,5 imported from astrocytes.

MTU10-11

Metabolism of mannose in cultured primary rat neurons

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Glucose is the main peripheral substrate for energy production in the brain. However, as other hexoses are also present in blood and cerebrospinal fluid, we have investigated whether neurons have the potential to metabolize, in addition to glucose, also the hexoses mannose, fructose or galactose. Incubation of primary cerebellar granule neurons in the absence of glucose or in the presence of fructose or galactose caused severe cell toxicity within 24 h, while the cells remained viable during incubation in the presence of either mannose or glucose. In addition, cultured neurons produced substantial and almost identical amounts of lactate after exposure to either glucose or mannose, while lactate production was low in the presence of fructose and hardly detectable during incubations without glucose or with galactose as carbon source. Determination of the K_M values of hexokinase in lysates of cultured neurons for the hexoses revealed values in the micromolar range for mannose (32 ± 2 μM) and glucose (59 ± 10 μM) and in the millimolar range for fructose (4.4 ± 2.3 mM), demonstrating that mannose is efficiently phosphorylated by neuronal hexokinase. Finally, cultured neurons contained reasonable specific activities of the enzymes phosphomannose isomerase, which is required for isomerization of the hexokinase product mannose-6-phosphate into the glycolysis intermediate fructose-6-phosphate. These data demonstrate that cultured cerebellar granule neurons have the potential and express the required enzymes to efficiently metabolize mannose, while galactose and fructose serve at best poorly as extracellular carbon sources for neurons.

MTU10-12

Rates oxidative metabolism in astrocytes and neurons are coupled to the glutamate-glutamine cycle in the tree shrew visual cortex

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Neuronal oxidative metabolism was shown to be coupled to the glutamate-glutamine cycle that represents glutamatergic neurotransmission. While metabolism in astrocytes is also stimulated by glutamatergic neurotransmission, it remains unclear whether the glutamate-glutamine cycle is coupled to glial oxidative metabolism. We took advantage of the columnar characteristics of the *Tupaia belangeri* primary visual cortex (V1) to measure metabolic changes induced by continuous stimulation of V1 using ^{13}C magnetic resonance spectroscopy (MRS) during infusion of [1,6-^{13}C]glucose *in vivo.*

Each animal under light isoflurane underwent the three MR modalities at 14.1T, namely blood oxygenation level-dependent functional magnetic resonance imaging (BOLD fMRI), ^{1}H and ^{13}C MRS localized in V1, either at rest (n = 4) or during stimulation (n = 5).

Visual stimulation resulted in a relatively large activated area in V1 that allowed localized MRS. Cortical brain activity resulted in a decrease in both brain glucose concentration (-17%; -0.34 μmol/g) and phosphocreatine/creatine ratio (−9%; −0.07) after 15 min of stimulation. At the individual level, close relationships between the neurotransmission rate (V_{NT}) and total cerebral metabolic rate of glucose oxidation ($CMR_{glc(ox)}$, R^2=0.68, P = 0.006), glial (V_{TCA}^g, R^2=0.66, P = 0.008) and neuronal (V_{TCA}^n, R^2=0.40, P = 0.066) oxidative metabolism were measured. At the group level, 20% increase in V_{NT} (+0.038 ± 0.042 μmol/g/min) resulted in a 24% (ΔV_{TCA}^g=0.063 ± 0.057 μmol/g/min) and 12% (ΔV_{TCA}^n=0.061 ± 0.032 μmol/g/min) increase in glial and neuronal TCA cycle activity, respectively, resulting in 14% increase in $CMR_{glc(ox)}$ (+0.058 ± 0.032 μmol/g/min).

We conclude that cortical brain activity resulted in a significant increase in cerebral metabolic rate of glucose, and an increase in both glial and neuronal oxidative metabolism. In both cells, the tricarboxylic acid cycle rate was correlated with the rate of the glutamate-glutamine cycle.

MTU10-13

Cortical neuronal glucose metabolism is impaired at mid stage in the hSOD1^{G93A} mouse model of amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a multi-pathogenic disorder mainly characterized by the selective loss of motor neurons

in brain and spinal cord. Although metabolic alterations have been reported in this disease, the specific biochemical changes in the energy producing pathways are unknown. We therefore investigated glucose metabolism in the superoxide dismutase ($hSOD1^{G93A}$) mouse model of ALS. Wild-type and $hSOD1^{G93A}$ mice (n = 11) at 17 weeks old were injected with 543 mg/kg [1-^{13}C] glucose and 504 mg/kg [1,2-^{13}C] acetate (i.p.) 15 minutes before sacrificed. Cerebral cortices were collected and extracted using methanol/chloroform. The labelled and unlabelled metabolites of amino acids were quantified using ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy and high performance liquid chromatography. Numerous metabolic alterations were found mainly related to neuronal glucose metabolism. Reductions in the amounts of glycolysis derived metabolites such as total and labelled lactate (by 28 and 54%) and total and labelled alanine (by 18 and 65%) respectively ($p < 0.05$) indicating impairments in glycolysis. Also, the incorporation of ^{13}C glucose via pyruvate dehydrogenase (PDH) enzyme into the 1st turn tricarboxylic acid (TCA) cycle metabolites such as [4-^{13}C] glutamate, [4-^{13}C] glutamine and [2-^{13}C] GABA was reduced by 42–54% ($p < 0.05$) showing less entry of glucose into the TCA cycle. The levels of some of the branched chain amino acids (BCAAs) such as isoleucine and leucine ($p < 0.05$) were reduced indicating a compensatory degradation of BCAAs mainly due to an increased energy demand and need for anaplerosis. There were minor changes in astrocytic metabolism including increased transfer of [1,2-^{13}C] acetate-derived glutamine towards the formation of GABA but no changes in the levels of [4,5-^{13}C] glutamate, [4,5-^{13}C] glutamine and [1,2-^{13}C] GABA or pyruvate carboxylase-derived metabolite levels. In conclusion, we found defective cortical neuronal glucose metabolism in the $hSOD1^{G93A}$ mouse model of ALS at symptomatic stage of the disease.

MTU11 Neuroimmunology

MTU11-01

Role of ck2 in t-cell differentiation and autoimmunity

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$CD4^+$ T cells are the major pathogenic cells in many autoimmune and inflammatory disorders, including Multiple Sclerosis (MS) and Experimental Autoimmune Encephalomyelitis (EAE), an animal model of MS. Th17 cells are important effector cells in the pathogenesis of MS, whereas regulatory T cells (Treg) are crucial in disease resolution. Current therapies for MS patients are incapable of stopping disease progression and are not curative. Thus, studies on the mechanisms of enhancing anti-inflammatory responses, such as enhancing trans-differentiation of pathogenic Th17 cells into beneficial Treg cells, may lead to new potential therapies for MS. Protein kinase CK2 (Casein Kinase II) is a constitutively active serine/threonine kinase composed of two catalytic subunits (alpha and/or alpha') and two regulatory beta subunits. CK2 is involved in the activation of multiple signaling pathways, including PI3K/AKT/mTOR and JAK/STAT, which are essential for the differentiation of $CD4^+$ T cells. However, little is known about the specific function of CK2 in T cells, and the consequences of CK2 inhibition during $CD4^+$ T cell differentiation and the pathogenesis of MS/EAE**.** Our data indicate that expression of the major catalytic subunit of CK2, CK2alpha, is induced in a time-dependent manner upon activation of $CD4^+$ T cells both *in vitro* and *in vivo*. Utilizing a small molecule CK2 inhibitor CX-4945 (Silmitasertib), we find that inhibition of CK2 kinase activity significantly ameliorates the severity of EAE disease, which is correlated with regulation of Th17 and Treg cell frequencies. Treatment with CX-4945 inhibits the differentiation of Th17 cells, while promoting the differentiation of Tregs. Our preliminary results indicate that conditional deletion of CK2alpha in $CD4^+$ T-cells inhibits Th17 differentiation and promotes Treg polarization, comparable to what was observed with CX-4945 treatment. Thus, we propose that CK2 kinase activity in $CD4^+$ T cells correlates with the pathogenesis of MS/EAE by promoting inflammatory Th17 cell responses and suppressing anti-inflammatory Treg cell development, thereby affecting the ratio of these two important $CD4^+$ T-cell subsets. Furthermore, inhibition of CK2 kinase activity may be a potential novel therapeutic treatment for MS patients.

MTU11-02

Generation of IL33 knockout mice and cell-based reporter assay for functional studies of IL-33 *in vitro* and *in vivo*

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Background: Interleukin-33 (IL-33), an IL-1 family cytokine, is a tissue-derived nucleus alarmin that drives inflammatory responses through binding to its receptor ST2. IL-33 is expressed by multiple tissues and released from nucleus upon tissue damage or inflammation.

Aims: In this study, we aimed to generate a cell-based functional reporter assay for screening of IL-33 activity. We also aimed to generate IL33 knockout mice for *in vivo* loss-of-function studies.

Methods and Results: We established the IL-33 reporter cells with stable expression of functional receptor complex for IL-33 (ST2L and IL-1RacP) and an AP-1/NF-kB/SEAP reporter cassette. The reporter cells secrete alkaline phosphates to the culture in response to IL-33 stimulation and the levels of the enzyme activity can be determined using colorimetric enzyme assay. Our results demonstrated the specificity of IL-33 in activation of ST2 signaling in the IL-33 reporter cells.

For generation of IL33 conditional deletion mice, the exon 5, exon 6 and exon 7 of the IL33 gene were flanked by two directional Lox-p sites. Germ line transmission and the establishment of IL33 floxed mice ($IL33^{fl/fl}$) were confirmed by allele-specific genotyping and diagnostic restriction analysis of PCR products. The $IL33^{-/-}$ were further generated from $IL33^{fl/fl}$ mice by crossing the mice to C57BL/6-Tg(UBC-Cre) to remove the loxP-flanked exons. Deletion of targeted exons was confirmed using allele specific PCR and RNA-sequencing analysis. Immunofluorescent staining and ELISA analyses also confirmed the deficiency of IL-33 protein in $IL33^{-/-}$mice.

Conclusions: We have established a cell-based reporter assay for functional analysis of IL-33 activity. We also generated $IL33^{-/-}$ mice and $IL33^{fl/fl}$ mice for conditional knockout studies. These materials will be useful for further investigation of the role of IL-33 in health and diseases.

MTU11-03

Curcumin reverses altered expression of GFAP astrocytes marker and hypercorticosolemia in depressed rats

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Astrocyte pathology has been consistently observed in Major Depressive disorders (MDD) and this could be seen in the altered expression of proteins for astrocyte markers such as the Glial Fibrillary Acid Protien (GFAP). Astrocyte deficit observed in MDD, astrocytes may become novel targets for antidepressant medication. The present study investigated the effect of curcumin and fluoxetine antidepressant drug on the expression of GFAP astrocyte marker and corticosterone (CORT) in depressed rats. Depression was induced in albino wistar rats using a modified Chronic Unpredictable Stress (CUS) method for 42 days. The rats were grouped into six groups of ten rats each. The Control received distilled water only. Depressed group received distilled water after CUS. Olive oil group received 0.8 ml/kg of virgin olive oil for 42 days after CUS. Curcumin group received 30 mg/kg of curcumin for 42 days after CUS. Fluoxetine group received 20 mg/kg of fluoxetine for 42 days after CUS. Fluoxetine+Curcumin group received 30 mg/kg of curcumin and 20 mg/kg of fluoxetine for 42 days after CUS. There was increased astrocyte pathology as indicated by the increased

intensity in expression of GFAP astrocyte marker, hypertrophy of the astrocyte cell bodies and processes as well as increased scar formation in the Prefrontal cortex, dentate gyrus and CA3 region of the hippocampus of depressed rats compared to the control group. On the other hand, there was reduced intensity of GFAP expression in astrocyte cell bodies and processes in the Prefrontal cortex, dentate gyrus and CA3 region of the hippocampus of curcumin treated rats as well as the curcumin+fluoxetine treated group and in the control group. The CORT level was significantly increased in depressed rats compared to the control group whereas there was no significant difference observed in the curcumin treated group and other treatment groups. Hence, curcumin has shown to ameliorate the increased astrocyte pathology and hypercortisolemia seen inMDD.

MTU11-04

c-Abl regulates rotenone-induced inflammatory response via the activation of NLRP3 inflammasome

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Multiple evidences support the hypothesis that exposure to pesticides increases the risk of PD. Emerging evidence indicates that intracellular inflammasome complex namely NLRP3 complex is involved in the recognition and execution of host inflammatory response. Thus in the present study we investigated the hypothesis that NLRP3 inflammasome activation is linked to rotenone- induced microglial activation which is dependent upon a priming stimulus by a pathogen associated molecular pattern (PAMP) or damage associated molecular pattern (DAMP) respectively. We employed primary microglia, BV-2 microglial cell culture, and an *in vivo* endotoxin model of neurodegeneration to address the stated hypothesis. We found that LPS priming accelerated rotenone-induced NLRP3 inflammasome activation that was associated with the activation of caspase-1 and subsequent proteolytic processing and release of IL-18 and IL1β as well as the release of TNFα and IL-6 as assessed via WB analysis and Luminex multiplex technology. Mechanistic studies revealed c-Abl/PKCδ kinase signaling axis as a proximal signal that exacerbated rotenone-induced NLRP3 inflammasome activation, that is mediated via mitochondrial and autophagolysosomal system (ALS) dysfunction and accompanying downregulation of TFEB, a lysosomal transcription factor. Intriguingly, gene silencing and pharmacological inhibition of c-Abl attenuated NLRP3 inflammasome activation, mitochondrial and ALS dysfunction and PKCδ activation; while, c-Abl overexpression potentiated that response in LPS primed rotenone treated microglial cells. Furthermore, using an *in vivo* LPS model of neurodegeneration we showed that c-Abl upregulation positively correlated with NLRP3 inflammasome activation and accompanying sickness-like behavior. Our findings demonstrate for the first time that c-Abl/PKCδ signaling axis is a key regulator of NLRP3 inflammasome activation which is mediated partly via dysregulation of ALS and mitochondrial function during rotenone-induced microglial activation (supported by NS088206).

MTU11-05

S-guanylation of SNAP-25 by 8-nitro- cGMP attenuates the interaction of snare complex with complexin

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Introduction: 8-Nitroguanosine 3′,5′ -cyclic monophosphate (8-nitro-cGMP) is an unique second messenger, which is generated in the nitric oxide/reactive oxygen species signal. This molecule covalently binds to protein thiol groups, called protein S-guanylation, and alters protein functions. On the other hand, exocytosis is catalyzed by soluble N-ethylmaleimid-sensitive factor attachment protein receptor (SNARE) proteins and is modulated by some modulatory proteins. Complexin is a small protein that binds to SNARE complex with high affinity and modulates exocytosis. Previously we reported synaptosomal-associated protein 25 (SNAP-25) as a target protein of S-guanylation, however, the neurophysiological functions have not been clarified. Here, we investigated the role of S-guanylation of SNAP-25 by 8-nitro-cGMP in neurons.
Material and methods: The localization of 8-nitro-cGMP and S-guanylated proteins in the Wistar rat brain were confirmed by immunohistochemistry using each specific antibody. To confirm the effect of 8-nitro-cGMP for the interaction between SNARE complex and complexin, we performed pull-down assay using GST-tagged complexin, co-immunoprecipitation and blue native (BN)-PAGE followed by western blotting. SH-SY5Y neuroblastoma cells were transfected with FLAG-tagged SNAP-25 (wild-type and C90A) or V5-tagged complexin, treated with 8-nitro-cGMP, and then used as samples.
Results and discussion: The result of the immunohistochemistry revealed that the 8-nitro-cGMP and S-guanylated proteins were localized in neurons in the brain. Pull-down assay revealed that the amount of SNAP-25 pulled-down by GST-complexin was decreased by S-guanylation of cys90 in SNAP-25. We could obtain almost the same results from co-immunoprecipitation. Furthermore, BN-PAGE followed by western blotting revealed that the amount of V5-complexin detected in high molecular mass was decreased by 8-nitro-cGMP treatment. Our results suggest that S-guanylation of cys90 in SNAP-25 attenuates the interaction of SNARE complex, which would form a large oligomer, with complexin.

MTU11-06

Apoptosis and host-defense peptide cathelicidins determine different outcomes of bovine alpha-herpesviruses neuropathogenesis

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Alpha-herpesviruses are closely related viruses that cause neurological disease in humans and cattle. Bovine herpesvirus

(BoHV) type 5 is an important cause of encephalitis in cattle. However, encephalitis by BoHV-1 occurs occasionally. It is unknown how the innate immune response contributes to their differences in neuropathogenesis. BoHV-5 specifically induced the expression of Toll-like receptors (TLRs) in bovine neural inflamed tissue (Marin *et al*., 2014). Antimicrobial cathelicidin peptides modulate TLR4 expression in epithelial cells although their function in the nervous system remains elusive. These innate factors together with specific BoHV-apoptotic potential could be determinant in the neuropathogenesis as they could promote or limit the inflammatory response. In this study, we determined apoptosis and the expression of cathelicidins in the bovine nervous system during BoHV-1 and 5 acute infections. Calves were inoculated with BoHV-1 Cooper or BoHV-5 97/613 strains ($10^{6.3}$ $TCID_{50}$) or inert culture medium (control). At 6 days post-infection (dpi), different regions of central nervous system (CNS) and trigeminal ganglion (TG) were collected for immunocytochemical detection of cleaved caspase 3 and messenger gene determination of bovine cathelicidins BMAP27 and BMAP28 (RT-qPCR). At 6 dpi, caspase 3-apoptotic neurons were detected in the TG of BoHV-1-infected calves, whereas fewer numbers of caspase 3 positive neurons were observed in BoHV-5-infected calves. Cathelicidins expression was up- and down-regulated in CNS from BoHV-1- and BoHV-5-infected calves, respectively. BMAP27 was noticeably up-regulated in TG from BoHV-1-infected calves, while BMAP28 was only detected in BoHV-5-infected calves. Our findings suggest that modulation of apoptosis and cathelicidin expression orchestrate the final inflammatory outcomes of alpha-herpesviruses infection of the nervous system. Inhibition of both factors might be responsible for neurological lesions in BoHV-5 infection. Further research will be required to determine the role of cathelicidins during infectious diseases of the CNS.

MTU11-07

Potential link between C5a receptor and mood disorders in mouse exposed to experimental malaria *in utero*

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In Africa, a large number of pregnancies are exposed to *Plasmodium falciparum* infection during pregnancy. The in-utero environment extremely influence childhood neurodevelopment and behaviour. The complement 5a receptor is linked to several disease conditions. However, the influence of *Plasmodium berghei* during pregnancy on maternal complement 5a receptor and subsequently on fetal behaviour is unknown. Pregnant mice were intra-peritoneal infected on gestational day 13 with $1.02x10^5$ infected red blood cells. Infected red blood cells used in this experimental infection were obtained from *in vivo* passage of *P. berghei* in mice when the percentage of iRBCs reached approximately 10-20%. A section of pregnant mice (both infected and uninfected) were allowed to deliver and the progenies monitored up to postnatal day 42 when depression-like behaviour was evaluated using tail suspension test model. The other pregnant mice were subjected to cardiac puncture on gestational day 19 for C5a receptor estimation using Elisa assay. We show that pregnant mice infected with *P. berghei* had elevated C5a receptor compared with uninfected pregnant females. We also show that *P. berghei*-exposed offspring presented a depressive like behaviour compared to unexposed controls. Our results demonstrates a pathogenic role of complement 5a receptor signaling and its possible role in mediating depression which is linked to *Plasmodium berghei* infection during pregnancy.

MTU11-08

Neonatal proinflammatory treatment affects the cognitive functions development and brain neuroplasticity-related gene expression

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Pro-inflammatory cytokines such as IL-1β, IL-6, and TNFα are the main mediators of the neuro-immune interactions and are known to affect learning and memory. Pro-inflammatory cytokines elevation during neonatal period is associated with high risk of neuropsychiatric symptoms in later life. However, their effects on the maturation of brain functions during early postnatal development are not completely defined.

We have investigated the effects of the treatment with IL-1β or bacterial mimetic lipopolysaccharide (LPS, pro-inflammatory cytokine inducer) during the 3rd week of life, which corresponds to human perinatal period in terms of the CNS development, on the development of behavior and brain gene expression in Wistar male rats. Early-life IL-1β injections impaired working memory, while LPS enhanced anxiety. Long-term memory in active avoidance and Morris water maze paradigms was affected in adult rats both after neonatal IL-1β and LPS treatment, whereas in adolescent animals, exploratory behavior and locomotor activity was changed. IL-1β treated animals had long-lasting changes in the D2 dopamine receptor, FGF2, TIMP1 and MMP9 mRNA expression in the medial prefrontal cortex and hippocampus in the region- and task-dependent manner. The treatment of rats with LPS during early life induced the short-term and long-term changes in the expression of AMPA и NMDA glutamate receptor subunits, TIMP1 and MMP9 genes in the hippocampus and medial prefrontal cortex.

The impairments induced by the elevation of pro-inflammatory cytokine level during the early postnatal period may be associated with the development of psycho-neurological decline of young and adult patients with attention deficit hyperactivity disorder or other cognitive dysfunctions, and the mechanisms of that may be explained by dysregulation of brain neuroplasticity-related gene expression. Supported by RFBR projects 17-04-02116 A, 16-34-00873 mol_a, 16-34-00316 mol_a.

MTU11-09

Neurotrophin-3 modulates microglial phenotype in the traumatized CNS

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Over two-thirds of spinal cord injury (SCI) patients have anatomical preservation at the injury site, yet this preserved tissue is typically completely or partially dysfunctional. Therefore, to provide improved function in these patients, strategies are needed that enhance the function of the remaining connections by enabling the inherent plasticity of the CNS. We found an unanticipated interplay between a neurotrophin and cellular immune processes that may provide a means to promote plasticity after SCI. Moreover, we have evidence that this interplay may be exploited to induce neuroplasticity in *chronic* SCI. Our earlier work showed that unilateral viral-vector mediated over-expression of Neurotrophin-3 (NT-3) in lumbar motoneurons induced axon growth from the contralateral corticospinal tract (CST) towards the source of the NT-3. Subsequently, we showed that there is an immune component to the NT-3-induced axonal sprouting and that microglia and T cells are likely involved. When the host animals were rendered immunocompetent by pharmaceutical or genetic manipulations the NT-3 did not induce sprouting. Grafting normal T cells into immunocompetent rats enabled NT-3-induced axonal sprouting. Recent data show that microglia and macrophage have the NT-3 receptor TrkC and that NT-3 re-programs inflammatory macrophages and microglia to a more pro-regeneration phenotype. These experiments suggest that future therapeutic strategies based upon manipulation of microglia may promote enhanced neuroplasticity and increase functional recovery in patients with chronic SCI. Supported by the Mission Connect a project of TIRR Foundation, The Dana and Christopher Reeve Foundation and the NIH-NINDS.

MTU11-10

Inorganic arsenic mediated disturbance in microglial glutathione metabolism leads to bystander death of immature neurons

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Low level environmental arsenic exposure has toxic effects on neurons leading to cognitive dysfunction. Inspite of this fact, microglial role in arsenic mediated neurotoxicity remain elusive. In this study, arsenic -exposed microglial (N9) culture supernatant induces bystander death of neuro-2a (N2a) cells, which resembles developing neurons; contrarily, arsenic-exposed N2a culture supernatant did not affect N9 viability. These results indicated that microglia create toxic environment for N2a in presence of arsenic. Screening for involvement of toxic factors like reactive oxygen species (ROS), nitric oxide (NO), interleukin 6 (IL-6) and tumor necrosis factor α (TNF-α) secreted from N9 cells in bystander N2a death is found to be negative. Arsenic exposure induces GSH synthesis in N9 cells by uptake of cystine from culture medium through cystine/glutamate exchanger (xCT) resulting in lower cystine and higher glutamate concentration compared to control culture supernatant. Cystine/glutamate imbalance is also enhanced by increased xCT expression in Nrf2 dependent manner in microglia. Bystander death of N2a is rescued by supplementation of 200 μM cystine to arsenic-exposed N9 culture supernatant. Simultaneous exposure of excessive glutamate and arsenic compromises N2a viability which is again rescued by cystine addition. Therefore, microglia executes bystander N2a death simultaneously by reduction of extracellular cystine concentration and competitive inhibition of cystine transport due to high extracellular glutamate levels. *Ex-vivo* microglia from gestationally arsenic exposed mice releases excessive glutamate and reduces cystine levels in culture supernatant as compared to control and n-acetyl cysteine co-treated group. Immunofluorescence staining of brain cryosections from treated group showed more apoptotic immature neurons. Interestingly, TUNEL fluoresecence did not merged with microglia specific Iba1. Finally bystander death of primary immature neuronal cultures using primary microglia arsenic treated culture supernatant is also confirmed. Collectively, xCT is indispensable for survival of immature neurons both *in-vitro* and *in-vivo* in presence of arsenic and microglia.

MTU11-11

One-two punch, combination of immune tolerance and myelin repair therapy to effectively target disease course and severity in MS

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The CNS autoimmune disease Multiple Sclerosis (MS) is characterized by demyelination and neurodegeneration. Available FDA-approved disease modifying therapies are global immunosuppressants and have limited efficiency. We have developed a novel method of inducing immune tolerance to selectively regulate known immune responses without compromising the entire adaptive immune system. We have demonstrated an effective means of ameliorating disease in a mouse model of MS through tolerance induction in autoreactive T cells using i.v. infusion of nanoparticles coupled with or encapsulating myelin peptides (Ag-PLG) that effectively reduces disease burden in relapsing-remitting (RR-EAE) and chronic-progressive (C-EAE) mouse models of experimental autoimmune encephalomyelitis. This works to prevent disease induction, but more importantly can stop disease progression in mice treated following the initial clinical episode resulting in antigen-specific blockade of disease relapses. At present, there are no available therapies marketed for myelin repair in MS. The objectives of the study were to prevent disease progression as well as to promote CNS repair and neuroprotection. We tested an FDA approved cardiac glycoside (Na^+/K^+ ATPase) and uncovered it promoted an increase in the oligodendrocyte cell lineage *in vitro* and *in vivo*, in the non-T cell-mediated Cuprizone model of demyelination/remyelination promoted a quicker restoration of myelin integrity, and improved clinical score throughout the autoreactive Th1/Th17 driven C57BL/6 Chronic EAE time course. Additionally, we tested the hypothesis that to effectively target disease course and severity in MS, regulated by autoimmunity and neurodegeneration, a combination of selective immune regulation and myelin repair therapy is required. Combination therapy using Ag-PLG immunoregulatory therapy and the cardiac glycoside completely

ameliorated clinical disease severity. Findings from these studies may not only prove a rapid and safe therapeutic strategy for EAE reversal, but will pave the way for future clinical studies in MS undertaking this combinatorial therapeutic approach.

MTU11-12

Role of MECP2 in neuroimmune interactions

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Rett Syndrome is an autism spectrum disorder (ASD) caused by mutations in Methyl Cytosine Binding Protein 2 (MeCP2) and mouse models of Rett have been widely used for studying ASDs. The main goal of our project is to use this monogenic model of ASD, in order to evaluate the role of altered immunity in the pathogenesis of this disorder. To this end we first evaluated the autoimmune response in the context of the experimental autoimmune encephalomyelitis (EAE). Male MeCP2 WT and MT mice, were immunized with MOG $_{35\text{-}55}$ peptide, scored daily for EAE symptoms and sacrificed at 12 dpi (acute stage) or at 30 dpi (chronic stage). When WT-EAE animals and MT-EAE animals were compared, we found that MeCP2 MT mice showed an accelerated onset of the disease and more severe clinical scores. Coronal sections of spinal cord were subjected to IHC to analyze the level of expression of Iba1 (microglia) and to assess CNS lymphocyte infiltration during EAE. MeCP2 MT animals showed increased levels of infiltrating cells and microgliosis compared to WT mice; this observation correlated with the individual clinical score reached by each animal. To determine the response of immune cells, we re-stimulated spleen mononuclear cells derived from MeCP2 WT and MT mice with MOG peptide *in vitro*. Proliferation index and cytokine production was assessed. We observed increased proliferation index in all EAE animals compared to CFA in response to MOG stimulus, with no significant differences between MT and WT MeCP2 mice. Nevertheless, when the cytokine response was analyzed, MT-EAE group showed increased IFN-gamma levels in response to MOG in comparison with WT-EAE animals. Our results showed a more severe neuroinflammation in the absence of MeCP2, suggesting that Mecp2 has an active role in regulating the immune response and maintaining the neuroimmune homeostasis.

MTU12 Cellular Mechanism of Alzheimer's Disease

MTU12-01

Effects of presenilin-1 mutations in mitochondrial dynamics

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Early stage of Alzheimer's disease reveals mitochondrial deficit and dysfunction. Mitochondrial dysfunction in Alzheimer's disease causes synaptic alteration, imbalance of lipid homeostasis, calcium homeostasis, and lack of ATP production. Familial Alzheimer's disease-linked Presenilin-1, catalytic subunit of γ-secretase, mutations cause early onset Alzheimer's disease. All mutation types have different pathological mechanism and ultimately break down cellular homeostasis. Our research shows more details about relationship between Presenilin-1 mutations (PS1A431E, PS1E280A, PS1H163R, PS1M146V, PS1ΔE9) and mitochondrial dysfunctions. All of PS1 mutants-expressing cells exhibited mitochondrial dysfunctions and reduced levels of proteins involved in mitochondrial dynamics without alteration of total mitochondrial biogenesis.

MTU12-02

Protective effect of periodic dietary restriction on behavior and hippocampal deficits in a mouse model of Alzheimer's disease

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Alzheimer's disease (AD) is a neurodegenerative pathology associated with progressive decline in cognition and brain functions. Accompanying amyloid β (AB) deposition, astrocytes and microglia lose their neuroprotective functions and induce pathways that amplify inflammation. Dietary restriction (DR) has been shown to decelerate the aging process and reduce the impact of age-associated diseases, probably modulating oxidative and inflammatory status, regulating autophagy and inducing cell protection.

Our objectives were to evaluate neuroprotective effects of DR in a model of familial AD and to parallelize *in vivo* results using an *in vitro* model of nutrient restriction on glial cells exposed to AB. We established a model of periodic DR in control and PDAPP-J20 transgenic mice. Daily food consumption was restricted to 60% for 5 days/week every one week for a total of 6 weeks.

At 8 months of age, cognitive deficits and anxious-like behavior were found in *ad libitum* fed transgenic mice and were prevented by DR. In parallel, hippocampal neurogenesis was decreased in transgenic mice under *ad libitum* diet whereas transgenic mice under DR showed a neurogenic status similar to controls. *In vitro* experiments were done on C6 astroglial cells exposed to AB with and without nutrient restriction (FBS 2% vs. 10% in RPMI). Serum deprivation and AB induced autophagy. Subsequently, conditioned media (CM) from C6 were used to stimulate BV2 microglia. Microglial NFkappaB nuclear translocation was increased when exposed to CM from C6 cells with ABeta but not from C6 cells exposed to AB and serum restriction. Our results suggest neuroprotective effects of nutrient restriction in the context of AD, with glial activation and autophagy as potentially involved pathways.

MTU12-03

Inhibition of DRP1 ameliorates mitochondrial fission and and cognitive impairment in Alzheimer's disease model

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Excessive mitochondrial fission is a prominent early event, and contributes to mitochondrial dysfunction, synaptic failure and neuronal cell death in the progression of Alzheimer's disease (AD). In the present study, we examine the role of Drp1, a key regulator of mitochondrial fragmentation, in mitochondrial and synaptic dysfunction-induced by Aβ, and AD-like neuropathology and cognitive functions in AD mice. Our results demonstrate that the inhibition of Drp1 alleviates mitochondrial fragmentation, loss of mitochondrial membrane potential, ROS production, and ATP reduction in neurons treated with Aβ oligomers. An inhibitor of Drp1 also significantly restores Ab-mediated depression of synaptic vesicle exocytosis. Furthermore, Drp1 inhibition significantly improves learning and memory, synaptic density, and prevents mitochondrial fission, lipid peroxidation, BACE1 expression and Ab deposition in an AD mouse model. These results provide evidence that Drp1 plays an important role in Aβ-mediated and AD-related neuropathology, and in cognitive function in an AD animal model. Thus, inhibiting excessive Drp1-mediated mitochondrial fission may be an efficient therapeutic avenue for AD.

MTU12-04

Untargeted 1 h-NMR spectrometry to detect central and systemic metabolic changes in a rat model of early Alzheimer's disease

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Characterization and comprehension of the Alzheimer's Disease (AD) presymptomatic stage would help to better understand disease progression, develop early detection methods and improve treatment. Our main goal was to perform untargeted NMR metabolomics in hemizygous McGill-R-Thy1-APP (TG±) rats which compiles several biochemical and neuropathological characteristics detected in presymptomatic human AD brain. Experiments were performed in male TG+/- (n = 8) and non-transgenic littermate (WT) (n = 8)

rats of 9 months of age. CSF, hippocampus, and plasma were collected to study changes at central and systemic levels. Hippocampus were homogenized in 80% methanol to extract soluble metabolites. Differences between TG+/- and WT were analyzed by 1D 1H-NMR, whereas metabolite identification was performed by 2D NMR and confirmed by spiking with standard compounds. Experiments were carried out on a Bruker Avance II spectrometer operating at 600.3 MHz. Only few metabolites changed between TG+/- and WT rats in each sample type (n = 2 in CSF, n = 1 in plasma, and n = 3 in hippocampus). In plasma, lactate levels were higher in TG+/- than WT ($p < 0.07$), resembling the already described increase in presymptomatic AD patients. By contrast to what was described using MRI in dorsal hippocampus of homozygous McGill-R-Thy1-APP rat, which mimic late stages of AD, we did not find significant decrements in the N-acetylaspartate, GABA, and Glutamate levels in TG+/- as compared to WT. However, we did observe increased levels of NAD/H ($p < 0.01$) and decreased levels of nicotinamide ($p < 0.01$). These two metabolites are part of the NAD^+ salvage pathway, which is essential to maintain the NAD^+ pool. Our results suggest that a rise in NAD^+ synthesis, probably to ameliorates mitochondiral disfunction, and/or an impairment in NAD^+ conssumig enzimes activity like sirtuins, affecting epigenetic and metabolic processes, are taking place in the brain at early stages of AD.

MTU12-05

Amyloidogenic processing of β-amyloid precursor protein promotes ferroptosis: implications for Alzheimer's disease

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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, amyloid precursor protein (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. Altering the proteolytic processing of APP at the cell surface by suppressing secretase expression or activity, expression of APP carrying familial AD mutations or disrupting lipid rafts, causes consequential changes in neuronal iron homeostasis. Enhancing the amyloidogenic pathway of APP processing leads to intracellular iron accumulation and contributes to oxidative stress and toxicity. Iron induced toxicity caused by increased amyoidogenic processing of APP appears to be mediated by the ferroptosis inhibitor ferrostatin-1. 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.

MTU12-06

Imbalance in BDNF and probdnf levels in serum and cerebroespinal fluid in untreated Alzheimer's disease patients

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Brain-derived neurotrophic factor (BDNF) is essential for the survival and differentiation of neurons, and it's considered a key target in the pathophysiology of various neurodegenerative diseases. There are not full consensuses regarding the serum levels of BDNF in Alzheimer's Disease (AD). In this study, we measured serum and CSF BDNF levels in patients with AD newly diagnosed without treatment with drugs that can upregulate the expression of BDNF. BDNF serum concentrations were lower in AD with depression follow to AD without depression, controls with depression and finally controls without depression. Apathy, MMSE, semantic verbal fluency and several depression scales also correlated with lower levels of BDNF. Contrarily to BDNF, pro-BDNF induce apoptosis through its interaction with p75NTR and its co-receptor, sortilin. In CSF the ratio proBDNF/mBDNF is increased compared to controls. In the hippocampus of human AD samples, proBDNF is modified by AGE/ALEs preventing its processing to the mature form and thus, increase the pathogenicity of the proform.

MTU12-07

The role of olfactory dysfunction in the pathogenesis of Alzheimer disease

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Alzheimer disease (AD) is a chronic disorder that affects millions of individuals worldwide. Olfactory dysfunction is a common symptom of several neurological disorders including AD. Studying the mechanisms underlying the olfactory dysfunction may lead to the discovery of potential biomarkers and/or treatments for neurodegenerative diseases. Objective: to determine if olfactory dysfunction predicts future cognitive impairment and to characterize the olfactory system in a murine model expressing a genetic factor of AD. For the human study, quantitative olfactory tests have been

done on 93 subjects from the NuAge cohort accepting to participate in the ORCA secondary study. The t-MMSE was used to assess cognition status, and an olfactory self-report collected. In a separate cohort, olfactory cortical volume was calculated using MRI from healthy old adults and AD individuals. Based on the self-report, 81% of our participants claimed to not suffer from any problem with olfaction. However, based on the UPSIT, 94% show olfactory dysfunction. We also detected a significant decrease in olfactory cortical volume in AD compared to controls. Murine study: Preliminary data demonstrate there is a significant decrease in expression of the proform of caspase-9, caspase-3 and the caspase substrate STK3, in the olfactory bulb of mice expressing human APOE4 compared with controls. The data also suggest that Iba-1 is increased in the olfactory bulb of APOE4 mice compared to wild type. The activation of caspase-3 may be the cause of the decreased levels of STK3 through caspase cleavage and may play role in the inflammation observed.

MTU12-08

The role of copper in ubiquitin-dependent protein degradation in Alzheimer's disease

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Disruption to copper homeostasis is a feature of Alzheimer's disease (AD). Recently it was discovered that copper reduces Amyloid Precursor Protein (APP) endocytosis from the plasma membrane and promotes its ubiquitination. The importance of this finding is underlined by studies that indicate that endocytosis is a key step in amyloidogenic processing of APP to form neurotoxic amyloid beta (Aβ) peptides. Ubiquitin plays a fundamental signalling role in proteasome-mediated protein degradation, endocytic protein sorting and targeting membrane proteins to lysosomes for degradation via autophagy. Our HYPOTHESIS is that APP amyloidogenic processing is modulated by copper-responsive ubiquitination of APP, signalling it towards a degradative pathway rather than an endocytic pathway where it encounters the enzymes responsible for Aβ generation, namely β- and γ-secretase.

SPECIFIC AIMS:: Aim 1: To determine the role of Cu-responsive ubiquitination of APP on its localization and degradation in cultured mouse neurons.

Aim 2: To compare Cu-responsive ubiquitination of APP in differentiated neurons that have been re-programmed from healthy and AD patient human fibroblasts using induced pluripotent stem cells (iPSCs).

Aim 3: To determine if mutations that cause familial AD affect Cu-responsive ubiquitination of APP using cultured mouse and human fibroblasts.

Aim 4: To identify novel Cu-responsive ubiquitin targets in AD-affected and healthy control fibroblasts using an 'ubiquitin-omics' approach.

We propose that copper is a physiological co-factor for the ubiquitination of APP, a neuroprotective mechanism that reduces the level of amyloidogenic processing.

MTU12-09

The relationship between the amyloid precursor protein and tar DNA binding protein 43 (TDP-43): links to Alzheimer's disease

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An increasing body of literature supports a role for TAR DNA binding protein 43 (TDP-43) in the pathogenesis of Alzheimer's disease (AD). Recent studies have founds TDP-43 inclusions present in the brains of substantial numbers of AD patients, with prevalence reported as between 19 and 57%. Other researchers have suggested that TDP-43 inclusions are correlated with greater disease severity as measured by Braak staging. In addition, TDP-43 has been suggested to modulate the expression and proteolytic processing of amyloid precursor protein (APP). In this study, co-immunoprecipitation and immunocytochemistry were used in SH-SY5Y cells and control induced pluripotent stem cell (iPSC)-derived human neurons to assess the possible direct interaction between the predominantly nuclear TDP-43 and the nuclear fragment of APP, namely AICD. We were unable to show any co-immunoprecipitation between TDP-43 and AICD. Furthermore, AICD and TDP-43 showed opposing nuclear localisation in SH-SY5Y cells and iPSC-derived neurons, suggesting a lack of direct interaction between TDP-43 and AICD. APP isoforms were subsequently over-expressed in SH-SY5Y cells, and TDP-43 expression was monitored by immunoblotting. Although no change was observed, preliminary data generated by targeting APP with siRNA suggest that APP may regulate TDP-43 expression in an indirect manner. In addition, TDP-43 over-expression was preliminarily shown to modulate the expression of the APP holoprotein and the β-secretase, BACE1. Taken together, these findings suggest that the correlation of the presence of TDP-43 inclusions with the severity of AD may derive, at least in part, from the ability of TDP-43 to regulate the expression of APP and BACE1. Furthermore, our data suggest that, in the case of APP and TDP-43, the relationship may be bidirectional. An improved understanding of the contribution of TDP-43 to the pathogenesis of AD could reveal novel therapeutic avenues.

MTU12-10

Lupeol isolated from betula alnoides inhibit phophodiesterase and ameliorates streptozotocin induced cognitive impairment and NEU

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Objective: To investigate the therapeutic potential of lupeol (isolated from *Betula alnoides)* in STZ-induced experimental dementia of Alzheimer's type in rats.

Material and methods: Lupeol was isolated from the *BA* and its phosphodiesterase (PDE) inhibitory activity was assessed through *in-vitro* PDE inhibitory assay. STZ was administered intracerebroventrically (ICV) on day 1 and 3 (3 mg/kg, ICV bilaterally) in rats. lupeol was administered (25, 50 and 100 mg/kg/day p.o.) 1 hr following 1st STZ infusion upto 21st day. Morris water maze (MWM), locomotor activity and object recognition task (ORT) were used to assess behavioral changes in rats. On 22nd day, animals were sacrificed and hippocampus were isolated for biochemicals

(acetylcholinesterase (AChE), lipid peroxidase (LPO), reduced glutathione (GSH and nitrite), neuroinflamatory (Tumor necrosis factor alpha (TNF-α), Interleukin 1 beta (IL-1β), and IL-6), neurotransmitters (NTs) analysis (dopamine, norepinephrine, serotonin, 3,4-Dihydroxyphenylacetic acid (DOPAC), Homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) and cyclic nucleotide (cAMP and cGMP) estimation. Histological estimation of the brain slides were carried out using Hematoxylin and eosin staining.

Results: STZ infusion significantly impaired memory as observed in MWM and ORT, increased oxidative stress (LPO, nitrite, AChE) and decreased enzyme (GSH), increased pro-inflammatory levels (TNF-α, IL-6 and IL-1β), altered NTs level (DA, NE, 5-HT and their metabolites) and decreased cyclic nucleotide levels. Lupeol showed potent PDE inhibitory activity in *in-vitro* PDE inhibitory assay and post treatment of lupeol (25, 50 and 100 mg/kg) significantly restore STZ induced behavioral, biochemical, NTs abnormalities and cyclic nucleotide levels in rat brain. Histological examination of lupeol treated brain show improvement in neuronal flora as compare to STZ treated rats.

Conclusion: The findings of the present study suggests that lupeol inhibit PDEs, reduced neuroinflammation via acting through multiple mechanisms and would be a used as a target molecule to curb cognitive decline associated with neurodegenerative disorders such as AD.

MTU12-11

Human TP53 ARG72PRO SNP dictates neuronal susceptibility to Aß-neurotoxicity upon CDK5-induced P53 stabilization in mitochondria

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Alzheimer's disease (AD) is a complex multifactorial disease in which neural death occurs predominantly by apoptosis. The p53 tumour suppressor protein functions as a key regulator of cell apoptosis and has been described to accumulate in affected brain areas from AD patients. However, the role of p53 in AD is controversial. This protein naturally occurs in humans in two functional variants with single nucleotide polymorphism (SNP) resulting in Arg or Pro at residue 72 that modulates the apoptotic activity of the p53 protein. Our objective was to evaluate the impact of the *Tp53 Arg72Pro* SNP on amyloid-ß (Aß)-induced neurotoxicity. Cortical primary neurons from humanized *Tp53 Arg72Pro* knock-in mice were treated with 10 mM Aß25-35 oligomers for 24 h and protein expression levels, mitochondrial function and apoptosis were evaluated. In some experiments, neurons were lipotransfected with siRNA against cyclin dependent kinase-5 (Cdk5) or plasmids containing apoe2, e3 and e4 variants. We found that Aß triggered Cdk5-induced p53 phosphorylation and stabilization in both Arg^{72}-p53 and Pro^{72}-p53-expessing neurons. However, neurons carrying the polymorphic variant Arg^{72}-p53 were more susceptible to Aß-induced mitochondrial dysfunction and apoptosis than neurons with the Pro^{72}-p53 variant. Moreover, Arg^{72}-p53 promoted p53 translocation to mitochondria after Aß treatment, whereas this effect was not found in Pro^{72}-p53 neurons. Then, the Arg^{72}-p53 variant promotes p53 accumulation in the mitochondria and increases neuronal vulnerability to Aß-induced neurotoxicity. Finally, the expression of apoe4, the well-known major risk factor for AD, in both neurons abrogated this effect, and Pro^{72}-p53 neurons became as vulnerable as Arg^{72}-p53 to Aß-induced neurotoxicity. In conclusion, the *Tp53 Arg72Pro* polymorphism modulates neuronal susceptibility to Aß toxicity and determines damage extent. These results make this SNP a possible biomarker of genetic risk and progression for AD. This study was funded by ISCIII grants –PI15/00473, RD16/0019/0018-, European Union -686009- and FEDER.

MTU12-12

Complement C3 and C3aR receptor promote tau pathology and Alzheimer`s disease

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Background: Besides the pathological hallmarks of β-amyloid (Aβ) plaques and neurofibrillary tangles (NFTs), increasing evidence suggests that neuroinflammation plays a significant role in AD pathogenesis. The complement pathway is a key regulator of innate immunity. Recent findings from our laboratory showed that the central complement component C3 is upregulated in astrocytes of AD patients and mouse models. Moreover, elevated astroglial C3 can modulate Aβ dynamics and pathology and reduce dendritic morphology and synaptic function through microglial and neuronal C3a receptor (C3aR), respectively. Giving the importance of the C3-C3aR axis in the CNS, we are interested in understanding whether the complement pathway also impacts the NFT pathology.

Methods and Results: First, we analyzed the expression patterns of complement pathway genes in AD, MCI and non-cognitive impaired brain samples obtained from the ROS-MAP cohorts and revealed a strong upregulation of complement pathway genes that is highly correlated with Braak staging and cognitive decline. Prominent complement upregulation was also observed in a PS19 mouse model of tauopathy in response to NFT pathology. Next, to study the functional effects of the C3-C3aR pathway on reactive gliosis and NFT pathology we manipulated C3aR expression in PS19 animals. AAV-driven neuronal overexpression of C3aR exacerbated tau/NFT pathology in PS19 mice. Conversely, knocking out C3aR in PS19 mice resulted in significant reduction of inflammation, phospho-tau levels and associated pathology and improved behavioral deficits of PS19 animals. Finally, we utilized RNA sequencing and reverse-phase proteomics (RPPA) to identify C3aR downstream signaling pathways that promote such effects and identified the Jak-Stat3 pathway as a potent regulator of reactive gliosis and tau pathology in PS19 mice downstream of C3aR.

Conclusions: Our data shows that C3-C3aR activation promotes reactive gliosis and tau pathology in PS19 mice through the activation of the Jak-Stat3 pathway and indicates that blocking C3aR can be therapeutically beneficial for AD treatment.

MTU12-13

The distribution and function of neuronal PSER727-stat3 is modify by mediators released by astrocytes in response to aβos

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Amyloid-beta oligomers (AβOs) have been found in Alzheimer's disease (AD) brains and there is vast evidence that supports a role of AβOS, which would trigger synapse failure and memory impairment. Astrocytes respond to AβOs through a process called reactive astrogliosis, which generates reactive oxygen/nitrogen species (ROS/RNS) and inflammatory cytokines that affect surrounding neurons. Stat3 is a crucial transcription factor involved in maintenance and function of nervous system and its deregulation has been implicated in AD. Growth factors induce serine-727 phosphorylation and this modification is associated with modulation of transcriptional activity of Stat3. The main goal in this work is determine if hippocampal neuronal Stat3 is affected by reactive astrocytes.

Methods: Primary hippocampal neuron and astrocytes cultures were used. Changes in pSerStat3 distribution were detected by immunocytochemistry. The oxidative tone and ROS production were evaluated by redox cytochemistry and Hyper strategy. Protein and mRNA levels were determined by Western blotting and qPCR, respectively.

Results: We found that AβOs induced no changes in protein, mRNA Stat3 levels and pSerStat3 cellular distribution, in neurons. However, AβOs treatment in mixed neuron-astrocytes cultures induced notorious neuronal pSerStat3 redistribution. Reactive astrocytes increased the expression of pro-inflammatory cytokines, and astrocyte-conditioned media treated with AβOs (ACM-AβOs) increased the neuronal oxidative tone. ACM-AβOs induced a decrease of Stat3 target genes (survival and antioxidant response) and an increase of pro-apoptotic Bax/Bcl2 ratio.

Conclusion: We propose that in hippocampal neurons, pSerStat3 is a sensor for stressor astrocyte-produced induced by AβOs activation.

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MTU12-14

Role of DRP1 in ad pathogenesis

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Alzheimer's disease (AD) is an age-related disorder characterized by deposition of neurotoxic form of beta-amyloid (Aβ) and degeneration of neurons. In the brain, accumulation of Aβ in the mitochondrial compartment has a responsible role in impairing mitochondrial physiological functions. Impaired regulation of mitochondrial dynamics, which shifts the balance towards fission, is associated with neuronal death in age-related neurodegenerative diseases, such as AD. We investigated the effect of inhibition of mitochondrial fission protein Drp1 (dynamin-related protein 1) by Mdivi-1 in AD mice and neuronal cells. Mdivi-1 reduced reactive oxygen species, oxidative stress level, BACE1 level, and amyloid plaques. The treatment of Mdivi-1 also improved memory and cognitive functions in AD mouse models. Mdivi-1 treatment delayed Aβ-mediated mitochondrial fragmentation and oxidative stress in neurons. These results indicate that inhibition of mitochondrial fission can be a therapeutic approach for AD.

MTU12-15

Critical roles of cathepsin b in Alzheimer's disease-like phenotypes following chronic systemic exposure to porphyromonas gingival

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A number of clinical and experimental studies have revealed a strong association between periodontitis and accelerated cognitive decline in Alzheimer's disease (AD), however, the precise mechanism of their association remains unclear. In this study, we found that chronic systemic exposure to lipopolysaccharide derived from Pg (PgLPS; 1 mg/kg, daily, i.p.) for five consecutive weeks induced learning and memory deficits with the intracellular accumulation of Aβ in neurons in the middle-aged (12 months old) wild-type (WT) mice, but not in young (2 months old) WT or middle-aged CatB-deficient ($CatB^{-/-}$) mice. PgLPS significantly increased the expression of cathespin B (CatB), a typical cysteine lysosomal protease, in both microglia and neurons in middle-aged WT mice, while increased expression of mature IL-1β and TLR2 was restricted to microglia in the hippocampus of middle-aged WT mice, but not in that of the middle-aged $CatB^{-/-}$ ones. In *in vitro* studies, PgLPS (1 μg/ml) stimulation upregulated the mean mRNA expression of IL-1β, TLR2 and downregulated the protein levels of IκBα in the cultured microglia. These PgLPS-induced responses were significantly inhibited by pharmacological or genetic inhibition of CatB.

Furthermore, the mean mRNA expression of APP and CatB were significantly increased in the primary cultured hippocampal neurons after treatment with conditioned medium from PgLPS-treated WT, but not from $CatB^{-/-}$, primary cultured microglia (MCM). Taken together, these findings indicate that chronic systemic exposure to PgLPS induces AD-like phenotypes, including microglia-mediated neuroinflammation, intracellular Aβ accumulation in neurons and impairment of the learning and memory functions in the middle-aged mice in a CatB-dependent manner.

We conclude that CatB plays a critical role in the initiation and exacerbation of neuroinflammation following chronic systemic exposure to *Pg*, leading to induce AD-like phenotypes. Therefore, CatB can be a potential therapeutic target for AD.

MTU12-16

HMGB1 promotes autophagy and increase amyloid-β clearance in Alzheimer disease models

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The accumulation of amyloid-β (Aβ) neuritic plaques and intracellular tau protein tangles are key pathological characteristics

of Alzheimer's disease (AD). There is evidence that the autophagosome-lysosomal degradation is a central role in AD, and disturbing the processing of autophagy lead to Aβ accumulation and provoke AD pathology. Previous studies showed that high-mobility group box 1 (HMGB1) promotes mitochondrial dysfunction–triggered striatal neurodegeneration via autophagy activation. In this study, we focused on whether the HMBG1 could regulate autolysosome formation and clearance of Aβ in AD models. We administrated HMGB1 inhibitor Glycyrrhizin in hippocampus with Aβ-treated mice by stereotaxic injection, then analyzed changes in expression of autophagic protein and phospho-c-Jun amino-terminal kinases (p-JNK). p-JNK and autophagic marker LC3-II were significantly reduced by the HMGB1 inhibitor glycyrrhizin. Glycyrrhizin also significantly inhibited survival of hippocampal neurons with Aβ-treatment. Moreover, neuronal death was replicated by exposing hippocampal neurons in culture to Aβ. Furthermore, to elucidate the role and mechanism of HMGB1 in clearance of Aβ, we investigated the impact of HMGB1 on autophagy activation and autolysosome formation *in vitro*. shRNA-HMGB1 inhibited cellular viability and reduced LC3-II expression compared with cells treated with Aβ only. Immunofluorescence revealed that HMGB1-shRNA in Aβ-treated neuronal cells increased the number of LC3-II-positive autophagosomes that were colocalized with the lysosomal marker. p-JNK expression was significantly reduced by shRNA knockdown of HMGB1, an effect that was reversed by exogenously increased expression of HMGB1. Furthermore, HMGB1-shRNA markedly reduced Aβ immunoreactivity colocalized within lysosomes and increased intracellular Aβ levels compared with Aβ-treated cells. These results suggested that HMBG1 regulated autolysosome formation and clearance of Aβ in AD models, and exerted a neuroprotective effect through modulation of Aβ clearance in Aβ-treated neuronal cells. HMGB1 would be a future strategy for AD treatment via autophagy pathway for clearance of Aβ in AD.

MTU13 Therapeutic Approaches of Parkinson's Disease

MTU13-02

PNR2B mediates the role of FYN kinase in levodopa induced dyskinesia in a mouse model of Parkinson's disease

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Levodopa (L-DOPA) induced dyskinesia (LID) is one of the undesired side effects of Parkinson's disease (PD) treatment. To reduce the development of LID, without affecting the positive restorative effect of dopamine stimulation, is one of the greatest challenge in this area. We have previously explored the pathway Pleiotrophin/RPTP ζ/b/Fyn at the postsynaptic density complex and found that Fyn-KO mice developed less LID than WT littermates. Fyn kinase modulates the N-methyl D-aspartate (NMDA) receptor through phosphorylation of the NR2B subunit and this could explain the role of Fyn in LID. The main goal of this work is to demonstrate the direct relation between Fyn and NMDA receptor in a paradigm of dyskinesia. We lesioned Fyn-KO and WT mice with 6-hydroxydopamine (6-OHDA) and treated them daily with L-DOPA to model LID. Postmortem dopaminergic denervation was confirmed by immunodetection of tyrosine hydroxylase in the substantia nigra pars compacta. Several molecular markers, in particular the amount and phosphorylation status of NR2B subunit of NMDA, were determined in the striatum by Western blot. As expected, in WT mice we found upregulated the transcription factor ΔFosB and ERK phosphorylation, both previously reported markers of LID, while Fyn-KO mice showed a significant reduction of LID accompanied by a downregulation of ΔFosB and NR2B phosphorylation (pNR2B). In conclusion, pNR2B is downregulated in dyskinetic Fyn-KO mice, what can explain a reduced NMDA signaling and therefore the observed reduced dyskinesia. In this sense, Fyn would be an attractive target to modify the NMDA signaling and a promising treatment to modulate LID without affecting the therapeutic efficacy of L-DOPA in PD.

MTU13-03

Brain preconditioning reprograms the inflammatory response to neuronal damage in rat model of Parkinson's disease

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Background: Neuroinflammation is considered as both cause and consequence of neuronal injuries and has a key role in the pathogenesis of neuronal disorders such as Parkinson's disease (PD). Based on this, modulation of inflammation in the brain can be offered as one of the therapeutic approach to control neuronal damage in PD. Recent studies have indicated that Lipopolysaccharide (LPS) preconditioning could be used as a potential alternative strategy to attenuate, postpone or even cure the deficits of neuronal disorders. The purpose of this study was to study the effect of the brain preconditioning with single low dose of LPS on inflammatory profile in 6-OHDA model of PD.

Methods: To clarify the neuroprotective effect of brain LPS preconditioning on inflammatory profile in PD rats, motor coordination and balance were evaluated using rotarod in all animals. In addition, the effect of the LPS preconditioning on neuronal damage were observed in the substantia nigra by using nissl staining. Finally, gene and protein expressions associated with different cascades induced by LPS preconditioning including TLR4 and inflammatory signaling pathways were analyzed using RT-PCR and Western Blot.

Results: Based on the behavioral assessments, preconditioned animals performed significantly better on the behavioral performance in 6-OHDA rat model of PD. Furthermore, neuroprotective effect of brain LPS preconditioning were consistent with the histological observation. In addition, our result showed that the brain LPS preconditioning reduced inflammatory response in the brain of PD rat by enhancing NFκB inhibitors (e.g. SHIP1 and TOLLIP) as well as anti-inflammatory cytokines. These findings were confirmed by western blot analysis.

Conclusion: Generally, reduction in inflammatory response through the brain LPS preconditioning may contribute to the induction of tolerance to neuronal damage in PD. This neuroprotection parallel the reprogramming strategy that leads to the synthesis of new markers to change molecular response against brain lesions. Altogether, our findings demonstrate that LPS preconditioning has a therapeutic effect on the modulation of neuroinflammation and this could suggests a promising therapeutic strategy for various neuronal disorders such as PD.

MTU13-04

Addressing the mechanism of priming of dopamine receptors and its effect on drug-induced dyskinesia

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The gold standard treatment for Parkinson's disease is still the use of L-DOPA, which after prolonged use induces severe motor complications known as L-DOPA-induced dyskinesia (LID). In contrast, D2 agonists are frequently used in clinical practice because its low propensity to induce dyskinesia. *Priming* is defined as the behavioral and molecular sensitization occurring after the first exposure to L-DOPA or a full DA agonist. Priming is not necessarily associated with dyskinesia but once it has occurred, lower doses of L-DOPA or dopamine agonists are enough to induce dyskinesia. Priming has been pharmacologically studied but the underlying molecular mechanism is not well understood. The aim of

this work is to understand the mechanisms of priming and evaluate the effect of D1/D2 receptor stimulation on subsequent DA agonist responses. C57BL/6 mice injected with 6-OHDA received a dyskinetogenic dose of L-DOPA or saline to induce priming, and then were treated with the D2R agonist Quinpirole. We compared Quinpirole-induced dyskinesia after increasing doses vs prolonged treatment with the maximum dose of Quinpirole, and observed higher dyskinesia scores in primed vs non-primed animals. Immunohistochemistry of striatal FosB and cFos, showed increased levels of both dyskinesia markers in primed mice compared to saline pre-treated. Furthermore, we analyzed selective cFos expression in D1R expressing cells, using bacterial artificial chromosome transgenic mice expressing tdTomato in striatal projection neurons that express D1 receptor. We reproduced L-DOPA priming and Quinpirole administration protocol, and observed significant cFos immunoreactivity in D1R expressing neurons in primed mice but was almost insignificant in non-primed mice. These results confirm once again the importance of behavioral sensitization and increased dyskinesia marker expression after D1/D2 receptor stimulation. Furthermore, they demonstrate that D2 receptor stimulation has a great impact onto D1R expressing cells only after priming and shows the important role of D2 receptor in dyskinesia development, even though current concepts emphasize the role of D1 receptor as the major player in dyskinesia induction.

MTU13-05

IGF-1 gene therapy in early parkinson′s disease

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Backgound: Insulin-like growth factor-1 (IGF-1) is an endogenous peptide transported across the blood brain barrier that is protective in several brain injury models, including in an animal model of Parkinson's disease (PD).
Objectives: To determine in an experimental model of the neuropathology if IGF-1 gene therapy could: 1) improve the cognitive dysfunctions and 2) induce changes in the neuronal activity of the affected brain areas.
Methods: Male Wistar rats were bilaterally injected in CPu with either the neurotoxic 6-hydroxydopamine (6OHDA rats), or vehicle (SHAM rats) as controls. Then they were divided into 6 experimental groups according to the gene therapy with adenovirus in hippocampus: G1) SHAM (vehicle-vehicle), G2)6OHDA (neurotoxic-vehicle), G3) SHAM-RAd-DS-Red, G4) SHAM-RAd-IGF-1, G5) 6OHDA-RAd-DS-Red, G6) 6OHDA-RAd-IGF-1. At 3 weeks post lesion with 6OHDA and injection with adenovirus the animals were tested for spatial memory with Y-maze test and for locomotor activity. At the end of the study the rats were perfused, the brains fixed and immunohistochemistry performed for TH and IGF-1. All data were compared by 2-way ANOVA ($p < 0.05$ considered as statistically significant).
Results: 6OHDA causes cognitive deficits in G2 compare to G1 ($p > 0.05$) indicated by a decreased in spontaneous alternation percentage. This effect could not be attributed to decreased motor activity, because the number of arm entries was not significantly changed and neither the number of cm performed after amphetamine administration. This effect was partially reverted with IGF-1 overexpression in G6 respected to G5 ($p > 0.05$). There were no significant changes in G2 respect G5 and in G1 respected to G3 and G4. Preliminary results showed that IGF-1 gene therapy induce an increase in TH expression in the nigrostriatal pathway.
Conclusions: our results suggest that IGF-1 could be an important neuroprotective molecule against neurodegeneration. Its effect on neuronal activity could explain in part the improvement in the cognitive symptoms that we observed in this animal model of PD.

MTU13-06

TRKB agonist provide neuroprotection via attenuating neuroinflammatory cascade and regulator of g-protein signaling 4 (RGS4)

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Parkinson disease is (PD) a debilitating motor disorder affected million populations worldwide. The objective of our study was to investigate the effect of TrkB agonist; 7,8-dihydroxyflavone (7,8-DHF) on 6-hydroxydopamine (6-OHDA) induced inflammation cascade in Neuro-2a (N2a) cells and MPTP-induced neuroinflammatory cascade connecting the link with RGS4 in striatum region of male swiss albino mice (weight 25–30 g). The Neuro-2a (N2a) cells treated with 6-OHDA of various concentrations (1.5–100 μM) for 24 hrs and the cytotoxicity (6-OHDA, 25 μM) was prevented by 7,8-dihydroxyflavone (3, 6 and 12 μM) evaluated by MTT and LDH assay. The neuroinflammatory markers such as NF-κB and Cox-2 protein expression were markedly upregulated by 6-OHDA treatment which further reduced with 7,8-DHF cotreatment. Further, animal studies, MPTP (30 mg/kg, i.p. for 5 days i.e. 10th day to 14th day) were conducted for 28 days study period. The 7,8-DHF (10 mg/kg) treatment for 28 days mitigated symptoms in MPTP-treated animals induced motor deficits as evaluated on rotarod test, open field test, and grip strength test. The biochemical oxidonitrosative stress markers such as lipid peroxidation, nitric oxide level and reduced glutathione (GSH) level, proinflammatory cytokines (TNF-α, IL-1β) level in striatum and substantia nigra were found to be high in MPTP-treated animals. Furthermore, the real-time PCR for the genes (iNOS, COX-2, NF-κB, Nrf2, PARP-1, RGS4) and western blot for the protein expression studies were found to be altered in the striatum (NF-κB, Nrf2, PARP-1, RGS4) and substantia nigra (NF-κB, Nrf2) of animals which got reversed by the 7,8-DHF treatment. Hence, 7,8-dihydroxyflavone significantly ameliorated the induced neuroinflammatory cascade in N2a cells and animals as well as downregulated the RGS4 activation in striatum region of mice model through its potential antioxidant activity. Thus, TrκB agonist may be the futuristic non-dopaminergic candidate to treat Parkinson's disease progression.

MTU13-07

Pathological α-synuclein transmission initiated by binding lymphocyte-activation gene 3

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Parkinson's disease (PD) is the second most common neurodegenerative disorder causing serious movement disability and cognitive impairment in those afflicted. Pathologically, PD is characterized by the accumulation of α-synuclein (α-syn) in Lewy bodies and neurites. There is dopamine neuron degeneration in the substantia nigra pars compacta which causes many of the major symptoms of PD. Emerging evidence indicates that the pathogenesis of PD may be due to cell-to-cell transmission of misfolded preformed fibrils (PFF) of α-syn. The mechanism by which α-syn PFF spreads from neuron to neuron is not known.

Here, we show that LAG3 (lymphocyte-activation gene 3) binds α-syn PFF with high affinity (dissociation constant = 77 nanomolar), whereas the α-syn monomer exhibited minimal binding. Tau-biotin PFF, β-amyloid-biotin oligomer, and β-amyloid-biotin PFF do not bind to LAG3, indicating that LAG3 is specific for α-syn PFF. α-Syn-biotin PFF binding to LAG3 initiated α-syn PFF endocytosis, transmission, and toxicity. Neuron-to-neuron transmission of pathologic α-synuclein and the accompanying pathology and neurotoxicity is substantially attenuated by deletion of LAG3 or by antibodies to LAG3. Lack of LAG3 substantially delayed α-syn PFF–induced loss of dopamine neurons, as well as biochemical and behavioral deficits *in vivo*. The identification of LAG3 as a receptor that binds α-syn PFF provides a target for developing therapeutics designed to slow the progression of PD and related α-synucleinopathies.

MTU13-08

Antidyskinetic effect of acute guanosine administration in reserpinized mice

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Dyskinesia is characterized as involuntary movements that affect several body parts. Several neurological disorders can exhibit this symptom, such as Parkinson's disease. Dyskinesia can be induced by the alkaloid reserpine that acts as an inhibitor of vesicular monoamine transporter (VMAT-2). The consequent monoamine neurotransmitters depletion induces hypolocomotion, muscle rigidity and involuntary movements. Guanosine (GUO), an endogenous nucleoside, has been evidenced as a neuroprotective agent, although the exact mechanism of GUO action is not fully characterized. This study evaluated the therapeutic potential of GUO as an antidyskinetic agent in mice treated with reserpine (1 mg/kg, *subcutaneously,* every other day). GUO (7.5 mg/kg p.o.) was administered 24 h after the last reserpine injection and 20 min before behavioral test. GUO prevented the increase of orofacial dyskinesia induced by reserpine. Additionally, the antidyskinetic effect of GUO was abolished by prior administration of the A_1 adenosine receptor antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 0.75 mg/kg). Reserpinized mice also showed a cataleptic state when evaluated in the bar test. Likewise, this behavior was prevented by GUO. Interestingly, DPCPX also abolished the anti-cataleptic effect of GUO, besides presenting an anti-cataleptic effect *per se*. Reserpine increased cells damage and reactive oxygen species (ROS) levels in the striatum of treated mice. GUO was effective in reducing the increase of ROS levels, but it did not alter cells damage induced by reserpine. This study shows for the first time an antidyskinetic effect of GUO and its effect of modulating motor and neurochemical impairments induced by reserpine.

MTU13-09

Sphingosine 1-phosphate receptors modulators decrease neuroinflammation and prevent Parkinson's disease symptoms in mptp mice

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Sphingosine-1-phosphate receptors (S1PR) may be an attractive molecular target to the treatment of neurodegenerative diseases. For instance, Fingolimod (FTY720), an immunomodulator that acts on S1PR, has been documented to display neuroprotective effects in multiple sclerosis and animal model of Alzheimer's disease. We postulated that the immunomodulatory effects associated to FTY720 treatments might also be beneficial to Parkinson's disease pathogenesis. Therefore, the present study investigates the effects of an oral FTY720 treatment (1 mg/kg/day, 14 days) on the behavioral and molecular alterations induced by the administration of MPTP (30 mg/kg/day, i.p., 5 days) in mice. We first established that FTY720 has the capacity to prevent the motor deficits observed in the Pole test after MPTP treatments. In the striatum of MPTP mice, Western blot analysis revealed diminutions of ~50% in the levels of tyrosine hydroxylase and dopamine transporter proteins following MPTP injections, which were both prevented by FTY720 treatments. In parallel, while striatal levels of phosphorylated extracellular signal-regulated kinases and S1PR subtype 1 were unaffected, tumor necrosis factor-alpha and glial fibrillary acidic protein levels were robustly increased in MPTP-treated mice, an outcome that was totally prevented by FTY720 treatments. Notably, FTY720 treatments was also able to prevent the reduction of brain-derived neurotrophic factor levels observed in the striatum of mice treated with MPTP. Altogether, our findings propose that oral FTY720 treatments halt the detrimental effects of MPTP on striatal dopamine terminals and motor behaviors. The mechanism of action may involve inhibition of inflammatory pathways and the modulation of brain-derived neurotrophic factor production. This study is providing novel evidence for the clinical utility of targeting S1PR in Parkinson's disease therapy.

MTU13-10

A novel approach to detect the presence of levodopa (l-DOPA) in the polypeptide chains of proteins

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Background: Deposition of protein aggregates in neurons is a hallmark of neurodegenerative diseases. Genetic mutations in proteins can result in the synthesis of non-native proteins that accumulate in cystosolic aggregates. Certain non-protein amino acids (NPAAs) can be mischarged onto tRNA and mistakenly inserted into the polypeptide chain of proteins resulting in the sporadic generation of non-native misfolded proteins [1,2]. We utilise a novel approach to investigate the ability of the therapeutic agent levodopa (L-DOPA) to replace L-tyrosine in proteins by applying mass spectrometry (MS) whereby we can sequence peptides to reveal the presence of DOPA.

Methods: We examine the ability of high resolution accurate mass mass-spectrometry (HRAM-MS) to detect DOPA in neuronal cell proteins avoiding the need for traditional protein hydrolysis and to allow individual proteins to be identified.

Results: We successfully tested a novel approach for the detection of DOPA in peptides generated from digestion of DOPA-containing proteins and validated this method using synthetic peptides. We showed that L-DOPA can be incorporated into cell proteins. The presence of L-DOPA in proteins resulted in a decrease in solubility. Specific structural and long-lived proteins were most affected. We are now applying this approach to biological samples.

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Poster sessions WTH Wednesday/Thursday

WTH01 Myelination and Demyelination

WTH01-01

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.

WTH01-02

Identification of the antigen recognized by RHIGM22, a remyelination-promoting human monoclonal antibody

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Recombinant human IgM22 (rHIgM22) binds to myelin and oligodendrocytes (OLs), and promotes remyelination in mouse models of multiple sclerosis. rHIgM22 preferentially reacts with sulfatide-positive ($O4^+$) OLs, and binding of rHIgM22 is abolished in CNS tissue slices from Cst (−/−) mice, suggesting that its binding requires the presence of a product of cerebroside sulfotransferase, possibly sulfatide, highly expressed in OLs and myelin. However the identity of the antigen recognized by this antibody remains to be elucidated. We tested the binding of rHIgM22 to purified lipids and lipid extracts from mouse brain, CNS myelin, mixed glial cells, and $O4^+$ OLs using TLC immunostaining and SPR with lipid monolayers. Our preliminary results show that rHIgM22 binds to sulfatide *in vitro*, while it does not bind to other myelin sphingolipids suggesting that sulfatide at the OLs surface might be important for the binding of rHIgM22 to these cells and to myelin. However, rHIgM22 does not bind structures expressing sulfatide outside the nervous system, so additional factors are likely relevant for the immunoreactivity of rHIgM22 in CNS. Indeed, in lipid extracts from different sources we found another lipid antigen selectively recognized by rHIgM22, whose identity is under investigation. This lipid is also present in the extracts from mixed glial cultures, which do not contain mature $O4^+$ OLs, suggesting that other glial cells in addition to OLs might be important in the response to rHIgM22.

WTH01-03

Essential role of endogenous fatty acid synthesis in CNS remyelination

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Remyelination requires adult oligodendrocyte progenitor cells (OPCs) to proliferate and subsequently differentiate into myelinating cells, hence calling for tremendous increase in lipid availability. Fatty acids are the primary constituents of cellular membrane lipids, and thus myelin itself. Furthermore, fatty acids are critical to a variety of fundamental cellular functions, including membrane targeting of proteins, energy storage, cell signalling and transcriptional regulation. While most cells are thought to mainly rely upon uptake to maintain their fatty acids pool, highly metabolically active and proliferative, i.e. cancer and precursor-/stem-cells are strongly functionally dependent on *de novo* synthesis, mediated by fatty acid synthase (FASN). The multifunctional enzyme FASN is strictly required for the synthesis of saturated fatty acids, mostly palmitate, from substrates acetyl-CoA and malonyl-CoA in the presence of NADPH as a cofactor. The relevance of this fundamental metabolic pathway during CNS remyelination has so far not been fully clarified. Thus, we addressed the functional role of *de novo* fatty acid synthesis in adult OPC-mediated remyelination. Using inducible Cre/lox system, we examined the specific effect of conditional depletion of FASN in adult OPCs on remyelination, following lysolecithin-induced demyelination. We show that FASN-mediated *de novo* fatty acid synthesis is critical to achieve efficient CNS remyelination and the maintenance of the remyelinating oligodendrocyte population. Our results add valuable information to the understanding of the regulation of the remyelination process in demyelinating conditions (including multiple sclerosis), a promising currently pursued drug target.

WTH01-04

VDR gene polymorphism BSMI in association with multiple sclerosis risk and progression in Slovak population

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Background and objectives: Vitamin D, that acts through the vitamin D receptor (VDR), has been found to be an important factor involved in the etiopathogenesis of Multiple Sclerosis (MS). Since single nucleotide polymorphism (SNP) BsmI in *VDR* gene can affect

the structure and function of VDR, we tried to identify whether this SNP is associated with the risk and progression of MS in Slovaks.
Methods: The group of examined individuals consisted of 270 MS patients and 303 healthy controls. The disease progression was evaluated by MSSS score. Genotyping was performed by polymerase chain reaction (PCR) and restriction analysis.
Results: We found that genotype BB (AA) of BsmI VDR gene polymorphism is decreasing the MS risk (recessive model, OR = 0.59, 95% CI = 0.39–0.90, $p_{\log} = 0.014$). We did not identify any association of BsmI VDR gene polymorphism with the disease progression.
Conclusion: Our findings suggest an association of VDR SNP BsmI with MS susceptibility in the cohort of Central European Slovak population. We propose the BsmI gene polymorphism to be one of the valuable genetic markers useful in the prediction of MS risk.

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WTH01-05

TSC function in CNS myelination independent of oligodendrocyte differentiation

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Our previous studies revealed that the mechanistic target of rapamycin (mTOR) promotes oligodendrocyte differentiation and developmental myelination in the CNS both *in vitro* and *in vivo* (Tyler et al., 2009; Wahl et al., 2014). Surprisingly though, recent studies showed that mice in which mTOR pathway is constitutively active in OPCs, due to a deletion of its negative upstream regulator tuberosclerosis complex (TSC), also display a hypomyelination phenotype instead of hypermyelination as originally predicted (Lebrun-Julien et al., 2014; Carson et al., 2015; Jiang et al., 2016). However, in all these studies, the deletion of TSC was introduced very early in development, during specification or differentiation of the oligodendrocyte cell lineage, and myelination independent of differentiation was not solely assessed.

The goal of this study is to assess how myelin production is affected when mTOR signaling is upregulated through deletion of TSC exclusively in the mature oligodendrocyte population, so that the differentiation process remains unperturbed. We hypothesized that loss of TSC at this later stage in oligodendrocyte maturation would lead to hypermyelination. To test this hypothesis we generated an inducible conditional knock-out mouse model for TSC1 using a tamoxifen-inducible cre recombinase expressed under the proteolipid protein (PLP) promoter. We induced deletion of TSC1 at postnatal day (PND) 7–10 and analyzed myelination in spinal cord at PND 17, 27 and 60. Our preliminary data suggest that loss of TSC in maturing oligodendrocytes has no effect on oligodendrocyte survival or differentiation. In addition, the expression levels of different myelin proteins were unchanged in the mice lacking TSC1, in sharp contrast to all previous studies showing a decrease in myelin proteins with early loss of TSC. In ongoing studies we are analyzing myelin thickness by electron microscopy to determine the impact of TSC loss in the process of myelination, independent of its effect on oligodendrocyte differentiation.

WTH01-06

A dual role for the PI3K-AKT-MTORC1 axis in peripheral nerve myelination

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Consistent with the intense anabolic challenge posed by myelination, the PI3K-Akt-mTORC1 axis has recently emerged as a fundamental player in the myelination of the peripheral and central nervous system. We and others have recently shown that genetic disruption of mTORC1 in SCs impaired myelination. However, it is not clear whether, conversely, hyperactivation of mTORC1 could augment myelin growth. Surprisingly, we show here that hyperactivation of mTORC1 following deletion of TSC1 and/or PTEN during early nerve development delayed SC myelination, rather than promoting it. Consistently, we found that mTORC1 negatively controls the expression of Krox20, a master transcription factor for the onset of SC myelination, via its downstream target S6K and that mTORC1 activity is physiologically high before the onset of myelination and declines as soon as SCs start myelinating. In sharp contrast to the outcome during early development, activation of the same pathway in SCs already committed to myelination resulted in radial hypermyelination. We, therefore, propose a unifying model according to which mTORC1 does not exert a univocal role in myelination, but at least two distinct functions: inhibition of the onset of myelination in not-yet myelinating SCs and promotion of myelin growth in myelinating SCs. The physiological decline in mTORC1 activity represents then a turning point allowing Krox20 expression to increase and SC myelination to begin, while supporting myelin growth through residual mTORC1 activity.

WTH01-07

TRKB agonists promote myelin repair in the brain

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Multiple Sclerosis is a neurodegenerative disease common in young adults, caused by an autoimmune attack against myelin. While current immune-directed treatments are successful in early disease, they do not directly stimulate myelin repair, and cannot fully prevent irreversible nerve damage, and subsequent disability. We have shown that brain-derived neurotrophic factor (BDNF) enhances myelination through activation of TrkB receptors on oligodendrocytes. To test if activation of TrkB receptors can promote myelin repair, we infused BDNF, and known TrkB agonists TDP-6, LM22A-4 and 7,8-dihydroxyflavone (DHF), into the brain of adult mice previously treated with 0.2% cuprizone to induce demyelination. Following 7 days of infusion, all TrkB agonists, but not BDNF, significantly enhanced remyelination above vehicle

controls, with increased MBP in the corpus callosum ($p < 0.001$). Each TrkB agonist had a distinct effect on oligodendrocyte populations, with TDP-6 and LM22A-4 treatment significantly increasing the density of post-mitotic oligodendrocytes ($p = 0.01$) and DHF infusion significantly increasing the density of oligodendrocyte progenitors ($p = 0.02$). Morphometry revealed TDP-6 and LM22A-4 increased the percentage of remyelinated axons ($p = 0.04$) and increased myelin sheath thickness ($p < 0.001$). BDNF treatment for 7 days did not alter oligodendrocyte density, or the percentage of remyelinated axons. The structural BDNF-mimetic TDP-6 had the greatest increase in myelin sheath thickness. To elucidate the divergent effects of the TrkB agonists, quantification of phosphorylated TrkB and Erk1/2 are being performed in conjunction with *in vitro* assays to examine signalling bias. To confirm that enhanced remyelination is an oligodendrocyte-driven effect, selected TrkB agonists have been infused into the brains of cuprizone-fed conditional knockout mice, where TrkB has been deleted from maturing oligodendrocytes. Importantly, the enhanced remyelination effect of TDP-6 and LM22A-4, in contrast to the poor response to BDNF, highlights that while native neurotrophins have poor therapeutic properties, selective modulation of neurotrophin signalling is a viable therapeutic strategy to promote myelin repair in the brain.

WTH01-08

Myelin-associated EPHRINB3 restricts schwann cell migration within central nervous system white matter

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Myelination, which allows rapid and saltatory nerve conduction, is provided by two different types of glial cells, oligodendrocytes in the central nervous system(CNS), and Schwann cells in the peripheral nervous system(PNS). These cells mainly differ in their remyelination capacity, Schwann cells being more efficient in the PNS than oligodendrocytes in the CNS. Since Schwann cells can occasionally invade the CNS under dys/demyelination conditions, these cells have been considered robust candidates for autologous cell therapy in some pathologies. However, Schwann cell survival and migration within the CNS is far from being optimal and the mechanisms involved in this restriction are poorly understood. The object of this work is to study the involvement of CNS myelin in this Schwann cell-CNS segregation. Particularly, we focused on the role of EphrinB3 as a myelin component, able to induce repulsion to other cell types. We demonstrate that Schwann cells present receptors for EphrinB3, and its presence on the Schwann cell surface impairs their adhesion on myelin and consequent migration capacities. We gained evidence that EphrinB3 response is mediated by EphA4 and EphB6 receptors. In addition, our *in vivo* studies showed that grafted Schwann cells are able to migrate towards focal demyelinated lesion in wild-type mice, but failed to mingle with CNS myelin. In contrast, these cells use blood vessels as scaffolds to pave their way towards the lesion, embedded in the extracellular matrix of endothelial cells. Finally, we show *in vitro* that EphrinB3 alters the adherence of Schwann cells to ECM components through integrin beta1. This suggest that Ephrin/Eph may act by a dual mode, repulsing Schwann cell from CNS myelin and enhancing their attraction to basal lamina, and therefore, directing their migration along CNS vasculature.

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WTH01-09

Role of RHIGM22, a remyelination-promoting antibody, in the regulation of acid sphingomyelinase activity in mixed glial cells

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Recombinant human IgM22 (rHIgM22) binds to myelin and oligodendrocytes (OLs), and promotes remyelination in mouse models of multiple sclerosis. The identity of its antigen is still under investigation but we have shown that different sphingolipids are potentially involved in its ability to bind at the cell surface. Literature strongly suggests that rHIgM22 biological activity is mediated by the reorganization of Lyn, integrin αvβ3 and PDGFαR at the cell surface to form a signaling complex triggering Lyn activation which, in turn, promotes oligodendrocyte precursor cells (OPCs) survival and proliferation[1]. However, rHIgM22-mediated OPC proliferation is only detectable in mixed glial cultures (MGC), but not in purified OPCs[2]. Previous studies in OLs showed that the anti-apoptotic effect of Lyn activation might be due to reduced activity of acid sphingomyelinase (ASMase) and consequent reduced ceramide generation[3]. Ceramide generated by the action of ASMase represents an important pro-apoptotic signal, but also a signal for the re-arrangement of sphingolipid-rich signaling platforms[4]. We observed that, in MGC, Lyn is enriched in sphingolipid-enriched membrane fractions, which are also enriched in ASMase[5]. We assessed ASMase activity in MGC following a single dose treatment with rHIgM22, for either 24 or 48 h. Two different non-immunogenic human IgMs were used as a negative control. The data we obtained show a significant decrease of total ASMase activity in MGC treated with rHIgM22, with respect to control. rHIgM22-mediated increased Lyn expression and activation could result in a decrease in ASMase activity and in ceramide generation, thus inhibiting pro-apoptotic signaling and/or the organization of sphingolipid-dependent signaling platforms.

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WTH01-10

THE anti-large myelin protein zero (L-MPZ) antibody in serum modifies the peripheral nerve demyelination in Lewis rat

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Large myelin protein zero (L-MPZ) is a recently identified novel isoform of P0, in which extra 63 amino acids are added at the C-terminus. Antibodies against L-MPZ were often found in the sera from the patients with chronic demyelinating polyneuropathy (CIDP), although pathophysiological role of these antibodies are still uncertain. To determine the relevance of anti-L-MPZ antibodies to neuropathic conditions, we examined rodent demyelinating nerves in the presence and absence of anti-L-MPZ antibody.

Lewis rats were immunized with antigenic peptide of L-MPZ (244–282aa) emulsified in Freund's complete adjuvant (FCA). For control treatment, phosphate-buffered saline was used instead of L-MPZ peptide (FCA-immunized). Remarkable elevation of the L-MPZ-specific antibody titers and the slight reduction in grip power were observed, however, histological examination showed no apparent neuritis in the immunized rats, suggesting that anti-L-MPZ antibody did not induce pathological condition in the peripheral nerves. Next, we carried out the induction of demyelination with lysolecithin in the L-MPZ/FCA-immunized, FCA-immunized and non-immunized animals. Demyelinated areas were examined in the sciatic nerves of days 7 and 14 after lysolecithin injection. In non-immunized group, demyelination was observed to be peak on the day 7 and almost disappeared at the day 14 after lysolecithin injection. Demyelinated areas of L-MPZ/FCA-immunized group were considerably smaller than non-immunized group, but larger than those of FCA-immunized group on the days 7. The infiltrated $CD68^+/CD206^+$ M2 type macrophages of the L-MPZ/FCA-immunized group at the day 7 were about 50% but decreased to 10–30% at day 14, although those of FCA-immunized group at day 7 and day14 were about 20%.

Thus, present results indicate that anti-L-MPZ antibodies may influence macrophages and modulate pathological conditions during demyelination.

WTH01-11

Dynein/dynactin is necessary for anterograde transport of MBP MRNA in oligodendrocytes and for myelination in vivo

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Myelin is the lipid rich sheath that surrounds axons and promotes rapid action potential propagation in the vertebrate nervous system. In the central nervous system (CNS), myelin is produced by specialized glial cells called oligodendrocytes and disruption of myelin causes neurological disorders. Through a large scale forward genetic screen, I identified a mutant that exhibits axon and myelin defects. The phenotype results from a mutation in the gene actr10, encoding the protein Arp11, a component of the dynactin complex necessary for retrograde transport by dynein. Interestingly, *in situ* hybridization for myelin basic protein (mbp) mRNA showed that actr10 mutants have reduced mbp expression in the CNS as well as a punctate mbp phenotype, reminiscent of kinesin kif1b zebrafish mutants, leading us to hypothesize that retrograde transport was influencing anterograde mbp transport. Pulldowns of a reporter protein that binds to Mbp mRNA from rat oligodendrocytes demonstrate that Mbp mRNA granules co-immunoprecipitate with dynactin and dynein. Furthermore, treatment of rat oligodendrocyte cells with ciliobrevin, a dynein inhibitor, resulted in arrest of Mbp transport in anterograde and retrograde directions. In combination with *in vivo* data obtained from actr10 zebrafish mutants, our data highlight an unexpected role for the retrograde motor complex in anterograde Mbp mRNA trafficking.

WTH01-12

Persistent cytokine production induced in the cerebral meninges in a rat model of MS gives rise to chronic cortical pathology

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The progressive phase of multiple sclerosis (MS) is characterised by accumulating grey matter (GM) pathology. The presence of immune cell infiltrates in the meninges is associated with lymphoid tissue development, greater cortical demyelination, shorter disease duration and significant neuronal loss. Analysis of isolated meninges of MS cases has shown an increased gene expression for the pro-inflammatory cytokines: tumour necrosis factor (TNF) and

interferon-γ (IFNγ). We aimed to test the hypothesis that chronic production of pro-inflammatory cytokines in the meningeal compartment and diffusion into underlying GM can drive MS GM pathology. To do this we stereotactically injected HIV-1 based VSV-g pseudotyped lentiviral transfer vectors into the sagittal sulcus of DA rats to deliver continuous transgene expression (TNF + IFNγ) in the meninges for chronic periods through efficient transduction of meningeal cells. A neuropathology analysis was conducted at time points up to 2 months, together with RT-PCR to determine changes to TNFR1 signalling molecules. Injection of these vectors induced the formation of large immune cell aggregates in the meninges by 28 dpi, which remained at 2 months, containing CD4+ and CD8 + T-cells, CD79a+ B-cells and Iba1 + macrophages. These aggregates extended the length of the SS and across the surface of the cortex. Subpial demyelination underlying these aggregates was accompanied by widespread microglial activation and a decrease in neurofilament and NeuN staining indicating areas of potential neuronal loss. TNF/TNFR1 interactions can initiate cell death by activating pathways involved in necroptosis. RT-PCR on cortical RNA at 28 dpi showed an increase in expression of TNFR1 and downstream necroptotic genes, RIP3 and MLKL, compared to eGFP vector control animals. RIP3 + and MLKL+ immunopositive cells with the morphology of neurons were present in TNF vector injected animals. Our results suggest that TNF in the presence of IFNγ is a potent inducer of meningeal inflammation and can activate TNF signalling pathways in cortical cells leading to neuronal death and subpial demyelination and thus may contribute to clinical progression in MS.

WTH01-13

Alterations in oligodendrocyte progenitor cell populations with loss of MTOR

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Oligodendrocyte progenitor cells (OPCs) undergo several distinct stages of differentiation to form mature myelinating oligodendrocytes. The process of differentiation is highly regulated through multiple signaling pathways. Two major pathways that promote CNS myelination are PI3K/Akt/mTOR and Mek/Erk1/2. Disruption of either of these pathways in mice compromises developmental myelination, however, their functions appear only partially overlapping.

Deletion of mTOR in OPCs results in a delay in oligodendrocyte differentiation and initiation of myelination as well as long-term hypomyelination of the spinal cord. In contrast, these mice have normal myelination of the brain implying variations in cellular response to loss of mTOR. We have identified the stage of differentiation where spinal cord OPCs are delayed and accumulating in the absence of mTOR. Percentages of O^{4+} late-stage progenitor cells and PDGFRα+ early-stage progenitor cells were measured by flow cytometry. We have found decreased numbers of late-stage progenitors in the developing mTOR knockout spinal cord, with a corresponding increase in early-stage progenitors, suggesting an accumulation of many early progenitors that are unable to progress to the O^{4+} stage when mTOR is deleted. To further define alterations in OPC populations and differentiating oligodendrocytes with loss of mTOR, we have initiated experiments using Drop-seq, an innovative technology for single-cell RNA sequencing. In initial studies, we used magnetic bead columns to isolate O^{4+} OPCs from mTOR knockout and control spinal cords and simultaneously analyzed the mRNA transcripts of thousands of individually identifiable cells. Transcriptional variation across the single cells can be used to define distinct populations. Sequence data from our Drop-seq experiment is currently under analysis.

Our long term goal is to further understanding of heterogeneity of OPCs and how it contributes to differences in oligodendrocyte function. Future directions will also include studying changes in specific cellular functions and pathways with loss of mTOR.

WTH01-14

Exploring the factors causing remyelination arrest through studying cystatin F gene expression regulatory mechanism

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Demyelinating diseases are series of disorders that damage the myelin sheath in the central nervous system. At an early phase of demyelinating diseases, demyelination is accompanied by remyelination forming the shadow plaque. However at a late stage, remyelination become arrested. Cystatin F, a papain-like lysosomal cysteine proteinase inhibitor, and its main target, cathepsin C, have been demonstrated to be crucial factors in regulating remyelination arrest. A chronic demyelination mouse model, named heterozygous proteolipid protein (PLP) transgenic 4e ($PLP^{4e/-}$) mouse, was used to study cystatin F function. We found cystatin F was upregulated in the early phase (2–4 months of age) and then decreased in the chronic phase (4–8 months of age) in the $PLP^{4e/-}$ mouse. To explore the remyelination arrest mechanism in the chronic demyelinating disorders, we clarified cystatin F gene regulatory mechanisms in this study. We used a mouse line (CysF-STOP-tetO::Iba-tTA) in which the cystatin F gene expression is drived by the tetO promoter. We surprisingly found the cystatin F gene is forced to be expressed but its expression was later decreased in CysF-STOP-tetO::Iba-tTA mouse. Together with other results, we proved cystatin F expression was post transcriptionally regulated. Then, we found the factor Embryonic lethal, abnormal vision, drosophila like RNA binding protein 1 (ELAVL-1), which is an AU rich elements binding protein, stabilized cystatin F mRNA. Its expression was downregulated together with cystatin F decreased level in both $PLP^{4e/-}$ mice CysF-STOP-tetO::Iba-tTA mice. *In vitro* study showed decrease of ELAVL-1 downregulated cystatin F expression. All of these data revealed the important role of ELAVL-1 in regulating cystatin F expression. It may provide a new insight in the therapy of demyelinating disorders.

WTH01-15

Remyelination from demyelinating lesions induced by multiple sclerosis antibodies

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Multiple sclerosis (MS) is an inflammatory demyelinating disorder of the central nervous system (CNS). Following myelin loss, remyelination may occur. Current models of remyelination rely on toxin or detergent-induced injuries that are not relevant to pathological mechanisms in MS. To address this issue, we have developed new inflammatory models of demyelination and remyelination using *ex vivo* organotypic mouse cerebellar slice cultures and *in vivo* spinal cord micro-injection using recombinant antibodies (rAbs) cloned from cerebrospinal fluid (CSF) plasmablasts of MS patients. Using our novel models, we measured axonal demyelination and remyelination, oligodendrocyte loss and repopulation, axonal integrity, and astrocyte gliosis by immunohistochemistry during injury and recovery. In the *ex vivo* model, transient treatment with myelin-specific MS rAbs induced robust complement-dependent oligodendrocyte cytotoxicity and rapid demyelination. The morphology and survival of astrocytes, oligodendrocyte progenitors and neurons were unaffected, and axons remained intact. After the rAb treatment was removed, oligodendrocyte cell bodies and processes repopulated with clear evidence of new myelin protein deposition along axons. In the *in vivo* model, a clear lesion boundary delineated by the loss of oligodendrocytes and the presence of myelin debris was observed 3 days after focal injection (DPI) of MS rAb plus human complement. At 14 DPI there was continued loss of oligodendrocytes, and clearance of myelin debris within the lesion site. Axons and astrocytes were preserved. Oligodendrocyte progenitor infiltration was detected in the lesion, suggesting commencement of active remyelination. At 28 DPI remyelinated sheaths appeared outside of axons. Our results indicate that MS rAbs with complement produce a well-controlled demyelinating lesion that remyelinates *ex vivo* and *in vivo*. These new models will advance our understanding of MS inflammatory demyelination and remyelination and aid in the development of successful strategies to promote myelin repair and restore neuronal function in affected patients.

WTH01-16

Alterations of synaptic terminals in the cerebellum of chronic demyelinating mouse model

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Normal brain function depends on integrity of complex networks among neurons through synapses. Loss or disruption of myelin sheath impairs fast saltatory conduction and axonal integrity, and leads to neurological deficits in demyelinating diseases. Although it is well established that demyelination alters structures and functions of demyelinated segments of axons, influence of demyelination to axon terminals is still poorly understood. In this study, we investigated alterations of axon terminals and related axonal organelles in mouse cerebellum, using a progressive demyelination model caused by overexpression of proteolipid protein ($PLP^{4e/-}$) [1]. Morphological and three dimensional ultrastructural changes of axonal terminals and organelles including mitochondria were analyzed using serial block face scanning electron microscopy (SBF-SEM) and immunohistochemistry. At 5 months of age, demyelinated axons and axons with abnormally thin myelin were prominent in the cerebellar white matter of hemizygous $PLP^{4e/-}$ mice. In the cerebellar cortex, number and height of climbing fiber terminals were significantly reduced while quantitative SBF-SEM results showed mitochondrial volume in the terminals was increased in the hemizygous $PLP^{4e/-}$ mice compared with age-matched wild-type (WT) mice. By contrast, the numbers and mitochondrial volume of the climbing fiber terminals were similar in $PLP^{4e/-}$ and WT mice at 1 months of age. To investigate the synaptic alterations in more detail, we established organotypic cerebellar slice culture system. In the organotypic slice cultures, robust myelination in WT slices and gradual loss of myelin in $PLP^{4e/-}$ slices were observed during the entire culturing period. These results demonstrated chronic synaptic loss and enlargement of presynaptic mitochondria upon myelin loss in demyelinated axons. The organotypic cerebellar slice culture is a useful tool to observe synaptic changes in the demyelination disease model.

[1] Kagawa et al. Neuron 13 (1994) 427–42.

WTH01-17

Protective role of kolaviron on cuprizone-induced demyelination in rat models of multiple sclerosis

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This study explored the efficacy of kolaviron (kv)-a biflavonoid complex isolated from the seeds of *Garcinia kola* in providing protection against Cuprizone (CPZ)-induced demyelination in both prefrontal cortex and hippocampus of Wistar rats. Thirty rats were treated to receive (A) 0.5 mL PBS (Control); (B) 0.5 mL corn oil; (C) 0.2% CPZ; (D) 0.2% CPZ and 200 mg/kg of kv and (E) 200 mg/kg of kv and 0.2% CPZ for 6 weeks each. Rats were assessed in the open field for exploratory functions and in the elevated plus maze for anxiety-like behavior, and thereafter euthanized and perfused transcardially using 4% paraformaldehyde. The brains were removed in fixed in the same fixative. Prefrontal and hippocampal thin sections were then stained in H&E and cresyl violet for Nissl bodies. CPZ-induced demyelination resulted into behavioural impairment as seen by reduced exploratory activities, rearing behaviour, stretch attend posture, center square entry and anxiogenic characteristics. Furthermore, degenerative changes including pyknosis, kharyorrhesis, neuronal hypertrophy and reduced Nissl integrity were seen in response to CPZ administration compared to control. However, rats treated with kv before or after CPZ administration showed significant improvement in behavioural outcomes and comparatively normal cytoarchitectural profile in neural tissues. This study showed that kv provides protective roles against CPZ-induced neurotoxicity through prevention of ribosomal protein degradation.

WTH01-18

Effect of novel readthrough agents on myelin P0 translation *in vivo*

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Large myelin protein zero (L-MPZ) is an isoform of myelin protein zero (P0), containing additional 63 amino acids at the C-terminus by stop codon readthrough mechanism (Yamaguchi et al., 2012). Our recent study showed that the adhesion activity of L-MPZ is weaker than P0, suggesting that the ratio of P0 and L-MPZ in myelin is important for normal myelin structure. Recently, readthrough agents have been developed to suppress nonsense mutations in the genetic disorders, including Duchenne muscular dystrophy. Before clinical use, however, influence of readthrough agents on proteins naturally produced by this mechanism should be clarified. In the present study, we examined the activities of novel readthrough agents, negamycin analogues (Taguchi et al., 2014), on P0 gene to choose the appropriate agent for *in vivo* experiments. G418 was used as a positive control. *In vitro* transcription/translation study using human P0 cDNA demonstrated that readthrough activity (relative % ratio of L-MPZ in total of L-MPZ and P0) was nearly 10% without any agents, indicating that P0 mRNA itself has relatively high readthrough activity. One of the analogues showed higher readthrough activity (approximately 30%) compared to negamycin without inhibition of protein synthesis. The readthrough activities were also examined using the cells with stable expression of human P0 mRNA. Percent ratios of L-MPZ-positive cells were increased to 60% (~ 10% in control) by three negamycin derivatives. One of these agents was directly injected in mouse sciatic nerves. The treated nerves showed 1.3-fold increase of L-MPZ/P0 ratio compared to vehicle control. Immunohistological analysis showed the increased signal of L-MPZ in Schmidt-Lanterman incisures and paranode as well as compact myelin, which was different from normal P0 distribution. Thus, it is important to examined physiological influence of translational readthrough in the PNS myelin using this agent.

WTH01-19

Targeting neuronal Nogo receptor 1 signaling in EAE preserves axonal transport and limits demyelination

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We have previously shown that deletion of the *ngr1* allele limits experimental autoimmune encephalomyelitis (EAE) severity by preserving central nervous system (CNS) axons. What is unknown is whether this preservation is governed by myelin being intact thereby protecting axons, or axonal degeneration is limited, preventing demyelination. In this study we investigated how targeting neuronal ngr1-dependent signaling, may prevent axonal degeneration during EAE. Conditional deletion of ngr1 in axons was produced by intraocular injection of AAV2 encoding Cre (AAV2-iCre-eGFP) in $ngr1^{flx/flx}$ mice. Conversely, conditional re-introduction of NgR1 in axons was produced by intraocular injection of AAV2 encoding full-length mouse NgR1 (AAV2-NgR1-eGFP) in $ngr1^{-/-}$ mice. All mice were EAE-induced with the myelin oligodendrocyte glycoprotein (MOG_{35-55}) peptide and culled at the peak stage of disease. We found that axonal degeneration is limited in AAV2-iCre-eGFP injected $ngr1^{flx/flx}$ whereas, significant axonal damage is found in AAV2-NgR1-eGFP injected $ngr1^{-/-}$ optic nerves during EAE. As a corollary, the preservation of myelin integrity was a prominent feature in AAV2-iCre-eGFP injected $ngr1^{flx/flx}$, whereas significant demyelination was found in AAV2-NgR1-eGFP injected $ngr1^{-/-}$ optic nerves. Furthermore, the interaction between the axonal motor protein, kinesin-1 (KIF5) and collapsin response mediator protein 2 (CRMP-2) was reduced in AAV2-NgR1-eGFP injected $ngr1^{-/-}$ optic nerves with significant stalling of cholera toxin transport within the diseased optic nerve. Our data suggest that NgR1 governs axonal degeneration in the context of inflammatory-mediated demyelination through phosphorylation of CRMP-2, abrogating axonal vesicular transport. Moreover, the axon-specific deletion of *ngr1* preserves axons blunting the induction of demyelination during EAE, thereby suggesting that NgR1-dependent neurodegeneration maybe a primary mechanism during neuroinflammation.

WTH01-20

TRPA1 receptor deficiency substantially diminishes the cuprizone-induced demyelination

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Our recent studies have presented evidence that Transient Receptor Potential Ankyrin 1 (TRPA1) receptor is expressed on astrocytes in the mouse CNS and its deficiency significantly attenuated cuprizone-induced demyelination by reducing the apoptosis of mature oligodendrocytes (Saghy et al. 2016). The aim of the present study was to investigate the time course of behavioural alterations and morphological changes in cuprizone-treated TRPA1 knock out (KO) mice.Demyelination was induced by feeding wild-type (WT) and KO mice with 0.2% cuprizone mixed into standard rodent chow for 6 weeks. For the open field test, animals were placed into an open arena and filmed with a digital camera. Recordings were evaluated for the determination of the time, distance and velocity of locomotion, while the number of rearings was counted manually. Spatial working memory was investigated by Y-shaped maze. The time course of demyelination was followed by Magnetic Resonance Imaging (MRI). Myelin decompaction was analysed by Luxol Fast Blue (LFB) staining and electronmicroscopy (EM).Cuprizone-treated mice spent more time with locomotion, their mean velocity was significantly higher and the distance they travelled was also consequently longer than untreated mice at weeks 2 and 3 of treatment. No statistical difference was detected between WT and KO mice in these parameters. On the other hand,

significantly increased rearing behaviour was induced in WT mice compared to TRPA1 KO animals. On the basis of MRI, FFB, and EM analysis reduced damage of the myelin was detected in TRPA1 deficient animals in each examined time point. Inhibition of TRPA1 receptors might diminish the degenerative pathology in multiple sclerosis and could be a promising therapeutic target in demyelinating diseases.Supported by National Brain Research Program-A (KTIA_NAP_13-1-2013-0001).

WTH01-21

Inflammatory demyelination induces ependymal modifications concomitant to activation of adult (SVZ) stem cell proliferation

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Ependymal cells (E1/E2) and ciliated B1cells confer a unique pinwheel architecture to the ventricular surface of the subventricular zone (SVZ), and their cilia act as sensors to ventricular changes during development and aging. While several studies showed that forebrain demyelination reactivates the SVZ triggering proliferation, ectopic migration, and oligodendrogenesis for myelin repair, the potential role of ciliated cells in this process was not investigated. Using conventional and lateral wall whole mount preparation immunohistochemistry in addition to electron microscopy in a forebrain-targeted model of experimental autoimmune encephalomyelitis (tEAE), we show an early decrease in numbers of pinwheels, B1 cells, and E2 cells. These changes were transient and simultaneous to tEAE-induced SVZ stem cell proliferation. The early drop in B1/E2 cell numbers was followed by B1/E2 cell recovery. While E1 cell division and ependymal ribbon disruption were never observed, E1 cells showed important morphological modifications reflected by their enlargement, extended cytoskeleton, and reinforced cell-cell junction complexes overtime, possibly reflecting protective mechanisms against ventricular insults. Finally, tEAE disrupted motile cilia planar cell polarity and cilia orientation in ependymal cells.

Therefore, significant ventricular modifications in ciliated cells occur early in response to tEAE suggesting a role for these cells in SVZ stem cell signalling not only during development/aging but also during inflammatory demyelination. These observations may have major implications for understanding pathophysiology of and designing therapeutic approaches for inflammatory demyelinating diseases such as MS.

WTH01-22

The effects of GUT microbiota on myelination and oligodendroglia in the BACHD model of huntington disease

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Structural and molecular myelination deficits occur in the early stages of Huntington disease (HD), an autosomal dominant neurodegenerative disorder, characterised by progressive motor, cognitive and psychiatric deficits. Recent evidence from germ-free (GF) animal models lacking gut-associated microorganisms have suggested the microbiome to play a role in neurodegenerative and neuropsychiatric disorders. In addition, the gut-brain bidirectional communication was shown to modulate the blood-brain barrier, neurogenesis and neuronal activity, microglia responsiveness, and more specifically to our interest, to be involved in the regulation of oligodendrocyte differentiation and myelination. In this study we aimed to investigate the impact of gut microbiota on HD-related white matter phenotypes of the BACHD mouse model, and the extent to which the status of the microbiota could modulate myelination and the expression of myelin-related genes. Three months old specific-pathogen-free (SPF) and GF mice of mixed sex and genotype (wild type and BACHD) were used. Preliminary findings revealed changes in body weight in GF BACHD compared to SPF BACHD animals, as well as a reduction in brain weight in the GF groups, both in WT and BACHD, compared to the SPF groups. Analysis of transmission electron microscopy images of the corpus callosum, quantifying the thickness of myelin sheaths, suggest alterations in axonal diameter and myelin thickness, particularly evident in the GF BACHD group. On-going assessments include examination of changes in oligodendroglia cell populations and expression of myelin related proteins. Our results on the effects of the gut microbiota on myelin plasticity, in both the HD and the healthy brain, may shed light on extrinsic mechanisms regulating oligodendroglia and have important implications for therapeutic approaches and interventions for HD.

WTH01-23

Unconventional myosin id is involved in the remyelination process after cuprizone-induced demyelination

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Myelin is a multilamellar structure that ensheathes an axon and is crucial for normal neuronal function. In the CNS, myelin is produced by oligodendrocytes (OLs) those extend processes and wrap their plasma membrane around multiple axons. The dynamic membrane trafficking system, which relies on motor proteins, is required for myelin formation and maintenance. Previously, we reported that Myo1d is enriched in the outer and inner cytoplasm-containing loops in the CNS myelin, and the knockdown of Myo1d expression using specific siRNA induces morphological changes and apoptosis, and impairs myelin proteolipid protein (PLP) transport in cultured OLs (Yamazaki et al., 2014; 2016). Myo1d possibly contribute to membrane dynamics either in wrapping or

transporting of myelin membrane proteins during myelination. However, the function of Myo1d *in vivo* is still unclear. In this study, we investigated the role of Myo1d in brain by using cuprizone (CPZ)-treated de- and remyelination mouse model. Immunofluorescence signals of Myo1d and myelin basic protein (MBP) were reduced compared to those in non-treated control mice in the demyelinated corpus callosum after 5 weeks of CPZ treatment. These changes were fairly recovered to the pretreatment levels during remyelination processes. To examine the importance of Myo1d during remyelination, we injected Myo1d-siRNA into the demyelinated corpus callosum using stereotaxic technique. Knockdown of Myo1d expression induced inhibition of MBP and PLP expressions during remyelination. However, the number of CC1-positive mature OLs was not altered by siRNA treatment. To examine whether Myo1d knockdown affects cell death, we calculated the number of caspase3-positive cells. The percentage of caspase3-positive cells to total cells tended to increase after Myo1d-siRNA transfection. Furthermore, Myo1d knockdown induced activations of microglia and astrocytes during remyelination. Therefore, Myo1d knockdown possibly promoted apoptosis in OLs, sustained demyelination, and delayed remyelination process at the final stage of OLs differentiation. These results indicated that Myo1d has an important role of regeneration process after demyelination.

WTH01-24

Effects of chronic TYPE 2 diabetes on expression of hippocampal proteins in rats

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In our previous study, we demonstrated that type 2 diabetes affects blood-brain barrier integrity and ultrastructural morphology in Zucker diabetic fatty (ZDF) rats at 40 weeks of age. In the present study, we investigated the possible candidates for diabetes-related proteins in the hippocampus of ZDF rats and their control littermate (Zucker lean control, ZLC) rats by using two-dimensional fluorescence difference gel electrophoresis (2D-DIGE) followed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF). Approximately 2756 protein spots were detected by 2D-DIGE, and an increase or decrease of more than 1.4-fold was observed for 13 proteins in the hippocampal homogenates of ZDF rats relative to those of ZLC rats. Among these proteins, we found four proteins whose levels were significantly lower in the hippocampi of ZDF rats than in those of ZLC rats: glial fibrillary acidic protein (GFAP), apolipoprotein A-I preprotein (apoAI-P), myelin basic protein (MBP), and rCG39881, isoform CRA_a. Among these proteins, apoAI-P protein levels were decreased most prominently in ZDF rats than in ZLC rats based on Western blot analysis. In addition, immunohistochemical and Western blot studies demonstrated that MBP, not GFAP, immunoreactivity and protein levels were significantly decreased in the hippocampus of ZDF rats compared to ZLC rats. In addition, ultrastructural analysis showed that ZDF rats showed myelin degeneration and disarrangement in the hippocampal tissue. These results suggest that chronic type 2 diabetes affects hippocampal function via reduction of MBP and apoAI-P levels as well as disarrangement of myelin.

WTH01-25

Axon initial segment cytoskeleton shows a more complicated pattern during brain development

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Axon initial segments (AIS) and nodes of Ranvier are highly specialized axonal membrane domains enriched in Na^+ channels. These Na^+ channel clusters play essential roles in action potential initiation and propagation. AIS and nodal Na^+ channel complexes are linked to the actin cytoskeleton through βIV spectrin. However, neuronal βIV spectrin exists as two main splice variants: a longer βIVΣ1 variant with canonical N-terminal actin and αII spectrin-binding domains, and a shorter βIVΣ6 variant lacking these domains. Here, we show that the predominant neuronal βIV spectrin splice variant detected in the developing brain switches from βIVΣ1 to βIVΣ6, and that this switch is correlated with expression changes in ankyrinG splice variants. We show that βIVΣ1 is the predominant splice variant at nascent and developing AIS and nodes of Ranvier, but with increasing age and in adults βIVΣ6 becomes the main splice variant. Remarkably, super-resolution microscopy revealed that the spacing of spectrin tetramers between actin rings remains unchanged, but that shorter spectrin tetramers may also be present. Thus, during development βIV spectrin may undergo a switch in the splice variants found at AIS and nodes of Ranvier.

WTH02 Ischemia and Oxidative Stress

WTH02-01

VEGF theranostic agent promotes neuroregeneration after experimental stroke

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Angiogenesis is a processes that occurs after stroke to help the function recovery in the affected tissue, but its effectiveness is limited. Therefore, the vascular endothelial growth factor (VEGF) has been proposed as a putative therapy aimed at vascular regeneration at the brain lesion. However, due to the side effects of pathologically elevated VEGF levels such as enhance vessel permeability and leakage, and disrupt blood-brain barrier integrity, no treatment using this compound has proved its efficacy. In stroke, the peri-infarct region is a key target region, a no man's land between severely affected tissue (infarct core), with a spreading front of mediators of damage, and healthy tissue, with mediators of remodeling and recovery coming from both sides. In this abstract we report the use of a liposome-based theranostic vehicles targeted to the peri-infarct region, that encapsulate VEGF to promote angiogenesis in controlled manner, potentially avoiding high peaking doses. Liposome-based theranostic agents were constructed by extrusion with DSPC, Cholesterol, DSPE-PEG, Rhodamine-PE and GdDTPA-BSA, and anti-HSP72, loaded with or without VEGF (25 μg/kg). VEGF was also administered intravenously (50 μg/kg). As animal models of stroke we used the intraluminal transient (90 min) MCA occlusion in male rats (MCAo). Treatments were administered 24 h after MCAo. To assess angiogenesis, we used contrast-enhanced magnetic resonance imaging. Briefly, ADC, T2 and T2*-weigthed images were acquired before and after injection of ultra-small paramagnetic iron oxides. N, Q and R maps were calculated according to Boehm-Sturm *et al.*, revealing higher microvessel density and relative vessel size at the peri-infarct region in the group treated with the VEGF-encapsulated liposomes with respect to VEGF administered systemically or empty liposomes. We have provided an alternative approach to VEGF-induced angiogenesis after stroke that enhances the angiogenic effect at the desired area with lower doses, apparently reducing its side effects.

WTH02-02

Nattokinase improves blood flow by inhibiting platelet aggregation and thrombus formation

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Anti-thrombotic effects of nattokinase, an enzyme produced by *Bacillus subtilis* during fermentation of soybeans, were investigated in comparison with aspirin (a blood flow enhancer) and tissue-type plasminogen activator (t-PA, a thrombolytic drug). Nattokinase inhibited platelet aggregation and thromboxane B_2 formation *in vitro*. As a preventive mode on the thrombus formation, oral administration of nattokinase delayed $FeCl_3$-induced arterial occlusion, doubling the occlusion time at about 160 mg/kg. Especially, a high dose (400 mg/kg) of nattokinase fully prevented the arterial occlusion, as achieved with aspirin (30 mg/kg). As a therapeutic mode for thrombolysis, intravenous injection of nattokinase blocked $FeCl_3$-induced arterial occlusion, fully inhibiting at 75 mg/kg, which was achieved with about 8.5 mg/kg of t-PA. In terms of adverse-effects, t-PA caused petechial hemorrhage in the lungs and thymus at 10 mg/kg, leading to extensive bleeding at 20 mg/kg. By comparison, intravenous injection of nattokinase induced pulmonary hemorrhage from 300 mg/kg. The safety margins for t-PA and nattokinase were estimated to be 1.2 and 4.0, respectively. In addition, dexamethasone (2 mg/kg) enhanced the efficacy and safety of nattokinase, in comparison with the beneficial effect on the safety of t-PA. Dexamethasone decreased the therapeutic dose of nattokinase to 50 mg/kg, but increase the hemorrhagic dose to 400 mg/kg, leading to the safety margin of 8.0. Dexamethasone also increased the safety margin of t-PA to 2.4. Therefore, it is suggested that nattokinase could be a good candidate as functional food and/or thrombolytic drug with relatively-low hemorrhagic risk for the improvement of blood flow.

WTH02-03

Cannabinoid receptors and TRPA1 on neuroprotection in a model of retinal ischemia

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Retinal ischemia is a patological event present in several retinopathies such as diabetic retinopathy and galucoma, leading to partial or full blindness with no effective treatment available. Synthetic and endogenous cannabinoids have been described as modulators of ischemic events in the central nervous system (CNS). Thus, the present study aimed to investigate the involvement of cannabinoid system in the cell death induced by ischemia in an avascular (chick) retina. Chicks (2–7 days post-hatched) retinal segments were randomly adressed to control or ischemic for 50 min. We observed that chick retinal treatment with a combination of WIN 55212-2 and cannabinoid receptors antagonists (either AM251/O-2050 or AM630) decreased the release of lactate dehydrogenase (LDH) induced by retinal ischemia in an oxygen and glucose deprivation (OGD) model. Further, the increased availability of endocannabinoids together with cannabinoid receptors antagonists also had a neuroprotective effect. Surprisingly, retinal exposure to any of these drugs alone did not prevent the release of LDH stimulated by OGD. Since cannabinoids may also activate transiente recpetor potencial (TRP), we investigated the involvement of TRPA1 receptors (TRPA1) in retinal cell death induced by ischemic events. We demonstraded the presence of TRPA1 in the chick retina, and observed an increase in TRPA1 content after OGD, both by western blot and immunohistochemistry. In addition, the selective activation of TRPA1 by mustard oil (MO) did not worsen retinal LDH release induced by OGD, whereas the blockage of TRPA1 completely prevented the extravasation of cellular LDH in

ischemic condition. hence, these result show that during the sichemic event there is an augment of TRPA1, and the activation of this receptor is important to evoke cell death. The data also indicate that metabotropic canabinnoid receptors, both type 1 and 2, are not involved with the cell death found in the early stages of ischemia. Therefore, the study points to a potencial role of TRPA1 as a target for neuroprotective approaches in retinal ischemia.

WTH02-04

Effect of punicic acid in a cerebral ischemia model in rat

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Stroke is one of the leading causes of death and disability in the world. Ischemic stroke accounts for approximately 80–85% of all cases and is caused by the obstruction of blood flow to the brain, followed by the subsequent restoration of perfusion and oxygenation. The interruption of the blood flow initiates a complex series of metabolic events that progress to cell death. The inflammation and the oxidative stress are fundamental mechanism of damage implicated in focal ischemic stroke. Punicic acid (PA) is a polyunsaturated fatty acid found in the seed of pomegranate fruit, and it has a wide array of health beneficial properties like antidiabetic, hypolipidemic, anti-inflammatory, anticancer, antioxidant activities, and antinephrotoxic activity. Mice modeling for genetic prion disease were treated with a nanodroplet formulation of pomegranate seed oil, the nano droplet possess neuroprotective effects via its antioxidant properties. The anti-inflammatory effects have been evaluated in models of the inflammatory bowel diseases, necrotizing enterocolitis and age related bone complications. The purpose of this work was to examine the effect of a nanodroplet formulation of pomegranate seed oil on neurological deficit and morphological alterations induced in a model of ischemia and reperfusion (IR), evaluating some antioxidant and anti-inflammatory markers. Animals were divided in 6 groups: control group (CT) received only vegetal oil; IR group submitted to 1 h of ischemia and 4 days of reperfusion; 3 groups submitted to IR and administered with nanodroplet (50, 100, 200 mg/Kg) for 5 days, i.g.; and 1 group submitted to IR and administered with a single dose of 200 mg/Kg i.p. before the reperfusion. Four days of onset reperfusion, the brain of each animal was obtained to evaluate the histological damage. The treatment with PA decreases the neurological deficit and the morphological alterations induced by IR. This work was supported by CONACYT (Grant 241655).

WTH02-05

Adult mouse neural stem cell-derived microvesicles: proteomic characterization and effects on brain ischemia

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Ischemic stroke is a neurological condition provoked by the sudden occlusion of blood flow in the brain. In response to this a series of cellular and molecular events take place to prevent neuronal death and the expansion of injury. One such event involves the proliferation of neural stem cells (NSC) in the neurogenic niches of the adult brain. It has been suggested that NSC proliferation could contribute to replenish the neuronal population that died following stroke, but several studies have shown that neuronal replacement might not be as important for recovery. Rather, NSC may actively communicate with neurons to mediate protection after ischemia. Like most cells, NSC release extracellular vesicles carrying regulatory proteins, lipids and nucleic acids that are able to modulate several functions in their target cells, this mechanism of intercellular communication may underlie some protective effects produced by the ischemia-stimulated NSC expansion. In this study we investigated the possible communication mediated by exosome-enriched microvesicles between NSC and neurons in cerebral ischemia using an *in vitro* model produced by the transient deprivation of oxygen and glucose (OGD). We tested the neuroprotective effect of NSC-conditioned medium on the neurodegeneration of primary cortical neuronal cultures subjected to toxic stimuli relevant in ischemic stroke, namely, glutamate excitotoxicity, oxidative stress and induction of apoptosis. Under these conditions NSC-produced factors are capable to decrease neuronal death; the mechanism underlying such protection might be mediated through the action of molecules released in microvesicles. Therefore, we collected NSC-derived microvesicles from cells subjected to OGD and control conditions and characterized their protein content by mass spectrometry, some of the molecular hits might be involved in promoting neuronal survival and their individual characterization is underway. These results highlight the complexity of NSC-mediated signaling in promoting neuronal survival after stroke. Supported by PAPIIT-DGAPA IN226617 and CONACYT 219542.

WTH02-06

Cyclooxygenase inhibition ameliorates hypobaric hypoxia induced spatial memory impairment in rats

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Background: Hypobaric Hypoxia (HH) is an environmental stress that leads to multiple pathophysiological consequences. Prostaglandin E2(PGE2) is derived from Arachidonic acid by sequential actions of cyclooxygenases (COX-1 and COX-2). Studies demonstrate that elevated PGE2, produced from COX activity is causal factor for memory deficits**.**

Aim: To temporal quantitate PGE2 related molecules in hippocampus and cortex and effect of COX inhibitors in HH induced deficits in spatial memory.

Methdology: Male Sprague–Dawley rats were exposed to different duration of HH exposure (0,1,3,7) at 25000 ft. Levels of PGE2, PGE synthase, COX-1, COX-2 was measured in day dependent manner in hippocampus and cortex. CV staining in day dependent manner in hippocampus was performed. We examined the effect of selective COX-1 inhibitor, Valeryl salicyclate (5 mg/kg/i.p) and COX-2 inhibitor, Celecoxib (20 mg/kg/i.p) on spatial memory during HH.

Results: We found spatial memory deficit post 7 HH exposure as compared to control ($p < 0.001$) with changes in PGE2 levels ($p < 0.01$) and no. of pyknotic cells in hippocampus. The results

indicate that HH evoked PGE2, PGE synthase, COX-1, COX-2 levels in hippocampus as early as day1, reached maximum at day 3 and starts droping back at day 7. Treatment with COX-2 ($p < 0.001$) and COX-1 ($p < 0.01$) inhibitor significantly reduced path-length and latency to reach platform and no. of pyknotic cells in hippocampus as compared to HH exposed animals.

Conclusion: This suggests that PGE2 considerably contributes to spatial memory deficits at day 7. However, the time course of PGE2 up regulation suggests that HH induced PGE2 response at day 3 precedes cognitive deficit at day 7 probably via both COX-1 and COX-2 pathway. Pretreatment with specific cyclooxygenase inhibitor during HH might be ameliorating inducible PGE2 and downstream signaling, results in improved cognitive performance.

WTH02-07

Feeding perilla oil improves atherosclerosis and ischemic stroke by controlling lipid metabolism

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Since plant oils are believed to be better than animal fats for cerebrovascular and cardiovascular diseases, the effects of perilla oil on atherosclerosis and ischemic stroke were investigated. In order to evaluate anti-atherosclerotic activity, hypercholesterolemia was induced in rabbits by feeding a high-cholesterol diet (HCD) containing 0.5% cholesterol and 1% corn oil, and perilla oil (0.1 or 0.3%) was added to the diet containing 0.5% cholesterol for 10 weeks. HCD greatly increased blood total cholesterol and low-density lipoproteins, and caused thick atheromatous plaques, covering 74% of the aortic wall. Hypercholesterolemia also induced lipid accumulation in the liver and kidneys, leading to lipid peroxidation. Perilla oil not only attenuated hypercholesterolemia and atheroma formation, but also reduced fat accumulation and lipid peroxidation in hepatic and renal tissues. To evaluate anti-stroke activity, Sprague–Dawley rats were fed a diet containing various oils (10%) including perilla oil, and then body weights, blood lipids, and effects on brain infarction and physical dysfunction induced by middle cerebral artery occlusion (MCAO) were analyzed. Plant oils and trans-fat, except perilla oil, significantly increased body fats and body weight gain. Sesame oil and trans-fat specifically increased blood cholesterols and triglycerides, respectively, while perilla oil decreased both the cholesterols and triglycerides. Only perilla oil not only attenuated the cerebral infarction, but also restored the physical function in locomotor activity and rota-rod performances of MCAO rats. The results indicate that perilla oil prevents atherosclerosis and fatty liver as well as ischemic stroke by controlling lipid metabolism, and that it could be the first choice oil to reduce the risk of dietary metabolic syndrome and ischemic stroke.

WTH02-08

VEGFR1-mediated neuroprotection in experimental cerebral stroke

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Cerebral stroke is a devastating disease that affects millions of people and it is the first cause of acquired disability in the word. Neuroinflammation is an important component of the pathophysiology of stroke and other neurodegenerative processes where microglia, the resident macrophages of the central nervous system, play a key role. Microglial activation can be modulated by a series of neurotrophic factors including vascular endothelial growth factor A (VEGF-A), which has been associated to neuroprotection in the acute phase of stroke, although the molecular mechanisms underlying such protection are not yet fully understood. The function of VEGF-A is mediated mainly by the activation of its canonical receptor 2 (VEGFR2), but also by receptor 1 (VEGFR1), which is preferably activated by other members of the VEGF family, such as VEGF-B. Both of these receptors are upregulated after stroke, but only VEGFR1 has been reported to be expressed in microglia. Here we studied the mechanisms of VEGF-A-mediated neuroprotection in the acute phase of stroke using an *in vivo* model produced by the transitory occlusion of the middle cerebral artery. Administering exogenous VEGF-A in the early phase after stroke, results in a significant reduction of infarct volume and increased neuronal survival. We found that the underlying mechanism of this protection involves the activation of VEGFR-1 and -2, but interestingly, pharmacologically inhibiting VEGFR2 in the presence of the exogenous ligand reduces infarct volume, limits edema, increases neuronal survival and improves neurological outcome to a greater extent than the simultaneous activation of both VEGFR1/2. Given the role of VEGFR1 on microglial responses to altered brain homeostasis, the underlying mechanisms of the VEGFR1-mediated protection could involve the modulation of the inflammatory response and microglial polarization to a neuroprotector phenotype. These results point towards VEGFR1 as an interesting therapeutic target for stroke worth of further investigation. Supported by PAPIIT-DGAPA IN226617 and CONACYT 219542.

WTH02-09

Probing nitrite and nitric oxide biochemistry in the brain *in vivo* by a novel sensing approach

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Communication between neurons and blood vessels in the brain is essential for cognition. This communication allows for an allocation of energy resources according to local demands imposed by neuronal activation, a process termed neurovascular coupling (NVC). During hypoxia, aging or neurodegeneration, NVC is compromised in part due to impaired bioavailability of nitric oxide (•NO). Nitrite has recently emerged as a metabolic precursor of •NO *in vivo* and may represent an alternative pathway for •NO

production, other than the nitric oxide synthases. The reduction of nitrite to •NO is favored via redox reaction of nitrite with ascorbate, which is present at high concentrations in the brain and is released to the extracellular medium upon glutamatergic stimulation. Thus, we hypothesized that modulation of nitrite concentration in the brain via diet may improve neurovascular coupling in emergency conditions by increasing •NO production. In this context, the development of tools to evaluate nitrite dynamics in the brain is of great interest. Here, we developed a novel sensing approach for nitrite monitoring in the brain in real-time by using fast-scan cyclic voltammetry (FSCV) associated with carbon fiber microelectrodes (CFMs). The local pressure-ejection of a nitrite solution resulted in the detection of transient signals when current was sampled at +1.1 V (*vs* Ag/AgCl). The signals had reproducible peak concentrations, rise times and decay rate constants. The developed method is a valuable tool for *in vivo* monitoring of nitrite dynamics in the brain. Furthermore, it will allow understanding the role of nitrite in neurovascular coupling and its modulation by diet.

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WTH02-10

Identification of synaptosomal receptor for extracellular protons

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Brain ischemia is accompanied by lowering of intra- and extracellular pH. Stroke often leads to irreversible damage of synaptic transmission by unknown mechanism. We investigated an influence of pH_i and pH_o lowering on free radical formation in synaptosomes. Three models of acidosis were used: pH_o 6.0 corresponding to pH_i decrease to 6.04; pH_o 7.0 corresponding to pH_i lowering to 6.92; 1 mM Amiloride corresponding to pH_i decrease to 6.65. We have shown that both types of extracellular acidification, but not intracellular acidification, increase DCF (2′,7′-Dichlorodihydrofluorescein) fluorescence that reflects free radical formation. These three treatments induce the rise of the dihydroethidium fluorescence that reports synthesis of superoxide anion. However, the impact of amiloride on superoxide anion synthesis was less than that induced by moderate extracellular acidification. Superoxide anion synthesis at pH_o 7.0 was almost completely eliminated by mitochondrial uncoupler CCCP (Carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone). Using fluorescent dyes JC-1 (5,5′,6,6′-Tetrachloro-1,1′3,3′-tetraethylbenzimidazolo-carbocyanine iodide) and Rhodamine-123, we confirmed that pH_o lowering, but not intracellular acidification, led to depolarization of synaptosomal mitochondria. This suggests the presence of receptor for protons on the plasma membrane of presynaptic terminals. In this effect may be involved ASICs (Acid-Sensing Ion Channels), ion channels permeable to calcium and sodium, and OGR1 (Ovarian cancer G protein-coupled Receptor 1), g-protein associated H^+-receptor. Cu^{2+} and Zn^{2+} in micromolar concentrations can block histidine residues of OGR1 receptor responsible for proton binding. Depolarization of synaptosomal mitochondria at pH_o lowering to 7.0 is partially blocked by10 μM Cu^{2+} and 10 μM Zn^{2+}, and by 1 μM Thapsigargin, that indicating on participation of OGR1 in signal transduction. Synaptosomal ROS accumulation and depolarization of synaptosomal mitochondria at the pH_o lowering is not dependent on the presence of Ca^{2+} in the incubation medium. Also, pH_o decrease does not lead to an increase in Sodium Green fluorescence. It shows a lack of activation Ca- and Na-specific forms of ASICs. These results indicate that the main receptor for protons on the plasma membrane of the presynaptic terminal at moderate acidification is OGR1.

WTH02-11

Redox state in modulation of neuroprotection in hypoxia

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In present study the changes in the redox state and free radical activity of the rat brain tissue were analysed in acute and prolonged hypoxia. Hypoxia was modeled by pressure chamber under 310 mm Hg during 1, 4, 7, 14, 28 days.The concentration of glucose in brain under acute hypoxia was in 2.4 higher then in intact animals. But after the 4th day of hypoxia the glucose concentration falls down till normal and remains at the same value till the 14th day, when it is slightly increased. A study of energy metabolism showed that lactate/pyruvate ratio in experimental animals was 3-fold higher than in intact specimens.The substrate relations in redox-pairs malate/oxaloacetate, NAD/NADH were increased in the same manner.The rate of tissue respiration, ADP phosphorylation were decreased essentially, oxidative phosphorylation kinetics was modified. Hypoxia decreased ATP content in brain tissue. Thus, acute hypoxia is accompanied by accumulation of reducing equivalents and inhibition of tissue respiration.Then in 2 weeks of hypoxia the redox state of brain tissue returns back to the value corresponding to intact animals and restores the origin balance of NAD dependent glycolysis and the Krebs cycle reactions, demonstrated the rise again by 28 day. Changes in the redox state of the cells are correlated to the intensity of free radical oxidation. Activation of free radical oxidation is accompanied by destabilization of the cell membranes, which results in the release of neuron-specific enzymes from damaged cells into the blood.Neuron-specific enolase in the group of animals exposed to acute hypoxia was 65% higher than in intact specimens, which reflects the severity of structural and functional cnanges in the neuronal membranes. The lowest results of the intensity of free radical reactions were noted at early (4 day) and late (28 day) of hypoxia.The antioxidant potential was the same as in intact animals increasing at 28th day. The different mechanisms of recovering of redox state of the nervous cells in acute and prolonged hypoxia were postulated.

WTH02-12

Evaluation of the potential neurotoxicity of gold nanoparticles in the different rat brain regions

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The present study aims to investigate the potential adverse effects of gold nanoparticles (Au NPs) in the cortex, hippocampus, striatum, midbrain, cerebellum and medulla of adult male Wistar rat through the estimation of some oxidative stress parameters and

acetylcholinesterase (AChE) activity. Rats were divided into two main experimental groups. Animals of the 1st and 2nd groups were intraperitoneally injected with a single dose (100 μg/kg body wt) of ~ 20 nm Au NPs and decapitated after 24 h and 2 weeks of injection, respectively. Control animals were injected with saline solution and sacrificed simultaneously with the treated groups. The present data revealed that Au NPs induced several significant changes in the levels of GSH and NO and GST activities in the brain areas investigated. These changes were more prominent after 24 h than after 2 weeks of injection and varied according to the brain region examined. However, these alterations did not induce lipid peroxidation except for the cerebellum and medulla after 24 h only. In addition, Au NPs induced significant decreases in cortical and hippocampal AChE activities after 24 h. However, significant increases in cortical and cerebellar AChE activities were recorded after 2 weeks. In conclusion, although most of the early biochemical changes induced by Au NPs injection were ameliorated after 2 weeks, careful must be taken into consideration in utilization of gold nanoparticles in biological applications especially with the particle size that can penetrate the BBB.

WTH02-13

There is not a simple linear relationship between increasing numbers of mild traumatic brain injury and increasing damage

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Following mild traumatic brain injury (mTBI), some patients go on to experience long-term cognitive impairments and additional mild impacts can exacerbate negative outcomes. To compare chronic damage and deficits following increasing numbers of repeated mTBIs, we used a clinically relevant closed-head weight-drop model of repeated mTBI to deliver 1, 2 or 3 mTBIs to adult female rats at 24 h intervals under anaesthesia. Outcomes were assessed at 3 months following the first mTBI.

Neurologic function was assessed using a modified neurologic severity score and no gross motor, sensory or reflex deficits were identified ($p > 0.05$), consistent with current literature. However, vestibulomotor deficits were observed following 3 mTBI ($p \leq 0.05$). Cognitive function assessed using a Morris water maze paradigm revealed chronic memory deficits following 1 and 2, but not 3 mTBIs compared to shams ($p \leq 0.05$). Oxidative stress was assessed immunohistochemically in various brain regions, quantifying immunoreactivity of indicators of lipid peroxidation, DNA oxidation and glycoxidation. Acrolein-mediated lipid peroxidation was increased in the dentate gyrus of the hippocampus following 1 mTBI ($p \leq 0.05$), while DNA damage indicator 8-hydroxy-2′-deoxyguanosine was increased in the corpus callosum following 2 but not 3 mTBIs, realtive to shams ($p \leq 0.05$). Glycoxidation, indicated by carboxymethyl-lysine, was increased in ventral brainstem following 3 mTBI ($p \leq 0.05$). Integrity of myelin ultrastructure in the corpus callosum was assessed using transmission electron microscopy, revealing that G ratio was decreased following 1 and 2 but not 3 mTBIs compared to shams ($p \leq 0.05$). Differences in damage and deficits following 1, 2 and 3 mTBI suggests that the effects of increasing numbers of mTBIs is not simply additive. The complex picture that has emerged warrants further studies exploring mechanisms of damage as well as chronic neuroregenerative responses that may facilitate the development of therapeutic strategies to limit long term functional deficits following repeated mTBI.

WTH02-14

NRF2 expression during ischemia with and without reperfusion in rat brain

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Cerebrovascular disease is a chronic-degenerative disorder that is divided into two types: ischemic (stroke), due to the interruption of the blood supply to a region of the brain, and hemorrhagic, which is generated by the rupture of an artery in some area of the brain, whereas ischemic stroke being the third leading cause of death and permanent disability in adults worldwide. Reperfusion to ischemic brain is currently the best way to save life and limit the development of cerebral infarction. However, it is believed that ischemia-reperfusion injury (IR) is another important clinical problem in the treatment of brain damage. In addition, the exact pathogenesis of cerebral IR is still not fully understood. Evidence has shown that inflammation, reactive oxygen species (ROS) and apoptosis are mechanism involved. Cerebral IR disrupts the balance between ROS production and its inactivation by antioxidant systems, which ultimately leads to an excessive accumulation of ROS, which contribute to brain tissue damage causing cellular dysfunction and cell death. Therefore, it is reasonable for patients suffering from an IR event to benefit from reduced ROS levels in therapy in case of stroke. Nrf2 is considered one of the master regulators of endogenous antioxidant defense. In response to oxidative stress, Nrf2 promotes the expression of a wide variety of antioxidant genes, including antioxidant and non-enzymatic enzymes, by their translocation to the nucleus, binding to antioxidant response elements (AREs) and regulation of transcription of target genes. Nrf2 appears to play an important role in the protection of brain cells against ischemic brain injury. The aim of this work was study the Nrf2 levels during ischemia with and without reperfusion. Animals were submitted to 15, 30, 60 and 120 min of ischemia using the middle cerebral artery occlusion model, and the Nrf2 levels were measured by western blot in striatum, frontal cortex and hippocampus. This project was supported by CONACyT (Grant241655).

WTH02-15

Intravenous injection of minoxidil reduced neuronal damage caused by transient focal cerebral ischemia

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Intracellular potassium ion level is higher than extracellular space, which is regulated by basically sodium pump. Potassium ion generates a resting potential on the cell membrane by passing

through the leak channels. Openings of the other potassium channels on the cell membrane induce hyperpolarization and it would cancel the excitation. Potassium channels are also on the mitochondrial inner membranes. Recent studies suggested that drugs, which open potassium channels in cellular and/or mitochondrial membrane protect neuronal tissues against neurodegenerative situation including transient ischemia. Here, we have demonstrated whether Minoxidil, a potassium channel opener reduced damage on neuronal tissues that was caused by transient focal cerebral ischemia.

Transient focal cerebral ischemia was induced in the 6-week old male C57/BL mice by a 1-h middle cerebral artery occlusion (MCAO) of left hemisphere and subsequent reperfusion. Thereafter, the mice were randomly divided into 4 groups, and either saline, edaravone (6 mg/kg bw) or minoxidil (0.5 or 5 mg/kg bw) was intravenously injected, immediately. One day later, the brain was taken out, and 5 slices in total were prepared from each mouse with a thickness of 1000 μm from the bregma, 2 on the front side and 3 on the back side, using a brain slicer. The slices were stained with 2,3,5-Triphenyl tetrazolium chloride (TTC staining) to detect cellular respiration activity.

Transient focal cerebral ischemia decreased TTC staining in ipsilateral striatal area by 50% compared to the opposite side, suggested that the operation damaged the neuronal tissues. The injection of edaravone immediately after the operation completely prevented the decrease. A similar prevention was observed with the injection with 5 mg/kg bw minoxidil instead of 0.5 mg/kg bw. Minoxidil was once developed for the treatment of hypertension, but now it is famous as a treatment for androgenic alopecia and it is applied for external application. We might need to think about injecting this medicine again in anticipation of the protective action of central nervous systems.

WTH02-16

BBB damage is reduced by blockade of BETA 2-adrenergic receptor-mediated HIF-1 alpha upregulation during acute cerebral ischemia

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Aims: Disruption of the blood brain barrier (BBB) within the thrombolytic time window is an antecedent event to intracerebral hemorrhage in ischemic stroke, however, mechanism underlying BBB damage at this acute stage is not well known. Since hypoxia-inducible factor-1 alpha (HIF-1α) was discovered as a mater regulator in hypoxia, we sought to investigate the roles of HIF-1α in BBB damage as well as factors regulating HIF-1α expression after acute ischemia stroke.

Methods: *In vivo* rat middle cerebral artery occlusion (MCAO) and *in vitro* oxygen glucose deprivation (OGD) models were used to assess the integrity if BBB.

Results: pretreatment with HIF-1α inhibitor YC-1 significantly inhibited 2-h MCAO-induced BBB damage accompanied by inhibition of occludin degradation, matrix metalloproteinase 2 (MMP-2) activity and vascular endothelial growth factor (VEGF) mRNA upregulation. Interestingly, blocking β2-AR reduced ischemia-induced BBB damage by regulating HIF-1α expression. HIF-1α was shown to be colocalized with neurons but not astrocytes or endothelial cells. Of note, *in vitro* results showed that HIF-1α inhibition with YC-1 or siRNA significantly prevented 2-h OGD-promoted upregulation of VEGF mRNA and secretion of VEGF and MMP-2 in neurons. More important, blocking β2-adrenergic receptor (β2-AR) inhibited 2-h OGD-induced HIF-1α upregualtion and reduced occludin degradation induced by OGD-neuron media.

Conclusion: Taken together, acute cerebral ischemia disrupts BBB by upregulating HIF-1α and activating the neurons to secret VEGF and MMP-2, while blocking β2-AR inhibited such change. These findings provide new mechanisms underlying BBB damage within thrombolytic time window and may help reduce thrombolysis-related cerebral hemorrhage.

WTH02-17

Maintenance of steady state H2S levels rescues hypobaric hypoxia-induced pathological effect

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Hypobaric hypoxia (HH) occurs at high altitude and is associated with multiple pathophysiological conditions including spatial memory loss Recently, the role of H2S in hypoxic cerebral autoregulation has been established, however, how the endogenous H2S production is regulated in HH not known. The present study was undertaken to investigate how H2S production is modulated at 1,3 and 7 day post HH exposure.Interestingly, we observed a significant lowering of H_2S levels after HH exposure. We next tested if HH induced any change in the expression of Cystathionine beta synthase (CBS) a critical enzyme for H_2S production in the brain, under these conditions employing western blot. We observed HH culminated in marked increase in the expression of CBS at all time points studied (Day 1, 3 & 7), suggesting that the decrease in production of H_2S was not due to reduced expression of CBS in the brain. To further investigate this condition, we quantitated various amino acids including L-Cysteine, employing HPLC, from brain extracts of animals exposed to HH. we clearly observed significant decrease in the level of L-Cysteine at all time points after HH exposure. Notably, the concentration of Methionine decreased significantly only at day 3 while that of Arginine at day 1 & 3. Taken together, we inferred that the reduced level of substrate (L-Cysteine) required for production of H2S by CBS could be a possible reason for HH-induced reduction in H2S levels in the the brain. Our experiments clearly suggest that the maintenance of endogenous H_2S levels through the administration of specific L-Cysteine donor counteracts the loss of spatial reference memory in response to HH. Taken together the steady state levels of H_2S is likely to serve as the key node for preservation of neuro-physiological functions during hypobaric hypoxia.

WTH02-18

Recurrent moderate hypoglycemia enhances brain injury induced by the hypoglycemic coma and leads to memory decline

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Most Type 1 Diabetes Mellitus (TIDM) patients who are under intensive insulin therapy suffer from repetitive episodes of moderate

hypoglycemia (RMH), which increase the risk for severe hypoglycemia (SH). SH can progress to the coma state, which induces neuronal death in vulnerable brain regions such as the cortex, the hippocampus and the striatum by a mechanism involving oxidative damage. However, the consequences of RMH on neuronal damage and cognitive function are not well understood, nor its effect on a subsequent period of hypoglycemic coma. The purpose of the present study was to investigate whether RMH can exacerbate neuronal damage and cognitive decline induced by a short (7–10 min) coma period in an *in vivo* model. Rats received an injection of insulin (6.5 insulin units, IU) during 7 consecutive days leading to moderate (40 mg/dl glucose) hypoglycemia. At day 8 animals received 32 IU to induce the hypoglycemic coma and were rescued with glucose after 7–10 min. Neuronal death and oxidative damage were assessed 24 after the coma by histological analysis and immunocytochemistry. Reduced glutathione (GSH) level glycogen were also assessed. Seven and 15 days after the coma, cognitive function was evaluated in two memory tests. Results show that previous RMH exacerbates oxidative damage and neuronal death induced by the hypoglycemic coma in the parietal cortex, the striatum but mainly in the hippocampus. These changes correlated with a severe decrease in GSH, glycogen increase and a significant spatial and contextual memory deficit. Results demonstrate that previous RMH enhances brain vulnerability to acute hypoglycemia by a mechanism involving decreased antioxidant defense and oxidative damage. They also highlight the relevance of an adequate control of moderate hypoglycemic episodes in TIDM.

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WTH02-19

Differential assessment of CB1R role in the neuroprotective effect of endocannabinoid system with different ways of its activation

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Endogenous cannabinoid system (ECS) regarded as a perspective target for ischemic brain injury correction. The aim of the investigation was to study the effect of ECS activation on cannabinoid receptors type 1 expression in normobaric hypoxia *in vitro*.

The experiments were carried out on primary hippocampal cultures obtained from CBA mice embryos (E18). Normobaric hypoxia was modeled on day 14 of culture development *in vitro* by replacing the normoxic cultural medium by a medium with a low oxygen for 10 min. N-arachydonoyl dopamine (10 mcM) (N-ADA) or antagonist of CB1 receptors – SR151716 (SR1) (1 mcM) or inhibitor of endocannabinoid degrading enzymes (monoacylglycerol lipase and fatty acid amide hydrolase) – JZL 195 (1 mcM) were applied into hypoxic cultural medium. The main parameters of spontaneous calcium activity, the viability of cells and the level of intravital CB1 mRNA expression by using SmartFlare RNA Detection Probes (Merck Millipore) were investigated.

ECS activation by N-ADA or JZL 195 prevented the cell death and reduction of spontaneous calcium activity in hippocampal cultures in the posthypoxic period. CB1 mRNA is synthesized by neurons and glial cells. Hypoxia caused an increase of CB1 mRNA expression. In cultures with N-ADA or JZL 195 application the number of CB1 mRNA positive cells doesn't differ from intact cultures.

Therefore, our studies revealed that activation of ECS has strong neuroprotective properties which implemented through CB1 receptors.

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WTH02-20

Zinc contributes to ischemia-induced blood-brain barrier disruption by activating mmps in cerebral microvessels

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Background: Zinc ions are stored in synaptic vesicles and cerebral ischemia triggers their release from the terminals of neurons. Zinc accumulation in neurons has been shown to play an important role in neuronal death following ischemia.
Objectives: in this study, we investigate whether zinc is involved in ischemia-induced blood–brain barrier (BBB) disruption.
Methods: We investigated the contribution of zinc to ischemia-induced acute BBB disruption and the possible molecular mechanisms using both cellular and animal models of cerebral ischemia.
Results: Zinc greatly increased BBB permeability and exacerbated the loss of tight junction proteins (Occludin and Claudin-5) in the endothelial monolayer under oxygen glucose deprivation conditions. In cerebral ischemic rats, a dramatically elevated level of zinc accumulation in microvessels themselves was observed in isolated microvessels and *in situ*, showing the direct interaction of zinc on ischemic microvessels. Treatment with a specific zinc chelator *N*,*N*,*N*′,*N*′-tetrakis(2-pyridylmethyl) ethylenediamine (TPEN), even at 60-min post ischemia onset, could greatly attenuate BBB permeability in the ischemic rats as measured by Evan's Blue extravasation, edema volume and magnetic resonance imaging. Furthermore, zinc accumulation in microvessels activated the superoxide/matrix metalloproteinase-9/-2 pathway, which leads to the loss of tight junction proteins (Occludin and Claudin-5) and death of endothelial cells in microvessels themselves.
Conclusions: Our findings reveal a novel mechanism of cerebral ischemia-induced BBB damage, and implicate zinc as an effective and viable new target for reducing acute BBB damage following ischemic stroke.

WTH02-21

Short chemical ischemia induces death of neuroblastoma SH-SY5Y cells but not glioblastoma T98G cells

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Both translation arrest and proteasome stress associated with accumulation of ubiquitin-conjugated protein aggregates were

considered as a cause of delayed neuronal death after transient global brain ischemia however exact mechanisms as well as possible relationships are not fully understood.

The aim of this study was to compare effect of chemical ischemia and proteasome stress on cellular stress responses and viability of neuroblastoma SH-SY5Y and glioblastoma T98G cells. Chemical ischemia was induced by transient treatment of the cells with sodium azide in combination with 2-deoxyglucose. Proteasome stress was induced by treatment of the cells with bortezomib. Treatment of SH-SY5Y cells with sodium azide/2-deoxyglucose for 15 min was associated with cell death observed 24 h after treatment while glioblastoma T98G cells were resistant to the same treatment. Treatment of both SH-SY5Y and T98G cells with bortezomib was associated with cell death, accumulation of ubiquitin-conjugated proteins and increased expression of Hsp70. These typical cellular responses to proteasome stress, observed also after transient global brain ischemia, were not observed after chemical ischemia. Finally, chemical ischemia, but not proteasome stress, was in SH-SY5Y cells associated with increased phosphorylation of eIF2α, another typical cellular response triggered after transient global brain ischemia.

Our results showed that short chemical ischemia of SH-SY5Y cells is not sufficient to induce both proteasome stress associated with accumulation of ubiquitin-conjugated proteins and stress response at the level of heat shock proteins despite induction of cell death and eIF2α phosphorylation.

WTH02-22

Developed human stem cells derived neuronal model of cerebral ischemia revealing the anti ischemic potential of trans-resveratrol

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Cerebral ischemia is the largest cause of long-lasting disability in humans, which occurs due to deprived blood supply to the brain. To screen the anti-ischemic potential of drugs, no suitable human specific tool is available. We explore the applicability of human cord-blood stem cell derived neuronal cells (hCBSCNCs) as a reliable tool for the purpose. To create a model the optimum time points for oxygen-glucose deprivation (OGD) and re-oxygenation (R) have been identified. The OGD of 6 h followed by a re-oxygenation period of 24 h could be recorded as optimum under our experimental conditions. Glucose concentration during re-oxygenation was found to be one of the major factors involved in growth and proliferation of hCBSCNCs. Re-oxygenation with 4–6 mg/mL glucose concentration in medium was found to be first statistically significant parameter. This OGD-R model increases Ca^{2+} influx, which triggered the hypoxic homeostasis transcription factors like-hypoxia induced factor-1 alpha (HIF-1α), Cav-beta 3 (Cav β3), signal transducer and activator of transcription 3 (STAT3) and heat shock protein 27 (hsp-27) and subsequently induces the ROS mediated apoptotic damages in the cells. The cells viability was assessed by trypan-blue exclusion and MTT assays. We further investigated the anti-ischemic potential of trans-resveratrol (RV) in this OGD-R model when exposed to biologically safe doses (5, 10 and 25 μM) of RV in three different exposure groups i.e., 24 h prior to OGD (pre-exposure); 24 h post OGD (post-exposure) and from 24 h before OGD to end of re-oxygenation period (whole exposure). Our findings demonstrated that RV has significant potential of increasing the viability of OGD-R insulted hCBSCNCs by decreasing ROS. The whole exposure group of RV is most efficient in decreasing hypoxia induced cell death through its antioxidant properties.

WTH02-23

Interplay between the ubiquitin-proteasome system and calpains in brain ischemia

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Ischemic stroke is characterized by overactivation of glutamate receptors (excitotoxicity) thus inducing a massive accumulation of $[Ca^{2+}]i$ in the postsynaptic cells. These events will lead to the activation of the Ca^{2+}-dependent calpains, and to an overall hypofunction of the Ubiquitin-Proteasome System (UPS), by mechanisms not fully elucidated. Herein, we show that cerebrocortical neurons subjected to Oxygen-Glucose Deprivation (OGD), an *in vitro* model mimicking transient global ischemia, for 1.5 h, display reduced chymotrypsin-like activity of the proteasome when evaluated 4 h after the insult. Unexpectedly, total polyubiquitin conjugates, a marker for proteasome dysfunction, were diminished under the same conditions, while no differences were observed in total free ubiquitin levels. The decreased proteolytic activity is correlated with a disassembly of the 26S proteasome into its major constituents, the 19S regulatory and 20S catalytic particles, as shown by NATIVE-PAGE. Two non-related *in vitro* assays with recombinant calpain, identified the ATPase ring (Rpt1,3,5) proteins, along with the Rpn2 and β3 subunits, as calpain targets. Six other proteasome proteins (Rpn1,3,5,12 and USP14) may also be cleaved by calpains since their amino acid sequence contains putative PEST sequences. Together, these results suggest that calpains may impair the UPS by acting on multiple targets. An increase in calpain activity was observed observed in cortical neurons subjected to OGD, and the active protease cleaved Rpn10, Rpt3, along with Rpt1 and possibly Rpn3, by a NMDAR-dependent mechanism. Incubation of cultured cerebrocortical neurons with the USP14 inhibitor IU1, a proteasome activator, fully prevented the OGD-induced calpain activation and neuronal death, when evaluated 24 h after injury. Therefore, proteasome activation in cerebrocortical neurons subjected to OGD may be exploited to develop novel neuroprotective strategies for ischemic stroke. (Supported by FCT [SFRH/BD/51967/2012], COMPETE and Mais Centro Program).

WTH02-24

Glycyrrhizic acid attenuates ROS mediated neurodegeneration and cognitive deficit in chronic cerebral hypoperfusion rats

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Chronic cerebral hypoperfusion, a major threat for cognitive health is linked to various vascular ailments and comorbidities such as diabetes, obesity and hypertension. The study primarily aims at explaining the mitigating effects of *Glycyrrhizic acid (GA)* on cognitive health challenged by chronic cerebral hypoperfusion. Adult male Sprague–Dawley rats were segregated into four groups (i) Sham (ii) Lesion (Bilateral common carotid artery permanent occlusion) (iii) GA treated (Lesion+GA) (20 mg/kg body wt, i.p.) and (iv)Lithium chloride (Lesion+Li) (Li 40 mg/kg body wt, i.p.) After a period of 30 days postoperatively the rats were tested for behavioral alterations through a repertoire of tests like Novel Object recognition (NOR), spontaneous exploratory drive through Hole board test and spontaneous alternation by T-Maze. The brain samples harvested were used for histological and biochemical parameters. The viable pyramidal cell density in the various subfields of dorsal hippocampus was counted. White matter rarefaction in the corpus callosum was also examined and dendritic spine status was also assessed. Antioxidant propensity of GA curtailed the ROS generation by restoring the activity of Mitochondrial complex I and IV. The treated group exhibited 200% more consumption of H_2O_2 through catalase activity, lipid peroxidation was curbed by 50% and 250% increase was seen in reduced glutathione. However, Lithium chloride (Li) (a standard inhibitor of choice for GSK3 beta) exhibited significantly lower antioxidant status when compared to GA. This strong antioxidant defence has led to considerable restoration of pyramidal neuron density, prevented myelin rarefaction and restored twice as much as dendritic spines in GA treated than Li treated. GA treated rats showed 200% rise in exploration in holeboard, the spontaneous alternation was 150% increased and the discrimination index was twice as much as the lesion rats. The outcome of this study clearly implies that GA treatment posses a promising edge over the conventional Lithium chloride treatment and could mitigate the pathogenesis of neurodegeneration.

WTH02-25

Folic acid provides neuroprotection by modulating hippocampal oxidative imbalance and astrocyte response of hypoxic-ischemic rats

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The aim of this work was to investigate the effect of folic acid (FA) in rats submitted to hypoxia-ischemia (HI) evaluating memory in the ox-maze task, antioxidant defenses by superoxide dismutase (SOD) and catalase (CAT) activities and astrocyte response in the rats hippocampus. Groups of pup Wistar rats: (i) control treated with saline (CTS); (ii) CTFA; (iii) HIS and (iv) HIFA. On 7th postnatal day (P7) pups were submitted to HI model and treated with FA (P7-P22). At P22 rats were evaluated in the Ox-maze (10 sessions during 10 days to find a food reward in four boxes containing different symbols). Thereafter, the enzymes activity and glial scar (Optical density of GFAP) were measured. The HIS group had poor performance in the ox-maze, displaying a higher time to complete the task and number of incorrect nose pokes. These behavior deficits were reduced by FA administration. Additionally, a lower SOD activity was found in the hippocampus in the HIS group when compared to the others groups; the HIFA group showed only a partial decrease of enzymatic activity. The Catalase activity decreased in the HIS and HIFA comparing with the CTA group. The GFAP density increased only in the HIS group at P22. Concluding, neonatal HI resulted in cognitive deficits and FA attenuated these effects. Such behavioral impairment was associated to the decreased CAT and SOD activities and increased immunoreactivity for GFAP in the hippocampus. Folic acid administration appears to reverse, at least, partially these neurochemical parameters. Altogether, our findings suggest a potential role of FA as a neuroprotective agent on the neonatal HI.

WTH02-26

Blockade of $GABA_B$ receptor endocytosis enhances neuroprotection

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Metabotropic $GABA_B$ receptors ($GABA_BRs$) are heterodimeric G protein coupled receptors composed of R1 and R2 subunits that mediate slow inhibitory signalling in the brain. Consistent with their roles in mediating neuronal inhibition, deficits in $GABA_BR$ function play significant roles in both neurological and psychiatric disorders. We have previously reported that $GABA_BRs$ are intimately associated with protein phosphatase 2A and directly dephosphorylate S783 in the R2 subunit to enhance $GABA_BR$ endocytosis (Terunuma et al., *PNAS*, 2010). Thus it was considered that the endocytosis of $GABA_BRs$ mediated by dephosphorylation is of significance in synaptic plasticity and pathological conditions characterised by prolonged activation of glutamate receptors such as ischemia.

To test the role that phospho-dependent modulation of $GABA_BRs$ play in neuronal activity, we generated a knock-in mouse in which S783 was mutated to alanine (S783A) to prevent S783 dephosphorylation and degradation. Using these knock-in mice, we identified that S783A mice express stable $GABA_BRs$ on the plasma membrane by reducing receptor endocytosis (Terunuma et al., *J Neurosci*, 2014). Oxygen glucose deprivation (OGD) induced neuronal death in the wild-type neurons but not in S783A neurons suggesting a strong neuroprotective role of $GABA_BRs$. We also found that $GABA_BR$ signalling regulate caspase-3 activity which may be a key mechanism for neuroprotection.

WTH02-27

HIF 1-dependent normalization of pentose phosphate pathway in rat brain as a neuroprotective mechanism of hypoxic postconditioning

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Postconditioning (PostC) is an exposure of the damaged organism to extreme factors of the mild intensity to mobilize endogenous protective mechanisms. Method of PostC, which consists of three sequential episodes of mild hypobaric hypoxia, has recently been developed and validated in our laboratory. This model of hypoxic PostC was shown to improve the rats` rehabilitation after injurious severe hypobaric hypoxia (SH) by preventing neuronal loss, normalizing the lipid peroxidation process, the activity of the endocrine system and animal behaviour. In particular, it was demonstrated that hypoxic PostC up-regulates hypoxia-inducible factor-1 alpha subunits (HIF1a) level in the CA1 field of the hippocampus. Here, we have tested the hypothesis that hypoxic PostC induces neuroprotection through HIF1-dependent stimulation of pentose phosphate pathway activity. We have proved that SH suppresses the glucose-6-phosphate dehydrogenase activity in rat hippocampus, which leads to attenuation of reduced NADPH, total and reduced glutathione levels. This data correlate with decreased total antioxidant activity of cytosolic and mitochondrial subcellular fractions of rat hippocampus in this group. Meanwhile, hypoxic PostC normalizes the activity of glucose-6-phosphate dehydrogenase, stabilizes the NADP reduction process and causes a significant grow in total and reduced glutathione quantity and the rise of total antioxidant activity in the rat hippocampus. The excess reactive oxygen species generation is considered to provoke hypoxia-mediated neuronal death. Therefore, stabilization of processivity of antioxidant systems can play a key role in preventing the consequences of reoxygenation.

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WTH02-28

Stimulation of GLP-1 receptor alleviates ischemic stroke injury by elevating cerebral angiogenesis

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The study focuses on an innovative treatment in post-ischemic stroke and its mechanisms for alleviating ischemic brain injury and shortening recovering period. Maintaining neuronal viability after stress insults or traumatic injuries, such as oxidative stress or ischemic stroke, is crucial for better recovery and reducing mortality. In this study, we observed administration of exendin-4 (EX-4), an agonist of Glucagon-like peptide-1receptors, significantly enhances viability of brain cells after middle cerebral artery occlusion induced ischemic stroke. In the mean time, we found administration of EX-4 upregulated expression of VEGF and nitrogen oxide synthase (NOS) and triggered brain vasodilation and agiogenesis. Furthermore, the phosphorylated form of NOS, including neuronal NOS, inducible NOS, and endothelial NOS, were dramatically enhanced by EX-4 treatment. All lines of evidences indicated that stimulation of the GLP-1R has function on neuroprotection after ischemic stroke.

Activation of GLP-1Rs triggers several signaling pathways, including phosphatidylinositol 3 kinase, Akt; protein kinase C, mitogen-activated protein kinase; and adenylate cyclase, MEK, and Erk. According to the results, we suggested that activation of GLP-1Rs has a protective function against focal ischemic stroke via inducing expression of VEGF and NOS to enhance vasodilation and angiogenesis, which both protective effects are mediated by the GLP-1R downstream signaling pathways. We therefore postulated the enhancement of vasodilation and angiogenesis is a potential strategy for therapeutic intervention in ischemic stroke.

WTH02-29

Autophagic flux is impaired after ischemia-reperfusion exposure via cpkcgamma-MTOR signaling pathway in cortical neurons of mice

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Autophagy dysfunction has been indicated to play a critical role in cerebral ischemia. Although the intervention targeted autophagy is testified effective against ischemic injuries in a lot of studies, its regulatory signaling pathway is mostly obscure. In addition, the real significance of autophagy in ischemic injuries requires a deeply exploration. In the current study, it was found that the conversion ratio of LC3II/LC3I was increased while SQSTM1/p62 accumulation occurred in the cultured cortical neurons from mice after the oxygen glucose deprivation (OGD)/reperfusion exposure, suggesting that autophagic flux was impaired during this process. Further, the accumulation of p62 wasn't observed in PKCgamma KO mice, indicating PKCgamma took part in maintaining the smooth autophagic flux. Meanwhile, it was investigated that PKCgamma-dependent phospho-mTOR was increased at ser2481 site with western blot assay, and the co-localization of LC3 and LAMP1 was decreased with the confocal imaging. At the same time, the cell viability was decreased in PKCgamma KO mice compared with that of wild-type mice, indicating the blockage of autophagic flux was beneficial for neurons under ischemia/reperfusion exposure. The protein level of STX was not changed significantly, but its co-localization with autopahgosomes was decreased during this stage. To sum up, we draw the following conclusions from the current study: ①Autophagic flux may be impaired during the ischemia-reperfusion stage. ② PKCgamma-mTOR signaling pathway is identified to regulate the fusion between autophagosomes and lysosomes by influencing the STX anchoring to autophagosomes. ③ The impairment of autophagic flux suppressed the ischemic injuries. ④ The phosphorylation site at ser 2481 of mTOR is essential for the fusion of autophagosomes and lysosomes, and independent of the phospho-mTOR at the ser2448 that has been testified important for the upstream regulation of autophagy. ⑤ the binding of PKCgamma and mTOR is increased after ischemia-reperfusion. Together, our study provides a new insight of downstream regulation of autophagy in ischemic injuries, which may be a promising target for ischemic stroke.

WTH03 Synaptic Plasticity

WTH03-01

Assessing KCC2 functions using transport-deficient mutants

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Many neurological and psychiatric disorders such as epilepsy, schizophrenia, autism and Rett syndrome are associated with reduced expression of the neuronal chloride/potassium co-transporter KCC2. In mature neurons, chloride extrusion through KCC2 maintains low intracellular concentrations thereby ensuring chloride influx and membrane hyperpolarization upon $GABA_A$ receptor activation. However, recent studies revealed additional, ion transport-independent functions of KCC2 at excitatory synapses. Thus, chronic KCC2 down-regulation in hippocampal neurons reduces the efficacy and compromises long-term potentiation of glutamatergic synapses through modifications of actin dynamics in dendritic spines. These effects involve KCC2 interactions with intracellular partners, such as 4.1N and b-PIX that influence actin anchoring to the plasma membrane and polymerization, respectively. Whereas diuretics may be used to compensate for the loss of chloride export in pathological conditions associated with KCC2 down-regulation, they would fail to rescue ion-transport independent deficits.

In order to distinguish the impact of transport-dependent vs. independent functions of the transporter, we generated and compared several putative transport-deficient recombinant KCC2 bearing C568S, L675A or C287S/C302L/C322S/C331L mutations. Although reduced chloride efflux has been observed in heterologous cells expressing these mutants, whether this results from altered membrane expression or transport function remains unclear. We therefore compared membrane trafficking, protein interactions as well as chloride transport function of wild-type vs. mutant recombinant KCC2 both in Neuro2a cells and hippocampal neurons *in vitro*. Our data reveal that all mutants are correctly targeted to the plasma membrane. In addition, 4.1N or bPIX interactions with KCC2 mutants were compared using co-immunoprecipitation experiments in Neuro2a and $[Cl]_i$ was assessed using a highly sensitive chloride sensor for quantitative analysis. Altogether, our results will help us identify key residues involved in KCC2 function and characterize genuine, transport-deficient KCC2 retaining normal membrane expression and protein interactions. These mutants may then be used to specifically restore KCC2 transport-independent functions in pathological conditions.

WTH03-02

Changes in synapses induced by GLUN2A knockdown

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Synaptic plasticity refers to long lasting changes in synapses that have been related to the structural bases of memory and learning processes. For several years, NMDA receptors (NMDAR) have been involved in those process through different approaches. NMDAR are composed of two GluN1 obligatory subunits and two regulatory subunits GluN2 (A-D) or GluN3 (A-B). In hippocampus and other memory related brain structures, GluN2A and GluN2B are the most expressed regulatory subunits. Expression of these subunits is highly variable; GluN2B is expressed in immature and/or unstable synapses while GluN2A is expressed in mature, stable synapses. To better understand the role of GluN2A during memory acquisition and plasticity induction, we built an AAV vector carrying a shRNA anti GluN2A (AAV-sh2A) and the sequence of the eGFP protein; we also built an AAV carrying a shRNA scramble as control (AAV-shSc). In this work we analyzed changes in pre and postsynaptic markers in primary neuron cultures infected with AAV-sh2A or AAV-shSc. We observed a GluN2A expression decrease in GFP+ neurons transduced with the AAV-sh2A, compared with those transduced with the AAV-shSc. Surprisingly, GFP- neurons in cultures treated with the AAV-sh2A showed an increased GluN2A expression when compared with GFP+ and GFP- in cultures treated with the AAV-shSc. Then, we decided to investigate a presynaptic marker as Synapsin (Syn). We observed an increase in Syn dots that impact with GFP+ neurons in cultures infected with the AAV-sh2A. Finally, we asked if this increase was reflected at the postsynaptic side. We found that every Syn dot corresponded with a spine in the GFP+ neurons and, moreover, AAV-sh2A GFP+ neurons showed an increase in immature spines. We hypothesized that GluN2A decreased expression causes synaptogenesis, driven by 1) the maturation impossibility of preexisting synapses, 2) the lack of efficient connectivity in AAV-sh2A GFP+ neurons or a restriction in the dendritic arborization in those neurons.

WTH03-03

Cued memory reconsolidation in rats requires nitric oxide

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It is known that the reactivation of consolidated fear memory under protein synthesis blockade results in an impairment of memory, suggesting that the reactivated memory is destabilized and requires synthesis of new proteins for reconsolidation. It was shown earlier in our lab that nitric oxide blockade during reminder in *snails* prevented memory impairment induced by protein synthesis blockade (Balaban PM et al., 2014). In this work we tested the hypothesis of nitric oxide (NO) involvement in memory destabilization during the reconsolidation process in *rats*.

On the training day the rats were placed in Context A and after 2 min exposure to context received two auditory conditioned stimuli (CS) presentations (5 kHz, 75 dB, 30 s) that co-terminated with a 0.4 mA, 2 s foot-shock unconditioned stimulus (US). 48 h later memory reactivation test was performed by administering a single 30 s CS in context B. Immediately after the reactivation session the rats were twice intraperitoneally and subcutaneously injected with either DMSO (100%)+DMSO (1%) in NaCl 0.9% (control, $n = 15$) or DMSO (100%)+cycloheximide in DMSO (1%) (protein synthesis inhibitor, $n = 23$), or 3-Br 7-NI in DMSO (100%)+ DMSO (1%) (nNOS blockade, $n = 12$), or 3-Br-7-NI+CXM (protein synthesis and nNOS blockade, $n = 13$). 48 h later memory testing by administering a CS in context C was performed.

ANOVA Repeated Measures revealed that protein synthesis blockade after reactivation significantly impaired the fear memory to the CS (sound). Administration of the nNOS selective blockers 3-Br-7-NI alone or with CXM did not affect the freezing level. We concluded that NOS blockade in the conditions of reactivation of memory under a protein synthesis blockade prevented destabilisation of fear memory to the conditioned stimulus. Obtained results support the role of NO signaling pathways in the destabilisation of existing fear memory triggered by reactivation, and demonstrate that the disruption of this pathway during memory reconsolidation may prevent changes in long-term memory. Supported by RSF grant 14-25-00072.

WTH03-04

α-Mangostin improves hippocampal cholinergic enzyme activities and cognitive impairment in scopolamine-induced amnesic rats

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Hippocampal acetylcholine (ACh) plays a role in synaptic plasticity, including learning and memory. Hippocampal cholinergic enzymes are markedly depleted in Alzheimer's disease (AD) which is associated with cognitive deficits. 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. We examined whether α-mangostin (α-MG) improved activity of hippocampal cholinergic enzymes, behavioral alterations and cognitive impairment, in rats induced by administration of scopolamine (SCOP), anticholinergic agent that blocks the activity of the muscarinic acetylcholine receptor which commonly used as a model for AD. The ability of α-MG to improve the learning and memory performance in the SCOP-induced neurodegenerative rats was assessed using the Morris Water Maze (MWM) test. Rats injected with SCOP showed cognitive impairment and daily administration of α-MG improved memory function and increased learning behaviors. In hippocampal cholinergic system, the result found that α-MG increased the activity of choline acetyltransferase (ChAT) whereas decreased the activity of acetylcholine esterase (AChE). These findings suggest that α-MG has potential therapeutic value in alleviating SCO-induced cognitive deficits in rat hippocampus which its mechanism might be involved in regulating the hippocampal cholinergic enzymes and facilitating learning and memory.

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WTH03-05

The level of PKMζ MRNA in neuronal soma decreases after chemical stimulation of helix lucorum neurons

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Atypical protein kinase M zeta is considered to be one of the key regulators of memory formation in vertebrate and invertebrate animals. It was shown that PKMζ is synthesized *de novo* in synaptic spines after their activation. It's supposed that PKMζ concentration is regulated mostly on translational level. However, another possible way to regulate PKMζ concentration is to change transcription rate of its gene (Prkcz).

The aim of our study was to assess changes in PKMζ mRNA expression level in neurons after chemical stimulation. We used gastropod *Helix lucorum* as a model organism (it has giant neurons, which makes it possible to estimate mRNA expression even in a single neuron). Isolated snail nervous systems were activated *in vitro* by serotonin/caffein mixture application, then total RNA was extracted from ganglia or individual giant command neurons. Differential expression of Prkcz gene transcripts in experimental and control neurons was measured using RT-PCR or RNA-Seq.

Sequencing data indicated that Prkcz gene expression level decreased after neuronal activation. Using RT-PCR we measured levels of 2 different splice isoforms of PKMζ mRNA (which we discriminated earlier using 5′-RACE method), and quantity of both isoforms decreased in activated neurons.

These data may at first seem contradictory to the increase of PKMζ protein concentration in postsynapses demonstrated earlier by other researchers. If we consider that the decrease in PKMζ mRNA level revealed in our experiments was observed in somata only, but not in neurites that were not analyzed, then it's possible to speculate that after neuronal activation PKMζ mRNA is actively transported from soma to postsynapses where it's needed for LTP processes. So, the decrease of PKMζ mRNA level in soma doesn't necessary mean that transcription of the Prkcz gene is decreased. Additional experiments with mRNA level measurement in neurites are necessary to test this hypothesis.

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WTH03-06

A single ketamine neonatal exposure induces acute hippocampal susceptibility and cognitive and motor changes in adult rats

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The exposure to general anesthetics has been associated with neuronal apoptosis and changes in morphology of dendritic spines in the developing brain. Ketamine, a noncompetitive *N*-methyl-D-aspartate receptor (NMDAR) antagonist, is widely used in pediatric patients to induce general anesthesia, analgesia and perioperative

sedation. This study investigated, in an acute or long-term protocol, the hippocampal and frontal cortical cellular viability of rats exposed at postnatal day (PND) 7 to a single dose of ketamine (20 mg/kg by subcutaneous route) or saline 0.9%. In addition, biochemical and behavioral parameters were evaluated in adulthood (60 days-old rats). Neonatal administration of ketamine (PND 7) decreased the hippocampal but not frontal cortex cellular viability, 24 h after the treatment. None biochemical alteration (propidium iodide incorporation and L-[^{3}H] glutamate uptake) was observed in both hippocampus and frontal cortex of adult rats. The brain structures from neonatal rats displayed tolerance to glutamate excitotoxicity, while adult brain showed susceptibility, evidenced by the cellular viability reduction. Importantly, a single ketamine neonatal exposure prevented the glutamate-induced excitotoxicity in the frontal cortex of adult rats. Regarding behavioral analysis, an improvement in the motor function and short-memory deficit, evaluated respectively in the rotarod and novel object recognition task, was observed in the ketamine group in adulthood. Altogether, our data indicate that the hippocampal cellular viability decrease induced by a single neonatal ketamine exposure to rats can be linked, at least in part, with alteration in motor performance and short-term memory impairment in adulthood.

WTH03-07

Structural neuroplasticity of identified microcircuits investigated by Novel EM Probe Technology

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Our studies combine novel electron microscopy (EM)-probe technologies with transgenic mouse lines, aim to explore at high spatial resolution the structural modifications associated with neuroplasticity. We have previously shown that altering circuit activation in the brain by photoperiod changes the numbers of dopamine neurons. Specifically, after 1 week of long-day photoperiod, neurotransmitters released by hypothalamic neurons in the PVN switched from dopamine to somatostatin. The plasticity in neurotransmitter expression was coupled to dopamine receptor matching in the post-synaptic CRF-releasing cells and affected stress responses. Nothing is known about ultrastructural changes, like terminal sprouting and new synapse formation that might occur between pre- and postsynaptic elements during neurotransmitter switching. To this aim, we implemented novel genetically targetable probes designed for correlated light and EM (CLEM). MiniSOG is the first fluorescent protein genetically engineered for CLEM. More recently, we adapted a correlative nanobody against GFP to visualize any GFP-fused protein by light and EM via the engineered enzyme ascorbate peroxidase (APEX2), which can oxidize DAB into an EM contrasting agent. We crossed a CRE-driver line, which expresses CRE recombinase in CRF+ cells, with a TH-GFP reporter mouse line. The TH-GFP line was used as historical marker of TH expression since GFP can be detected in PVN neurons after neurotransmitter switching (when TH protein is no longer detected). The pre-synaptic cells were labeled using a nanobody against GFP fused to APEX2 introduced via viral infection. To label the post-synaptic cells, we introduced a floxed farnesylated MiniSOG via viral infection in the PVN. Next, we will reconstruct the pre-and post-synaptic cells using Serial Block face Scanning Electron Microscopy.

We expect to achieve a level of analysis on neuronal circuits with unprecedented details. The resulting tools could provide applications aimed at monitoring changes in microcircuit connectivity associated with neuroplasticity of any brain region.

WTH03-08

Autism-associated CASPR2 regulates synaptic AMPA receptors in the context of homeostatic synaptic plasticity

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During development and learning/memory-related events, the mammalian brain undergoes constant changes that can compromise its function. To prevent this, homeostatic synaptic plasticity mechanisms come into play, allowing experience-based adaptations to occur while maintaining neuronal network activity in-balance for proper brain function. One fundamental mechanism to achieve neuronal homeostasis is the dynamic regulation of AMPA receptors at glutamatergic synapses.

Herein, we describe a novel role for the cell-adhesion molecule Caspr2, implicated in autism and other neuropsychiatric disorders, in the regulation of synaptic AMPARs in the context of homeostatic plasticity. We demonstrate that loss of Caspr2 not only decreases the basal synaptic content of GluA1-containing AMPARs in cortical neurons, but also hinders the triggering of homeostatic synaptic scaling of AMPARs during prolonged neuronal inactivity. Accordingly, Caspr2 is further required for experience-dependent plasticity *in vivo*, since its loss in the mouse visual cortex (V1) prevents the scaling of AMPAR-mediated mEPSC amplitudes following chronic visual deprivation. Caspr2 is also a target antigen in autoimmune synaptic encephalitis. Remarkably, *in vitro* or *in vivo* incubation with patient-purified Caspr2 autoantibodies significantly decreases synaptic GluA1-AMPARs in cortical cultures and mEPSC amplitudes in V1.

Overall, we uncover a novel function for autism-associated Caspr2 in the regulation of synaptic AMPARs and homeostatic plasticity. Importantly, this evidence hints at a potential disruption of neuronal homeostasis following Caspr2 dysfunction in the context of disease, which is consistent with accumulating data implicating glutamatergic synapse dysfunction and impaired neuronal homeostasis as common underlying pathologies of several cognitive disorders, including autism.

WTH03-09

Identification of O-glcnacome/phosphoproteome interplay of synaptosome-associated proteins in sensorimotor cortex

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In human, a chronic reduction in neuromuscular activity through prolonged body immobilization alters motor task performance through a combination of peripheral and central factors. Studies performed in a rat model of sensorimotor perturbation have shown morphological and biochemical changes in sensorimotor cortex. However, the underlying mechanisms are still unclear. It is well known that phosphorylation regulates a wide field of the synaptic activity leading to neuroplasticity. Another post-translational modification that interplays with phosphorylation is O-linked-*N*-acetylglucosaminylation, termed OGlcNAcylation. This glycosylation is atypical, reversible and dynamic, and is involved in essential cellular and physiological processes such as synaptic activity, neuronal morphogenesis, learning and memory. Moreover, the interplay between phosphorylation and O-GlcNAcylation has been shown to play a critical role in neurodegenerative diseases. The objective of this study is to characterize the modulation of phosphoproteome/O-GlcNAcome interplay of synaptosomeassociated proteins in sensorimotor cortex after sensorimotor perturbation by differential proteomic analysis. Sensorimotor cortex synaptosomes were separated by sucrose gradient in order to isolate a subcellular compartment enriched in proteins involved in synaptic functions. Then, a multiplexed proteomic strategy was used to detect O-GlcNAcylated proteins, phosphoproteins, and the whole proteome within the same bidimensional gel. The O-GlcNAcome was revealed by the way of the Click chemistry and the azide–alkyne cycloaddition of a fluorophore on O-GlcNAc moieties. The phosphoproteome was stained by "Phospho-Tag phosphoprotein gel stain", while the whole proteome was visualized through Sypro Ruby staining. This method permitted, after sequential image acquisition, the direct in-gel detection of O-GlcNAcome, phosphoproteome, and whole proteome of synaptosome-associated proteins. Moreover, differential proteomic analysis of O-GlcNAcylated/phosphorylated proteins balance allowed us to identify key markers of synaptic plasticity induced by a period of sensorimotor perturbation.

WTH03-10

Prolonged changes in polysaccharide components of the brain extracellular matrix following photothrombotic stroke

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Perineuronal nets (PNNs) are brain extracellular matrix structures surrounding subset of GABAergic neurons. They stabilize neuronal connections, thus limiting synaptic plasticity. Decrease in PNNs densities was observed after stroke and it can be considered an attempt to create neuroplasticity conditions. We hypothesize the role of polysaccharide modifying enzymes in observed phenomenon, as PNNs are composed mainly of sugar moieties. Therefore, we investigated the expression of genes coding for enzymes directly involved in hyaluronic acid and chondroitin sulfate metabolism in mice subjected to photothrombotic stroke.

The expression of genes was analyzed in the perilesional area at earlier (4 h, 24 h, 7d) and later time points (1 month and 3 m) after unilateral photothrombotic ischemia. To investigate spatiotemporal mRNA expression qPCR method was employed. Immunohistochemical staining was used to analyze cellular localization of investigated enzymes.

We observed only ipsilateral changes, no changes in contralateral homotopic cortex were found. Analysis of early time points revealed increases in mRNA level of degrading enzymes that were accompanied with decrease in mRNA coding for some of the synthesizing enzymes. The increase in hyaluronidase 1 (HYAL1) expression was detected 24 h post-stroke and was still observed 1 month after photothrombosis. Prominent increase of hyaluronan synthase 2 (HAS2) mRNA level was detected 24 h after stroke. Elevated expression of HAS1 and HAS3, but not HAS2, was observed 1 month after stroke. At 1 month post-stroke, increased mRNA level of enzyme involved in chondroitin sulfate chain elongation, chondroitin sulfate synthase 3 (ChSy3), was observed. No change in mRNA level of enzymes modifying polysaccharide components of extracellular matrix in perilesional area was detected 3 months after stroke. Interestingly, elevation of protein level of investigated enzymes, which mRNA level change was observed at early time points, was still detected at 1 month post-photothrombosis.

Obtained data indicate prolonged changes in polysaccharide component of the brain extracellular matrix after stroke that may affect neuronal plasticity.

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WTH03-11

Development of a novel antiepileptic therapy for dravet syndrome by targeting the EEF2K/EEF2 pathway

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eEF2K is an ubiquitous Ca^{++}/Calmoduline-dependent kinase that regulates protein translation by catalyzing the phosphorylation of eEF2 (eukaryotic Elongation Factor 2). We have recently demonstrated that eEF2K/eEF2 pathway inhibits the synthesis of certain proteins involved in the function of brain inhibitory synapses (Heise et al., 2016). Thus, mice deleted of the eEF2K gene ($eEF2K^{-/-}$ mice) show potentiated GABAergic synapses and are less susceptible to drug-induced seizures than non-mutated mice. In order to investigate whether the inhibition of eEF2K can be a possible target for epilepsy treatment, we crossed $eEF2K^{-/-}$ mice with $Scn1a^{+/-}$ mice, a model of Dravet syndrome that is a genetic disease characterized by pharmaco-resistant epileptic seizures, cognitive impairment, elevated mortality and ataxia. Our preliminary data demonstrate that the phosphorylation of eEF2 in $Scn1a^{+/-}$ mice is higher compared to wild type mice, indicating a possible contribution of eEF2K/eEF2 pathway in altering excitatory/inhibitory balance in these mice. Electroencephalographic and electrophysiological experiments confirm that double $Scn1a^{+/-}$/$eEF2K^{-/-}$ mice are protected from epileptic seizures either under basal condition or under thermal stress by the increased of GABAergic transmission

(the frequency of spontaneous inhibitory post synaptic currents is higher in double $Scn1a^{+/-}$/eEF2K$^{-/-}$ mice than $Scn1a^{+/-}$ mice). Moreover our behavioral experiments suggest that also cognitive impairments of $Scn1a^{+/-}$ mice are rescued by the genetic deletion of eEF2K. Given that eEF2K inhibition is efficacious in reverting epileptic phenotype in another epilepsy model (Heise et al., 2016), our data suggest eEF2K as a possible new pharmacological target for the treatment of genetic form of epilepsy.

Heise C., Taha E., Murru L. *et al.,* 2016, *Cereb Cortex*:

WTH03-12

HCN channels in cerebellar and hippocampal neurons

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Amongst the various ion channels that contribute to initiation, coordination and modulation of neuronal signals, hyperpolarization-activated and cyclic nucleotide-gated (HCN) channels play an essential role in the determination of biophysical properties of membranes. In contrast to typical voltage-dependent channels, these channels are activated at negative membrane potentials and their activation can additionally be modulated by direct binding of cAMP.

HCN channels affect neuronal excitability throughout the murine CNS. Here, we focus on two specific CNS regions, *i. e.* the hippocampus as the relay center for novel information during learning and memory processes and the cerebellum as the center of motor learning and motor control. Both regions are appealing targets for the study of synaptic plasticity due to their distinct cellular organization and defined pathways of signal relay.

First, we assessed the expression profiles of HCN channels in cerebellar and hippocampal neurons. We could show distinct expression patterns of the subunit isoforms HCN1, HCN2 and HCN4 on the RNA level as well as on the protein level. For in-depth analysis, we established cultivation of primary neurons, which retain the biochemical and electrophysiological properties of neurons *in vivo*. We addressed expression of HCN isoforms in primary neurons using super-resolution microscopy in order to gain insight on HCN isoform localization on the subcellular level.

Furthermore, we established recombinant adeno-associated viral (rAAV) vectors as efficient tools for the modification of neuronal cell function based on RNAi. Applying RNAi-mediating viral vectors *in vitro* and *in vivo* will allow us to investigate the functional contribution of individual HCN isoforms to hippocampal and cerebellar processes including neuronal excitability and learning behavior.

WTH03-13

Triclosan impairs hippocampal neuronal function

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Triclosan, an antibacterial and antifungal agent, is present in toys and in commonly used household products, such as toothpaste, detergents and soaps. However, the efficacy of triclosan is controversial and it has potential harmful effects; in the US the FDA has banned its use starting September, 2017. Nevertheless, further research on the effects of triclosan is required. Here, we investigated the effects of triclosan on a range of hippocampal functions. Addition of 1 uM triclosan to hippocampal neurons in primary cultures decreased the enhancement in spine density produced by the neurotrophin BDNF. Pre-incubation with the same concentration of 1 uM triclosan inhibited by 40% long-term potentiation induced by theta burst stimulation (CA3 to CA1) of rat hippocampal slices; higher concentrations of triclosan exerted a more drastic inhibitory effect. In addition, daily bilateral injections for 3 consecutive days of triclosan (1 ul, 10 uM) into the hippocampal CA3 area markedly reduced the ability of rats to perform a spatial navigation task. We propose that triclosan, at very low concentrations, has significant noxious effects on hippocampal function. Financial support: FONDECYT-1140545, FONDECYT-11140580 and BNI P-09-015F.

WTH03-14

Nitric oxide and ampa receptor trafficking

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The α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) are composed of four types of subunits, designated as GluR1, GluR2, GluR3, and GluR4. GluR2 subunit blocks calcium influx into the cell, therefore the GluR2-lacking AMPARs are calcium-permeable (CP) channels and GluR2-containing AMPARs are calcium-impermeable (CI) channels. It has been demonstrated that long-term potentiation (LTP) in CA1 hippocampal pyramidal neurons causes rapid incorporation of GluR2-lacking calcium-permeable AMPARs: CP-AMPARs are present transiently, being replaced by GluR2-containing AMPARs ~ 25 min after LTP induction (Plant et al. 2006). A number of molecules is involved in this process including the nitric oxide (NO). Different hypotheses concerning the role of NO in AMPARs trafficking exist. One of them implies that NO regulates incorporation of GluR2-containing AMPARs into the cell membrane.

We tested whether it's true by blocking the NO-synthase (NOS) and GluR2-lacking CP-AMPARs. Experiments were performed using standard whole-cell patch clamp recordings from CA1 pyramidal neurons in acute hippocampal slices from 14-18 day old rats. CP-AMPARs blockade by adding PhTx-74 5-10 min after the LTP induction decreased the EPSC amplitude down to baseline. To our surprise, PhTx-74 application along with the NOS blockade by L-NAME did not lead to LTP reduction. Moreover, the nNOS inhibition by another blocker 3-brom-7-nitroindazole with

simultaneous CP-AMPA blockade did not reduce LTP either. Also, by measuring the rectification index we found that the balance of two types of AMPARs after LTP induction under NOS blockade is different in the control neurons. Obtained results can be explained by the fact that NO regulates GluR2-lacking AMPARs incorporation into the postsynaptic membrane.
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WTH03-15

Dopamine D1 receptor activation prevents the loss of long-term spatial memory in mice

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Hippocampal synaptic plasticity, in the form of long-term potentiation (LTP) and long-term depression (LTD), enables spatial memory formation. Dopamine, released from the ventral tegmental area particularly under conditions of reward, acts on the hippocampus, and may specifically influence the encoding of information into long-term memory. D1/D5 dopamine receptors are importantly involved in the regulation of synaptic plasticity thresholds in the CA1 region of the hippocampus and determine the direction of change in synaptic strength that occurs during novel spatial learning. Here, we explored whether D1/D5-receptors influence memory persistence without further stimulation. Using the Barnes maze paradigm, we found that mice would persist their spatial learning within 14 days, however, on the 21st day after training, they could not remember the spatial memory. Following the dopamine D1 agonist treatment, mice can remeber 40% further compared to the vehicle-treated control groups. This type of memory persistence would disappear upon dopamine D1 antagonist treatment. These findings suggest that the dopaminergic system, acting via D1/D5-receptors, influences spatial memory persistence and modulates the direction of change in synaptic strength that underlies information storage in the hippocampus. Memory reconsolidation is especially dependent on D1/D5-receptor activation. Thus, dopamine acting on D1/D5-receptors is likely to support specific experience-dependent encoding, and may influence the content of hippocampal representations of experience.

WTH03-16

Chronic treatment of combined chemotherapeutic agents alters neuronal architecture in the mouse hippocampus

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There is accumulating clinical evidence that chemotherapeutic agents induce long-term side effects, including cognitive impairment and mood disorders, in breast cancer survivors who have undergone chemotherapy. Although several preclinical studies have investigated the behavioral changes associated with hippocampal dysfunctions induced by anti-cancer drugs, the precise mechanism of chemotherapy-induced alterations in the anatomical structure of hippocampal neurons remains unknown. In this study, we investigated the detrimental effect of chronic treatment with doxorubicin (Adriamycin) and cyclophosphamide (AC) combination on neural architecture of the hippocampus in female mice. 4 weeks after chronic AC administration, histological changes in neuronal complexity and dendritic spine density and morphology in dentate gyrus (DG) granule and cornu ammonis (CA)1 pyramidal neurons were quantified using Golgi staining. Treatment of AC combination modified the dendritic morphology of hippocampal neurons, showing decreases in the total dendritic length and reduction of dendritic complexity in area CA1 apical and DG. However, AC treatment did not alter dendritic morphology in the CA1 basal dendrites. AC combination significantly reduced spine density and mature dendritic spines in the CA1, but did not alter dendritic spine density and morphology proportion in the DG. These findings indicate that AC treatment leads to alterations in micromorphometric parameters in the hippocampus in region specific manner. Thus, the alteration of neuronal architecture may be related with hippocampal dysfunctions due to anti-breast cancer chemotherapy.

WTH03-17

Hypobaric hypoxia dysregulates fear conditioning response by modulation of synaptic strength and dendritic morphology

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Fear learning is essential for survival but its generalization leads to disorders. This study aims to explore the effects of hypobaric hypoxia (HH) on synaptic plasticity correlates together with dendritic morphology in limbic brain regions and its consequence on fear conditioning.

Methods: Sprague–Dawley (SD) rats were divided into ten groups ($n = 7$): Normobaric Normoxia (NN) and HH [1, 3, 7, 14 and 21 days] each. Animals were trained for fear conditioning paradigm and exposed to HH under a simulated condition in an animal decompression chamber. Animals were tested for changes on cued and contextual fear conditioning. Synaptic plasticity in Medial Prefrontal cortex (mPFC), hippocampus, amygdala and dendritic morphology in amygdala were studied through immunohistochemistry and Golgi cox method respectively under HH.

Results: Results of the present study revealed significant decrement in fear memory as evident from decreased freezing time during 1 day ($p < 0.05$) and 3 day ($p < 0.0001$) of HH exposure, whereas no significant difference was found on day 14 and 21 of HH when compared to control group. Concurrently exposure to 3HH leads to reduction in synaptic strength by decreasing expression of PSD95 ($p < 0.05$), synaptophysin ($p < 0.05$) in hippocampus and mPFC, whereas amygdala showed increased synaptic strength by increasing expression ($p < 0.001$) of these markers. Additionally, synaptic morphology i.e. dendritic arborization, dendritic length, branch intersection and spine density significantly decreased ($p < 0.0001$) in basolateral amygdala on 3 day HH as compared to NN.

Conclusion: HH dysregulates fear conditioning which may be attributed to a differential role on synaptic strength in hippocampus, mPFC, and amygdala.

WTH03-18

Functional role of hyaluronan receptor CD44 palmitoylation in hippocampal neurons

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The extracellular matrix (ECM) and its modifiers function as important regulators of neuronal morphology and synaptic plasticity contributing to physiological processes such as learning and memory. One important player in ECM signalling is the hyaluronan receptor CD44 which has been proposed to regulate myelination, axonal growth, dendritic arborisation, synaptogenesis as well as neuronal excitability. Localisation and signalling properties of CD44 can be modified by its post-translational modifications. Palmitoylation is the most common post-translational lipid modification of proteins which represents the reversible attachment of the C16 saturated fatty acid palmitate to cysteine residue(s). Even though CD44 is known to be palmitoylated, the functional consequences of CD44 palmitoylation in the brain have not been studied yet.

Here we investigated the molecular mechanism of CD44 palmitoylation and its role in CD44-mediated regulation of neuronal morphology and synaptogenesis. We demonstrated that CD44 undergoes palmitoylation in different regions of the rodent brain. In rat hippocampal neurons, we found CD44 to be either non-palmitoylated or mono-palmitoylated. Using site-directed mutagenesis, we identified the cytoplasmic cysteine residue 298 as single palmitoylation site in rat CD44. Furthermore, by silencing endogenously expressed CD44 accompanied with the over-expressing a palmitoylation-deficient CD44 mutant we studied the effects of this lipid modification on CD44 function in hippocampal neurons.

WTH03-19

Regulation of the neuronal glycine transporter GLYT2 by P2X purinergic receptors

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Glycinergic inhibitory neurons of the spinal dorsal horn exert critical control over the conduction of nociceptive signals to higher brain areas. The neuronal glycine transporter 2 (GlyT2) is involved in the recycling of synaptic glycine from the inhibitory synaptic cleft and its activity modulates intra and extracellular glycine concentrations. In this report we show that the stimulation of P2X purinergic receptors with βγ-methylene adenosine 5′-triphosphate induces the rapid up-regulation of GlyT2 transport activity by increasing total and plasma membrane expression and reducing transporter ubiquitination. We identified the receptor subtypes involved by combining pharmacological approaches, siRNA-mediated protein knockdown, and dorsal root ganglion cell enrichment in brainstem and spinal cord primary cultures. Up-regulation of GlyT2 required the combined stimulation of homomeric $P2X_3$ and $P2X_2$ receptors or heteromeric $P2X_{2/3}$ receptors. By measuring spontaneous glycinergic currents in response to $P2X_3$ receptor agonists and glycine release and GlyT2 uptake in parallel, we could integrate GlyT2 modulation within the response of glycinergic neurotransmission to $P2X_3$ receptor activation. The recognized pro-nociceptive action of $P2X_3$ receptors suggests that the fine-tuning of GlyT2 activity may have consequences in nociceptive signal conduction.

WTH03-20

Effect of neurotrophin-4 in hippocampal synaptic plasticity induced by testosterone

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Background of the study: Neurotrophins are powerful molecular mediators of hippocampal synaptic plasticity and its electrical properties shape the structural organization of the synapse. Among these, Neurotrophins-4 (NT4) has emerged as having key roles in the neurobiological mechanisms related to learning and memory. Young hypo-gonad men, with low endogenous testosterone, are diagnosed with anxiety or depressive disorders and exhibit aberrant performance in some cognitive tasks. Indicating the sustenance or activation effect induced by testosterone towards enhances the spatial ability and the possible role of NT4 in adult hippocampus seems to be a prospective area to explore.
Method: Adult male Wistar rats were divided into four groups (n = 9): Sham, Orchidectomized (ODX), ODX+Testosterone (T) (5 mg/kg body weight) and sham+T. Animals were subjected to eight-arm radial maze (RAM) trial to evaluate the working and reference memory task (WME). Paraffin processed hippocampus tissues sections were stained with H&E, CFV, modified Trichrome and TUNEL staining. Dendritic arborizations of hippocampal pyramidal neurons were analyzed by Golgi-cox technique. Antioxidant level estimated biochemically and gene expression of NT4 signaling pathway via RT-PCR.
Results: In the RAM trial, ODX group showed a high number of WME and RME throughout the period. Whereas, T supplementation group showed significantly reduces WME and RME at the end of the trial sessions. Orchidectomized rat hippocampal cells showed altered cellular morphology, however, these changes were absent in ODX +T group. In the ODX rats, there was a reduced expression of NT4 and this has been restored in ODX+T supplemented rat hippocampus. These parameters were better in Sham+T rats.
Conclusion: Spatial memory impairment in ODX rats after T depletion confirms its positive effect on spatial learning and memory. Further, these results indicate that T presence reciprocally up-regulates the mRNA levels of NT-4. Thus, indicating the crucial role played by T in controlling NT4 expression and hippocampal neuronal plasticity, an essential modification for learning and memory.

WTH03-21

Expression of LTP-specific PRPS in prelimbic cortex region of the brain is necessary for the formation of long term memory

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Memory is one of the most fundamental processes of brain and we have not yet explored the complete notion of its underlying mechanism. Here we aimed to investigate the underlying process of "Behavioural Tagging" in long term memory (LTM) formation and to find the key factors playing role in consolidation of LTM. Behavioural tagging is a process which explains how short-term memory induced by a weak stimulus transforms into LTM when exposed to a novel environment in a critical time window. Here we have shown that how the process of "Behavioural Tagging" provides the necessary plasticity related proteins (PRPs) to stabilize LTM in adult Wistar rats. Therefore LTP-specific PRPs have been shown to play a significant role in both maintaining long term potentiation (LTP) and memory storage. For that intracerebroventricular (ICV) infusion of LTP-specific PRPs synthesis inhibitor in adult Wistar rats was done into the prelimbic cortex region of the brain which inhibited the activity of these PRPs and thus LTM which suggested that the inhibition of these LTP-specific PRPs in the prelimbic cortex region can disrupt consolidation of LTM. The results here indicate that memory consolidation-like events take place in the prelimbic cortex and these LTP-specific PRPs are essential components in LTM formation.

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WTH03-22

Lack of SEZ6 family proteins affects neuronal physiology and behaviour

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Excitatory synapse maturation and maintenance is a complex process that begins in early development and continues throughout life to facilitate learning. Characterisation of Seizure-related gene 6 (Sez6) knock-out (KO) mice revealed a role for Sez6 in patterning the dendritic arbor as well as in the development of excitatory synapses. Sez6 and related family members, Sez6 Like and Sez6 Like 2, are all expressed in neurons and have been found to have partially overlapping spatial and temporal patterns of expression indicating the possibility of functional compensation by these proteins. Additionally, it has recently been determined that all Sez6 family members are cleaved by the Alzheimer's protease β-APP cleaving enzyme 1 (BACE1). In order to investigate the roles played by Sez6 family proteins in the brain, a triple KO (TKO) mouse model was used in which all Sez6 family members are lacking. As previously reported, these Sez6 family TKO mice exhibited motor deficits on the accelerating Rotarod and reduced movement was seen in the elevated open field test. They also had behavioural deficits that indicate an impairment in cognitive flexibility. The TKO mice failed to extinguish their fear response in the context fear extinction paradigm and had difficulty changing their search strategy in the reversal portion of the Morris water maze. Along with the behavioural deficits of the TKO mice, alterations in the functional properties of neurons in the pre-limbic cortex were observed using electrophysiology. Additionally, pyramidal neurons in the somatosensory cortex of TKO mouse brains had altered dendritic spine structure with a shift away from the mature, mushroom shaped spines in favour of thin and stubby spines. Together these data reveal that the Sez6 family proteins are crucial for the structural and functional excitatory synapse plasticity that underlies flexible behaviour.

WTH03-23

Seizure-related gene 6 (SEZ6)family proteins and their influence on excitatory synapse function, motor co-ordination and cognition

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The Seizure-related gene 6 (Sez6) family of proteins, which inckudes Sez6, Sez6L and Sez6L2, are major β-secretase1 (BACE1) substrates. This protease is a therapeutic target in Alzheimer's disease and, while blocking BACE1 would be predicted to reduce toxic Ab production, it may also negatively influence Sez6 family protein dependent mechanisms. Sez6 is required for the normal development of excitatory neurons and constitutive Sez6 knockout (KO) mice display dendritic and synaptic abnormalities as well as motor and cognitive deficits. The persistence of Sez6 expression, particularly in the cortex and hippocampus, suggests ongoing roles for these proteins in the mature brain although it is not possible to separate these effects from those caused by abnormal development in the constitutive Sez6 KO mouse line. To investigate the functional roles of Sez6 family proteins, we have adopted a range of approaches utilizing an inducible Sez6 conditional KO model, Sez6L and Sez6L2 knockout mouse lines and a triple knockout (TKO) mouse model for Sez6 family proteins.

Using tamoxifen feed, we achieved near complete loss of Sez6 protein in calcium-calmodulin protein kinase II (CaMKII)-expressing hippocampal and cortical neurons. Behavioural tests indicate that Sez6 is required for normal expression of contextual fear memory in adult mice. Compared to controls, fear memory was enhanced in Sez6 cKO at 24 h and 7 days post-shock which is reminiscent of the persistent strong fear seen in Sez6 family TKO mice. Sez6L KO, Sez6L2 KO and Sez6 TKO mice exhibit deficits in behavioural tests of motor and memory function We conclude that Sez6 family proteins play important roles in the developing and adult brain particularly in learning and memory.

WTH03-24

TGF-β-sensitive neurons in the hypothalamus regulate food intake and body weight

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Regulation of feeding behavior is essential for survival and any deregulation can lead to metabolic pathologies such as obesity. In mammals, the hypothalamus is well known to orchestrate most metabolic processes including feeding. However, the specific neuronal populations and networks that control appetite and satiety are not fully understood. Here, we describe the role of distinct TGF-β-sensitive neurons in three hypothalamic regions: the paraventricular nucleus of the hypothalamus (PVN), the arcuate nucleus (ARC) and the lateral hypothalamic area (LHA) in the regulation of food intake and body weight. First, we find high numbers of TβR1- and Smad3-positive cells (TβR1: TGF-β receptor 1, Smad3: downstream transcription factor) in the PVN, ARC and LHA. To directly investigate the role TGF-β-sensitive neurons in the regulation of feeding and body weight, we used a combination of genetically engineered mice (TβR1 flox/flox, Smad3 flox/flox) and stereotactic viral injection (AAV-hsyn-GFP-Cre) that allows targeted deletion of TβR1 or Smad3. Loss of TβR1 and Smad3 in PVN neurons ($T\beta R1^{PVN}KO$ and $Smad3^{PVN}KO$) resulted in significant body weight gain and fat mass increase over time due to an increase in food intake. TβR1 deletion in the ARC ($T\beta R1^{ARC}KO$) resulted in a significant increase in body weight and food intake, but not to the same extent as the PVN. Further, Smad3 ablation in the LHA ($Smad3^{LHA}KO$) also resulted in an increase in body weight. We find that most TβR1- and Smad3 positive cells localized in the PVN express either oxytocin (OXT) or vasopressin (AVP). In the ARC, most TβR1 and Smad3-expressing neurons colocalize with POMC neurons. To further define the role of these neurons, we are conditionally activating TGF-β signaling using a "gain of function" model (LSL-TβR1CA) in the same regions. Taken together, these studies establish the importance of hypothalamic TGF-β-sensitive neurons in the central mechanisms of feeding behavior with implications to obesity pathogenesis.

WTH03-25

Presynaptic spike timing-dependent long-term depression in the CA1 region of the hippocampus

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Spike timing-dependent plasticity (STDP) is a model of synaptic plasticity that may underlie learning and memory. The aim of our research was to investigate the signalling pathway for the induction of spike timing-dependent long-term depression (t-LTD) in the hippocampus. Whole-cell recordings were made from individual CA1 cells in hippocampal slices prepared from P12-P18 mice. We have previously shown in the hippocampus that a post-before-pre pairing protocol (pairing postsynaptic action potentials with EPSCs at 0.2 Hz) produced robust input-specific t-LTD and that the induction of this form of LTD was completely blocked by D-AP5, by the broad spectrum mGluR antagonist MCPG, as well as by mGluR1 and mGluR5 selective antagonists, by phospholipase C (PLC) inhibitors and by the CB1 receptor antagonist AM251. The blockade of postsynaptic NMDARs by application of MK-801 (1 mM) through the patch pipette did not affect the induction of t-LTD (76 ± 8%, $n = 12$). We have also determined that this t-LTD requires astroglial signalling as is completely prevented by loading astrocytes with 20 mM BAPTA (129 ± 7%, $n = 5$ vs. interleaved slices 54 ± 6%, $n = 5$). Fluctuation, failures and paired-pulse ratios analysis all indicated a presynaptic locus of expression of this t-LTD. To further support a presynaptic locus of induction and expression of this form of LTD we recorded in the same neuron miniature responses before and after a post-pre pairing protocol. The frequency of miniature responses decreased from 0.4 ± 0.1 Hz (baseline) to 0.17 ± 0.06 Hz (after LTD) with no changes in mEPSCs amplitude. These results show that whereas t-LTP induction depends on postsynaptic NMDARs, the induction of t-LTD is independent of postsynaptic activation of NMDARs and likely requires presynaptic NMDA receptors. The results also show that the induction and expression of t-LTD at CA3-CA1 synapses is presynaptic.

WTH03-26

Role of matrix metalloproteinase-9 (MMP-9) in a chemically-induced synaptic plasticity

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Neuronal synapses are maintained by a complex network of adhesion molecules. MMPs known as extracellular proteases are able to modify synaptic function of which MMP-9 is of particular importance because of being able to change spine morphology by cleavage of postsynaptic adhesion molecules. However, it still remains largely unknown how MMPs may contribute to pre- and postsynaptic function. The aim of this study was to evaluate the effect of proteolytic activity of MMP-9 on the synaptic function. To test this theory, we pharmacologically inhibited the activity of MMP-9 in cultured hippocampal organotypic slices. Using whole-cell patch-clamp technique, AMPA receptor-mediated miniature excitatory postsynaptic currents (mEPSCs) were recorded from CA1 pyramidal cells. To induce synaptic plasticity, a cholinergic agonist named carbachol triggering rhythmic activity, which causes a lasting synaptic enhancement, was applied. One hour of carbachol treatment followed by an overnight incubation showed significant increase in frequency of mEPSCs. Interestingly, we observed that using MMP-9/13 inhibitor I along with carbachol further enhanced frequency of mEPSCs compared to carbachol and inhibitor I alone. Moreover, we observed the same result in MMP-9 knockout animal. Furthermore, evaluation of gelatinase activity by gel zymography in conditioned cultured medium indicated remarkable increase in the level of MMP-9 but not MMP-2 compared with control 24 h after carbachol treatment. In addition, preliminary result showed increase in spine density of group which we observed enhancement of mEPSCs frequency. Collectively, our results indicate that MMP-9 proteolytic activity can have a great impact on the synaptic plasticity.

WTH03-27

Protein methyltransferases 8 (PRMT8) restricts proteins associated with synaptic maturation in murine visual cortex

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The brain adapts to dynamic environmental conditions by altering its epigenetic state, thereby influencing neuronal transcriptional programs. An example of an epigenetic modification is protein methylation, catalyzed by protein arginine methyltransferases (PRMT). One member, *Prmt8,* is selectively expressed in the central nervous system during a crucial phase of early development, but little else is known regarding its function. We hypothesize *Prmt8* plays a role in synaptic maturation during development. To evaluate this, we used a proteome-wide approach to characterize the synaptic proteome of *Prmt8* knockout versus wildtype mice. Through comparative network-based analyses, proteins and functional clusters related to neurite development were identified to be differentially regulated between the two genotypes. One interesting protein that was differentially regulated was Tenascin-R (TNR). Chromatin immunoprecipitation demonstrated binding of PRMT8 to the *tenascin-r* (*Tnr*) promoter. TNR, a component of perineuronal nets (PNNs), preserves structural integrity of synaptic connections within neuronal networks during the development of visual-somatosensory cortices. On closer inspection, *Prmt8* removal increased net formation and decreased inhibitory parvalbumin positive (PV+) puncta on pyramidal neurons, thereby hindering the maturation of circuits. Consequently, visual acuity of the knockout mice was reduced. Our results demonstrated *Prmt8*′s involvement in synaptic maturation and its prospect as an epigenetic modulator of developmental neuroplasticity by regulating structural elements such as the PNNs.

WTH03-28

PER1-dependent molecular mechanisms behind daytime-dependent plasticity in mouse hippocampus

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The ability to convert transient stimuli into long-term changes of brain function is central to the capacity of an animal to adapt to a dynamic environment by learning. Coping with periodically recurring harmful or rewarding stimuli requires their efficient prioritization and a molecular machinery that is capable of associating, retaining, and recalling timing information. Hippocampus integrity ensures proper memory acquisition, consolidation, and retrieval. Notably, hippocampus-specific cellular and molecular dynamics that are associated with long-term memory (LTM) formation are clearly molded by time-of-day and depend on proper output from the master circadian (circa: about; dies: day) clock in the suprachiasmatic nucleus. We show that time-of-day-dependent LTM formation is tightly coupled to post-translational modifications and/or *de novo* gene expression of plasticity-related proteins, relies on intact cAMP/PKA/PKC/CREB signaling and requires chromatin remodeling. In addition, compelling evidence suggests that hippocampus-dependent LTM formation is mirrored in the plasticity of long-term potentiation (LTP): LTP efficiency, structural synaptic plasticity, synaptic excitability and the responsiveness to synaptic input follow a similarly clear circadian rhythm, depending on a dynamic expression of the clock gene PER1 in mouse hippocampus. These observations argue for an intricate interplay between the circadian system and memory, the mechanisms of which are not yet well understood. We here reveal in addition a PER1-dependent modulation of cytoplasmic-to-nuclear signaling in the murine hippocampus, providing a molecular explanation for how the circadian system potentially shapes a temporal framework for memory performance dependent on time-of-day, and adds a novel facet to the versatility of the clock gene protein PER1.

WTH03-29

PSD lattice and scaffold-adaptor protein model for PSD structure

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Postsynaptic density (PSD) is a dynamic structure, which is localized immediately underneath the postsynaptic membrane and works an essential devise for synaptic transmission and synaptic plasticity. A well-known model for architecture of PSD of type I excitatory synapse comprises of several scaffolding proteins including shank, PSD-95, GKAP and homer, to which various molecules involved in postsynaptic signaling are associated (scaffold/adaptor protein model). On the contrary, "PSD lattice" has been identified in the preparation obtained after treatment of synaptosome, SPM or Triton X-100-PSD with deoxycholate, a relatively strong detergent, and has been considered to be a basic backbone of type I PSDs before the proposal of scaffold/adaptor protein model. However, major constituents of the PSD lattice and the relationship between the PSD lattice and the scaffold/adaptor protein model have not been known. It is essential to know the details of molecular architecture of PSD for full understanding the mechanisms, at the molecular level, of dynamic nature of PSD, one of basis of synaptic plasticity. We purified a fraction that contained PSD lattice-like structures. The structure was recovered in the fraction slightly lighter than the pellet that contained PSDs. The lattice-like structure was planar, of which diameter was similar to PSD, sparser than PSD, and contained mesh-like woven fibers when observed in thin-section electron micrograph. Components of the structure were examined by Western blotting, immuno-dot blotting and immunogold negative staining electron microscopy. This study will give a new insight on the molecular architecture of type I excitatory PSD and new architecture model will be discussed.

WTH03-30

LMTK1 is a novel membrane bound kinase involved in anxiety and depression

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Lemur kinase 1 (LMTK1) is a novel Ser/Thr kinase, which is highly expressed in mammalian brain. There are two isoforms,

LMTK1A and LMTK1B; while LMTK1A binds to recycling endosome via myristoylation of the N-terminal cysteines, LMTK1B has transmembrane sequences at the N-terminal region. We report that LMTK1 regulates axon and dendrite elongation negatively through the transport of Rab11-positive recycling endosomes. Knockdown or knockout of LMTK1 enhances axonal outgrowth and dendrite arborization. We think that LMTK1 prevents overgrowth of axon and dendrites by controlling the supply of membrane components to the tip of neurites. The overgrowth of neurites caused by dysfunction of LMTK1 would result in developmental retardation or psychiatric diseases. Therefore, it is important to understand the mechanism regulating the neurite outgrowth by LMTK1. In this study, we analyzed expression of LMTK1 in mouse brains and behavior of LMTK1 knockout mice.

LMTK1 was expressed in neurons of cerebral cortex, hippocampus, cerebellum and olfactory bulb at postnatal day 5 and its expression increased gradually with aging. Quantitative PCR indicated almost equal expression of LMTK1A and LMTK1B in developing and adult brains. In adult brains, LMTK1 appeared to be also expressed in glial cells. Brain structures looked normal in LMTK1 knockout mouse when they were observed at the light microscopic level but when examined by an electron microscopy the synaptic vesicles in presynaptic terminus was more abandant in LMTK1 knockout mouse than wild-type mouse. Further, when dendritic arborization was examined in cultured cerebellum Purkinje cells, Purkinje cells of knockout mouse displayed larger dendrite expansion. LMTK1 knockout mouse exhibited abnormal behaviors, such as hyperactivity, reduced anxiety behavior and depression-like behavior. These results indicate that LMTK1 plays an important role in dendrite arborization and synaptic activity, and is involved in anxiety and depression.

WTH03-31

Remember or forget – dopaminergic modulation of auditory memory persistence in the presence or absence of alpha-synuclein

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Frequency-modulated tone discrimination (FMTD) learning of rodents induces elevated dopamine responses in auditory and prefrontal cortices during initial conditioning. We hypothesized that during early stages of FMTD learning cortical dopamine determines the efficiency of subsequent learning and memory formation. For the auditory cortex, we previously showed that local activation of D1/D5 dopamine receptors induces distinct proteome changes (including the nerve terminal-enriched protein alpha-synuclein), facilitates the stabilisation of newly acquired memory, and supports anterograde memory formation. To address the role of prefrontal dopamine, we now have utilised local infusion of D1/D5 receptor agonists into the murine medial prefrontal cortex shortly after the initial FMTD conditioning; treatment effects were monitored on subsequent 15 training days and after a training intermission of 4 weeks. Compared to vehicle-controls, prefrontal agonist treatments did not cause differences in the ascending section of the learning curve. However, at the asymptotic curve region and, in particular, during re-learning after the 4-week conditioning-free period of spontaneous forgetting, FMTD performance in D1/D5 agonist-treated mice was significantly affected. Interestingly, in mice of the C57BL/6JOlaHsd substrain, displaying a spontaneous deletion of the alpha-synuclein encoding *snca* gene, and in C57BL/6J mice with no such deletion, local post-acquisition activation of prefrontal D1/D5 receptors affected late memory performance in opposite directions, causing impairments in C57BL/6JOlaHsd mice and improvements in C57BL/6J mice. This is in line with our recent findings of substrain-dependent learning curve differences after systemic dopaminergic interference. Together, the results on the discrimination of complex sounds suggest that dopamine acting at D1/D5 receptors in different regions of the cerebral cortex during initial encoding may control both the temporary storage/retrieval of recent memories and a predisposition of neural networks essential for remote memory storage/retrieval. Opposite actions of pharmacological treatments in alpha-synuclein deficient and non-deficient mice imply a role of this protein in the dopaminergic modulation of memory consolidation.

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WTH03-32

Mechanisms of CDC42 palmitoylation in respect to neuronal functions

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Dynamic S-palmitoylation arose as an important regulator of signalling-protein functions critically involved in synaptic plasticity. The reversible attachment of the C-16 saturated fatty acid (palmitoylation) can modulate membrane insertion and sub-compartmentalisation of proteins. Palmitoylation emerged as pivotal modification of synaptic proteins, affecting key player of neuronal morphology and synaptogenesis. Here we investigated molecular details of the brain-specific isoform of small the GTPase Cdc42-palm. As a key modulator of cellular morphology, it plays an important role in regulating spine structural plasticity. We addressed the palmitoylation in more detail and explored functional consequences of neuronal signalling pathways. After identifying palmitoyltransferase DHHC5 as a protein responsible for Cdc42 palmitoylation, we could show that the enzyme favours a single cysteine residue at position 188 within Cdc42-palm as a site of palmitoylation. We also found that Cdc42-palm directly interact with the C-terminus of DHHC5. Functionally, DHHC5-mediated mono-palmitoylation of Cdc42 is necessary for the ability of Cdc42-palm to modulate dendritic morphology of hippocampal neurons and to regulate gene activation.

WTH04 Neuronal Polarity

WTH04-01

P2 receptors control the migration of medial ganglionic eminence-derived interneurons

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Cortical interneurons migration is a fundamental event for the development of the cerebral cortex. Defects in this process may underlie neurological and psychiatric conditions (Nat. Rev. Neurosci. 13(2):107-20). Thus, it is mandatory the identification of the mechanisms governing interneurons migration. It was recently shown that adenosine A_{2A} receptors contribute to interneurons migration (Sci. Trans. Med. 5:197ra104). We now found that P2Y1Rs are also expressed at mid-late stages of embryogenesis, coincident with the onset of interneurons migration. They are predominantly present in proliferative regions of mice developing telencephalon (E13), including the medial ganglionic eminence (MGE), and particularly in MGE-derived interneurons assessed by immunoreactivity and functionally identified by Ca^{2+} transients induced by the pharmacological activation of P2Y1R (MRS2365, 100 nM). In MGE explants cultures, the selective blockade of P2Y1R (MRS2179, 10 μM) significantly decreased the migration of interneurons from the MGE explants. This effect was mimicked by apyrase, which catabolizes ATP and ADP into AMP, being the migration restored upon the pharmacological activation of P2Y1R (MRS2365). This was arrested by the presence of a PKC inhibitor (BIM-1, 500 nM). These data shows that P2Y1Rs are expressed and promote MGE-derived interneurons migration through PKC activation. In contrast, the P2X-preferring agonist BzATP (1–100 μM) inhibited MGE-derived interneurons migration in a concentration-dependent manner, an effect prevented by the P2Rs antagonist PPADS (10 μM), by the selective antagonist o P2X1R, NF279 (1 μM), but not by the P2X7R antagonist, A438079 (10 μM). Moreover, we could detect immunoreactivity for P2X1R, but mostly in βIII-tubulin positive migratory neurons opposed to the predominant expression of P2Y1Rs in proliferative zones. Altogether, these evidences raise purinergic signalling in interneurons migration, supporting a scenario in which P2Y1R promotes interneurons migration as a motogenic factor in the MGE, while the P2X1R should be contributing for their guidance by transducing ATP as a repulsive cue.

WTH04-02

Novel mechanisms involving redox biology are essential to support axonal growth

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Physiological levels of ROS are important for several process in the nervous system, ranging from neuronal precursors proliferation, to axonal guidance and neurotransmission. ROS also support neurite outgrowth and axonal specification, but the mechanisms by which ROS are able to shape neurons remain unknown. We recently showed that NADPH oxidase activity is essential to sustain axon growth. Now, we report that Ca^{2+} release from the endoplasmic reticulum (ER) is coupled to ROS signaling dependent on NOX2. In this work, we explore the contribution of the link between NOX and RyR-mediated Ca^{2+} release towards axonal specification of rat hippocampal neurons. Using genetic approaches, we find that NOX activation promotes both axonal development and Rac1 activation through a RyR-mediated mechanism, which in turn activates NOX through Rac1, one of the NOX subunits. Collectively, these data suggest a feed-forward mechanism that integrates both NOX activity and RyR-mediated Ca^{2+} release to support cellular mechanisms involved in axon development. Finally, we explore the contribution of calcium entry from the extracellular milieu as a triggering factor to promote the concerted functions of NOX and RyR2 during axon specification.

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WTH04-03

Cortical principal neurons migration entails A2A receptor-driven neuronal polarization and axon formation

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Neuronal migration is a fundamental process in brain development. Indeed, impairment in neuronal migration is one of the major causes of cortical malformation (Neuron 60:273-84). Hence, it is of upmost importance to unravel the mechanisms driving neuronal migration. It was recently demonstrated that adenosine A_{2A} receptor ($A_{2A}R$) controls interneurons migration (Sci. Trans. Med. 5:197ra104). We now aimed to evaluate if $A_{2A}R$ is also involved in the migration of cortical principal neurons. We found that embryos lacking the $A_{2A}R$ ($A_{2A}R$-KO mice) showed a delayed migration of cortical principal neurons at embryonic day 17 (E17), in comparison to their wild-type littermates. Similarly, embryos exposed to the $A_{2A}R$ antagonist SCH58261 (daily 0.1 mg/kg i.p. injection in pregnant females E13-E16) presented delayed migration when compared with embryos exposed to vehicle. These effects should be due to $A_{2A}Rs$ expressed by migratory neurons, as *in utero* electroporation of plasmid encoding shRNA specific for $A_{2A}R$ at the same developmental stage (E14-E17) also delays migration. This delay in neuronal migration occurs mostly at the intermediate zone (IZ), where it is required a transition from a multipolar to a bipolar shape and the establishment of an axon-like leading process in order for neurons to proceed their migration towards the cortical plate (Nat. Neurosci. 12:1693-700). Accordingly, we found that mice

primary cortical neurons cultured in the presence of the $A_{2A}R$ antagonist SCH58261 (50 nM) leads to a reduction in the number of axons and in their length (DIV0-3). Altogether, these results demonstrate that $A_{2A}R$ is required for proper cortical principal neuronal migration, in particular for the transition from the intermediate zone into the cortical plate by controlling the establishment of neuronal polarity and axon formation.

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WTH05 Animal Model of Neuropsychiatric Disorders

WTH05-01

Changes in adult neurogenesis in chronic unpredictable mild stress and early-life inflammatory stress models in rats

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Chronic stress is a widespread condition involved in development of multiple brain disorders including depression and post-traumatic disorder. The exact way of long-term action of stressful impacts remains not completely understood. Here we provide the data concerning possible involvement of altered adult neurogenesis in the development of stress-associated brain pathology. We applied two paradigms of the chronic stress: chronic unpredictable mild stress (CUS) and early-life inflammatory stress (ELIS). In CUS paradigm, the rats were subjected to a series of stressful events including food and/or water restriction, cage tilt, crowded housing, isolation, and inversion of the light-dark schedule. Stressors were changed twice a day and presented randomly during 2 months. In the middle of CUS protocol, rats were injected with BrdU to assess the long-term effects of stress on differentiation of cells in the hippocampus. After completion of CUS protocol, behavior was analyzed, and animals were sacrificed for analysis of neurogenesis. In ELIS paradigm, bacterial lipopolysaccharide was administered intraperitoneally to rat pups on postnatal days 3 and 5 followed by BrdU injections, and behavior and neurogenesis were assessed later in adulthood, at the age of 3 months.

Two models of stress were accompanied by different changes in neurogenesis. The proliferation of precursor cells after completion of stress, assessed by PCNA staining, was unaffected in both paradigms. However, the neuronal differentiation assessed by doublecortin staining was suppressed by ELIS and enhanced by CUS. On the other hand, the number of new neurons and astrocytes generated from the cells which were born during early-life inflammation was increased in the dentate gyrus of rats subjected to ELIS. Our results suggest the difference between the ways in which these two stress paradigms influence the process of postnatal neurogenesis in the hippocampus of rats.

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WTH05-02

Optogenetic activation of striatonigral pathway is sufficient to induce obsessive-compulsive disorder-like behaviors

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fMRI studies in Human with obsessive compulsive disorder (OCD) evidenced an over-activity of the orbitofrontal cortex (OFC) to the ventral striatum (VS) projections. A recent study succeeded the modeling of OCD-like behavior in mice on the basis of clinical evidence; selective and repetitive activation of VS projecting OFC neurons induced a chronic, but not acute, over-grooming, which is relevant to human OCD phenotype. The next challenge of OCD-related circuit genetics is to clarify which cell type is involved in the pathogenesis of OCD-like behaviors. Indeed, VS projecting OFC neurons terminate on two distinct populations, called striatonigral and striatopallidal projection neurons, but which cell type mediates the pathogenesis is unknown. In this study, we hypothesized that the overactivation of striatonigral neurons caused the chronic over-grooming seen in study presented above. To selectively activate striatonigral neurons, we first generated transgenic mice in which step function channelrhodopsin2 (ChR2 (C128S)) was expressed by both striatal projection neurons (i.e. D1 receptors expressing medium spiny neurons and D2 receptors expressing medium spiny neurons). We then inserted an optic fiber onto the left ventral mesencephalon, enabling the selective illumination of striatonigral neurons axon terminals. Blue light illumination induced a contralateral rotation, suggesting the successful activation of striatonigral neurons. To further prove the opto-activation of striatonigral neurons, we examined the firing of putative connected neurons in the ventral mesencephalon. Fifty millisecond blue light illumination blocked the firing for over 15 seconds, indicating that optogenetically activated striatonigral neurons released GABA. Five second blue light illumination was given every minute, 5 times per day, for 5 consecutive days. Similarly to an OFC-VS projecting neurons overactivation, repeated activation of striatonigral neurons was sufficient to induce chronic over-grooming behaviors.

WTH05-03

Investigating mitochondrial biomarkers and function using MRS at 14.1 Tesla in a mouse model of mood disorders

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In vivo magnetic resonance imaging(MRI) and spectroscopy (MRS) are non-invasive techniques of choice for investigating and monitoring brain metabolic changes related to mitochondrial function and health. Mitochondria have been associated with many brain disorders and, among them, mood disorders. Defining and understanding mitochondrial MRI/MRS biomarkers related to mood disorders can help better characterizing endophenotypes of these psychiatric illnesses.

In this study we have investigated the MRI/MRS profile of a mouse model of mood disorders lacking an important brain

plasticity gene, *Crtc1* (CREB-regulated transcriptional coactivator 1).

Metabolic and volumetric profile alterations were determined with T_2-weighted MRI together with ^{1}H-MRS of prefrontal cortex (PFC) and dorsal hippocampus (HDors). Results indicated age-dependent alterations of glutamate and GABA levels in *Crtc1* KO mice PFC together with a constant reduction in phosphocreatine (PCr) energy metabolites in the dorsal hippocampus (PFC: Glu ($-12 \pm 3\%$), GABA($-26 \pm 11\%$); HDors: PCr($-20 \pm 8\%$)). qPCR experiments revealed no changes in electron transport chain (ETC.) gene expression but increased creatine kinase(CKMt and CKB) levels in the dentate gyrus of KO mice, confirming neuroenergetic deficiency in HDors. MtDNA copy number quantification revealed a reduction of mitochondrial mass in the dentate gyrus, which could explain the observed energetic dysfunction. Finally, preliminary ^{1}H[^{13}C]-MRS results upon infusion of [U-^{13}C] glucose suggested metabolic differences in the HDors with reduced glucose uptake or an increased glycolytic rate in KO animals. Together, these results suggest that CRTC1 might be an essential regulator of brain energy metabolism in the mouse HDors. Further investigations will aim at clarifying the mitochondrial failure of these mice and monitor its evolution with its associated MRI/MRS profile.

WTH05-04

Fluvoxamine maleate effects on dopamine signaling in the prefrontal cortex of stressed parkinsonian rats: a cognition implication

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Parkinson's disease (PD) also affects extra-striatal midbrain cells resulting in reduced extrinsic supply of dopamine (DA) to the prefrontal cortex (PFC). In the present study, we investigated the effects of reduced DA presence in the PFC on cognitive function and whether treatment with Fluvoxamine maleate (FM) attenuated these effects. Maternal separation was used to develop an animal model for early life stress that has chronic effects on brain and behavior. Sprague–Dawley rats were treated with the antidepressant FM prior to 6-hydroxydopamine (6-OHDA) lesion to model motor deficits in rats. The Morris water maze (MWM) and the forelimb use asymmetry (cylinder) tests were used to assess learning and memory impairment and motor deficits respectively. Blood plasma was used to measure corticosterone concentration and prefrontal tissue was collected for lipid peroxidation, DA, and serotonin (5-HT) analysis. Our results show that animals exposed to early life stress displayed learning and memory impairment as well as elevated basal plasma corticosterone concentration which were attenuated by treatment with FM. A 6-OHDA lesion effect was evidenced by impairment in the cylinder test as well as decreased DA and 5-HT concentration in the PFC. These effects were attenuated by FM treatment resulting in higher DA concentration in the PFC of treated animals than in non-treated animals. This study suggests that FM may ameliorate cognitive impairment in PD by preserving DA and 5-HT transmission in the PFC.

WTH05-05

Zebrafish larvae model: a novel approach to study autism

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Autism Spectrum Disorder (ASD) is a complex neurodevelopmental disorder of early onset, highly variable in its clinical presentation. It includes the development of abnormal cortical circuitry that underlies autistic cognitive processes, social impairment and other behaviors. Although the animal models for autism do exist but have several disadvantages, which motivate us to design a new model for high throughput screening on autism. The aim of our study was to develop a cost and time effective model with a robust parameters to understand autism using zebrafish larvae, to overcome the shortcomings of rodent model on autism. Zebrafish embryos were treated with valproic acid and a battery of behavioral tests (markers of anxiety, fear, social impairment and irritability) was performed on larvae at seventh day post fertilization. This model shows a significant behavioural impairment in valproic acid treated larvae in comparison to control which was again supported by alteration in few marker genes and proteins involved in autism. This model was further validated using positive control drugs available for autism which reverts the phenotypic abnormalities. Thus we postulate that our 7 days larval model for autism can help in high throughput screening of new molecules on autism.

WTH05-06

Maternal and offspring MTHFR genotype contribute to autistic-like behavior in mice

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Mthylenetetrahydrofolate-reductase (MTHFR) has high prevalence of polymorphism (MTHFR677C > T) in autistic patients. We previously reported that in mouse model, maternal MTHFR-deficiency delayed morphogenesis and reflex development.

We hypothesized that maternal Mthfr$^{+/-}$ genotype increase the risk for autistic-like behavior in the adult mouse. That was tested by analyzing behaviors associated with core symptoms of autism. Susceptibility to PTZ-seizure was also tested due to high co-morbidity with epilepsy.

Three groups of 90-days old Mthfr$^{+/+}$ (Wt) and Mthfr$^{+/-}$ (Het) mice, representing maternal and offspring genotype, were tested; Wt-Wt, Het-Wt, Het-Het.

In the *open field* test, the frequency mice entered the center/center+margin was used to estimate their anxiety. Het-Het male exhibited lower anxiety compare to Wt-Wt and Het-Wt 0.33 ± 0.06; 0.23 ± 0.08; 0.18 ± 0.04, respectively ($p < 0.01$). Conversely, Het-Het female showed higher anxiety (0.14 ± 0.06; 0.22 ± 0.08; 0.26 ± 0.06; respectively, $p < 0.05$).

Mthfr$^{+/-}$ genotype interfered with recognition memory as tested in the object recognition test; offspring to Het dams spent shorter time exploring the new object versus Wt-Wt group (female-Het-Het-$25 \pm 6\%$ vs. $56 \pm 1\%$, $p < 0.03$; and male-Het-Wt $6 \pm 3\%$ vs. $29 \pm 9\%$ $p < 0.02$). In female, maternal-genotype had an additive

effect to the offspring-genotype, whereas in male it canceled the impact of offspring-genotype.

Sociability. Het-Wt male spent longer time exploring the empty versus mouse chamber whereas Wt-Wt and Het-Wt preferred the mouse chamber ($p < 0.01$). Preference to familiar versus novel mouse in Het-Het versus Wt-Wt female was indicated by longer delay to enter the unfamiliar mouse chamber (71 ± 21sec vs. 25.5 ± 8sec, $p < 0.04$). In the *resident-intruder test*, intruder Het-Wt male performed more aggressive behaviors, compared to Wt-Wt and Het-Het ($p < 0.05$).

Higher susceptibility to *PTZ-induced seizure* was obtained in Het-Wt male ($p < 0.01$ vs. Wt-Wt and Het-Het), as indicated by higher convulsions score and higher number of events ranging between head- and forelimbs-myoclonus to generalized tonic-clonic seizure.

Maternal- and offspring-Mthfr$^{+/-}$ genotype contribute to autistic-like behavior, however, results suggests the presence of some compensatory mechanism in Mthfr$^{+/-}$ offspring due to in-utero exposure to the deficiency.

WTH05-07

Ondansetron reverses depressive phenotype in diabetic mice by normalizing hippocampal neuronal atrophy and reduced bdnf levels

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It is well established that persistent diabetes may lead to neuronal atrophy, characterized by loss of synaptic connections in key limbic brain regions, implicated in depression. This is thought to occur, in part, via decreased expression and function of growth factors, such as brain-derived neurotrophic factor (BDNF), in hippocampus. We previously found that ondansetron, a selective 5-HT3 receptor antagonist ameliorated depressive phenotype evoked in streptozotocin (STZ)-induced diabetic mice. However, the plausible mechanism of its action remains unknown. Therefore, this study aimed to determine whether ondansetron was able to reverse diabetes-induced neuronal atrophy and low BDNF levels in hippocampus, along with depression-like behavior in mice. Ondansetron (0.5–1 mg/kg/day, intraperitoneally) was given to 8-week (STZ-induced) diabetic mice for 28 days followed by tail suspension test (for depression-like behavior) and open field test (for anxiety-like behavior). 24 hrs after behavioral assays, brains were collected and hippocampi were isolated, which were then subjected to Golgi-Cox stain procedure for dendritic morphological changes quantified by Sholl method and enzyme-linked immunosorbant assay for determination of BDNF levels. The results showed that STZ-induced diabetic mice exhibited a significant reduction in dendritic length and number of intersections in pyramidal neurons of CA1 region and BDNF levels of hippocampus along with pronounced depression and anxiety-like behavior. Chronic ondansetron treatment significantly reversed these behavioral, neurochemical and morphological perturbations in diabetic mice. Our results extend the previous findings demonstrating neuronal atrophy and reduced neurotrophic factors signaling associated with depression in diabetes and evidence that these processes correlate to antidepressant-like effects of ondansetron.

WTH05-08

The oligodendroglial abnormalities in the postmortem brain from human patients and monkey depression model

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Recent postmortem brain studies of patients with psychiatric disorders have revealed several abnormalities in neurons and glia. We have developed a FACS method to count numbers of neuronal (NeuN+), oligodendroglial (Olig2 +), and astroglial/microglial (NeuN−/Olig2−) cells, which enables us to find that the number of oligodendroglia is decreased in the frontopolar cortex of postmortem brain from patients with major depressive disorder (Hayashi et al., Mol Psychiatry, 2011). The oligodendroglial progenitor cells exist in the adult mammalian brain, which are proliferating at least in rodents, and therefore, it would be reasonable to consider that the oligodendroglial abnormalities play critical roles in the pathogenesis of psychiatric diseases. In this study, in order to clarity the oligodendroglial abnormalities in major depressive disorder, we first analyzed the oligodendrocyte progenitor cells in the adult primate brain and found that Ki67 + /Olig2 + proliferating oligodendrocyte lineage cells in both gray and white matter in the cortex of macaque monkey (*macaca fascicularis*) and human. Second, we have tried to establish a non-human primate model of major depressive disorder by chronic administration of interferon-alpha, which often causes depression in human patients with hepatitis and cancer. Some behavioral changes were observed in the depression model monkeys such as the time of food intake and body shaking and preference of position in a cage after chronic administration. The oligodendroglial DNA was extracted from the monkey brain by FACS sorting and was subjected to the methylation analysis of Sox10 promoter region, which is associated with oligodendroglial differentiation. We observed a trend of hypomethylation in the Sox10 promoter of oligodendroglial DNA in the depression model monkeys as compared with that of controls.

WTH05-09

5-HT2A receptor deficiency alters the metabolic and transcriptional, but not the behavioral, consequences of CUS

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Chronic stress enhances risk for psychiatric disorders, and in animal models is known to evoke depression-like behavior accompanied by perturbed neurohormonal, metabolic, neuroarchitectural and transcriptional changes. Serotonergic neurotransmission, including serotonin$_{2A}$ (5-HT$_{2A}$) receptors, have been implicated in mediating specific aspects of stress-induced responses. Here we investigated the influence of chronic unpredictable stress (CUS) on

depression-like behavior, serum metabolic measures, and gene expression in stress-associated neurocircuitry of the prefrontal cortex (PFC) and hippocampus in 5-HT_{2A} receptor knockout (5-$HT_{2A}^{-/-}$) and wild-type mice of both genders. While 5-$HT_{2A}^{-/-}$ male and female mice exhibited a baseline anxiolytic state, this did not alter the onset or severity of behavioral despair during and at the cessation of CUS, indicating that these mice can develop stress-evoked depressive behavior. Analysis of metabolic parameters in serum revealed a CUS-evoked dyslipidemia, which was abrogated in 5-$HT_{2A}^{-/-}$ female mice with a hyperlipidemic baseline phenotype. 5-$HT_{2A}^{-/-}$ male mice in contrast did not exhibit such a baseline shift in their serum lipid profile. CUS evoked gene expression changes in specific stress-responsive genes (*Crh*, *Crhr1*, *Nr3c1, and Nr3c2*), trophic factors (*Bdnf*, *Igf1*) and immediate early genes (IEGs) (*Arc*, *Fos*, *FosB*, *Egr1–4*) in the PFC and hippocampus, with the pattern altered in 5-$HT_{2A}^{-/-}$ mice both under baseline and CUS conditions. Our results support a role for the 5-HT_{2A} receptor in specific metabolic and transcriptional, but not the behavioral, consequences of CUS, and highlight that the contribution of the 5-HT_{2A} receptor to stress-evoked changes is sexually dimorphic.

WTH05-10

Reduced interneuron density in the hippocampus and anti-despair-like behaviors in RALBP1-mutant mice

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Inhibitory interneurons in the hippocampus play an important role in the control of the network stability and the hippocampal output. Mice deficient of RalBP1, a downstream effector of the small GTPase RalA and RalB, display reduced synaptic inhibition in the hippocampus. However, the cellular mechanisms and behavioral role of reduced synaptic inhibition in the hippocampus of RalBP1-mutant mice are unknown. Here we show that RalBP1 deficiency induces reduction of interneurons in the hippocampus and anti-despair-like behaviors in mice. RalBP1-mutant mice exhibit reduced density of GABAergic interneurons in the hippocampus and decreased immobility during both the tail-suspension and forced swim tests. However, deficiency of RalBP1 does not induce anxiety and anhedonia. $GABA_A$ agonist muscimol reverted anti-despair-like behaviors in RalBP1-mutant mice. In addition, anti-despair-like behaviors were induced by suppressing GABAergic transmission in CA1 neurons of WT mice. These results suggest that inhibitory synaptic transmission in the hippocampus may regulate behavioral despair.

WTH05-11

Gestational vitamin d treatment blocks behavioural phenotypes relevant to schizophrenia induced by maternal immune activation

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Maternal infection and developmental vitamin D (DVD) deficiency are well-validated risk factors for developmental neuropsychiatric disorders such as schizophrenia. Growing evidence from animal models of maternal immune activation (MIA) using poly (I:C), a viral mimic double-stranded RNA, and DVD-deficiency suggests a shared pathophysiological pathway, altered dopamine neurodevelopment, persistently leading to behavioural phenotypes relevant to schizophrenia in offspring. To test this hypothesis, we administrated the active form of vitamin D, $1,25(OH)_2D_3$, subcutaneously to C57BL/6 mouse dams at gestation day (GD) 9 that simultaneously received intravenous injection of poly (I:C). Vitamin D treatment abolished the MIA-induced schizophrenic behavioral abnormalities in offspring, including positive symptoms (amphetamine induced hyperlocomotion and prepulse inhibition), negative symptoms (social interaction deficit) and cognitive symptoms (fear conditioning). To investigate vitamin D's protective mechanism, we considered its well-known immune regulatory functions. However, vitamin D had no effects on MIA-induced elevations of pro-inflammatory cytokines (interlukin-6, interlukin-1 beta or tumor necrosis factor alpha) in maternal plasma or fetal brain. Secondly, we assessed vitamin D's actions on the ontogeny of dopamine neurons at GD11, the earliest time point of dopamine neuronal differentiation in mice. We established an automated image analysis method using the CellProfiler software and state-of-the-art spinning disk confocal microscopy. Quantitatively immunochemistry data revealed that MIA altered the ratio of mature to immature dopamine neurons (DAs) in fetal midbrain (two-way ANOVA, $F(1,22)=14.923$, $p < 0.01$), which was restored by vitamin D (two-way ANOVA, $F(1,22)=6.536$, $p < 0.05$). In addition, we found that vitamin D increased the expression of a key dopamine differentiation factor, the nuclear receptor related 1 protein (Nurr1), and the dopamine synthesis enzyme, tyrosine hydroxylase in individual post-mitotic DAs in both control and poly (I:C)-treated fetal midbrain (two-way ANOVA, $F(1,22)=15.478$, $p < 0.01$). Taken together, these findings suggest vitamin D promotes the development of midbrain DAs and that such actions may be neuroprotective for DAs when subjected to a maternal stressor. This may account for vitamin D's ability to restore normal behaviors regulated by dopamine, and raises the possibility for future prophylactic strategies for schizophrenia using dietary vitamin D.

WTH05-12

Neuroadaptations in the dorsal striatum and escalation of methamphetamine self-administration across adolescence

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Methamphetamine (meth) is a powerful psychostimulant and the second most common abused illicit drug worldwide. In Australia, age of Meth use has significantly dropped, where two percent of Australians aged 12–14 have experimented with it recently. As adolescence is a period known for its vulnerability to develop addiction, we used the intravenous self-administration paradigm to compare meth abuse-related behaviours in adolescent and adult rats. We first observed a consistent escalation of meth intake in adolescents with dose increase following acquisition (ps < 0.01). Therefore, we hypothesised that meth during adolescence causes more drastic neuroadaptations compared to meth during adulthood.

To test this hypothesis, we performed genome wide transcriptome analysis following acquisition of meth self-administration in the dorsal striatum, which is involved in the transition from goal directed to habitual behaviour. A list of 30 differentially expressed genes (ps < 0.01, fold change > 2), in the adolescent meth compared to saline, was generated. No gene expressions were significantly changed in adult rats. Of particular interest were the downregulation of SLC18A1 (that codes vesicular mono-amine transporter 1 (VMAT1)), and upregulation of GFRA1 (that codes GDNF family receptor alpha 1). Polymorphisms in SLC18A1 are linked to schizophrenia, bipolar disorder and anxiety in humans. GFRA1 is expressed in dopamine neurons and involved in injury response in the substantia nigra. Western blotting replicated the downregulation of VMAT1 at the protein level in the dorsal striatum in meth administering adolescent rats ($p < 0.05$). GDNF protein levels are yet to be determined. Taken together, my research has developed a novel behavioural and genetic model of adolescent vulnerability to meth addiction and has found potential new targets for treatment.

WTH05-13

Neuroplastin ablation causes retrograde amnesia and circuit-dependent deficits correlated to loss of neuroplastin-PMCA complexes

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We investigate the role of Neuroplastin in memory acquisition, consolidation, storage and retrieval, which are critical processes affected in psychopathological disorders, but mechanistically not sufficiently understood. Neuroplastin cell recognition molecules are implicated in activity-dependent synaptic plasticity and intellectual abilities and essential for associative learning in conditioning paradigms e. g. two-way active avoidance and fear conditioning. In addition, neuroplastin-deficient mice reveal profound physiological and behavioral deficits, some related to depression and schizophrenia, illustrating neuroplastins' essential functions. By inducible ablation of *neuroplastin* gene expression specifically in neurons of adult mice ($Nptn^{lox/loxPrp1CreERT}$ mice), we elicit retrograde amnesia of learned associative memories and show that neuroplastins are indispensable for access and retrieval of previously acquired associative memories. In contrast, Np ablation selectively in glutamatergic neurons ($Nptn^{lox/loxEmx1Cre}$ mice) causes particular behavioral deficits indicating hippocampal, striatal, and sensorimotor dysfunctions, but intact associative learning. These results reveal that neuroplastin expression in distinct neuronal sub-types and circuits commands particular behaviors. Furthermore, neuroplastin expression by GABAergic interneurons appears to be essential for associative learning in conditioning paradigms. Potentially, neuroplastin participates in disinhibition of GABAergic cortical interneurons that is required for associative fear conditioned learning. Neuroplastin-deficient mice display reduced levels of Plasma Membrane Ca^{2+} ATPases (PMCAs), an essential regulator of the intracellular Ca^{2+} concentration ($[iCa^{2+}]$) and neuronal activity. Altered hippocampal and cortical activities correlate with reduction of distinct PMCA paralogs in $Nptn^{lox/loxEmx1Cre}$ mice and increased $[iCa^{2+}]$ in cultured mutant neurons. Human and rodent Neuroplastin enhance the post-transcriptional expression of and co-localized with PMCA paralogs in the plasma membrane of transfected cells. Our results show that Neuroplastin is essential for PMCA expression in neurons allowing proper $[iCa^{2+}]$ regulation and normal circuit activity. Neuron-type-specific Neuroplastin ablation empowers the investigation of circuit-coded learning and memory and identification of causal mechanisms leading to cognitive deterioration.

WTH05-14

Repeated ascorbic acid treatment produces antidepressant-like effect and modulates cell survival signaling pathways in swiss mice

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This study aimed to investigate the ability of a 21-day ascorbic acid administration to produce an antidepressant-like effect in the mouse tail suspension test (TST). Additionally, we examined the effect of this vitamin on hippocampal and cerebrocortical brain-derived neurotrophic factor (BDNF) immunocontent, phosphorylation of protein kinase B (AKT), extracellular signal-regulated kinase (ERK), $p38^{MAPK}$ and c-Jun N-terminal kinase (JNK) by Western Blotting. Female Swiss mice received a daily oral (by gavage) administration of ascorbic acid (0.1 and 1 mg/kg) or fluoxetine (10 mg/kg, positive control) for 21 days. The TST was performed 24 h after the last drug administration. Five minutes after the TST, the same group of animals was evaluated in the open field test as a control of general locomotor activity. Immediately after behavioral observations, hippocampi and cerebral cortices were dissected for neurochemical evaluation. Ascorbic acid (0.1 and 1 mg/kg) or fluoxetine (10 mg/kg) administration elicited an antidepressant phenotype in the TST, with no change in locomotor activity in the open fiel test. Ascorbic acid at 1 mg/kg caused an increase in AKT phosphorylation in the cerebral cortex of mice. Ascorbic acid treatment (1 mg/kg), similar to fluoxetine, decreased hippocampal $p38^{MAPK}$, but did not alter ERK or JNK phosphorylation. This study explored, for the first time, the intracellular pathways involved in the antidepressant properties of repeated ascorbic acid administration. We demonstrated that ascorbic acid anti-immobility effect in TST is accompanied by a modulation of AKT and $p38^{MAPK}$, but not BDNF, ERK and JNK, suggesting that these targets play a significant role for its behavioral response in the TST.

WTH05-15

Serotoninergic depletion generates aggressive behaviour in male Sprague–Dawley rats

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The serotoninergic system modulates appetitive, motivational and aggressive behaviour. The 5-HT synthesis reduction by drug increase aggressive behaviour in species and strains prone to it.

In this study we attempted 1- to generate aggressive behaviour with para-chlorophenylalanine (pCPA) by inhibiting tryptophan hydroxylase, and 2- to examine dose/day response related to 5-HT depletion.

Male Sprague–Dawley 60 days old rats were used. A resident/intruder paradigm was applied. Animals were divided into 5 groups: Naïve, pCPA treated rats (300 mg/kg, i.p) evaluated on the 3rd and 6th day after the administration, and the respective saline control.

Offensive behaviour (OB) was measured as attempted mounts, lateral threats and footsteps. Persecution latency time (PLT) was examined as a different parameter of OB. Bites, clinch and clinch attacks were considered as aggressive behaviour (AB). We also measured non-social interaction (freezing, lying, sitting and grooming), social interaction (sniffing and heterogrooming) and locomotor activity. The test was recorded and the videos were analysed with Kinovea 0.8.15 software. 5-HT levels were measured in plasma, olfactory bulb and raphe nucleus with HPLC fluorescence. All data were analysed by ANOVA I and Tukey *post Hoc* test.

We observed a significant decrease in PLT ($p \leq 0.001$) and a significant increase ($p \leq 0.001$) in OB between the treated, control and naïve groups. There was a significant increase ($p \leq 0.05$) of AB between the treated group tested on the 3rd and 6thday. There were no significant differences in social interaction, non-social interaction and locomotor activity. The treated groups showed a significant decrease of 5-HT levels in plasma, olfactory bulb and raphe nucleus versus control and naïve groups depending on the day of evaluation. On the 3rd day the difference was $p \leq 0.01$ and on the 6th day $p \leq 0.05$.

The depletion of 5-HT affects OB, PLT and AB. This is related to dose/day response, suggesting that a certain 5-HT level is necessary to generate aggressive behaviour.

WTH05-16

Administration of allopregnanolone and S-norfluoxetine upregulate reelin expression and improve depressive/anxiety-like behaviors

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Neuroactive steroids, including the GABA-A receptor active, allopregnanolone (ALLO) and its isomer, pregnanolone are downregulated in major depression and post-traumatic stress disorder (PTSD). SSRIs normalize their levels in treatment responders. Likewise, animal models of depression and anxiety, such as the social isolation and the forced swimming test (FST), suggest that ALLO is involved in emotional and cognitive behavioral dysfunction and its levels increase improves these deficits. Social isolation induces several other neurochemical alterations, including a corticolimbic downregulation of reelin expression. The amygdaloid nuclei are part of a crucial brain circuitry involved in emotional regulation and experimental intervention that increase ALLO in this region show antidepressant and anxiolytic effects. The aim of the present study was to compare the antidepressive and anxiolytic effects of ALLO administration with those of the neurosteroidogenic drug, S-norfluoxetine (2 weeks, twice daily) after social isolation in mice, and to analyze the corticolimbic expression of reelin mRNA after those treatments. We further investigated the behavioral effects of the bilateral intra-amygdala infusion of recombinant reelin. Social isolation in mice induced depressive-like effects (FST), aggression, and anxiety-like behavior that were improved by S-norfluoxetine and ALLO. ALLO or S-norfluoxetine also increased the expression of reelin mRNA in the hippocampus, amygdala and frontal cortex of socially isolated mice. Infusion of reelin in the amygdala induced a long-term (2 weeks) improvement of anxiety and aggression. These results suggest that the long-term improvement of emotional behavior of ALLO and S-norfluoxetine may be mediated by upregulation of reelin expression, which suggest new neural target for treatment of depression and PTSD.

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WTH05-17

Proteomic analysis of rat saliva proteins for stress biomarkers after mental and physical stress loading

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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 for qality of life. Quite recently, we examined rat saliva marker proteins for mental stress by proteome using a rat mental stress model. The increased proteins by mental stress were subjected to liquid chromatography-mass spectrometry/mass spectrometry. We detected the known enzymes and secretary proteins with MW of 20–70 KDa in rat saliva proteins. In the present study, we analyzed the biomarkers for physical stress by proteome after treadmill running loading to rats. After the separation by SDS-PAGE, the increased proteins by physical stress were used for LC-MS/MS and we further analyzed the saliva proteins using a comprehensive proteomic analysis (isobaric Tags for Relative and Absolute Quantitation, iTRAQ). Finally, we might find biomarkers for mental and/or physical stress. This study (No.25350824) is supported by Grant-in-Aid for Scientific Research (KAKENHI); Grant-in-Aid for Scientific Research(C) from 2013 to 2015.

WTH05-18

Transgenerational impact of paternal exercise: rhoa gtpase family proteins and regulatory micrornas in offspring hippocampus

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There is growing evidence that perinatal paternal environments and lifestyle factors influence offspring metabolic and behavioural phenotypes. Our lab previously reported that perinatal paternal exercise is associated with impeded reinstatement of fear memory in juvenile male offspring (Short et al., Transl Psychiatry 2017). Paternal exercise has also been separately reported to enhance hippocampal-dependent learning and memory abilities to male offspring. Our lab has reported that specific microRNAs (miR-19b

and miR-133a) are differentially expressed in the sperm of C57Bl/6 mice after 4 weeks of voluntary wheel-running (Short et al., Transl Psychiatry 2017). Functional annotation analysis of validated gene targets of the microRNAs revealed significant over-representation of KEGG pathways related to axon guidance (FDR $p = 0.035$), chemokine signalling (FDR $p = 0.05$), focal adhesion (FDR $p = 0.07$) and regulation of the actin cytoskeleton (FDR $p = 0.1$). We identified two members of the small GTPase family proteins, RhoA and cdc42, that are common to those pathways. We assessed the expression of these GTPases and cytoskeletal proteins in the hippocampus of PND15 male F1 offspring using Western blot. However, we found no significant changes to RhoA, cdc42 and Rac1 protein levels in the hippocampus. We also found no significant differences in the cytoskeletal protein b-actin and the post-synaptic scaffold protein PSD-95. Thus, we conclude that the male offspring phenotype is not due to changes in GTPase family expression, and that microRNA changes in sperm are not necessarily predictive of changes to downstream gene targets in the offspring brain.

WTH05-19

Evidences of hypoconnectivity in the valproic acid rat model of autism spectrum disorder

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Autism spectrum disorders (ASD) are classified as synaptopathies and characterized by impairment in social interaction, verbal and nonverbal communication and repetitive and stereotyped behaviors. Hypoconnectivity has been suggested in ASD patients, particularly in the corpus callosum (CC). In the valproic acid (VPA) animal model of ASD, we have previously postulated local hipocamppal hypoconnectivity based on the decrease in synaptic protein synaptophysin (SYN) seen in these animals. The aim of this work was to characterize the CC structure of VPA animals and evaluate neuronal differentiation and synaptic formation of hippocampal neurons from VPA animals. Valproic acid (500 mg/kg) or saline were prenatally administered on E 10.5 (control and VPA animals, respectively). At DIV 3–5, primary hippocampal neurons from VPA animals exhibited increased complexity in dendritic and filopodia development along with increased SYN immunostaining. As differentiation proceeded (DIV 14), SYN puncta area and number as well as PSA-NCAM immunoreactivity in the VPA group were lower than in controls. Labeling of presynaptic boutons with FM4-64 dye revealed a diminution of functional synapses in the VPA group at this stage. The anterior region of CC from VPA animals showed a disorganized cellular arrangement in the absence of changes in GFAP (astrocytes) or Iba-1 (microglia) immunostainings. Immunoreativities for CC1 (mature oligodendrocytes) and myelin basic protein were reduced. Myelin of axonal tracts from VPA animals exhibited an unorganized disposition. Our results suggest that neuronal changes and myelin defects in the hippocampus and CC of VPA animals, respectively, could underlie altered connectivity postulated for these brain areas in ASD.

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WTH05-20

GLUD1 deficient mouse as a model animal of depression-like behavior

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The delta family consisting of glutamate receptor GluD1 and GluD2 has been classified as an ionotropic receptor subunit, but recent studies have revealed that GluD2 contributes to synapse formation occurring between parallel fibers and Purkinje cells in cerebellum. GluD1 is widespread in adult mouse brains, with abundant expression in the cerebral cortex, striatum, limbic regions, and cerebellar cortex. Like GluD2, GluD1 binds to neurexins via the Cbln family and their interaction induces synaptogenesis *in vitro*. Although the functional significance of GluD1 is inferred from human genetic studies reporting that the*GRID1* is a strong candidate gene for schizophrenia, bipolar disorder, major depressive disorder, and autism spectrum disorder, the relationship between molecular function of GluD1 and the onset mechanism of these diseases has been still unknown. To approach the issue, we generated GluD1 knockout mice from C57BL/6N strain RENKA ES cells with pure genetic background for behavioral analysis. GluD1 knockout mice showed increased locomotor activity in the open field test and decreased social interaction in the three-chamber test, but no significant change in anxiety-like behaviors in the light and dark test and elevated plus maze test. Under the baseline conditions, the immobility of GluD1-KO mice in the forced swim test was significantly longer than that of wild-type mice. To examine the depression-like behavior of GluD1 deficient mice, several antidepressants were administered and a forced swimming test was conducted. Interestingly, by intraperitoneal injection of saline alone, the immobility of GluD1 deficient mice lasted longer than naive GluD1 deficient mice. The decreased baseline immobility in GluD1-KO mice was remedied by pretreatment with imipramine (serotonin and norepinephrine reuptake inhibitor) and fluoxetine (selective serotonin reuptake inhibitor), whereas no difference was observed in the immobility, when treated with desipramine (selective norepinephrine reuptake inhibitor). There results indicate that GluD1-deficient mice are vulnerable to stress, and there is a possibility that the serotonin signaling system may have been involved in the depressive behavior.

WTH05-21

Synaptic proteome alterations in chronic toxoplasma GONDII-infected mice suggest interference with glutamatergic neurotransmission

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Background: Chronic infection with the intracellular parasite *Toxoplasma gondii* affects approximately 30–50% of the human population and has been implicated in the risk for psychiatric disorders like schizophrenia or major depression. The mechanisms, by which *Toxoplasma gondii* can alter neural function, behavior and disease risk, are yet incompletely understood. Here we employed a proteomic approach to investigate potential influences of latent toxoplasmosis on synaptic protein composition.
Methods: Female C57BL/6 mice received either *Toxoplasma gondii* (ME49 type II strain) or sham infection at 8 weeks of age, and brains were harvested after another 8 weeks. Synaptosomal fractions of the hippocampus and neocortex were isolated via ultracentrifugation in a sucrose gradient and submitted to mass spectrometry (MS)-based protein identification. Furthermore, in a candidate-based approach, expression levels of key synaptic proteins were compared using immunoblotting and immunofluorescence.
Results: The synaptosomal protein composition as identified with MS showed infection-related alterations of the synaptic proteome in *Toxoplasma*-infected mice, with the majority of proteins being down-regulated. Candidate-based analysis further revealed a down-regulation of the excitatory amino acid transporter (EAAT2), the vesicular glutamate transporter (VGLUT1), and postsynaptic scaffolding proteins from the Shank family (ProSAP1/Shank2, ProSAP2/Shank3) in both hippocampus and somatosensory neocortex. Immunofluorescence further revealed that the astrocytic marker protein GFAP was up-regulated in both structures.
Conclusion: Our results provide evidence for profound alterations of glutamatergic synapse composition in Toxoplasma-infected mice, with a down-regulation of key proteins involved in glutamatergic neurotransmission. Future research should assess whether these alterations are a direct effect of the parasite or rather a consequence of resulting chronic neuroinflammation.

WTH05-22

Neurotoxic effects of prenatal hyperhomocysteinemia in rats

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Adverse impacts on the maternal organism during pregnancy can lead to serious consequences on embryogenesis and postnatal development of the offspring, and their nervous system is the most vulnerable to the harmful factors. Increased serum levels of homocysteine (HC) are a risk factor for neurodegenerative diseases. It has been shown that accumulation of the products of methionine metabolism including HC in the organism is accompanied by oxidative stress and impaired catecholamine metabolism. Due to the ability to pass through the placental barrier HC may have an adverse effect on developing embryos. The content of nerve growth factors in the serum and brain structures can serve as a marker of neurodevelopmental disorders. The present work was designed to analyze the changes in the content of a neurotrophic factor NRG1 and the levels of biogenic amines in the brain of rats during embryonic and postnatal periods as well as formation of different types of memory in adult female rats subjected to prenatal hyperhomocysteinemia (PHHC). Our results demonstrate that PHHC has resulted in increased HC levels in blood serum of newborn rats and in a significant increase of NRG1 in the brain of fetuses at E20. However, despite the fact that HC content in the blood and NRG1 level in the hippocampus of female rats subjected to PHHC returned to control values by 2–2.5 months after birth, the negative effects of PHHC could still be observed when they were tested using novel object recognition and elevated 8-arm maze tests which revealed disruption of different types of memory. There was also a decrease of noradrenaline and serotonin content in the hippocampus of these rats which can underlie dysregulation of functions associated with biogenic amine transmission in the brain. Supported by RFBR 14-04-00776 and 16-04-00694, Russian State Budget for 2013-2017 (01201351571).

WTH05-23

Chronic stress induces specific responses in neurotrophin systems in brain of rats with different behavior in forced swim test

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Chronic mild unpredictable stress (CUMS) induces depressive-like behavior in laboratory animals. It has been hypothesized that depressive-like features are associated with significant modifications in the system of neurotrophic factors in the brain. In the present study we examined how the system of neurotrophic factors may impact the development CUMS-induced depressive-like behavior in rats, which are initially differed in immobility duration in the forced swim test (FST). Prior to stress exposure, male Wistar rats were

tested in the FST and divided into low immobile (LI) and high immobile (HI). Then, half of animals of each group was exposed to CUMS for eight weeks. After the end of exposure, the duration of immobility significantly decreased in stressed HI rats compared to the initial level and slightly increased in stressed LI rats. Both LI and HI exhibited increased anxiety whereas anhedonia (lower sucrose preference) was observed in the LI group only. Control LI and HI rats significantly differed in the level of BDNF mRNA in the hippocampus, but not in frontal cortex with higher level in the LI rats. LI and HI rats were similar in the expression of NGF in both structures studied. Expression of Ntrk1, Ntrk2, and Ngfr mRNA were similar in the hippocampi of LI and HI rats; however, expression of Ntrk1 mRNA was higher in the frontal cortex of the HI group. Exposure to CUMS significantly decreased the BDNF mRNA content in the hippocampus of the LI group whereas expression of NGF mRNA increased in hippocampus of the HI rats. Interestingly, in the frontal cortex of LI rats, the level of Ntrk1 mRNA increased after CUMS exposure. Thus, the initially LI rats exhibited higher expression of BDNF in the hippocampus; however, the effects of CUMS exposure (anhedonia, lower BDNF mRNA in hippocampus, and higher Ntrk1 mRNA in frontal cortex) were more expressed in this group of rats as well.

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WTH05-24

Mangiferin alleviates sleep deprivation-induced anxiety- and depressive-like behaviors, and memory deficits in mice

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Sleep is an important to strengthen immunity and functioning of nervous system thus plays central role in learning and memory consolidation. Sleep influences various predisposing factors including inflammation, oxido-nitrosative stress, excitotoxicity and Amyloid-β proteins, which are involved in the pathogenesis of anxiety, depression and memory deficits. Sleep deprivation causes release of reactive oxygen and nitrogen species which induce oxidative damage especially in hippocampus activating inflammatory process which further leads to neurobehavioral and biochemical alterations. Mangiferin (MGF), a C-glucosylxanthone, has shown to possess activities including antioxidant, anti-inflammatory, anti-anxiety, antidepressant and neuroprotection. Therefore, present study evaluated the alleviating effect of MGF pre-treatment on SD-induced anxiety- and depressive-like behaviors, and memory deficits in mice. Moreover, SD-induced changes in pro-inflammatory cytokines, ROS&RNS and BDNF level in mice were also studied to confirm their role in the pathophysiology of these disorders. Mice ($n = 10$) were pre-treated with MGF (40 mg/kg, p.o) for 14 days including 5 days of SD protocol. After SD protocol animals were subjected to Elevated Zero maze (EZM), Tail suspension test (TST) and Novel object recognition test (NOR) to assess anxiety- and depressive-like behaviors, learning and memory. Following behavioral studies, mice were sacrificed to isolate hippocampus for the analysis of IL-1β, TNF-α, BDNF, MDA, GSH and nitrite level. Results showed that chronic SD significantly decreased open arms entries and duration in EZM ($p < 0.01$), increased the immobility time in TST ($p < 0.001$) and decreased recognition index in NOR ($p < 0.001$) in mice which was significantly ($p < 0.01$) attenuated by MGF pre-treatment. Hippocampal IL-1β, TNF-α, MDA & nitrite level increased significantly ($p < 0.001$) after SD in mice which were reversed by MGF pre-treatment. Furthermore, MGF pre-treatment improved SD-induced decrease in hippocampal GSH & BDNF level. In summary, results suggested that MGF provided alleviating effect against SD-induced neurobehavioral and neurochemical alterations by impeding neuroinflammation and oxido-nitrosative stress.

WTH05-25

Role of MMP-9 in schizophrenia-like behaviors in rodents

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Schizophrenia is recognized by 3 symptoms, classified as positive, negative and cognitive. Positive symptoms may be modelled in experimental animal models by hyperlocomotion, whereas in negative symptoms lack of interest in rewards and problems in social behavior can be demonstrated. Finally, poor working memory may correlate with cognitive symptoms of schizophrenia. Herein, we employed mouse models of schizophrenia for positive, cognitive and negative symptoms and investigated the role of diminished MMP-9 in pathogenesis of schizophrenia in these animals. Mice with genetically lowered MMP-9 levels in heterozygotes (+/−, MMP-9 HET) were employed, along their wild type (WT, +/+) littermates. Since early-life stress is regarded as a factor promoting schizophrenia, we subjected the mice, in some experiments, to daily (for 21 days) encounter with an aggressive conspecific. The results indicate that alterations in the level of active MMP-9 in the brain result in increased sensitivity to locomotor hyperactivity induced by MK-801. On the other hand chronic stress, potentiates negative symptoms of schizophrenia in MMP-9 Het mice such as depressive behaviors and social behaviors impairment. Cognitive symptoms such as poor working memory can be seen in MMP-9 HET control mice. These results support the notion that MMP-9 alterations in brain may play a role in schizophrenia.

WTH06 Molecular Mechanism of Alzheimer's Disease

WTH06-01

The Alzheimer's disease transcriptome mimics the neuroprotective signature of IGF-1 receptor-deficient neurons

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Diminishing insulin-like growth factor 1 (IGF-1) signaling delays aging and alleviates neurodegeneration in several species including mammals. We previously demonstrated in a mouse model of Alzheimer-like pathology that neuroprotection can be significantly sustained by long-term suppression of IGF-1 receptor (IGF-1R). Here, we aim to decipher molecular pathways underlying the specific role of neuronal IGF-1R in neuroprotection. In the present study, we showed that suppression of IGF-1R in neurons of the aging brain efficiently protects from neuroinflammation, anxiety and memory impairments induced by intracerebroventricular injection of amyloid β oligomers. The suppression of IGF-1R signaling also invariably led to small neuronal soma size, indicative of profound changes in cellular homeodynamics. To gain insight into transcriptional signatures leading to Alzheimer's disease-relevant neuronal defense, we performed genome-wide microarray analysis on laser-dissected hippocampal CA1 after neuronal IGF-1R knockout, in the presence or absence of APP/PS1 transgenes. Functional analysis comparing neurons in early-stage Alzheimer's disease with IGF-1R knockout neurons revealed strongly convergent transcriptomic signatures, notably involving neurite growth, cytoskeleton organization, cellular stress response and neurotransmission. Moreover, in Alzheimer's disease neurons, a high proportion of genes responding to amyloid pathology showed a reversed differential expression when IGF-1R was deleted. Interestingly, the neurofilament medium polypeptide *Nefm* stood out consistently in genome wide comparisons. We found that NEFM accumulated in hippocampus with amyloid pathology, and decreased to control levels under IGF-1R deletion, suggesting that reorganized cytoskeleton likely plays a role in neuroprotection. These findings demonstrated that significant resistance of the brain to amyloid β can be achieved lifelong by suppressing neuronal IGF-1R and identified IGF-dependent molecular pathways that coordinate an intrinsic program for neuroprotection against proteotoxicity. Our data also indicate that neuronal defenses against Alzheimer's disease rely on an endogenous gene expression profile similar to the neuroprotective response activated by genetic disruption of IGF-1R signaling. This study highlights neuronal IGF-1R signaling as a relevant target for developing Alzheimer's disease prevention strategies.

WTH06-02

Investigating the role of vitamin d receptor signaling in a cell-based model of neurodegeneration

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Objectives: Sporadic Alzheimer's disease (AD) has a multifactorial etiology with interplay of genetic, environmental, metabolic and endocrine factors, although the exact pathologic mechanisms still remain obscure. Epidemiological studies from different labs including ours have reported lower serum 25OHvitamin D levels in AD patients. Further, we observed a significant association of Apa1 polymorphism on vitamin D receptor (VDR) in AD patients. Thus, it is intuitive to postulate that VDR confers genetic susceptibility to AD by modulating neuronal survival and APP processing. This study attempts to investigate the involvement of VDR/Vitamin D signaling on amyloid beta metabolism and neuronal survival in a cell based model of sporadic AD and to determine whether VDR knockdown can alter the amyloid homeostasis.

Methods: SHSY5Y human neuroblastoma cell-line was treated with oxidative/transition metal stress(ferric ammonium citrate, 20–200 μM) with or without 10–100 nM 1,25(OH)2 Vitamin D for 24–48 h. Commercially available SiRNA was used to knock down the vitamin D receptor (VDR) expression in SHSY5Y cells. Cell death, oxidative stress markers, mitochondrial function(Membrane potential, O_2 consumption rate, ATP production) VDR expression(realtime RT PCR) and changes in amyloid beta metabolism (APP, BACE expression and amyloid beta accumulation) were examined.

Results: Vitamin D was found to improve mitochondrial functions and protect the cells from iron induced death of SHSY5Y cells as measured by trypan blue exclusion, LDH release assay and propidium iodide-Hoechst staining, inhibition of NFkB and ROS production. Fe induced increase in APP and BACE expression was inhibited by vitamin D treatment. The effects of VDR knock-down on control and iron- treated cells with respect to cell viability, mitochondrial dysfunction and APP expression are currently under study.

Conclusion: Our results indicate that impaired vitamin D receptor signaling may have a role in neural cell death, and vitamin D is a putative neuroprotective agent and therapeutic candidate for AD.

WTH06-03

Profile of sumo conjugation and neuronal death in Alzheimer's disease

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Alzheimer's disease (AD) is the most common cause of chronic dementia among the elderly, with an estimated ~ 40 million patients diagnosed worldwide, a number predicted to almost double every 20 years. Therefore, the mechanisms underlying neuronal death in AD are the focus of intense research. Mitochondrial dysfunction has

been identified as a central element in the pathology of AD. The dynamic attachment of small ubiquitin-like modifier (SUMO) to target proteins is essential in all eukaryotes because it acts as a biochemical switch that alters target protein localization, stability and/or function. SUMOylation is a post-translational modification that regulates both pre- and postsynaptic function and plasticity. Recent studies suggest that protein SUMOylation can interfere with mitochondrial dynamics, which is essential for neuronal function, and may play a pivotal role in AD pathogenesis. Here we investigated global changes in protein SUMOylation, and changes in relevant proteins to mitochondrial dysfunction and neuronal death in an *in vitro* model of AD. Our data indicate that perturbations in global SUMOylation occur alongside mitochondrial impairment. In addition, the increase in SUMO2/3 conjugation (by decreasing the deSUMOylating enzyme SENP3) is an exciting potential strategy to reduce and/or prevent the neuronal death that may be initiated by mitochondrial fragmentation. In conclusion, global SUMOylation may play an important role in the mechanisms underlying AD. The identification of a SUMO substrate and the elucidation of its function and regulation by SUMOylation will lead to important insights into the pathophysiology of AD and therapeutic intervention.

WTH06-04

Phytochemical allylguaiacol exerts neuroprotective effect in hippocampal cells and ameliorates memory impairment in a mouse model

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Allylguaiacol is a phytochemical occurring in various plants such as cloves, cinnamon, basil, and nutmeg. Pharmacological activities of allylguaiacol have been reported on anti-microbe, anti-inflammation, anti-cancer, antioxidant, and neuroprotection. Although allylguaiacol has been known to have neuroprotective effects, there is no report on its regulatory mechanisms at the molecular level. In our present study, we investigated the mechanisms of allylguaiacol as an antioxidant and neuroprotective agent using hydroperoxide (H_2O_2)-treated HT22 hippocampal cells. Allylguaiacol increased the scavenging activities of free radicals 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH), and enhanced expression of antioxidant enzymes manganese superoxide dismutase (MnSOD) and catalase. In addition, allylguaiacol inhibited H_2O_2-induced damage of HT22 with increasing production of brain-derived neurotrophic factor (BDNF) and phosphorylation of phosphoinositide 3-kinase (PI3K) and cAMP response element-binding protein (CREB). In a memory impairment mouse model, allylguaiacol (2.5 or 5 mg/kg) significantly ameliorated scopolamine-mediated cognitive impairment in the passive avoidance task. In addition, allyguaiacol significantly increased the expression of TrkA and B in the hippocampus from scopolamine-treated mice. Taken together, our findings suggest that allyguaiacol exerts the neuroprotective effect through the antioxidant activation and phosphorylation of PI3K and CREB. Furthermore, the ameliorating effect of allyguaiacol may be useful for treatment of memory impairment in Alzheimer's and its related diseases.

WTH06-05

Role of circulating irisin and adiponectin in probable Alzheimer's disease

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Objectives: The hippocampus is considered as one of the principal regions affected by Alzheimer's disease (AD) and that exercise causes neurogenesis in humans reducing the risk of AD. Irisin, a novel exercise-induced myokine, has been suggested to regulate energy homeostasis and insulin sensitivity and may have a plausible role in the pathogenesis of the disease. On the other hand, adiponectin, an adipocytokine known to regulate energy and glucose metabolism, insulin sensitivity etc. has altered levels in AD which is still unclear. None of the studies has been done on irisin and thus our study attempts to explore the role of circulating irisin and adiponectin, their association with cognitive decline and their inter-relationships in AD pathogenesis.
Methods: The preliminary case-control study included 52 persons with AD who were matched for age, sex and body mass index (BMI) and 38 healthy control subjects. Non fasting serum levels of adiponectin, and irisin were measured by commercially available immune assay kits, and routine biochemical parameters were analyzed in both the study groups.
Results: The results show statistically significant lower levels of serum Irisin and higher serum adiponectin levels in AD subjects with respect to controls. The changes in the serum adiponectin were found to be positively correlated while serum irisin was inversely correlated in AD subjects with the cognitive decline. Moreover, an inverse correlation was also observed between serum adiponectin and serum Irisin in AD subjects.
Conclusion: Our results indicate the association of these hormones might act as a significant predictor in the progression of AD. Moreover, the role of serum Irisin is promising and might potentially act as a meaningful drug target in the pathogenesis of AD.

WTH06-06

The role of stress-inducible protein 1 (STI1) in cellular resilience and Alzheimer's disease

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Stress-inducible protein 1 (STI1) is a cochaperone for Hsp70/Hsp90 and secreted STI1 can signal via the Prion protein (PrP^C).

Deletion of STI1 in mice is lethal and STI1 haplo-sufficient neurons are less resistant to insults by oxidative stress and β-amyloid oligomers (AβO). AβO, a major toxin in Alzheimer's disease (AD), bind to PrP^C triggering neuronal toxicity, which can be attenuated by extracellular STI1 treatment, or overexpression of STI1. We generated several mouse lines targeting STI1, including an overexpressing mouse line (TgA) and a line with hypomorphic alleles (dTPR1), lacking exons 1 and 2. We crossed these lines with the 5XFAD (FAD) mouse model of familial AD that overexpresses mutant Amyloid precursor protein. We used biochemical and cell biology assays to characterize these lines and investigated hippocampal amyloidosis and neurodegeneration. Surprisingly, mice overexpressing STI1 presented faster and increased amyloidosis. Immunostaining suggests accumulation of extracellular STI1 and Hsp90 around plaques of TgAFAD mice. dTPR1 mice have 75% less mutated STI1 protein and show significant reduction in levels of several STI1-Hsp90 clients, including glucocorticoid receptor and tau. Cells derived from these mice are also less resistant to stress. Our preliminary results indicate a complex role for STI1 in cellular resilience and reveals a novel role for Hsp90/STI1 chaperone machinery in amyloidosis. The mouse lines we generated will be critical to understand physiological and pathological roles of STI1 in several models of protein misfolding diseases.

WTH06-07

Crosstalk between endocannabinoid and cholinergic systems in a rat model of basal forebrain cholinergic lesion

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The cholinergic hypothesis of Alzheimer's disease (AD) is based on the vulnerability of basal forebrain cholinergic pathways as responsible of learning and memory impairment. We have observed altered endocannabinoid (eCB) signaling and changes in brain lipid profile in AD patients. The objectives were to explore the crosstalk between eCB and cholinergic systems in a rat model of AD, inducing a basal forebrain cholinergic dysfunction by 192IgG-saporin. Acetylcholinesterase staining demonstrated extensive cholinergic denervation. CB_1 receptor autoradiography showed no changes in cortical [^{3}H]CP55,940 binding and up-regulation in basal forebrain (180 ± 13 and 364 ± 63 fmol/mg; $p < 0.05$) of 192IgG-saporin-treated rats. [^{35}S]GTPγS autoradiography revealed enhanced eCB signaling in several cortical regions (i.e. entorhinal: 156 ± 17% and 277 ± 30%, $p < 0.01$; somatosensory: 131 ± 29% and 218 ± 11%, $p < 0.05$) and in basal forebrain (103 ± 18% vs. 142 ± 9%, $p < 0.05$) of lesioned rats. The use of the novel and powerful MALDI-Imaging mass spectrometry (IMS) technique, allowed us to detect specific alterations of phospholipid species such as phosphatidylcholines (PC) and phosphatidylethanolamines (PE) including: [PC(36:1), 14.62 ± 0,30% and 21.64 ± 1.53%, $p < 0.01$; PC(36:4), 10.32 ± 1.01% and 18.61 ± 2.71%, $p < 0.05$; PC(40:6), 0.88 ± 0.17% and 2.25 ± 0.40%, $p < 0.01$; PE(14:1/20:4), 15.21 ± 0.70% and 11.87 ± 1.35%, $p < 0.05$]. Furthermore, the passive avoidance and Barnes Maze tests were used to evaluate learning and memory in this model. The behavioral data were compared with the specific regulation of CB_1 receptors, the degree of cholinergic damage and the relative abundance of phospholipid precursors of choline in the lesioned neurotransmission pathway.

In conclusion, CB_1 receptor-mediated signaling is potentiated in brain areas where cholinergic neurotransmission is deregulated and PE(14:1/20:4) and PC(36:4) may serve as lipid precursors for the synthesis of eCB, as well as for *de novo* synthesis of choline. A link between eCB and cholinergic systems in the CNS under neurodegenerative conditions is proposed.

WTH06-08

P2Y1 receptor is required for Aβ-induced synaptic loss, plasticity deficits and memory impairment

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Early Alzheimer's disease (eAD) is characterized by memory impairment associated to a synaptic loss in cortical and hippocampal regions (Science 298:789). These early morphological traits, already evident in mild cognitive impairment, correlate with the increased levels of Aβ oligomers (Science 297, 353–56). Moreover, there is growing evidence that extracellular levels of ATP are increased in different brain insults/pathologies and several reports have shown the involvement of P2 receptors (P2Rs) in neurodegeneration in different pathological conditions (*Front. Neurosci. 9:148*). We now found that the pharmacological blockade of P2Y1R (MRS2179, 10 μM) prevented the neuronal death of rodent hippocampal neurons exposed to $A\beta_{1–42}$ (0.5 μM, 48 h) and the initial synaptic loss observed at 24 h gauged by a decrease in the immunoreactivity of synaptic markers (e.g. synaptophysin), reflecting a reduction in synaptic density gauged by morphological analysis and by a decrease in the frequency of mEPSCs. Moreover, we found an increased density of P2Y1R in hippocampal terminals of both rats and mice 2 weeks after administration of $A\beta_{1–42}$ (2 nmol, *icv*) at a time where they displayed a mnemonic deficit (Y maze) and synaptotoxicity (reduced levels of synaptic markers) but no neuronal death (Fluoro-Jade C staining). Indeed, P2Y1R-KO mice did not display mnemonic deficit or hippocampal synaptic markers loss 2 weeks after administration of $A\beta_{1–42}$, as displayed by wild-type mice. Also, the micro-infusion of the P2Y1R antagonist MRS2500 (≈ 1 nmol/day *icv*) prevented $A\beta_{1–42}$-induced memory loss (modified Y-maze; Object displacement and Object recognition), LTP impairment (hippocampal CA1) and hippocampal synaptic loss. These data show that P2Y1Rs are responsible or contributing to synaptic dysfunction/loss underlying the cognitive deficits associated to eAD.

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WTH06-09

Oxidative stress and inflammation induced by acute and subacute ultrafine particles exposures: contribution to Alzheimer's disease

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Alzheimer's disease (AD) is a neurodegenerative illness affecting the elderly population, characterized by plaques of $A\beta_{42}$ aggregates, neurofibrillary tangles and neuronal loss (Allsop, 2000). Several epidemiologic and experimental studies suggest that air pollution, mainly ultrafine particles (UFPs), may exert adverse effects on central nervous system (CNS) functions (Block and Calderón-Garcidueñas, 2009). Inflammation and oxidative stress have been suggested as primary mechanisms by which UFPs exert their harmful action on CNS (Genc et al., 2012). Therefore, the aim of this work was to evaluate the activation of oxidative stress and inflammation in mice exposed to UFPs and to elucidate putative physiopathological correlations with neurodegeneration.

Male BALB/c mice were selected as in-vivo model to study oxidative stress and inflammation induction in the brain after acute and subacute intratracheal administration of UFPs from two anthropogenic sources: BC (biomass combustion generated emissions) and DEP (EURO 4 diesel engine emission) (50 μg). In parallel, control mice were always considered (sham). After treatments, RoB (rest of brain), cerebellum and hippocampus were screened for oxidative stress (HO-1, Hsp70, and Cyp1b1), inflammation (iNOS) and AD-related markers (P-APP Thr668, APP and BACE1). Moreover, sham and treated mice were submitted to fluorescence molecular tomography and brain histopathological evaluations.

BC and DEP acute peripheral exposure was able to induce oxidative stress and inflammation in mouse brain, while sub-acute exposure sustained these mechanisms and additionally it induced increase of PAHs metabolism and alteration of APP processing. Interestingly, BC resulted generally less effective than DEP in inducing the above described alterations.

In conclusion, our results suggest that both acute and subacute UFPs peripheral administration are able to induce the response of the CNS.

WTH06-10

Amyloid precursor protein level and acetylcholine esterase activity changes in neuronal cell cultures and rat brain after hypoxia

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The amyloid precursor protein (APP) and acetylcholinesterase (AChE) are multi-faceted proteins with a wide range of important functions. They are also crucially linked with the pathogenesis of Alzheimer's disease (AD). APP is the precursor of the Aβ peptide, which is the causative pathological agent in AD. AChE is linked to AD pathogenesis either by increasing cholinergic deficit or by exacerbating Aβ fibril formation and toxicity. As such, both proteins are the main targets in AD therapeutics. In our studies we have demonstrated an important interrelation in functioning of these proteins. Both can be released from the cell membrane. As we have shown AChE shedding involves a metalloproteinase-mediated mechanism which, like the α-secretase dependent cleavage of APP, is stimulated by cholinergic agonists or inhibited by batimastat, a metalloproteinase inhibitor. Overexpression of the neuronal specific isoform APP695 in neuronal cells substantially decreased levels of AChE mRNA (Hicks et al., JBC, 2013, 288:26039). In human neuroblastoma cells SH-SY5Y and NB7, AChE activity negatively correlates with the levels of their endogenous APP expression. Cultivation of NB7 cells under hypoxic conditions resulted in APP up-regulation and reduction in AChE activity. Similarly, acute hypoxia in adult rats (7% O_2, 3 h) resulted in increased APP protein levels in their cortex and reduction in AChE activity. Hence, APP influences AChE physiology under normal and pathological conditions but precise mechanisms still require elucidation. Supported by ARUK, RFBR 16-04-00694 and Russian state budget (01201351571).

WTH06-11

Aβ-induced inhibition of protein prenylation causes autophagic flux blockade

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Alzheimer's disease (AD) represents one of the most serious health problems for which treatments are urgently needed. To develop therapies we need a better understanding of the events that lead to AD. We discovered that amyloid beta peptide ($oAbeta_{42}$) inhibits the mevalonate pathway that synthesizes cholesterol and isoprenoids farnesylpyrophosphate(FPP) and geranylgeranylpyrophosphate (GGPP). FPP and GGPP are used for protein prenylation. As a consequence, protein prenylation is impaired in neurons exposed to Abeta. Moreover, protein prenylation is reduced in brains of the AD mouse model TgCRND8.

Among the several cellular processes that may be affected by hypoprenylation, autophagy is particularly susceptible because it relies heavily on prenylated proteins, particularly Rabs. Autophagy is dysfunctional in AD and reversing autophagy dysfunction in TgCRND8 mice improves the pathophysiology and rescues memory performance. Yet, the nature and cause(s) of autophagy dysfunction in AD are unclear, which prevents the development of autophagy-targeted strategies with disease-modifying value.

We determined the nature of autophagy dysfunction by examining autophagic flux using mCherry-GFP-LC3 in neurons treated with Abeta and *in vivo* in TgCRND8 mouse. We found that autophagic flux is blocked. To demonstrate that autophagy dysfunction is caused by inhibition of prenylation we tested the ability of GGPP to normalize autophagic flux and the function of Rab7. GGPP prevented Abeta-induced autophagy dysfunction. Rab7 is required for autophagy progression and is altered in AD. In Abeta-treated neurons Rab7 is hypoprenylated and its localization

to autophagosomes is reduced. GGPP corrects Rab7 prenylation and subcellular localization. Our data indicate that autophagic defects in AD are due, at least in part, to inhibition of protein prenylation, which occurs as consequence of decreased isoprenoids synthesis. Restoration of protein prenylation in cultured cells normalizes autophagy and *in vivo* could improve the pathophysiology and behavior in AD.

WTH06-12

Methylation of Aβ derived in exosomes is the diagnosis marker in Alzheimer disease

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It is a major global concern for the high and increasing incidence Alzheimer Disease (AD) worldwide. There is still limited effective treatment for AD patients, thus the early and accurate diagnosis would be the goal for retarding AD. The accumulation of amyloid-β (Aβ) levels is the symbol for AD progression, and DNA methylation of Aβ is a key epigenetic mechanism in AD. Exosomes are nanovesicles that detectable in human plasma, and they including methylated Aβ could be considered as a diagnostic marker for the development of AD. In this study, we purified the plasma exosomes of 56 AD patients by high-speed centrifuge and analyzed the contents of methylated Aβ by PCR and Western blotting. The data showed that the methylated Aβ level in exosomes of AD patients were higher than control group (health adult) ($p < 0.01$), and the methylation of Aβ increased with the development of AD ($p < 0.01$). The similar result was showed in exosomes of AD mice model that the methylated Aβ level in exosomes of AD mice were higher than control mice ($p < 0.01$). Furthermore, the treatment of methylation inhibitor 5-Aza-Cd decreased the methylation level in exosomes of Aβ in AD mice model compared with that in non-treated AD mice ($p < 0.01$). Interestingly, the survival time of the AD mice with the treatment of 5-Aza-Cd was longer (131 ± 35 days) than non-treated AD mice (101 ± 12 days). Thus, exosomes could be the diagnosis maker for AD, and inhibition of methylation of Aβ would be the target for treatment of AD also prevent the occurrence of AD development. This study could be beneficial to clinical surgical treatment for AD diagnosis as early as possible, and it would be important theoretical foundation for clinical treatment and treatment guidance.

WTH06-13

The effects of abeta oligomers on regulators of protein synthesis

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Synapse loss is a key pathophysiological feature of Alzheimer's disease (AD) and the best correlate of cognitive decline in AD patients. Nevertheless, the specific mechanisms that mediate reduction of synaptic proteins levels and, ultimately, synapse elimination in AD, remain to be fully understood. Decreased protein synthesis is a well-known feature of AD that could explain the reduction on synaptic protein levels. However, the interplay between the many regulators of protein translation are not yet well established on the course of the disease. There is also a controversy in the literature on the levels of major regulators of protein translation in AD and their role in the disease. A systematical analysis on translational regulators in different time-points is yet lacking. Here, we have investigated the levels of major regulators of protein synthesis using RT-PCR and Western Blotting in experimental models of AD such as hippocampal neuronal culture treated with Aβ oligomers (AβOs) and hippocampi extracted from mice that received intracerebroventricular (i.c.v.) injection of AβOs. We found that AβOs, increasingly recognized as proximal synaptotoxins in AD, trigger decrease in the levels of p eIF4E, p 4E-BP1, p S6K, p S6, p ERK, p mTOR and an increase on the levels of ATF4 in the hippocampi of mice 7 days after receiving an i.c.v. injection of AβOs, but not after 24 h. We also report an increase on FMRP expression and levels in neuronal cultures treated for 24 h with AβOs and in synaptosomes isolated from the hippocampi of mice 7 days after receiving i.c.v. injection of AβOs. We will further expand these analyses on APP/PS1 mice and in AD patients, but so far we show that many positive regulators of protein synthesis are inhibited, while translational repressors are enhanced in our AD models. It may be very interesting to further correlate these findings with protein translation inhibition observed in AD and further investigate the specific role of each of these proteins on the outcome protein synthesis modulation in AD.

WTH06-14

Potential crosstalk between autophagy and apoptosis in amyloid-β-induced neuronal death

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Amyloid-β (Aβ) induced neuronal death plays important role in the pathogenesis of Alzheimer's disease (AD). Among several death modalities, autophagy and apoptosis play important roles in Aβ-induced neuron death suggesting that there may be regulatory mechanisms that initiate both cell death pathways. In our study, we find that tribbles pseudokinase 3 (Trib3, an ortholog of Drosophila *Tribbles*), a novel ER stress inducible gene, is upregulated in neurons, both *in vivo* and *in vitro* upon Aβ treatment. Increased Trib3 levels inhibit the activity of Akt by interacting with it. As a result, forkhead box O1 (FoxO1), a transcription factor that is negatively regulated by Akt, is activated, translocates to the nucleus, and induces the pro-apoptotic gene *BCL2 like 11* (Bim). This leads to apoptotic death of cells via activation of caspases. We also observe that overexpression of Trib3 leads to increased accumulation of p62 and autophagic vacuoles indicating abnormal autophagy flux. Thus suggesting a role of Trib3 in autophagy. We further find that Beclin1, an autophagy induced protein is cleaved upon Aβ treatment. Studies reveal that autophagy induced Beclin1 is cleaved by active caspases, which may thwart further autophagy and induce apoptosis. Interestingly, we also find a physical interaction between Bim and Beclin1, this interaction reduces upon Aβ treatment. Thus our study reveals that induction of Trib3 leads to increased expression of Bim via the transcription factor FoxO1, which leads to apoptotic death of cells. On the other hand there is release of Beclin1 from physical interaction with Bim upon Aβ treatment. The free Beclin1 leads to enhanced autophagy, but is subsequently cleaved by caspases turning autophagy to apoptosis. We find

inhibition of both autophagy and apoptosis leads to better survival upon Aβ treatment. Most importantly, silencing endogenous Trib3 strongly protects neurons from Aβ insult. Thus, Trib3 may serve as potential therapeutic target for AD.

WTH06-15

Role of zinc in regulating calcium dependent signaling pathways during aluminium induced neurodegeneration

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Alteration of metal homeostasis has been perceived as major risk factors in the progression of neurodegeneration. Aluminium (Al) has been regarded as the third abundant element in the earth's crust (comprises nearly 8%) and has been linked to several neurodegenerative disorders including Alzheimer's disease. Despite of its abundance in nature, the molecular basis of its interaction with the physiological system are rather sparse. On the other hand, zinc (Zn) is an essential trace element that regulates a number of biological activities in our body. The objective of the present study was to explore the role of zinc, if any, in regulating calcium dependent signal transduction pathways during aluminium induced neurodegeneration in rat. 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. All the treatments were carried out for a total duration of 8 weeks. Al treatment caused impairment in the cognitive behaviour of rats, whereas zinc supplementation caused an improvement in the learning and memory of animals. Al exposure increased the levels of cAMP, intracellular calcium and calcium content in both the cerebrum and cerebellum, which however were modulated upon Zn supplementation. Further, Al treatment also decreased the Ca^{2+}ATPase activity in different regions of brain, which was found to be increased on zinc supplementation. Western blot of proteins phospholipase C (PLC), inositol triphosphate (IP3) and protein kinase A (PKA) were also found to be significantly elevated after Al treatment, which however were reversed following Zn treatment The light and electron-microscopic analysis of brain revealed alterations in neuro-histoarchitecture in the form of calcium deposits, chromatin condensation as well as mitochondrial swelling, which were appreciably improved upon zinc supplementation. Therefore, the current study suggests that zinc plays a vital role in regulating calcium dependent signal transduction pathways during aluminium induced neurodegeneration.

WTH06-16

Phosphorylation and isoform expression of tau are regulated independently during mouse brain development

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The microtubule-associated protein tau is a principal component of NFTs found in brains of Alzheimer's disease (AD). Tau in NFTs is hyperphosphorylated, but it is not known why and how those tau are hyperphosphorylated. There are 6 isoforms in tau, which are produced by alternative splicing. Interestingly, both phosphorylation and isoforms of tau are changed during development. Because phosphorylation levels of fetal tau are similar to the major hyperphosphorylated tau species in AD, it is important to understand the mechanism of high phosphorylation of fetal tau. However, it is not addressed how the isoform and phosphorylation changes are regulated during neuronal development and how it contributes mechanistically to development of AD or tauopathies. Here, we investigated regulation of the isoform and phosphorylation changes during early postnatal development in mouse.

At first, we confirmed that 3R tau was replaced by 4R tau gradually from postnatal 9 (P9) to P18 and in the similar time course as the high phosphorylation was changed to the low phosphorylation. It is known that hypothyroid delays brain development. We examined whether changes of isoforms and phosphorylation are separated by hypothyroid, which was induced by a thyroid hormone synthesis inhibitor 2-mercapto-1-methylimidazole (MMI). MMI delayed the day of dephosphorylation but did not affect the conversion of tau isoforms, indicating that the changes of isoforms and phosphorylation are not necessarily linked. Further, we examined phosphorylation of the single isoform of human tau, 3R or 4R, knocked in mouse brain. Human tau, either 3R or 4R, reduced phosphorylation levels during development even though the isoform did not change. These results show for first time that the phosphorylation and isoform changes are differently regulated during development. Our results would contribute to the understanding of the roles of tau during development but also the pathogenesis of tauopathy.

WTH06-17

Degeneration of septal cholinergic neurons caused by immunotoxin 192IGG-saporin alters gene expression in the hippocampus

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It is well known that Alzheimer disease is associated with degeneration of septal cholinergic neurons. To investigate the role of cholinergic innervation in the regulation of gene expression in the hippocampal cells, we induced degeneration of cholinergic septal neurons by intracerebroventricular injection of toxin 192 IgG-saporin. To validate effect of the toxin, we performed behavioral testing, immunohistochemical staining of septal slices and quantitative RT-PCR in the hippocampus.

Administration of the immunotoxin led to a significant increase in the total swam distance and higher velocity in the Morris Water Maze. Furthermore, during probe trial when the platform was removed from the maze, saporin-treated rats spent significantly less time in the target quadrant and swam shorter distance in it compared to the control.

Staining of brain slices from the animals that were treated with toxin showed that intracerebroventricular administration of saporin resulted in a significant decrease in the number of cholinergic neurons in the medial septum and diagonal band of Broca ($p < 0.05$). We analyzed Ig-saporin-induced changes in the mRNA expression of ribosomal genes (*mrpl27, rps23, rpl28*), microglia-

specific *slc2a5*, and *fgf1* in the hippocampus. In Ig-treated rats, *rps23* expression increased whereas expression of *mrpl27 and rpl28* was not altered. The expression of *slc2a5* significantly increased whereas the expression of fgf1 decreased. Our results suggest that Ig-saporin-induced degeneration causes not only alterations in behavior but also strongly alters expression of genes.

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WTH06-18

Caspase-3 activity in early ontogenesis is essential for regulation of neprilysin and transthyretin expression in rat brain

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Pathogenesis of the late-onset Alzheimer's disease is to a great extent linked to impaired amyloid-β peptide (Aβ) clearance from the brain caused by various pathological factors including brain ischemia and hypoxia. Epigenetic changes caused by prenatal stress are known to increase the risk of neurodegeneration in later life. Our studies in rats subjected to a single episode of prenatal hypoxia (PH) in the critical period of brain development (E14, 7% O_2, 3 h) demonstrated alterations in Aβ metabolism, impaired synaptic plasticity and cognitive deficit during postnatal development. Along with increased amyloid precursor protein (APP) expression in rat brain caused by PH we also observed activation of caspase-3 and reduced levels of a major amyloid-degrading enzyme, neprilysin (NEP), correlating with decreased levels of a transcriptional regulator AICD (C-terminal fragment of APP produced alongside Aβ) which is readily cleaved by caspases. Manipulating caspase-3 activity in PH rat brain by intraventricular injection of an inhibitor, Ac-DEVD-CHO, on P20 resulted in restoration of AICD levels, NEP activity, the number of synaptic spines in the cortex and hippocampus, and improved cognitive functions. PH hypoxia also resulted in significant changes in the levels of a transport protein transthyretin (TTR) in the choroid plexus and other brain regions whose levels also increased after injections of the caspase inhibitor. Since both NEP and TTR play an important role in Aβ clearance, alterations of their expression might lead to disruption of Aβ homeostasis leading to impaired brain functions. Understanding amyloid clearance mechanisms in the brain is of particular importance for designing strategies for prevention of neurodegeneration caused by Aβ accumulation. Supported by RFBR (16-04-00694), Alzheimer's Research (UK).

WTH07 Neurodegenerative Disease

WTH07-01

Validation of metabolic neuroimaging biomarkers in huntington disease

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Background: Evidence suggests that energy deficit plays a critical role in the pathophysiology of Huntington disease (HD). There is however lack of robust biomarkers for testing therapeutic strategies targeting brain energy metabolism. This study therefore aims at measuring dynamic parameters of brain energy metabolism in HD.

Methods: Phase one of the study involved recruiting 10 healthy individuals for method validation. The second phase – consisting in scanning 10 presymptomatic individuals, 10 patients at the early stage of HD and 10 controls – has been initiated and will be completed by May. Following our observation of abnormal energy profile in the occipital cortex, we wish to assess the rate of brain creatine kinase using 31P magnetization transfer (31P MT). Diffusion weighted spectroscopy (DWS) is also used to evaluate the diffusion properties of metabolites that reflect distinct metabolic compartments, i.e. neuronal versus glial. Furthermore, resting state functional MRI (rsfMRI) intends to capture the impact of functional connectivity on neurometabolism and vice versa.

Preliminary Results: We have successfully finished the validation phase in 10 healthy volunteers. 31P MT data showed that we fully saturated gamma-ATP without directly saturating the PCr signal. Our DWS analyses displayed similar findings to other studies performed in healthy individuals. Furthermore, the rsfMRI protocol was well tolerated with no induction of peripheral nerve stimulation and the data was of high quality. We are now actively scanning HD carriers and controls. Data analyses and interpretation will be available in August.

WTH07-02

Regulation of COPI vesicle transport via SCYL1 methylation in the pathogenesis of ER-stress induced neurodegenerative diseases

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Cumulative evidences have shown the importance of ER-stress in pathology of neurodegenerative diseases, such as Alzheimer's disease, Amyotrophic lateral sclerosis, etc. Previous studies have indicated that accumulation of unfolded protein response (UPR) by ER-stress is related to the pathology of neurodegenerative diseases. To elucidate the pathogenesis of neurodegenerative diseases from the point of view of ER-stress, we investigated the altered gene expression in SK-N-SH cells under the condition of tunicamycin-induced ER-stress by the gene fishing method. As the result, we found that Protein arginine N-methyltransferase 1 (PRMT1) is up-regulated in SK-N-SH cells under ER-stress. Based on this result, we examined the role of PRMT1 in the ER-stress pathway. PRMT1-knockdown cells showed the abnormal Golgi formation and increased UPR. To elucidate the mechanism of these alterations, we screened the methylated proteins under ER-stress condition by immunoprecipitation-mass spectroscopy, and identified Scy1-like protein 1 (Scyl1). Scyl1, a member of the Scy1-like family of catalytically inactive protein kinases, was recently reported to function in retrograde COPI-mediated intracellular transport. Interestingly, Scyl1 has also been identified as a gene product that is lost in an animal model of motor neuron disease, the muscle-deficient mouse. In the motor neuron of the above model animal, the protein circulation system between ER and Golgi apparatus was abnormal due to dysfunction of COPI transport. In consequence, UPR may be accumulated. Thus, we present the effect of Scyl1 arginine methylation on the COPI vesicle transport. This study provides novel insights into the pathogenesis of neurodegenerative diseases by UPR accumulation.

WTH07-03

Hypothalamic dysfunction and metabolic dysregulation in AD animal models

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Clinical and epidemiological studies have shown that Alzheimer`s Disease (AD) is related to diabetes. AD patients have a high risk of developing Type 2 Diabetes (T2D) and/or impaired glucose metabolism. Amyloid Beta Oligomers (ABOs), toxins that build up in AD patients brains, are known to induce ER stress and impair insulin signaling in the hippocampus of mice. In the current study we aim to investigate whether ABOs can also impact the hypothalamus and trigger peripheral metabolic dysregulation. WT mice and macaques intracerebroventricular (icv) injected with ABOs were evaluated. We further used transgenic mice models of AD (APPPS1 and CRND8) that overexpress mutant human APP and/or PSEN1 and generate high levels of human Amyloid Beta 42 (AB42) in this study. Glucose Tolerance Test (GTT) and Insulin Tolerance Test (ITT) were performed to access glucose homeostasis. Plasma insulin levels were assessed by Elisa. Levels of hypothalamic and plasma AB42 were measured in the transgenic AD mice models. Hypothalamic levels of proteins related to insulin pathway were quantified by Western Blotting and markers of inflammation were analyzed by Immunohistochemistry. Our results show that icv-infused AβOs trigger hypothalamic inflammation and impaired insulin signaling leading to glucose intolerance and insulin resistance. In the transgenic mice model we also identified glucose intolerance but not peripheral insulin resistance. These results provide evidences of metabolic dysregulation in AD models and suggest impaired hypothalamic insulin signaling as a shared molecular mechanism between AD and T2D.

WTH07-04

Ubiquitin proteasome system dysfunction in ALS patient IPSC-derived motor neurons

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Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative condition that results from the progressive death of upper and lower motor neurons. Dysfunction in the ubiquitin-proteasome system, the primary selective degradation system of the cell, is a common feature of ALS aetiology. Mutations in cyclin F, a protein central to the ubiquitin proteasome system, have recently been identified to cause ALS. Very little is known regarding the function of cyclin F in either healthy or ALS-affected motor neurons. Due to the difficulties related to culturing primary neurons, we have used patient-iPSC derived motor neurons to investigate the effects of endogenous mutant cyclin F. We aimed to utilise these motor neurons to investigate ubiquitin proteasome system dysfunction in cells derived from ALS patients with mutations including $CCNF^{S621G}$ and $SOD1^{E101G}$, compared to healthy controls. Analysis of the ubiquitin proteasome system by degron assay, a fluorescent proteasome reporter assay, indicated a significant deficit in protein degradation in ALS patient-derived motor neurons. Western blot analysis confirmed an increase in endogenous Triton X-insoluble proteins including phosphorylated TDP-43 as well as cyclin F. Identification of this disease phenotype in our motor neuron models provides a viable target for the development of future therapeutics.

WTH07-05

Regulation of neurofilament proteins by ALS-linked MIRNAS

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Neurofilaments are the most abundant cytoskeletal component in neurons. Neurofilaments determine axonal caliber and promote axonal growth, but also organize the cytoplasm to form a stable 3-dimensional lattice that supports the organization of organelles. Neurofilament protein assembly requires the correct stoichiometry among the three subunits: NFL (light), NFM (medium) and NFH (heavy). Dysregulation of neurofilament heteropolymers has been established as a cytopathological hallmark of amyotrophic lateral sclerosis (ALS), a neurodegenerative disease of motor neurons. Alterations of neurofilaments in ALS include selective suppression of *NEFL* mRNA in the human spinal cord, neurofilament proteins sequestration in inclusions and axonal swelling, and expression of variants of the *NEFH* gene. MiRNAs are evolutionary conserved non-coding RNAs that post-transcriptionally regulate the expression of most mammalian genes and play a critical role in degeneration. We have studied the expression profile of miRNAs in the spinal cord of ALS patients and the role of ALS-associated miRNAs in the regulation of NFL, NFM and NFH. We observed a massive down-regulation of miRNAs in ALS patients that was subsequently shown

to be specific to motor neurons. We developed a list of conserved miRNAs with miRNA recognition elements within human NEFL, NEFM and NEFH 3′UTRs. MiRNA *in vitro* studies showed that a total of 8 miRNAs dysregulated in spinal cord of ALS patients regulate the expression of NFL, NFM and NFH and are expressed in human spinal motor neurons. This observation is highly relevant because it implies that the alteration of a small group of miRNAs in ALS could potentially change neurofilament levels, impair its organization and induce the formation of pathological inclusions. Our results are relevant for future studies to identify novel therapeutics for ALS.

WTH07-06

Characterization of a presymptomatic stage in a drosophila Parkinson's disease model: unveiling compensatory mechanisms

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Parkinson disease (PD) is a degenerative disorder characterized by several motor symptoms including shaking, rigidity, slow movement and difficult walking, which has been associated to the death of nigro-striatal dopaminergic neurons. More than 90% of PD patients also present olfactory dysfunction. Although the molecular mechanisms responsible for this disease are not clear, hereditary PD is linked to mutations in specific genes, including the PTEN-induced putative kinase 1 (PINK1).

In this work we provide for the first time a thorough temporal description of the behavioral effects induced by a mutation in the PINK1 gene in adult *Drosophila*, a previously described animal model for PD. Our data suggests that the motor deficits associated to PD are fully revealed only by the third week of age. However, olfactory dysfunction is detected as early as the first week of age. We also provide immunofluorescence and neurochemical data that let us propose for the first time the idea that compensatory changes occur in this *Drosophila* model for PD. These compensatory changes are associated to two specific components of the dopaminergic system: Dopa decarboxylase, the enzyme responsible for the last step in dopamine biosynthesis, and the Dopamine transporter, a plasmatic membrane protein involved in maintaining dopamine extracellular levels at physiologically relevant levels.

Thus, our behavioral, immunofluorescence and neurochemical data help define for the first time presymptomatic and symptomatic phases in this PD animal model, and that compensatory changes occur in dopaminergic neurons in the presymptomatic stage.

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WTH07-07

Neuromyelitis optica immunoglobulin G targets AQP4 expressed in retinal Müller cells affecting cell volume homeostasis

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The current study evaluates if the water channel AQP4, highly expressed in Müller cells in the retina, is a pathogenic ocular target of specific serum immunoglobulin G autoantibody (NMO-IgG) produced in patients with Neuromyelitis Optica. Particularly we investigated the consequences of NMO-IgG binding to AQP4 on plasma membrane water permeability and cell volume homeostasis. Studies were performed in a human retinal Müller cell line (MIO-M1), a good model that maintains important functional characteristics of Müller cells. To avoid or to facilitate AQP4 down-regulation, cells were exposed to inactivated control or positive NMO-IgG sera in two different situations (1 hr at 4°C or 12 hr at 37°C). AQP4 expression was detected by immunofluorescence studies using a polyclonal anti-AQP4 antibody and the water permeability coefficient and cell volume regulation capacity were evaluated by fluorescence videomicroscopy. Our results showed that immediate NMO-IgG binding to AQP4 is not enough to affect water channel′s activity. However, long-term exposure to NMO patient sera clearly induced a loss of AQP4 signal from plasma membrane, along with a significant reduction of water permeability and the capacity to regulate cell volume after an osmotic swelling (RVD), a key function of Müller cells. These data demonstrate that NMO-IgG targets Müller cells AQP4, affecting its expression and its function, and subsequently cell homeostasis. Therefore, we propose that water permeability reduction after NMO-IgG binding to AQP4 in Müller cells contributes to retinal cell damage and tissue edema observed in NMO patients.

WTH07-08

The role of the autophagic pathway in muscle cell models of spinal and bulbar muscular atrophy

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Spinal and bulbar muscular atrophy is a motor neuron disease caused by an aberrant CAG expansion in the exon1 of the androgen receptor (AR) gene. This stretch is translated into a polyglutammine tract (polyQ) in the coded AR protein (ARpolyQ) that eventually prevent AR from folding correctly after binding to testosterone. Misfolded ARpolyQ may become toxic to cells and form aggregates. The protein quality control (PQC) system is in charge of protein homeostasis. It is composed by a chaperone network that tries to refold proteins; if this system fails proteins are brought to the two degradative systems: ubiquitin-proteasome system (UPS) and macroautophagy. Recently, some studies unrevealed a key role for muscle cells in SBMA. In this work we investigated ARpolyQ behaviour in muscle cells. We used muscle C2C12 stably

transfected with ARQ24 and ARQ100. We performed a filter retardation assay (FRA) (a technique that allow the detection of aggregates) on PBS extracts of both cell lines cultured in presence of testosterone. We observed that ARQ100 insoluble species retained on the membrane were higher than that of ARQ24. Interestingly, we found that ARpolyQ expression does not modify expression of PQC system-related genes measured by rtqPCR. We then performed FRA on samples previously treated with proteasome or autophagy inhibitors. We noted that only ARQ100 accumulates after autophagy inhibition. We then overexpress HspB8 a small heat shock protein that in complex with Hsp70 and Bag3 led misfolded protein/aggregates to autophagy. HspB8 overexpression counteracted ARQ100 aggregation in presence of testosterone. We then tested trehalose, an mTOR independent autophagic activator; we observed that trehalose increased HspB8 overexpression and reverted the testosterone dependent aggregation of ARpolyQ. Based on these results we hypothesized that muscle cells are a site for ARpolyQ aggregation, and that modulation of autophagy could reduce ARpolyQ aggregation in these cells.

WTH07-09

Loss of glutamine synthetase initiates a sequence of neuropathological events that culminate in epilepsy and neurodegeneration

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The enzyme glutamine synthetase (a.k.a. glutamate ammonia ligase, Glul) is enriched in astrocytes and serves as the primary pathway for synaptic glutamate clearance and brain ammonia detoxification. Loss of astrocytic Glul has been implicated in several CNS disorders, such as epilepsy; however, the mechanism by which Glul deficiency might cause disease is not understood. Thus, we selectively deleted Glul in the hippocampus and neocortex of mice to study the consequences of Glul deficiency. At 2 weeks of age, the brain cytoarchitecture and behavior of Glul deficient mice were largely unremarkable; however, the brain chemistry, microglial cells and blood vessels were altered. At 4 weeks of age, other changes became apparent, such as slowed brain growth, altered functional connectivity, reduced cerebrovascular reactivity, behavioral abnormalities, epileptic seizures and progressive neuron loss that resembled hippocampal sclerosis. Thus, loss of astroglial Glul initiates a series of molecular and cellular events that culminate in neurodegeneration and epilepsy.

WTH07-10

Ageing and oxidative stress contribute to neurodegenerative diseases-related proteins in human red blood cells

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Ageing represents the strongest predictor for developing of Neurodegenerative diseases (NDs), which are characterized by selective dysfunction and loss of neurons, associated with protein aggregates in human brain and peripheral tissues[1,2]. In particular, NDs commonly present misfolding and aggregation of one or more proteins, including primarily β-Amyloid (Aβ), α-synuclein (a-syn) and tau[1].

ND etiology remains to be fully elucidated, however increased oxidative stress seems one of the potential common factor. Oxidative stress, accumulating in ageing and NDs, has been related to impaired mitochondrial activity, lipid peroxidation, protein modification, DNA damage and apoptosis[3].

Herein, red blood cells were used as peripheral cellular model to investigate the correlation between ND-related proteins and the extent of the antioxidant capability (AOC), a key marker of oxidative stress in ageing-related pathologies. In particular, the content of a-syn, Aβ and tau and of their oligomeric/phosphorylated forms were determined by immunoenzymatic assays in a cohort of 110 human subjects.

Both plasma AOC toward hydroxyl radicals and total α-syn content were reduced in older subjects; in contrast, tau and Aβ accumulated in elderly subjects and showed an inverse correlation with hydroxyl AOC.

The positive correlation between antioxidant capability and reduced protein accumulation was confirmed by these data, and suggest that peripheral content of ND-related proteins should be further investigated as potential markers of neurodegeneration. In this respect, preliminary data on patients affected by Parkinson's disease will be shown.

[1]Biochem Pharmacol. 2017; pii:S0006-2952(17)30047-3.
[2]Int. J. Mol. Sci. 2016;7:82.

WTH07-11

Altered glycogen metabolism in the brain of insulin-resistant Goto-Kakizaki rats: a 13C magnetic resonance spectroscopy study in V

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Impaired insulin signalling affects brain structure and function leading to behavioural and cognitive alterations. The role of glycogen in the diabetic brain remains to be elucidated. In the present study we investigated insulin resistance-induced alterations of brain glycogen metabolism in the living brain by means of magnetic resonance spectroscopy (MRS). MRS experiments *in vivo* were performed on a 14.1 T spectrometer using a home-built surface coil. [1-^{13}C]glucose was infused into adult Wistar and insulin-resistant Goto-Kakizaki (GK) rats under isoflurane anaesthesia.

Localised ^{13}C MRS was performed in a volume of 600 μL within the brain with a modified SIRENE pulse sequence. The ^{13}C MRS experiment measured brain glucose and glycogen signals over at least 8 h. Then, rats were sacrificed with a focused microwave fixation device, and the brain was stored for extraction of glycogen and water-soluble metabolites. Fractional enrichment (FE) and content of glucose and glycogen were determined by MRS *in vitro*. Time courses of glycogen ^{13}C labelling measured *in vivo* were modelled together with FE and concentration determined in brain extracts to estimate glycogen turnover. The glucose infusion rate was adjusted to reach similar glucose levels and FE in the plasma of both GK and Wistar rats (~17 mM). Under such conditions, brain glycogen concentration was similar (5.5 ± 0.9 and 5.0 ± 0.4 μmol/g in Wistar and GK rats, respectively), the rate labelling incorporation from [1-^{13}C]glucose into glycogen was 0.24 ± 0.05 and 0.48 ± 0.09 μmol/g/h in GK and Wistar rats ($p < 0.05$), respectively. Taking in account brain glycogen concentration, glycogen turnover time τ was 26.4 ± 4.9 h in GK rats and 14.5 ± 1.7 h in Wistar rats ($p < 0.05$). In sum, we demonstrate that brain glycogen mobilisation is slower in insulin resistance despite normal brain glycogen content, which may have implications for the adequate support of neuronal function.

WTH07-12

Co-localization of RGNEF with TDP-43 into micronuclei-like structures induced by cellular metabolic stress

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Amyotrophic lateral sclerosis (ALS) is an adult-onset progressive disorder characterized by degeneration of motor neurons. Although the cause of the disease remains elusive, a common neuropathological hallmark is the formation of neuronal cytoplasmic inclusions (NCIs) in motor neurons, which include RNA-binding proteins such as TDP-43 and Rho Guanine Nucleotide Exchange Factor (RGNEF). Cellular stress seems to be highly relevant to the pathogenesis of ALS. Oxidative and osmotic stress have been extensively studied in regards to the role of stress granules in the formation of NCIs in ALS. However, the role of cellular metabolic stress in this pathology has yet to be explored. Previously, we determined that the Leucine-rich domain in the amino terminal region of RGNEF is critical for proper regulation of its RNA-destabilizing activity. Considering the fact that the Leucine-rich domains are commonly involved in protein-protein interactions, its presence could be critical in the regulation of protein complexes where RGNEF is involved.

We hypothesized that under cellular metabolic stress there is formation of cytoplasmic inclusions containing ALS-related proteins and the Leucine-rich domain of RGNEF is critical for the recruitment of RGNEF into those inclusions.

Interestingly, under cellular metabolic stress we observed the formation of cytoplasmic inclusions containing TDP-43 which resemble micronuclei structures. The nature of this micronuclei-like structures was confirmed using the nuclear markers SIRT-1, Histone H1 and NPCP (Nuclear Pore Complex Proteins). Flag-RGNEF-L-rich protein forms intracellular inclusions in stressed HEK293T cells which co-localized with endogenous TDP-43 into micronuclei-like structures. Endogenous RGNEF was also observed to co-localize with endogenous TDP-43 in micronuclei-like structures.

This is the first cellular model of recruitment of endogenous TDP43 into cytoplasmic inclusions *in vitro* induced by cellular metabolic stress. Notably, these inclusions were micronuclei-like structures. Also, our results indicate a critical role for the Leucine-rich domain of RGNEF in the formation of RGNEF inclusions under pathological conditions. This study suggests that the metabolic stress could have an important role on the pathogenesis of ALS.

WTH07-13

Modulation of tau isoforms by RNA reprogramming: analysis of phenotypic rescue in a mouse model of tauopathy

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Tauopathies are major neurodegenerative diseases characterized by the presence of intraneuronal aggregates of the Tau protein in insoluble neurofibrillary tangles. Tau is a microtubule-associated protein expressed in neurons, involved in cellular functions such as microtubule stabilization and axonal transport, is encoded by the MAPT gene. The Exon 10 (E10) can be alternatively spliced, giving rise isoforms with three (3R) or four (4R) repeats of microtubule binding domains, both isoforms are expressed in equal amounts in the normal adult human brain. Several tauopathies are associated with mutations in the MAPT gene which modify E10 alternative splicing, leading to an imbalance between the 3R and 4R Tau isoforms. Correction of that imbalance might represent a therapeutical approach for those tauopathies. We evaluate the phenotypes of mice carrying a human Tau transgene with an abnormal ratio of Tau isoforms (hTau mice), proposed as a model. Htau mice have an excess of 3R Tau in several brain areas. We sought to correct that Tau isoforms imbalance and analyze if such restoration produces a phenotypic rescue. We used RNA pre-trans-splicing molecules (PTM) to promote the inclusion of E10 in the endogenous Tau transcript. PTMs were delivered into specific areas of the mouse brain by lentiviral vectors. Cognitive performance was tested in the novel object recognition task. The content of 3R and 4R Tau isoforms was determined by qPCR and Western blot. The presence of hyperphosphorilated Tau was detected by immunohistochemistry, the content of insoluble forms of Tau was measure by western blot and the neuronal firing was recorded. Htau mice rescued by trans-splicing restored some cognitive and neurochemical phenotypes, indicating that RNA reprogramming is a suitable tool to achieve a phenotypic recovery. Our results raise perspectives about using this technique to treat tauopathies.

WTH07-14

Reduction of mutant huntingtin in oligodendroglia rescues myelination and behavioural deficits in a model of Huntington disease

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Clear evidence from human and animal studies indicates that white matter structures are profoundly affected in Huntington disease (HD). Although its etiology is not fully understood, white matter atrophy appears very early in the disease course suggesting that it may be a primary event preceding neuronal loss. We hypothesize that abnormalities in white matter reflect dysfunction that involves the direct effects of mutant huntingtin (mHTT) on oligodendrocytes, the myelinating cells of the central nervous system. Using the BACHD mouse model of HD, which expresses full-length human mHTT and mimics many of the behavioural and neuropathological features of the human condition, we genetically reduced mHTT expression in oligodendroglial cells by crossing BACHD mice to NG2-Cre mice. Using electron microscopy analysis of myelinated fibers of the corpus callosum at 1 and 12 months of age, we show that myelin sheaths are thinner and less compact in BACHD mice. Reduction of mHTT expression in oligodendroglial cells rescues the deficits in thickness and compactness of myelin sheaths, supporting cell intrinsic effects of mHTT on oligodendrocytes. We further show that silencing mHTT in oligodendroglia improves aspects of behavioural dysfunction in the HD mice, including motor and psychiatric-like phenotypes. Our findings suggest that the expression of mHTT in oligodendrocytes contributes to myelin abnormalities and certain behavioural manifestations in HD. Our study provides novel insights into the etiology of white matter pathology in HD.

WTH07-15

Evaluation of selective PKR inhibition in mouse models of memory deficits and neurodegeneration

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The pro-apoptotic Protein Kinase RNA-activated (PKR) is activated by auto-phosphorylation, which in turn phosphorylates translation initiation factor eIF2α, in response to Alzheimer's disease pathogenic mechanisms, e.g. high levels of beta-amyloid species, neuro-inflammation, or presence of the ApoE4 risk-promoting allele. The activity of a potent and selective small molecule PKR inhibitor (SAR489883) was evaluated for target engagement *in vivo* in two animal models: transgenic mouse model expressing human APOE4 (APOE4 knock-in mice), and mice with intracerebral ventricular (ICV) injection of toxic Ab oligomers (AbOs). After oral treatment in ApoE4 KI mice and in C57Bl/6 mice in which AbOs were administered ICV, PKR target engagement was evaluated from brain samples by measuring both PKR occupancy, using KiNativ™ technology, and brain levels of PKR substrate (phospho-eIF2α/eIF2a) using semi-automated Simple-Western). In AbOs-injected mice, effects of SAR489883 on synapse loss were assessed by measuring brain levels of synaptic biomarkers SNAP25, PSD95 and synaptophysin, a hallmark of neuroinflammation, brain IL1β was investigated using ELISA. The effects of SAR489883 were characterized on cognitive functions using the Barnes maze test in ApoE4 KI mice and the Morris water maze test in AβO-injected mice. In ApoE4 KI mice, 1-week oral BID administration of SAR489883 dose-dependently reduced learning and memory deficits. In AβOs-injected mice, a 2-week administration of SAR489883 in the diet also dose-dependently reduced cognitive impairment, rescued deficits of synaptic proteins and reduced IL-1b. In both mouse models, the effects of SAR489883 were associated with a dose-dependent PKR occupancy and reduction of brain levels of peIF2a. An innovative combination of recent technologies (ActivX/KiNativ, Simple-Western), provides evidence of specific and robust PKR engagement by SAR489883 in brain which can be applied in blood cells and may be a promising approach to monitor these biomarkers in human.

WTH07-16

Shrinkage of peripheral nerve fibers in KCC3-T991A mutant mice

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KCC3 is an electroneutral transporter mediating the efflux of K^+, Cl^-, and obligatory water molecules to maintain cell volume. Loss-of-function mutations in KCC3 result in Agenesis of the Corpus Callosum with Peripheral Neuropathy (ACCPN). ACCPN is prevalent in a region of Quebec affecting approximately 1 in 2000 live births. Individuals suffering from ACCPN are homozygous for a mutation that truncates KCC3, rendering it non-functional. Patients exhibit severe sensorimotor neuropathy. Analysis of peripheral nerves in patients and a mouse model of the disease demonstrated swelling of axons. The relationship between axonal volume and the neuropathy remains unknown. The activity of KCC3 is regulated by phosphorylation/dephosphorylation of two key residues, Thr991 and Thr1048, located in the cytosolic C-terminus of the protein. Phosphorylation of these residues results in loss of KCC3 activity, whereas dephosphorylation by phosphatase results in its activation. We recently documented the case of a young boy exhibiting a *de novo* mutation in the KCC3 gene substituting Thr991 into alanine (T991A). The patient exhibits a greater motor neuropathy than sensory and displays none of the ACCPN brain deficits. We created a mouse model recapitulating the mutation of this patient and observed that only the homozygous mice display severe locomotor deficits. To determine the size of the nerve fibers, we isolated the sciatic nerve of wild-type, heterozygote, and homozygote mice, separating the proximal (close to spinal cord) and distal portions (between knee and foot) of the nerves. We fixed these samples for transmission electron microscopy analysis and observed that nerve fibers are significantly shrunken in both heterozygous and homozygous mice compared to wild-type mice ($p < 0.001$), with greater shrinkage in homozygote versus

heterozygote animals ($p < 0.036$). These data are consistent with a gain-of-function mutation rendering KCC3 constitutively active, resulting in shrinkage of the nerve fibers, in contrast to loss-of-function mutations in KCC3 that lead to swelling of the fibers. Our data highlight the critical role of KCC3 in cell volume homeostasis for the integrity of peripheral nerve fibers.

WTH07-17

Evaluation of pridopidine in the transgenic YAC128 mouse model of Huntington disease

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Pridopidine is currently in clinical development for the treatment of Huntington disease (HD) and investigations to increase the understanding of its therapeutic benefit and mode of action are ongoing. Here we aim to investigate the efficacy and mechanism of action of pridopidine using the transgenic YAC128 mouse model of HD. Pridopidine was administered to animals starting at early (1.5 months of age) or late stages of disease (8 months of age). In the early treatment cohort, animals were divided into three groups receiving 0, 10, or 30 mg/kg of pridopidine for a period of 10.5 months. In the late cohort, animals were divided into two groups receiving either 0 mg/kg or an escalating dose of pridopidine (10 mg/kg in week 1, 20 mg/kg in week 2, and 30 mg/kg in weeks 3–8). Pridopidine-treated animals were evaluated using a battery of behavioural tests. Our analysis reveals that chronic treatment with pridopidine improves behavioural measures including motor learning, motor performance and affective phenotypes in the YAC128 HD mice. Specifically, pridopidine improved motor learning in the rotarod test, and motor performance in the accelerating rotarod and climbing tests, reduced immobility in the forced swim test of depression, and decreased anxiety-like behaviour in the open field test and elevated plus maze. Assessment of neuropathology revealed no effect of pridopidine on striatal and corpus callosum volumes, or forebrain weights. Finally, RNA-Seq analysis revealed that pridopidine treatment results in significant reversal of transcriptional deficits in YAC128 HD mice and highlighted potential mechanisms of pridopidine-mediated functional improvements. Overall, our study supports continued clinical development of pridopidine for HD.

WTH07-18

RAGE inhibition in substantia nigra of rats prevents 6-OHDA-induced parkinsonism

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The receptor for advanced glycation end products (RAGE) is a pattern-recognition receptor associated with inflammation in most cell types. RAGE up-regulates the expression of proinflammatory mediators and its own expression via activation of NF-kB. Recent works have proposed a role for RAGE in Parkinson's disease (PD). In this study, we used the multimodal blocker of RAGE FPS-ZM1, which has become available recently, to selectively inhibit RAGE in the substantia nigra (SN) of rats intracranially injected with 6-hydroxydopamine (6-OHDA). FPS-ZM1 (40 mg per rat), injected concomitantly with 6-OHDA (10 mg per rat) into the SN, inhibited the increase in RAGE, activation of ERK1/2, Src and nuclear translocation of NF-kB p65 subunit in the SN. RAGE inhibition blocked glial fibrillary acidic protein and Iba-1 upregulation as well as associated astrocyte and microglia activation. Circulating cytokines in serum and CSF were also decreased by FPS-ZM1 injection. The loss of tyrosine hydroxylase and NeuN-positive neurons was significantly inhibited by RAGE blocking. Finally, FPS-ZM1 attenuated locomotory and exploratory deficits induced by 6-OHDA. Our results demonstrate that RAGE is an essential component in the neuroinflammation and neurodegeneration induced by the parkinsonian agent 6-OHDA in the SN. Selective inhibition of RAGE may offer perspectives for therapeutic approaches.

WTH07-19

Characterisation of EIF2B bodies in vanishing white matter disease

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Leukoencephalopathy with Vanishing white matter (VWM) is a fatal neurological disorder. It arises through autosomal recessive mutations within eukaryotic initiation factor 2B (eIF2B); a guanine nucleotide exchange factor (GEF) for the protein eukaryotic initiation factor 2 (eIF2). eIF2B provides a critical control point in the regulation of protein synthesis. Although eIF2B is a global regulator of protein synthesis, the phenotypic effect of VWM mutations is predominately observed in oligodendrocytes and astrocytes. Structurally, eIF2B is composed of five subunits: α, β, δ, γ and ε. Over 150 mutations, within all subunits of eIF2B, have been identified as causative of VWM. Although VWM predominately affects infants, disease onset, course, and severity, is highly variable amongst patients, and currently no genotype-phenotype link has been established. Using cell biology techniques we are developing a model to assess the functional effects of VWM mutations *in vivo*, offering scope for more accurate patient prognosis. Localisation studies in Yeast have shown that eIF2B localises to specific cytoplasmic foci, termed eIF2B bodies. eIF2 shuttles through these bodies, suggesting they are sites of GEF

activity. Using a GFP tagged eIF2Bε subunit, we have identified eIF2B bodies in mammalian glial cells through live cell imaging studies. The distribution of eIF2B bodies in these cells revealed heterogeneous populations, differing in size and abundance. Co-localisation studies of all subunits of eIF2B, via immunocytological techniques, identified these different bodies as subcomplexes of eIF2B; correlating with those recently identified via MS. Furthermore, utilising fluorescent recovery after photobleaching (FRAP) technology, we have evidence to suggest that populations of eIF2B bodies possess different levels of GEF activity, complementing previous biochemical assays. Additionally, the various eIF2B body populations display diverse responses to cellular stress, demonstrating differing levels of regulation within the body populations. Investigation into eIF2B body populations in various cells types, demonstrates the importance of this regulation to individual cell function. These data suggest that different compositions of eIF2B bodies allow cells to have varying characteristic responses to stress.

WTH07-20

Phenotypic differences between brain- and liver-specific glutamine synthetase knockout mice

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Hepatic encephalopathy (HE) is a common manifestation of hyperammonemia in patients with severe liver disease. In the brain, the astroglial enzyme glutamine synthetase (a.k.a. glutamate ammonia ligase, Glul) is vital for ammonia detoxification and neurotransmitter inactivation. Hyperammonemia is associated with inactivation of brain Glul via protein tyrosine nitration, and it has been suggested that such inactivation worsens the symptoms of HE (Gorg et al., 2010). Further, lack of Glul in the liver can also lead to chronic hyperammonia (Qvartskhava et al., 2015). Here we created conditional knockout mice lacking GS in the liver (L-Glul cKO) and cerebral cortex (C-Glul cKO), respectively, and compared the phenotypes of the two lines. The L-Glul cKO exhibited normal locomotor activity and innate rodent behaviors such as digging and sheltering by the open-field, nest-building and marble-bury tests. In contrast, the C-Glul cKO exhibited altered locomotor activity and impairments in digging and sheltering behaviors. Furthermore, the L-Glul cKO mice had unremarkable gross brain histology, whereas C-Glul cKO had altered microglia, astrogliosis and neurodegeneration. Thus, the knockout phenotypes are markedly different, with a greater complexity of alterations caused by the loss of Glul in the brain.

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WTH07-21

ALS mutant SOD1 affects pathological modifications 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; mutant SOD1 form abnormal aggregates. Recently, phosphorylated and truncated TAR DNA-binding protein 43 (TDP-43) is a principal component of ubiquitinated cytoplasmic inclusions in neuronal and glial cells in ALS. However, it remains unclear whether these ALS-linked proteins partly have a shared pathogenesis. The main purpose of this study was to determine the association between mutant SOD1 and TDP-43 in SOD1 G93A transgenic mice model, ALS cell line model, and spinal cord tissues and induced pluripotent stem cells (iPSCs) derived motor neurons from familial ALS patient. We examined the pathological TDP-43 modifications in SOD1 G93A transgenic mice model, ALS cell line model, and spinal cord tissues and iPSCs-derived motor neurons from familial ALS patient. In the present study, we demonstrated an age-dependent increase in TDP-43 C-terminal fragments and phosphorylation at serine 409/410 in spinal cord motor neurons and glial cells of ALS transgenic mice and a similar increase in TDP-43 modifications in spinal cord glial cells of patients with ALS. The cytoplasmic mislocalization of TDP-43 was also observed in iPSCs-derived motor neurons from familial ALS patient. Moreover, we observe that mutant SOD1 interacts with TDP-43 by co-immunoprecipitation assays using WT-hSOD1 and mutant (G93A) hSOD1-transfected motor neuronal cell lines. Mutant SOD1 over-expression led to an increased amount of mutant SOD1 and its interacting proteins including TDP-43 C-terminal fragments and phosphorylation in the detergent-insoluble fraction in the spinal cord of SOD1 G93A transgenic mice and familial ALS patient. These findings suggest that mutant SOD1 could affect the solubility/insolubility of its interacting TDP-43 through physical interactions and pathological modifications of TDP-43 in glial cells may be involved in motor neuron death in the spinal cord of SOD1 G93A transgenic mice and familial ALS patient.

WTH07-22

Oxidative aggregation of AMPK underlying age-related impairment of selective autophagic clearance in the hippocampus

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AMP-activated protein kinase (AMPK) regulates energy states and autophagy in response to various stresses. Aging-associated disturbance of autophagy is closely related to impaired neuronal function and survival. Although AMPK is thought to be critical for maintaining autophagy and the clearance of damaged organelles in the aged brain, little is known about AMPK-associated changes in the brain during aging. Therefore, the aim of this study was to elucidate the impact of aging on AMPK physiology and signaling in

the hippocampus of aged mice. Young (8–10 weeks) and old (18 months) C57BL6 mice were used and acute restraint stress was given to them for 6 h. The changes in AMPK signaling and selective autophagic clearance were investigated through western blot analysis, immunofluorescent staining, co-immunoprecipitation and electron microscopic observation. We found that AMPK formed oxidized aggregates in the hippocampus of old mice, which occurred alongside a decline of thioredoxin 1 (Trx1). Moreover, old mice showed abnormal perinuclear mitochondrial clustering that colocalized with oxidized AMPK aggregates in hippocampal pyramidal neurons and impaired AMPK-mediated selective autophagic capacity. Interestingly, the oxidative aggregation of AMPK and perinuclear mitochondrial clustering were ameliorated by overexpression of Trx1, suggesting that age-related changes in AMPK physiology and consequent accumulation of dysfunctional mitochondria are reversible. These findings could be used to develop new therapeutic strategies for overcoming age-related hippocampal dysfunction and dementia.

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WTH07-23

MIR-18B dysregulation triggers apoptosis by inhibition of MCTP1 and RARB in fALS linked SOD1 mutation

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MicroRNAs (miRNAs) are endogenous noncoding RNAs that regulate gene expression at the post-transcriptional level and key modulators of neurodegenerative disease. Overexpressed miRNAs have an important role in amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). However, the pathogenic mechanisms of dysregulated miRNAs are still unclear. Here, we aimed to determine dysfunction of RNA metabolism including miRNAs in fALS. We compared transcriptional profiling of NSC-34 and NSC-34 (G93A) mutant cell lines and indentified upregulation of hypoxia inducible factor 1 alpha (Hif1α) and myocyte specific enhance factor 2c (Mef2c) and downregulation of multiple C2 and transmembrane domain containing protein 1 (Mctp1) and retinoic acid receptor beta (Rarb) in NSC-34 (G93A) mutant cell lines. We demonstrated that Hif1α which is increased by miR-18b dysregulation is associated with Mef2c expression. Decreased both Mctp1 and Rarb were directly regulated by miR-206 which also is increased by Mef2c. Thus, the simultaneously down regulation of Mctp1 and Rarb accelerates Bax which is apoptotic regulatory proteins in NSC-34 (G93A) mutant cells. The inhibition of Mctp1 and Rarb induce intracellular Ca^{2+} level and reduce cell differentiation, respectively. This finding suggested that miR-18b dysregulation is involved in apoptosis cell death in SOD1 mutation. Furthermore, miR-18b signaling pathway was precisely discovered in G93A TG mice, and SOD1 ALS patients as NSC-34 cells. Taken together, our data indicate that SOD1 (G93A) mutation decreases miR-18b which is sequentially regulates several genes (Hif1α, Mef2c, Rarb and Mctp1) and miR-206. These results strongly suggest new insights into the dysregulation of miRNAs dependent pathogenic mechanism in ALS and FTLD.

WTH07-24

Vitamin A (retinol) protects against neurodegeneration in a 6-hydroxydopamine rat model

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Introduction: Vitamin A (retinol) exerts fundamental role in cellular processes regulation, such as growing, cell division and apoptosis. In recent years, several studies have been proposing an anti-inflamatory and antioxidant effect of retinol, however, series of pro-oxidant actions have been shown in different *in vivo* and *in vitro* conditions, demonstrating a dualistic redox activity of this molecule. The role of vitamins as antioxidants in neurodegenerative diseases has also been extensively debated, and some results showed a positive correlation between serum levels of retinol and reduced cognitive decline. However, the effect of dietary intake of vitamin A on the risk of Parkinson's disease (or its prevention) is uncertain as available epidemiological data are limited.
Objective: To investigate the preventive role of vitamin A supplementation against 6-hydroxydopamine (6-OHDA) neurotoxicity in a Parkinson's disease rat model.
Results: Rats received oral supplementation of retinol as retinyl palmitate (3000 IU/Kg/Day^{-1}) for 30 days prior to 6-OHDA injection into substantia nigra (SN). Rotatrod test, imunohistochemistry and western blot analyses were performed 15 days after 6-OHDA injection. Retinol supplementation significantly protected against 6-OHDA-induced locomotory deficit in rotarod test. The decreases in TH^+ cells as well as TH protein levels in SN were prevented by retinol supplementation. Serum analysis revealed that retinol was able to reduce the amount of IL-1β and TNF-α pro-inflammatory cytokines, and also was able to reduce the levels of carboxymethyl-lysine, an advanced glycation end product, in cerebrospinal fluid, suggesting a possible involvement of receptor for advanced glycation endproducts (RAGE) in oxidative/inflammatory process.
Conclusions: Vitamin A supplementation had a preventive property in 6-OHDA-induced motor deficit, dopaminergic degeneration and induction of pro-inflammatory cytokines.

WTH07-25

SC79, a AKT activator, rescues Alzheimer's disease-associated memory impairments and aberrant synaptic plasticity

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Aβ is as a key mediator for synaptic dysfunction and cognitive impairment observed in Alzheimer's disease (AD). However, the

precise mechanism of the effect of Aβ is still not complete. Akt is known to be aberrantly regulated in AD brain. However, its possibility in therapeutic target for AD-associated memory impairment is not studied. Here we examined the effects of direct Akt activator, SC79, in hippocampal-dependent memory using Aβ-treated and 5XFAD mice AD models. We found that pharmacological activation of Akt rescued memory impairments and aberrant synaptic plasticity in both of Aβ-treated and 5XFAD mice. These results suggest that Akt might be a therapeutic target for memory impairment observed in AD.

WTH07-26

PTUPB, dual inhibitor of soluble epoxide hydrolase and cyclooxygenase-2, mitigates rotenone induced neurotoxicity

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Epoxyeicosatrienoic acids (EETs), are the metabolites of arachidonic acid cascade, plays a crucial role in cytoprotection by attenuating oxidative stress, inflammation and apoptosis. EETs are rapidly metabolised *in vivo* by soluble epoxide hydrolase (sEH). Elevating the half life of EETs by inhibiting sEH is a novel strategy for neuroprotection and the simultaneous inhibition of COX-2 will have an added advantage in neuroprotection. In the present study, PTUPB was evaluated for its anti-Parkinson activity against rotenone induced mitochondrial dysfunction, oxidative stress and neuroinflammation in N27 dopaminergic cell lines and *Drosophila melanogaster* model of Parkinson disease (PD). The *in vitro* neuroprotective efficiency was evaluated by measuring cell survival assays, oxidative stress parameters (intracellular ROS, protein oxidation, lipid peroxidation, and mitochondrial membrane potential), inflammatory markers (IL-6, COX-1 and COX-2), and apoptotic markers (c-jun, P-c-jun, JNK, P-JNK, pro and active caspase-3). Further, *in vivo* neuroprotective efficiency was confirmed by measuring Survival rate, negative geotaxis, dopamine and its metabolites (LCMS) and oxidative stress parameters. PTUPB pre-treatment significantly improved cell viability, through amelioration of ROS production, proteins oxidation and lipids peroxidation. It also attenuated the mitochondrial damage by improving mitochondrial membrane potential and complex I activity. PTUPB normalizes the altered mRNA expression levels of inflammatory markers and antioxidant enzymes as assessed by RT-PCR. PTUPB also decreased the phosphorylation of apoptotic markers like JNK and c-jun leading to alleviated levels of cleaved caspase-3. These results were in corroboration with *in vivo* results with improved survival rate, negative geotaxis, dopamine levels, antioxidants and anti-inflammatory status in *Drosophila* model of PD. These results substantiate the neuroprotective efficiency of PTUPB indicating its potential therapeutic benefits in the treatment of PD.

WTH07-27

Surveillance of human prion diseases in Brazil data from 2005 to 2017

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Global surveillance of CJD and its subtypes was recommended by WHO for investigate the presence of the variant of Creutzfeldt Jakob Disease, for a better understanding of the iatrogenic causes as well as the distribution of hereditary CJD forms. The compulsory notification of diseases caused by prion began in Brazil in 2005 as an initiative of the Ministry of Health. So far, we have received 620 blood samples from notified cases of suspected CJD. They were analyzed by genomic sequencing to identify mutations and polymorphisms in the *PRNP* gene. The average age of our patients was 60.6 years (10-94y). PRNP polymorphisms analysis at codon 129 showed that 50% of cases were homozygous for methionine, 31% 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 and exams evaluation, the notified cases were classified according to the WHO criteria. Among of them, 8% were classified as sporadic CJD, 42% as probable CJD, 20% as possible CJD, 4,5% as genetic prion disease, 18.5% as suspected CJD and 7% 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.

WTH07-28

Trimethyltin-induced hippocampal neurodegeneration

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Trimethyltin (TMT), a toxic organotin compound, induces neurodegeneration selectively involving the limbic system and especially prominent in the hippocampus. Neurodegeneration-associated behavioral abnormalities, such as hyperactivity, aggression, cognitive deficits, and epileptic seizures, occur in both exposed humans and experimental animal models. Previously, TMT had been used generally in industry and agriculture, but the use of TMT

has been limited because of its dangers to people. TMT has also been used to make a promising *in vivo* rodent model of neurodegeneration because of its region-specific characteristics. Several studies have demonstrated that TMT-treated animal models of epileptic seizures can be used as tools for researching hippocampus-specific neurotoxicity as well as the molecular mechanisms leading to hippocampal neurodegeneration. This review summarizes the *in vivo* and *in vitro* underlying mechanisms of TMT-induced hippocampal neurodegeneration (oxidative stress, inflammatory responses, and neuronal death/survival). Thus, the present review may be helpful to provide general insights into TMT-induced neurodegeneration and approaches to therapeutic interventions for neurodegenerative diseases, including temporal lobe epilepsy.

WTH07-29

Aberrant expression and possible pathogenic role of S100B-RAGE in ALS

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Neuroinflammation is one of the major players in amyotrophic lateral sclerosis (ALS) pathogenesis, and astrocytes are significantly involved in this process. S100B, a calcium-binding protein mainly expressed by astrocytes in the CNS, can be released in pathological states, activating the receptor for advanced glycation end-products (RAGE). In ALS, different indications point to an aberrant expression of S100B and RAGE; this work provides a comprehensive picture of their localization, expression timing and possible roles in SOD1G93A models of ALS. We observed that S100B and RAGE are progressively upregulated selectively in astrocytes of diseased rats with a timing pattern that may be linked to the level of neurodegeneration. Also the expression of the full length and soluble RAGE isoforms, which display antagonistic functions, is likely correlated to the features of tissue damage. Moreover, we showed that the mere presence of mutant SOD1 is able to increase the intracellular levels and release of S100B from astrocytes, suggesting that increased astrocytic expression of S100B might be an early event during the progression of the disease. Finally, our findings indicate that the protein may exert a pro-inflammatory role in the disease, since its inhibition in astrocytes derived from SOD1G93A mice dowmregulates the expression of reactive/ pro-inflammatory genes (GFAP, TNFalpha, CCL6, CXCL10), indicating that the protein migkt promote a pro-inflammatory phenotype in astrocytes.. Thus, our findings candidate the S100B-RAGE axis as an effective contributor to the pathogenesis of the disease, so that its blockade may be regarded as a rational target for therapeutic intervention in ALS.

WTH07-30

Role of G quadruplex rna structure in ALS/FTD

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Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are multisystem disorders with overlapping functional and genetic causes. Errors in the production of the DNA/RNA binding proteins Tar-DNA binding protein 43 (TDP-43) and fused in sarcoma/translocated in liposarcoma protein (FUS/TLS) are causative of ALS and FTD, as these proteins are the major protein components in over 90% of ALS and over 50% of FTD inclusions. In 2011 it was discovered that a hexanucleotide GGGGCC expansion in the *C9ORF72* gene is a common genetic cause for ALS and FTD. The mechanisms by which the mutations in FUS and the GGGGCC expansion lead to ALS/FTD are not known, hindering the development of therapeutic agents against these diseases. In this study we advance the hypothesis that the G-quadruplex RNA structure plays an essential role in the pathogenic mechanisms of both FUS and C9ORF72 hexanucleotide expansion in ALS and FTD. We show by biophysical methods that the GGGGCC expansion adopts preferentially the G quadruplex over an extended hairpin structure. Moreover, wild type FUS and ALS causing FUS mutants bind G quadruplex forming mRNAs, including the GGGGCC expansion using the C-terminal arginine-glycine-glycine motif.

WTH07-31

Postnatal exposure augments neurotoxicity of ZINC/ paraquat exposed adult rats on oxidative stress, monoamine transporters, apoptosis

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Pesticides and heavy metals are established as the major environmental risk factors for Parkinson's disease (PD). Developmental exposure to pesticides enhances the susceptibility for dopaminergic neurodegeneration in re-challenged adult rats. However, the effect of postnatal pesticide exposure on the heavy metal-induced neurotoxicity or vice versa is not yet clearly explored. The present study aimed to investigate the effect of postnatal exposure of zinc (Zn) or paraquat (PQ) on the nigrostriatal dopaminergic neurodegeneration in re-challenged adult rats. Male Wistar rats were treated with Zn/PQ during post natal (5-19) days followed by re-exposure upon adulthood (twice weekly) for 12 weeks. Striatal dopamine content, oxidative stress indicators and expression of tyrosine hydroxylase (TH), dopamine transporter (DAT) and vesicular monoamine transporter-2 (VMAT-2) were measured in the control and treated groups. Besides, the mitochondrial cytochrome-c release and caspase3/9 activation were also analyzed. A marked reduction was obtained in the striatal dopamine and glutathione content with concomitant increase in lipid peroxidation and protein carbonyls in adulthood exposed and postnatal + adulthood-exposed groups. While significant reduction in the expression of VMAT-2 and TH was observed, the mitochondrial cytochrome-c release and caspase-3/9 activation along with DAT expression were found to be elevated in adulthood exposed groups.

The changes were more pronounced in postnatal + adulthood exposed groups as compared with adulthood alone. Postnatal exposure per se did not show any noticeable change in any of the aforementioned parameters. Results of the study demonstrate that postnatal pre- exposure of Zn/PQ augments oxidative stress, alters DAT/VMAT-2 expression and induces intrinsic apoptosis leading to dopaminergic neurodegeneration in re-challenged adult rats.

WTH07-32

Functional impact of phosphorylation of mutant HTT at serine-421 in human neurons

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Huntington disease (HD) is a neurodegenerative disease caused by an autosomal dominant mutation in a single gene, Huntingtin (*HTT*), which results in an elongated polyglutamine tract in the HTT protein. The phosphorylation at serine-421 (pS421) of mutant HTT (mHTT) downstream of IGF-1/Akt signaling axis was previously reported to be neuroprotective. Though certain molecular mechanisms have been described, the effects of pS421 on mHTT toxicity and the exact cellular processes involved remain incompletely understood. Here we demonstrate that pS421 of mHTT ameliorates certain mitochondrial alterations seen in HD human neural cells. Using genome editing, we generated isogenic HD human pluripotent stem cell (hiPSC) lines in which the S421 site in mHTT has been mutated into a phosphomimetic glutamic acid (S421D) or phospho-resistant aspartic acid (S421A). We observed significant differences in mitochondrial function and structure for hiPSC-derived neural cells with the S421D compared with S421S and S421A forms of mHTT. We further observed amelioration of transcriptional changes in mitochondrial-related genes in neural cells with S421D but not the S421A form of mHTT. Our results show that post-translational modification at S421 may modulate the toxicity of the full-length mHTT protein at least in part by affecting HD-associated mitochondrial alterations. Our study highlights a facet of the relationship between mHTT and mitochondrial dysfunction in the context of human physiology, with potential relevance to the pathogenesis of HD.

WTH07-33

Ankyring malfunction and neurodegenerative process in HuC KO mice

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Hu proteins (the neuronal Elav-like: nElavl) are the mammalian homologue of the Drosophila Elav, an RNA-binding protein expressed in the nervous system. Hu proteins bind to target RNAs and regulate alternative splicing and translational process.

Almost all of the brain regions express HuC together with HuB and/or HuD. However, cerebellar Purkinje cells (PCs) express only HuC. Then if HuC gene is knocked out, PCs specifically become all of the neuronal Hu null. Dysfunction of PCs in HuC KO mice leads the intentional tremor, gain abnormality and ataxia. Prior to the onset, the axons of PCs underwent morphological changes; swollen and retracted at the cerebellar nuclei. To reveal the mechanisms of the axonal degeneration, components of the spheroids were investigated with histological analysis. Most spheroids were accumulated with mitochondria and endoplasmic reticulums. Surprisingly, cytosolic organelle such as nuclei and ribosomes were also observed in the spheroids. These abnormal distributions of cytosolic organelle were thought to be caused by dysfunction of selective filter between soma and axon. Based on these data, AnkyrinG was suspected to be a causing factor. In neurons, AnkyrinG is located in the axon initial segment (AIS) and forms the selective filter between soma and axon. This system is required to define the delicate protein distribution in neuron. Our studies showed that HuC regulates the alternative splicing of AnkyrinG, and the splicing process was disrupted in HuC KO cerebellum. Moreover, particular splicing variant, which increased in HuC KO cerebellum, was identified as embryo-specific variant of AnkyrinG. Furthermore, the embryo-specific variant exhibited differential binding affinity to Spectrin compared to adult variant. These data indicate that HuC maintains the homeostasis of axons probably through controlling alternative splicing of AnkyrinG.

WTH07-34

A novel contrast agent to detect apoptotic cells in stroke and Alzheimer's disease

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Apoptosis-related neurodegeneration is directly linked with the loss of brain function in stroke and Alzheimer's disease (AD). So far there are no clinical means to detect apoptotic cells *in vivo*. Here we present a new contrast agent (CA), bearing a fluorescent and a radioactive labels, that is designed to accumulate in apoptotic cells due to increased cleaved caspase-3 (CC3) activity. In neuronal culture models of neurodegeneration, including oxygen-glucose deprivation, camptothecin and beta-amyloid oligomer toxicity, CA accumulated predominantly in apoptotic cells. In live animals, positron emission tomography (PET) showed CA accumulated on the injury side of stroke mouse brains (transient middle cerebral artery occlusion, MCAO). Similarly, PET showed CA accumulation in forebrain of AD mice (5xFAD), but not in wild-type controls. Confocal microscopy on excised post-injection brains confirmed the spatial distribution of CA in both cases. In MCAO brains CA fluorescence correlated with the increase in CC3-positive cells on the operated side. In optically cleared MCAO brains CA and CC3

were co-localized on the injury side. In 5xFAD brains, CA fluorescence was elevated in hippocampus and cortex, compared to wild-type controls. Co-staining with thioflavinS revealed that CA accumulated in single cells and around amyloid plaques. Our studies in brain disease models show that this CA targeting caspase-3 activity could become an important tool for *in vivo* detection of apoptosis-affected regions in neurodegenerative diseases or other conditions or therapies associated with apoptosis, providing a proof of principle for its potential utility in humans.

WTH07-35

Neuroactive steroid levels in sciatic nerve: effects of blunted *de novo* fatty acid synthesis

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Neuroactive steroids are cholesterol-derived molecules that function as protective agents in central and peripheral nervous system. We recently described that a genetic model of reduced fatty acid synthesis, the sterol regulatory binding factor-1c knock-out mice (Srebp-1cKO), developed peripheral neuropathy over time. At 10 months of age, we found that Srebp-1KO sciatic nerves showed an apparent hypermyelination of small-caliber fibers due to changes in myelin periodicity resulting in myelin instability and Remak bundle degeneration. We decide to evaluate the levels of neuroactive steroids in plasma and in sciatic nerve of Srebp-1cKO male mice at 2 and 10 months of age using LC-MS/MS. At 2 months of age, we found an increase of pregnenolone, associated with decrease of progesterone and further metabolites. The levels of testosterone were also increased. Interestingly, changes in pregnenolone, progesterone and testosterone were not observed in plasma but were restricted to sciatic nerve. These results were further corroborated by gene expression analysis. The expression of P450scc, the enzyme involved in the first step of steroidogenesis, was increased. Moreover, both 5a-reductase (5a-R) and 3b-hydroxysteroid oxidoreductase (3a-HSOR) mRNA levels were also induced.

At 10 months of age, the neuroactive steroid profile showed further differences. Indeed, the levels of pregnenolone were decreased while those of dihydroprogesterone, tetrahydroprogesterone and isopregnanolone were increased. Furthermore, testosterone and its metabolites were decreased. Moreover, plasma levels of neuroactive steroids were unaffected confirming that the observed changes occurred in the sciatic nerve. At this age, we also found a significant decrease of P450scc gene expression associated with an increase of 5a-R and 3a-HSOR mRNA levels.

Altogether, our data support the concept that the cross-talk between fatty acid synthesis and neuroactive steroids, may represent a possible therapeutic strategy for peripheral neuropathy.

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WTH07-36

Comparative analysis of cerebral 64CU and F-18 FDG uptake in APPSWE TRANSGENIC MOUSE MODELS OF ALZHEIMER'S DISEASES WITH PET/CT

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Objectives: Copper is required for brain development and function. Copper deficiency causes malfunction and loss of neurons in Menkes disease and excess copper accumulated in brain tissue causes neuronal damage in Wilson's disease. To explore cerebral ^{64}Cu uptake as a biomarker in pathophysiology of AD, the objective of this study was to compare ^{64}Cu and F-18 FDG uptake in the brains of APPSWE transgenic mice measured with sequential $^{64}CuCl_2$-PET/CT and F-18 FDG PET/CT.

Methods: APPSWE Tg2576 transgenic mice (n = 5) were subjected to sequential PET/CT after intravenous injection of $^{64}CuCl_2$ and F-18 FDG as a tracer, respectively. A quantitative PET analysis was conducted to compare ^{64}Cu and F-18 FDG uptake in the brains of APPSWE transgenic mice, in comparison to ^{64}Cu and F-18 FDG uptake in the brains of control C57BL/6 mice (n = 5) after intravenous injection of $^{64}CuCl_2$ as a tracer.

Results: Different biodistribution of $^{64}CuCl_2$ and F-18 FDG in APPSWE Tg2576 transgenic mice and wild type C57BL/6 mice was visualized on PET/CT images, showing high F-18 FDG and low ^{64}Cu uptake in the brains of the mice. There was increased ^{64}Cu uptake in the brains of young adult APPSWE Tg2576 transgenic mice (0.71 $\pm$ 0.13%ID/g) compared to ^{64}Cu uptake in the brains of young adult C57BL/6 mice. In contrast, there was no difference of F-18 FDG uptake in the brains of APPSWE mice (5.0 $\pm$ 0.72%ID/g) and C57BL/6 mice (5.6 $\pm$ 1.30%ID/g).

Conclusion: Increased ^{64}Cu uptake was detected in the brains of young adult APPSWE Tg2576 transgenic mice compared with the ^{64}Cu uptake in the brains of C57BL/6, supporting further investigation of age-dependent changes of ^{64}Cu and F-18 FDG uptake in the brains of APPSWE Tg2576 transgenic mice, in comparison to control C57BL/6 mice.

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WTH07-37

Cytosolic CU(II) is modulated by glutathione

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Copper is an essential micronutrient that regulates several cellular processes, including mitochondrial oxidative phosphorylation, iron metabolism, free radical detoxification and neurotransmission. Copper is a redox-active transition metal that readily oxidizes from Cu(I) to Cu(II) in aerobic solutions. Several studies indicate that under physiological conditions, the main copper redox state in the cytosol is Cu(I), which is maintained by reducing agents,

hypothesized to predominantly glutathione. Brain glutathione depletion as well as copper dyshomeostasis, are features of neurodegeneration. We hypothesized that intracellular Cu(II) is elevated by the loss of glutathione in these diseases. There is no previous direct evidence for the normal presence of Cu(II) in the cellular cytoplasm. In this study, using XANES and a Cu(II)-sensor, we demonstrate for the first time that Cu(II) is present in the cytosol. Moreover, our results show that a decrease of intracellular glutathione by *N*-ethylmaleimide or L-buthionine sulfoximine, increases the intracellular levels of Cu(II), indicating that the copper redox-state is indeed regulated by glutathione. Furthermore, we show that under oxidative stress conditions, such as H_2O_2 or glutamate treatments, Cu(II) intracellular levels are also increased. Together, our results indicate that Cu(II) can emerge in the cytosol under oxidative stress that is typical of neurodegenerative diseases. Loss of Cu(I) and the emergence of redox-catalytic Cu(II) may exacerbate the metabolic injuries in these disorders.

WTH07-38

3-Hydroxykynurenine and 3-hydroxyanthranilic acid enhance the toxicity induced by copper in rat astrocytes culture

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Copper is a heavy metal and an integral component of various enzymes and biological functions;however, excess copper is neurotoxic and has been implicated with neurodegenerative diseases as Alzheimer. This metal is able to modify the cellular redox environment. In this context, kynurenine pathway (KP) is modulated by the redox environment and produces some metabolites with redox properties as 3-hydroxykynurenine (3-HK) and 3-hydroxyantrhanilic acid (3-HANA). The imbalance in the production of these kynurenines is related with some neuropathologies, in which the common factors are oxidative stress, inflammation and cell death. The aim of this study was to evaluate the effect of these kynurenines on the cupper toxicity in astrocytes cultures. First, we evaluated the $CuSO_4$ (0–500 μM) effect on MTT reduction, ROS production, mitochondrial membrane potential (MMP) and cell viability on primary cultured astrocytes. Then was evaluated the effect of the co-incubation of $CuSO_4$ (350 μM) whit metabolites (100 μM) in the same parameters that were previously tested, also GHS levels and the chelating copper effect of 3-HK and 3-HANA. Our results showed that $CuSO_4$ decreased MTT reduction and MMP, while it increased ROS production and cell death in a concentration-dependent manner. The co-incubation with metabolites enhances the toxic effect of copper in MTT reduction, MMP and cell death. Copper also decreased GSH levels around 50% and co-incubation with the kynurenines decreased 70% GSH levels. However, the increase in ROS production by copper was abolished by metabolites. Both metabolites are able to chelate cupper in a concentration dependent manner. These data suggest that 3-HK and 3-HANA increased copper toxicity in an independent manner to ROS production; however, their effect on GSH levels could play an important role in the potentiation of cell damage induced by copper.

WTH07-39

Lysosomal impairment causes the onset of neurodegeneration in mouse granule neurons: the side effect of sphingolipid metabolism

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Several lines of evidence implicate lysosomal dysfunction in the neuropathology associated with Lysosomal Storage Diseases. Nevertheless, the mechanistic link between the altered lysosomal homeostasis and the neuronal damage is still unknown. In this context, promising results obtained in a fibroblast-model of lysosomal impairment suggest a possible role played by the alteration of sphingolipid metabolism. With the aim to investigate the effects of lysosomal impairment in neurons, I developed a new *in vitro* model of lysosomal engulfment represented by differentiated mouse cerebellar granule neurons loaded with sucrose. Interestingly, in sucrose loaded neurons I found an increased lysosomal biogenesis due to the nuclear translocation of the Transcription Factor EB, master regulator of lysosomal genes. Furthermore, sucrose loading induces the activation of autophagy and a decrease in cell viability accompanied by the onset of neurodegeneration. Remarkably, after sucrose loading I found an alteration of sphingolipid composition characterized by the reduction of polysialoganglioside and sphingomyelin contents followed by the increase of ceramide level. These findings suggest that sucrose loading induces an activation of the sphingolipid catabolism as confirmed by the increased activities of the main sphingolipid hydrolytic enzymes, both intracellularly and at the plasma membrane level. These results let to speculate that sucrose loading causes an augmented fusion between lysosomes and the cell surface resulting in the increase of sphingolipids hydrolases *in-situ*. The relationship between these events and the production of ceramide at the plasma membrane level unveils a new role of sphingolipid metabolism in the activation of downstream pathways responsible for the onset of cell damage and neurodegeneration.

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WTH07-40

Locus coeruleus lesion by 6-hydroxydopamine induces recognition memory deficits in rats: involvement of prefrontal cortex

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Locus coeruleus (LC) degeneration, the main source of cerebral noradrenaline (NA), has been related with neurodegenerative disorders such as Alzheimer's (AD) and Parkinson's diseases (PD). Growing evidence support earlier NA deficiency in several brain areas resulting from selective degeneration of LC neurons. Additionally, LC projections to the prefrontal cortex (PFC) have a critical role on the cognitive functions. Therefore, here we evaluated the learning and memory of rats after a 6-hydroxydopamine (6-OHDA)-induced selective noradrenergic lesion in LC and the involvement of the likely resulting NA deficit in the PFC. For this,

adult male Wistar rats received stereotaxic bilateral injections of 6-OHDA (5 μg/side) into the LC and two stainless-steel guide cannulas were implanted aimed at the PFC. This 6-OHDA dose did not cause any motor alterations. SHAM group received just vehicle (0.2% ascorbic acid in saline). Selective noradrenergic lesion was reached by nomifensine administration (10 mg/kg/ml, i.p.) 1 h before of 6-OHDA infusion. LC lesion caused short- and long-term recognition memory impairments addressed on object recognition test 14 days after 6-OHDA administration. Moreover, LC slices from 6-OHDA-injected rats showed an elevation in the mitochondrial membrane potential. The propidium iodide (PI) incorporation and the nitroxidative stress production were not altered in the LC slices from lesioned rats. Importantly, PFC slices from 6-OHDA group exhibited cell damage evaluated by PI incorporation, nitroxidative stress production increase and mitochondrial membrane potential disruption when compared to the SHAM group. These outcomes suggest that the irregular action of LC neurons caused neurochemical changes in the PFC. Corroborating with the hypothesis of PFC noradrenergic deficit, bilateral NA infusion (1 μg/side) into this region immediately before of the training session restored the 6-OHDA-induced recognition memory dysfunctions. Thus, our results suggest that the LC lesion and the consequent dysregulation network with the PFC could be involved in the recognition memory impairments observed in both AD and PD patients.

WTH07-41

Participation of MAPK signaling pathway in a model of neuronal degeneration in striatal of rat

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Quinolinic acid (QUIN), an endogenous metabolite from kynurenine pathway, acts as a competitive agonist on NMDAr and its intrastriatal administration to rats has been used to reproduce some alterations similar to those observed in some chronic-degenerative disorders. Oxidative stress-depended signaling pathways, such as MAPK, are related with the physiopathology of some brain diseases, promoting cell death (JNK and p38 activation) or declining survival cell (ERK1/2 inhibition). Besides, MAPK activation has been related to changes on some systems antioxidants such as Nrf2 and CREB and to suitable levels of neurotrophins. In this study, we evaluate the activation of MAPK in the cell death in the striatum induced with QUIN, and its participation on transcription factors related to antioxidant response. Animals were intrastriatally infused with QUIN (30, 60, 120 and 240 nmol/ml). Right striatum was dissected at 2 h, 24 h and 7 days after QUIN injection. MAPK levels were detected by western blot and IHC. Histological analysis was done by H&E and FJ-B at 7 days after QUIN injection. Motor evaluation was done 6 days after operation. We found that QUIN (120 and 240 nmol/ml) significantly increased the activation of pathways related to death cell (p-JNK y p-p38) and decreased the activation of pathways that promote survival cell (p-ERK1/2) at 7 days. The sustained activation of p-JNK with QUIN 120 nmol from 2 h to 7 days could be involved in the onset of morphological alterations observed up to 7 days. Moreover, cell death in the striatum could also be due to decreased levels of Nrf2, CREB and BDNF levels. The decrease of preserved cells may be the cause of the deficit in motor evaluation with higher doses with QUIN. These data suggest that activation of p-JNK could be participating in the mechanism of damage of QUIN in the striatal cells of rats.

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WTH07-42

Validation of histocultures from adult human brain as a tool to study age-associated neurodegenerative diseases

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Histocultures from adult human brain are a powerful model to study cellular and molecular aspects of neurologic diseases. The advantages of this approach over 2-D cultures include the preservation of brain cytoarchitecture and neuronal connectivity. Few studies so far have assessed the morphofunctional state of adult human brain-derived tissue along the days in culture. Here, we have evaluated cell survival and function of cortical slices from adult human brains (median age 39 ± 11) along several days in culture. Tissues were obtained from patients submitted to partial lobectomy for the treatment of pharmaco-resistant temporal lobe epilepsy (Ethics Committee HCRP17578/15). Only cortical tissue resected to get access to the hippocampus was used. Fragments (ca. 1 cm^3) were collected at the surgical room, immersed in ice-cold, oxygenated buffered saline, sliced using a vibratome, and cultured. Tissue integrity was not affected by processing, as revealed by HE staining. MTT assay indicated no significant reduction in cell viability up to day 4. Immunohistochemistry revealed neuronal and astrocyte number stability along the days *in vitro*. Importantly, neurons remained synaptically active throughout the period in culture, as probed by ERK phosphorylation and neurotransmitter release after KCl-induced depolarization. The attack of Alzheimer's disease-associated Abeta oligomers to cultured slices was assessed by both IHC and ELISA. A massive binding of oligomers was evident, suggesting that this histoculture is amenable for modeling neurodegenerative diseases. This protocol may facilitate the application of histocultures from adult human brains in studies on new therapeutics for neurodegenerative disorders.

WTH07-43

Chronic stress triggers tau pathology through autophagy inhibition and induction of stress granules

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Imbalance of neuronal proteostasis associated with misfolding and aggregation of Tau protein is a common neurodegenerative feature in Alzheimer's disease (AD) and other Tauopathies. Consistent with suggestions that lifetime stress maybe an important precipitating factor of AD, we previously reported that environmental stress and high glucocorticoid (GC) levels evoke accumulation of aggregated Tau; however, the underlying molecular mechanisms remain unclear. We now demonstrate that chronic stress and GC trigger an mTOR-dependent inhibition of autophagic process, the cardinal clearance pathway for aggregated proteins, leading to accumulation of Tau aggregates and cell death in mice and cells stably expressing P301L-Tau. Considering the interplay of autophagy with Stress granules (SGs) dynamics, we also show that environmental stress/GC stimulate the induction of SGs, recently shown to promote Tau misfolding, aggregation and neurotoxicity. Notably, pharmacological intervention that stimulates autophagic process (Temsirolimus) attenuates the GC-driven elevation of Tau, SGs and cell death. This work provides novel insights into the mechanisms through which neuronal cells convey the detrimental impact of prolong environmental (HPA-related) stress to intracellular "stress" signaling, causing Tau-driven brain pathology.

WTH07-44

Activation of NRF2 in striatum is oxidative stress independent and dependent of P62 and DPP3 proteins

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Nrf2 is the most important protein that regulates the expression of genes involved in the celular redox homeostasis. Its activation in a non-canonical pathway, involves the disruption of Keap1-Nrf2 complex by direct interaction of some proteins with Keap1, like p62 and DPP3. It has been reported that quinolinic acid (QUIN), a selective agonist of NMDAr, it is capable to induce an oxidative/nitrosative state in the cell, so its intrastriatal administration has been used as an excitotoxic/pro-oxidant model. Unexpectedly, we found that QUIN administration increase the Nrf2 activation 30 min after its injection in the striatum, without increase of ROS production. In this work we propose to evaluate the effect of different doses of QUIN on Nrf2/Keap1 interaction in an *in vivo* model in the rat striatum. We administrated 1 μL of isotonic saline or QUIN (15, 30, 60, 120 and 240 nmol) in the striatum of male Wistar rats (260–300 g) and at 30 min after injection, striatum was collected. The Nrf2, Keap1, p62 and DPP3 levels was measured by western blot, the oxidative stress was evaluated by dihydroethidium oxidation and Xantine Oxidase (XO) and NAD(P)H oxidase (NOX) activity. Finally, the localization of protein expression (Nrf2 and p62) was identified by Immunofluorescence and the evaluation of protein interaction between Keap1 and p62 or DPP3 by Immunoprecipitation. Total amount of p62 and DPP3 proteins at 30 min, shows no change with all doses of QUIN, whereas total Keap1 are increased. However, an increase in p62, p-p62 and Nrf2 nuclear levels was observed. Dihydroethidium oxidation, XO and NOX activity showed not changes. The interaction between Keap1 and DPP3 or p62 increases and we found that this process is carried out in striatal neurons. These results suggest that at 30 min, the activation of Nrf2 is associated with the Keap1-Nrf2 disruption by DPP3 and p62 in neuronal cells, and this effect is oxidative stress independent. CONACyT (Grant): 241655.

WTH07-45

Ankyrin-R is required for cerebellar purkinje cell survival

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Ankyrin (Ank) proteins, are found throughout the body and act as the primary link between the spectrin-based cytoskeleton and the cytoplasmic domain of many membrane-associated proteins. Although AnkG and AnkB are well recognized as important domain organizers within the nervous system, few studies have investigated AnkR's role. Our lab recently showed AnkR can compensate for a loss of AnkG and cluster Na^+ channels at nodes of Ranvier. Additionally, multiple studies have indicated various neurological disturbances have disruptions in AnkR, including cerebellar dysfunction. However, the role of AnkR in the nervous system remains poorly understood. Our expression analyses show, unlike the other ankyrin proteins found widely throughout the brain, AnkR is only present in a subset of neurons, including cerebellar Purkinje cells. To elucidate the role of AnkR in these cells, I have examined AnkR knockout mice (AnkRpale/pale). Data reveals AnkRpale/pale mice have progressive Purkinje cell degeneration marked by increasing amounts of abnormal protein accumulation and dystrophic axons, resulting in cell loss in aged mice. Additionally, gait analyses show ataxia in null animals. Interestingly, mutations of bIII spectrin underlie spinocerebellar ataxia type 5 (SCA5), characterized by disrupted gait and progressive Purkinje cell degeneration, phenotypically similar to AnkRpale/pale mice. Although the precise molecular mechanisms underlying these changes remain unknown, our data confirms AnkR and bIII spectrin interact in the brain. Taken together, these data suggest AnkR plays an important role in stabilizing the spectrin cytoskeleton in Purkinje cells. Future studies using our novel AnkR cKO will further explore the role of AnkR in Purkinje cells, and other neuronal populations of interest.

WTH07-46

A novel model: optical stimulation causes axonal degeneration mediated by axoplasmic calcium

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Axonal degeneration contributes to neurological deficits in nervous system disorders, and is characterized by axonal swelling. Since energy failure and Na^+/Ca^{2+} overload play central roles in the axonal degeneration, abnormal functions of mitochondria are implicated in the axonal degeneration. However spatio-temporal changes of mitochondrial dynamics in relation to degenerative alterations of axons are still poorly understood. In this study, we investigated morphological changes and underlying mechanisms in the acute degeneration of sensory nerve axons observed with optogenetic stimulations, which enables spatio-temporal regulation of stimulations causing degenerative changes. Mixed dorsal root ganglion (DRG) cultures were obtained from rat embryo, and codon-optimized ChIEF (oChIEF), a channel rhodopsin variant, conjugated with red fluorescent mCherry was introduced into the DRG neurons with lentiviral vectors. Simultaneously, green Ca^{2+}-indicator, GCaMP3 or mitochondria-targeted green fluorescent Dendra2 (mitoDendra2) was introduced with other lentiviral vectors. Dominant negative mutant of Drp1, a mitochondrial fission protein (Drp1K38A), was also introduced in some cultures. Optogenetic stimulation of oChIEF caused axonal swelling following by axonal fragmentation in a manner dependent on duration of stimulation. GCaMP3 imaging demonstrated that axoplasmic Ca^{2+} increase precedes the axonal swellings, and treatments with Ca^{2+} chelators and Ca^{2+} channel blockers ameliorated the axonal swellings. Inhibition of mitochondrial fission by overexpression of Drp1K38A elongated stationary mitochondria, inhibited mitochondrial fragmentation upon optogenetic stimulation and decreased axonal swelling. During photo-stimulation, some axons exhibited rapid amelioration of axonal swelling, and the amelioration accompanied simultaneous decrease of GCaMP3 fluorescence. These results suggest that optical stimulation of channel rhodopsin variants causes axonal degeneration mediated by axoplasmic Ca^{2+} increase in sensory axons, and mitochondrial fission mediated by Drp1 exacerbates the initiation of axonal degeneration. Furthermore, intrinsic mechanisms reversing axoplasmic Ca^{2+} increase may be beneficial for axonal survival during Ca^{2+} induced axonal degeneration.

WTH07-47

Identification and characterization of novel dystonia musculorum mutant mice

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We identified a novel spontaneous mutant mouse showing motor symptoms that are similar to those of *dystonia musculorum (dt)* mouse. The observations suggested that the mutant mice inherited the mild *dt* phenotype as an autosomal recessive trait. Linkage analysis showed that the causative gene was located on chromosome 1, which are close to the dystonin (*Dst*) gene locus. To investigate whether *Dst* is the causative gene of the novel mutant, we crossed the mutant with *Dst* gene trap (Dst^{Gt}) mice. Compound heterozygotes showed a typical *dt* phenotype. Mutation analysis indicates a nonsense mutation in the spectrin repeat of plakin domain. The novel mutant mouse was named $Dst^{dt-23Rbrc}$. Histological analyses showed abnormal neurofilament (NF) accumulation in the nervous system of $Dst^{dt-23Rbrc}$ mice, which is characteristic of the *dt* phenotype. We mapped the distribution of abnormal NF-accumulated neurons in the brain and found that they were located specifically in the brainstem, spinal cord, and in regions such as the vestibular nucleus, reticular nucleus, and red nucleus, which are implicated in posture and motor coordination pathways. Therefore, we have identified a novel mutant allele of *dt*, which causes histological abnormalities in the central nervous system that may account for the abnormal motor phenotype. This novel spontaneously occurring mutant may become a good model of hereditary sensory and autonomic neuropathy 6, which is caused by mutations in human *DST* gene.

WTH07-48

Inhibition of HDAC4 with sodium butyrate does not prevent AMPA-induced excitotoxic degeneration of spinal motoneurons *in vivo*

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Selective motoneuron (MN) loss is the pathological hallmark of motoneuron diseases (MND). Chronic excitotoxicity is a fundamental mechanism proposed to explain this selective MN loss, which causes muscle denervation and atrophy. Muscle-nerve communication at neuromuscular junctions (NMJ) requires the synthesis and secretion of muscle-derived growth factors, and histone deacetylase 4 (HDAC4) activity in muscle has been

established as a critical regulator of NMJ maintenance (*Science* 326:1549, 2007). Furthermore, HDAC4 is overexpressed in skeletal muscle under conditions that result in NMJ disruption, such as nerve injury (*J Biol Chem* 282:33752, 2007) or MN disease (*Brain* 136:2359, 2013). These data suggest that HDAC4 inhibition might have protective effects against NMJ degeneration. Therefore, we studied the effects of the pan-HDAC inhibitor sodium butyrate (BA) in a model of chronic spinal MN death and paralysis induced by the chronic infusion of AMPA into the spinal cord of healthy rats using osmotic minipumps (*J Neuropathol Exp Neurol* 66:913, 2007). BA was administered intraperitoneally once daily for 6 days, beginning 1 day after osmotic minipump implantation. We observed that BA did not prevent paralysis, as assessed by two motor behavioral tasks, and did not reduce MN loss. In spite of this lack of protection, BA treatment induced an increase in histone H3 K9-14 acetylation in hindlimb muscles and spinal cord tissue, as determined by Western blots, suggesting HDAC inhibition by BA. These results indicate that either HDAC4 has only a minor role in excitotoxic MN death, or that BA inhibits other potential beneficial HDACs. In fact, recent reports have pointed out the relevant role of other HDAC members, such as HDAC7, in NMJ maintenance (*Muscle Nerve* 52:109, 2015). This work was supported by DGAPA, UNAM (Project IN204516) and CONACYT, México (project 240817). MP-G is recipient of a scholarship from CONACYT.

WTH07-49

Novel UBQLN2 mutations linked to amyotrophic lateral sclerosis and spastic paraplegia through defective proteolysis

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Amyotrophic lateral sclerosis (ALS), characterized by the degeneration of upper and lower motor neurons in the cortex, the brainstem and the spinal cord, is fatal, usually within 5 years. Frontotemporal dementia (FTD), due to the loss of frontal and temporal neurons, occurs in 15% of ALS patients. Most ALS cases are sporadic (SALS) whereas ~10% are familial (FALS). Mutations in *UBQLN2* have been associated with X-linked juvenile and adult forms of ALS and ALS/FTD. Ubiquilin-2 is a component of the ubiquitin inclusions detected in ALS spinal cord. We performed genetic analysis of 400 FALS and 770 SALS and identified three novel mutations in the PXX repeat domain of UBQLN2, a hot spot domain for ALS/FTD mutations. One of these mutations was also identified in patients with spastic paraplegia, affecting only the upper motor neurons of the limbs. These mutations, predicted to be deleterious, were absent from control databases. Experiments performed on patient lymphoblasts carrying these mutations showed that proteolysis pathways were improperly regulated. Our results confirm the role of PXX repeat in ALS pathogenesis, expand the clinical spectrum of *UBQLN2* mutations to spastic paraplegia phenotype and underline the pivotal role of ubiquilin-2 in proteolysis regulation pathways.

WTH07-50

Association between mitochondria and endoplasmic reticulum in dysmyelinated axons

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Myelin ensheathment maintains axonal integrity and enhances the transmission of electrical impulses along the axons. Diseases of myelin lead to axonal degeneration, but the underlying mechanisms are still unclear. Mitochondria associated membranes (MAM), the structural connections between endoplasmic reticulum and mitochondria, are important for physiological functions, such as Ca^{2+} signaling, mitochondrial lipid metabolism, and autophagy. Disruption of MAM has been implicated in mitochondrial dysfunction, which has been proposed as a major contributor of axonal degeneration in diseases of myelin. In this study, we investigated mitochondrial changes and the association with MAM in a chronic demyelination model. We used three dimensional ultrastructural analyses with serial block-face scanning electron microscopy (SBF-SEM). In mouse model of a chronic demyelination caused by extra-copies of proteolipid protein (PLP4e), most axons are myelinated at 1 month-old (mo) but chronic demyelination is observed at 5 months. Quantitative SBF-SEM analyses demonstrated that mitochondrial volume and surface areas and the total MAM areas of individual mitochondria were larger in the demyelinated axons of 5 months PLP4e compared with myelinated axons of 5 months wild-type mice. The increase of total MAM areas in demyelinated axons of PLP4e was attributable to enlargement of individual MAM, since MAM density (number of MAM / mitochondrial surface area) was similar. In the myelinated axons of 1 month PLP4e and wild-type mice, sizes of individual MAM and MAM density were similar. These results demonstrate usefulness of SBF-SEM in observing MAM and suggest that enlargement of MAM is caused by chronic loss of myelin. We propose increased MAM is beneficial for the mitochondrial functions and axonal survival in demyelinating diseases.

WTH07-51

Oligodendroglial conditional knockout of DARS2 results in white matter atrophy and neurobehavioral changes in mice

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Leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation (LBSL) is caused by mutations in *Dars2*, a gene encoding the mitochondrial enzyme aspartyl-tRNA synthetase. LBSL results in a rare, progressive, neurological disease that manifests as white matter signal abnormalities in the cerebral white matter and spinal cord, as well as slowly progressive dorsal column spasticity, dysarthria, and ataxia. To date, no animal model or treatment exists. Previous attempts to develop an animal model of LBSL through the complete or conditional neuronal knock-out of *Dars2* have been unsuccessful. Here, to mimic the clinical presentation in white matter, we developed a conditional knock-out of *Dars2* expression using Cre-lox recombination in *Pdgfrα*-expressing oligodendrocyte precursors. *Pdgfrα*$^{Cre+}$;*Dars2* $^{fl/fl}$ animals show a slight progressive behavioral phenotype with a reduction in both locomotor activity and rearing in open field over time, consistent with slowly progressive ataxia seen in LBSL. Preliminary data from these animals also suggests a reduction in oligodendrocyte transcription factor (OLIG2)-expressing cells per area in the corpus callosum and an overall reduction in corpus callosum area relative to age-matched control littermates, consistent with white matter abnormalities observed in the clinic. This novel mouse model has the potential to elucidate mechanisms of LBSL and may allow for translation to clinical discoveries for the treatment of LBSL.

WTH07-52

Quantification of GABA, glutamate and glutamine in a single measurement by magnetic resonance spectroscopy in human subjects

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Purpose: GABA and glutamate (Glu) are the major inhibitory and excitatory neurotransmitters in the brain and can be measured by magnetic resonance spectroscopy (MRS) *in vivo*, though GABA requires an additional measurement using the MEGA-PRESS editing sequence. Glu and glutamine (Gln) co-edit with GABA providing the possibility of measuring all three from a single MEGA-PRESS acquisition. Here we evaluate using phantom data whether Glu and Gln separation in GABA MEGA-PRESS can be achieved and use MEGA-PRESS spectra acquired *in vivo* to identify quality criteria of spectra from which Glu and Gln can be reliably estimated.

Method: Phantoms containing Glu, Gln, GABA and *N*-acetylaspartate at different concentrations were scanned using MEGA-PRESS optimized for GABA in a 3T *Philips Achieva* scanner. Spectra were also acquired *in vivo* from 5 different brain regions from 36 healthy volunteers. Quality assessment was performed on the data to determine the characteristics that were shared by spectra which returned Glu/Gln ratios in the physiological range after quantification using the QUEST routine in the jMRUI software package.

Results: Glu and Gln were estimated accurately in all phantoms with a linear relationship between measured and true concentration, R^2= 0.95 for Glu and R^2= 0.91 for Gln. The quality assessment framework was based on measurements from the spectra which, after quantification, returned physiological ratios of Glu/Gln (70% of all spectra). The signal-to-noise of the edited GABA signal, the linewidth of the *N*-acetylaspartate signal and the Cramer-Rao lower bound of the composite Glu + Gln signal were measured using AMARES in jMRUI. Data from the remaining 30% of spectra which had a Glu/Gln ratio outside the physiological range were from spectra which failed at least one of the quality criteria.

Conclusion: Glu and Gln can be reliably quantified from GABA optimized MEGA-PRESS acquisitions provided spectra meet certain quality criteria.

WTH08 Psychiatric Disorders and Drug Abuse

WTH08-01

Effects of nicotine on glia activation and dopaminergic system neurotoxicity induced by mdma in adolescent BALB/c mice

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The interaction between MDMA and Nicotine affects multiple brain centers and neurotransmitter systems (serotonin, dopamine and glutamate) involved in motor coordination and cognition. In this study, we elucidated the effect of prolonged (4 weeks) MDMA, nicotine and a combined Nicotine-MDMA treatment on motor-cognitive neural functions. In addition, we have shown the correlation between the observed behavioural change and neural structural changes induced by these treatments in BALB/c male mice. 20 Male periadolescent BALB/c mice (P.21) were treated for a period of 4 weeks with the vehicle, Nicotine, MDMA and Nicotine + MDMA. The control group received normal saline (subcutaneous, s.c). MDMA was administered subcutaneously to a set of 5 animals (2 mg/Kg body weight) at 2 days interval. The Nicotine treated group received 2 mg/kg BW of Nicotine daily while the Nicotine-MDMA group was treated with Nicotine (2 mg/Kg BW; s.c.) daily and MDMA (2 mg/ Kg; s.c.) at 2 days interval. We observed that MDMA (2 mg/Kg body weight; s.c) induced a decline in motor function, while Nicotine (2 mg/Kg body weight; s.c) improved motor function in male mice. In combined treatment, Nicotine reduced the motor function decline observed in MDMA treatment, thus no significant change in motor function for the combined treatment versus the control. Nicotine or MDMA treatment reduced memory function and altered dopaminergic and serotonergic, microglia and astrocytes activities in striatum and nucleus accumbens (core and shell). Similarly, a combined Nicotine-MDMA treatment reduced memory function when compared with the control.

WTH08-02

Proteomics and immunocytochemistry of rat neural stem cells, neurons and astrocytes exposed to alcohol: implications for FASD

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Neural stem cells obtained from rat embryos were exposed to various concentrations of ethanol (25 to 100 mM) for up to 96 h. There were no significant changes in the morphology of the cells but the numbers of neuron-like i.e. MAP- (microtubule associated protein 2-) expressing cells were reduced by ethanol in a dose-dependent manner, especially at 50 and 100 mM. The protein composition of the neural stem cells was analysed by proteomics (MALDI-TOF/Mass Spectroscopy) and, in the case of selected proteins, the changes were verified by western blotting. In the proteome analysis a total of 29 identified proteins were altered by ethanol (50 mM for 96 h) relative to ethanol-free control. Of these proteins some were related to cytoskelet (dihydropyrimidinase related proteins 2 and 3 involved in neural development and remodelling), others were involved in transcription/translation (e.g. nucleophosmin, dead end homolog protein, heterogeneous nuclear ribonucleoproteins H and C, and spliceosome RNA helicase bat1), energy metabolism (e.g. enolase-α and ADP-ribosylarginine hydrolase), signal transduction (e.g. RAB GDP dissociation inhibitors α and β, serine/threonin protein phosphatase and ras homolog gene family, member G) and oxidative stress (glutathione-S-transferase and heat shock proteins HSP 60, 70 and 90). Two of the proteins, nucleophosmin (NPM) and dead end protein homolog 1 (DND1) were further studied by immunocytochemical techniques in cultured neurons and astrocytes. NPM and DND1 displayed a similar pattern of ethanol-induced changes in both types of cells. Thus the ethanol exposure may alter and disturb a range of mechanisms needed for the normal function of neural stem cells eventually leading to the proliferation of seriously impaired neurons and glia. The processes of development, differentiation and repair using such cells in ethanol-exposed brains could result, *inter alia*, in abnormalities such as those typically encountered in the foetal alcohol spectrum disorder and/or in alcoholism later in life.

WTH08-03

Determination of neurosteroids in patients with internet addiction disorder by liquid chromatography-tandem mass spectrometry

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Neurosteroids are synthesized in the nervous system from cholesterol or steroidal precursors and regulate various functions such as development, neuronal plasticity, cognition, mood control, and social behavior in the central and peripheral nervous system. In this study, to investigate the alteration of neurosteroids in urine from patients with internet addiction disorder, an improved analytical method was developed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Urine samples were extracted by an Oasis HLB extraction cartridge after enzymatic hydrolysis with β-glucuronidase/arylsulfatase cocktail. Neurosteroids were separated using Waters ACQUITY @ BEH Phenyl column (2.1 × 100 mm, 1.7 μm) and a mobile phase consisting of eluent A (0.1% acetic acid in 95% water) and eluent B (0.1% acetic acid in 95% acetonitrile) with a gradient program at a flow rate of 0.4 mL/min and were monitored in Multiple Reaction Monitoring (MRM) mode by tandem mass spectrometry (MS/MS). The alteration of neurosteroids in human urine and excretion pattern may play important role to understanding probable internet addiction disorder, and the described methods could be used to evaluate and monitor patients with internet addiction disorder.

WTH08-04

Impairments in laterodorsal tegmentum to VTA projections underlie glucocorticoid triggered reward deficits

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Ventral tegmental area (VTA) activity is critical for motivated behaviours and reinforcement. Importantly, VTA activity is tightly modulated by afferents arising from the laterodorsal tegmentum (LDT). Disruption of this circuit can ultimately increase the risk for the development of neuropsychiatric disorders, including those associated with reward deficits, such as depression, anxiety, obsessive-compulsive disorder, obesity, addiction or antisocial behaviour. Additionally, the VTA region is particularly vulnerable to the effects of stress/glucocorticoids (GCs). Previous studies revealed that *in utero* exposure to glucocorticoids (iuGC) triggers prominent reward deficits later in life but nothing is known about the impact of this exposure in the LDT-VTA circuit.

Here, we show that iuGC animals have long-lasting changes in the expression of cholinergic markers in the LDT, and *in vivo* singe-cell electrophysiology revealed that LDT basal activity was decreased.

Interestingly, we observe a bidirectional effect in LDT-VTA inputs: upon LDT stimulation, iuGC animals present a decrease in the magnitude of excitation and an increase in the magnitude of inhibition in the VTA. While in control animals most of the inhibitory responses arise from putative GABAergic neurons, in iuGC group there is a shift in the type of cells presenting inhibitory responses, with a significant increase in the number of dopaminergic neurons.

In agreement with LDT-VTA dysfunction, we show that iuGC animals present motivational deficits that are rescued by selective optogenetic activation of this pathway. Importantly, we also show that LDTVTA optogenetic stimulation is reinforcing, and that iuGC animals are more susceptible to the reinforcing properties of LDT-VTA stimulation.

WTH08-05

Cannabidiol (CBD) treatment decreased the sensitized locomotor response to repeated methamphetamine exposure in male rats

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Background: Cannabidiol (CBD) is a non-psychoactive component of the cannabis plant and is showing potential as a promising treatment for mental disorders, such as psychosis. An animal model that is commonly used to mimic the neurochemical changes underlying psychosis is methamphetamine (METH) sensitisation, where repeat administration of the psychostimulant progressively increases locomotor activity. The ability of CBD to modulate METH-induced psychosis within a preclinical setting has not yet been examined. The aim of this study was to determine, in a preclinical psychosis model, whether CBD could attenuate locomotor activity in methamphetamine (METH)-induced sensitisation rats.
Methods: Male Sprague Dawley rats (n = 38) were subjected to daily METH (1 mg/kg days 2 and 8, 5 mg/kg days 3–7; i.p.) or saline (1 mg/kg; i.p.) injections for 7 days. After 3 weeks of withdrawal, METH-induced locomotor sensitisation was examined across 3 challenge days, whereby rats received a CBD injection (0, 40 or 80 mg/kg; i.p.) followed by a METH (1 mg/kg) or saline injection 30 min later. Locomotor activity was then measured for 60 min.
Results: Rats pretreated with METH showed a significant sensitised locomotor response on the veh + METH challenge day when compared to saline controls. Furthermore, METH-induced sensitisation was reduced following CBD treatment at both 40 and 80 mg/kg. In rats chronically treated with saline, CBD administration at 40 mg/kg significantly reduced acute METH-induced locomotor activity.
Conclusion: These results demonstrate that 40 and 80 mg/kg doses of CBD were able to reduce locomotor activity in METH-sensitised rats and may provide avenues of drug development for the reduction of behaviours associated with chronic METH abuse.

WTH08-06

Methamphetamine and modafinil elicit differential epigenetic and functional profiles in the mouse medial prefrontal cortex

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Methamphetamine (METH) addiction presents with specific behavioral alterations that suggest long-lasting changes in gene regulation within brain nuclei of the reward circuitry, including the medial prefrontal cortex (mPFC). METH negatively impacts the mPFC function, leading to decreased function and longstanding cognitive decline both in humans and animal models. Given the persistence of the addiction phenotype at both behavioral and transcriptional levels, increasing evidence implicate epigenetic mechanisms of gene regulation behind the neurobehavioral adaptions induced by psychostimulants. Also, psychostimulant drugs are known by their pro-cognitive effects, in part by its ability to increase PFC function, such as modafinil. Interestingly, modafinil has shown little abuse liability. The aim of the present study is to identify differential markers of METH and modafinil actions on epigenetic and functional targets in the mPFC, that may help identify pathways associated with addictive versus cognitive enhancing traits of these stimulants. Mice received METH (1 mg/kg) or modafinil (90 mg/kg) *single dose acute treatment* (sacrifice 1 hr later) or *subchronic daily7 days-treatment* (sacrifice withdrawal day 4). METH single dose treatment induced paired-pulse facilitation of EPSCs in D1-expressing layer V pyramidal neurons (patch clamp in BAC-Drd1a-tdTomato), suggesting reduced presynaptic probability of glutamate release, whereas modafinil had no effect. We found reduced dopamine receptors Drd1a and Drd2 mRNA expression after METH, whereas modafinil increased expression of Drd2 and c-

Fos compared to controls. Both stimulants acutely decreased H4ac and increased H3ac, HDAC2 and NMDA GluR1, compared to controls. H4ac, HDAC2 and GluR1 effects were blocked by D1 antagonist pretreatment, whereas H3ac effect was not. Subchronic METH and modafinil decreased H4ac and GluR1 expression, whereas only METH showed decreased H3ac and HDAC2. These differences could be related to METH-dependent detrimental effects on mPFC versus the pro-cognitive profile induced by modafinil in experimental and clinical settings.

WTH08-07

Differential role of CB1 receptors within accumbal subregions in stress-induced reinstatement of cocaine-conditioned preference

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Stress is considered one of the most important factors known to induce relapse in human addicts and in animal models of drug addiction. Research from our laboratory demonstrates that, using a conditioned place preference (CPP) paradigm, an acute restraint stress exposure triggers reinstatement of cocaine-CPP. With regard to the neurobiological mechanisms underlying relapse, there are numerous evidences for the participation of the endocannabinoid system (ECS), primarily through their actions at the widely distributed CB1 receptors (CB1R). Nevertheless, the role of ECS in stress-induced reinstatement has not been extensively studied. Considering that subregions of the Nucleus Accumbens (NAc) contribute significantly, but differently, to the impact of drug and stress on addiction, the present study has been designed to evaluate the involvement of CB1R within Core and Shell compartments of NAc in a restraint stress–induced reinstatement model. Male Wistar rats (220–300 g) that extinguished cocaine-CPP were microinjected into the Core or into the Shell of NAc with a CB1R agonist (ACEA; 0.001 or 0.01fmol/side) or a CB1R antagonist (AM251; 5 or 10ug/side), subsequently assigned to the different treatments of restraint stress exposure and then tested for reinstatement of cocaine-CPP. Results show that the intra-Core administration of AM251 abrogated restraint stress-induced reinstatement, and ACEA facilitated reinstatement after a non-reinstatement stress exposure. Moreover, the facilitating effect of ACEA was prevented by pretreatment with a microinjection of AM251. Interestingly, these effects were not observed after CB1R ligands microinjection into the NAc Shell compartment. Our results support the hypothesis of the preferential influence of CB1R within NAc Core, but not Shell, in the reinstatement of cocaine seeking behavior. This conclusion is in accordance with previous results of our lab that demonstrate the preferential role of glutamatergic transmission within NAc Core in the same model. Futures studies will attempt to confirm a possible glutamate dependent mechanism underpinning the effects of CB1R ligands on the restraint stress-induced reinstatement of cocaine-CPP responses.

WTH08-08

Effects of 4-phenylbutyric acid in abnormal behavior-displayed mice

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Valproic acid (VPA) is known as an anti-convulsant and a mood stabilizer. However, it has shown that exposure to VPA in pregnant causes high risk of autism and cognitive deficits in offspring mice. Previously, we reported that the offspring is under high endoplasmic reticulum stress. In addition, we found that the neuron of the offspring might suppress neurite outgrowth such as axon and dendrite in the process of embryonic development. 4-Phenylbutyric acid (4-PBA) is known as a chemical chaperone and a histone deacetylase inhibitor. In this study, we investigated the effects of 4-PBA in brain of the offspring. We injected VPA into pregnant mice at 12.5 days gestation. The offspring born from VPA-treated mothers were subjected to the experiment as abnormal behavioral mice. Prenatal exposure to 4-PBA did not improve abnormal behaviors such as locomotor activity and social communication in the VPA-treated offspring. However, 4-PBA led to improvement of decreased postsynaptic Shank3, density protein 95, neuroligin 1 and cell adhesion molecule 1 expression in cerebral cortex. These molecules have relevance to the pathogenesis of autism spectrum disorders (ASD). Therefore, prenatal exposure to 4-PBA have no influence on abnormal behaviors induced by VPA but, nevertheless, 4-PBA improved expression of the synaptogenic factors which related to ASD in cerebral cortex.

WTH08-09

Translational control by EIF2ALPHA regulates acute and persistent effects of cocaine

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Drug addiction is a major global mental health problem; however, the underlying neurobiological mechanisms remain elusive. While the effects of drugs of abuse require *de novo* protein synthesis, the translational control mechanism(s) targeted by drugs of abuse are not known. Our goal is to understand: a) how acute exposure to drugs of abuse usurp specific translational control mechanism(s), and b) how it leads to persistent changes in the reward circuits in the brain to cause maladaptive reward learning and reinforce compulsive drug-seeking behavior.

We discovered that translational control by phosphorylation of eukaryotic initiation factor 2 α-subunit (p-eIF2α) regulates the vulnerability to the effects of cocaine. We found that cocaine reduces p-eIF2α levels in the ventral tegmental area (VTA)—a key reward center in the brain—more readily in adolescent mice compared to adults. Specifically, in adolescent mice but not in adults, a sub-threshold dose of cocaine reduced p-eIF2α levels and potentiated synaptic inputs onto dopaminergic neurons in the VTA, and elicited drug-reinforced behavior (place preference).

Strikingly, in a series of gain- and loss-of-function experiments, we found that increasing or decreasing p-eIF2α levels genetically or pharmacologically render mice more resistant and more vulnerable, respectively, to the acute effects of cocaine. Consistent with these

findings, metabotropic glutamate receptor-mediated long-term depression—whose disruption is postulated to increase vulnerability to addiction—was impaired in the VTA of both adolescent mice and adult mice with reduced p-eIF2α-mediated translation.

Moreover, we also found that genetically or pharmacologically reducing p-eIF2α-mediated translation facilitates the progression of the transient effects of acute cocaine to a more persistent one. Taken together, our data suggest that: a) cocaine hijacks p-eIF2α-mediated translational program to elicit synaptic potentiation in VTA dopaminergic neurons that contributes to addiction-related behavior, and b) p-eIF2α-mediated translation could be a key mechanism gating the progression from transient to persistent effects of cocaine. Thus, modulating p-eIF2α mediated translation could be a therapeutic approach to prevent the persistent effects of drugs of abuse and may hold promise for new treatments for addiction.

WTH08-10

Investigating the role of dopamine receptor- and parvalbumin-expressing neurons in extinction and retrieval of conditioned fear

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Pavlovian fear conditioning and extinction have been studied extensively in the laboratory to understand learning and memory processes. There is significant interest in finding ways to enhance the strength of extinction learning, as it forms the basis for exposure therapy used to treat many anxiety disorders. A better understanding of the neurobiological basis of extinction is a critical step in achieving this goal. The aim of the present study was to examine the pattern o activation of neurons that express dopamine receptors 1 and 2 (D1R and D2R), and parvalbumin (PV) in mice that underwent extinction or retrieval of a fear memory. Adult male transgenic mice expressing D1R or D2R tagged with green fluorescent protein (GFP) were fear conditioned with 6 tone-shock pairings. The following day they were randomly divided into one of four experimental groups: handled, context, retrieval or extinction. Extinction groups were exposed to 45 tone presentations, retrieval groups were exposed to 5 tone presentations and the context groups were exposed to the extinction context without any tones. 90 min following their assigned treatment, mice were perfused and brain tissue processed for Fos/GFP/PV immunohistochemistry. The number of Fos, GFP and PV expressing cells were quantified in the prelimbic cortex (PrL), infralimbic cortex (IL) and basolateral amygdala (BLA). Extinction led to increased Fos expression in the IL and a decrease in the number of D2R+ cells in the IL compared to all other groups. Fear memory retrieval resulted in increased activation of D2R+ cells in the PrL compared to all other groups. These results highlight the complexity of dopamine's involvement in fear retrieval and extinction learning, and provide nuanced insights into the roles of specific dopamine receptor subtypes. This will be valuable for informing future research that aims to strengthen extinction learning via dopaminergic mechanisms.

WTH08-11

Sexually dimorphic function of dopamine in the postnatal neurodevelopment

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Many neuropsychiatric disorders share both developmental and dopaminergic hypotheses. Several studies showed that dopamine (DA) regulates neurogenesis, and we recently showed that DA is involved in GABAergic system development. However, these are early neurodevelopmental processes and it is possible that DA has different functions in postnatal brain development as well. The first five postnatal days of mouse brain development are important for synaptogenesis. Because of this, we investigated if DA imbalance at this developmental window affects prepubertal mouse behavior.
Objective: Evaluate the prepubertal behavioral consequences of dopaminergic imbalance in the first five postnatal days of mice.
Methods: Newborn Swiss mice were i.p. daily treated with saline, L-DOPA/benserazide (10/5 or 50/25 mg/kg) or quinpirole (0,5 mg/kg) from P1 to P5. Naïve and sham groups were also used as control. At 1 month of age, animal's behavior were investigated by open field test (OFT) and elevated plus maze (EPM). Another group, that were also treated with saline or L-DOPA/benserazide from P1 to P5, received an acute treatment (saline or L-DOPA/benserazide) 30 min before going through the tests. Data were analyzed using One Way ANOVA/One Way ANOVA on Ranks and Tukey test.
Results: We observed a decrease of total distance in all females treated, but not males (OFT). We also observed an increase in the number of rearings in females treated with Quinpirole and males treated with L-DOPA (OFT). In EPM test, only females treated with Quinpirole showed a decrease in the number of entries and time in open arms. Males previously treated with L-DOPA and acutely challenged with L-DOPA before the tests showed a higher decrease in the number of rearings in OFT and EPM, but not females.
Conclusion: Our data suggest a sexually dimorphic function of DA in the postnatal neurodevelopment. The DA challenge also suggest a sexually dimorphic consequence in the dopaminergic signaling when there is DA imbalance in early postnatal development. However, further studies are necessary to understand the molecular and biochemical mechanisms involved in this regulation.

WTH08-12

Ethanol withdrawal affects the mechanism of fear memory labilization in the basolateral amygdala complex: effect of D-cycloserine

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Contextual fear memory formed under withdrawal from chronic ethanol consumption is resistant to pharmacological interference with the reconsolidation process; indicating a resistance to the occurrence of the labilization phase after recall. In addition, pre-retrieval D-cycloserine (DCS) administration facilitates the reconsolidation interference of this resistant memory. The molecular mechanisms underlying the influence of ethanol withdrawal or DCS on fear memory labilization have not been established yet. Thus, here we evaluated the ubiquitin-proteasome system (UPS) activity in

the basolateral amygdala complex (BLA) after fear memory retrieval in ethanol withdrawn (ETOH) animals, and the influence of DCS on this molecular pathway. For this, we examined the polyubiquitinated proteins levels by Western Blot and proteasome chymotrypsin-like activity by enzymatic assay. Male Wistar rats were made dependent via an ethanol containing liquid diet (6% v/v) for 14 days. The respective control (CON) group was pair-fed with the same diet without ethanol. Contextual fear conditioning was performed on day 3 of withdrawal. Seven days after, rats were subjected or not to memory retrieval and were sacrificed 60 min later. In addition, separated CON and ETOH animals received DCS (5 mg/kg, i.p) or saline (SAL) injection 30 min before retrieval and were sacrificed 60 min later. Our results indicated that the retrieval only induced an increase in polyubiquitinated proteins expression and proteasome activity in the BLA from CON rats, whereas those effects were not observed in ETOH rats. These animals showed UPS activity patterns similar to those of the groups not subjected to retrieval. In the second experiment, we observed that ETOH rats treated with DCS before retrieval displayed elevated and similar UPS activity to CON rats after recall. In summary, ethanol withdrawal affects the neurobiological mechanisms involved in the fear memory labilization and DCS favors this molecular pathway in ethanol ETOH rats.

WTH08-13

Cocaine reward susceptibility is related to pubertal risk-taking behaviour in prenatally stressed offspring

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Gestational stress induces long-lasting neurochemical changes in the offspring and increases vulnerability to drug-seeking behaviour during adulthood. Since sensitivity to drug-induced reward is highly heterogeneous among individuals there is an urgent need to recognize predictive factors of drug reward vulnerability for a proper diagnosis and development of effective treatments. The aim of the present study was to identify early behavioural traits related to an adult increased vulnerability to cocaine reward. Employing a prenatal restraint stress model in rats, we evaluated novelty response, anxiety-like and risk-taking behaviours during puberty and its relationship with individual differences in cocaine-induced conditioning place preference during adulthood. Our results show that prenatal stress impacts differently in the pubertal offspring behaviour leading to two different populations: a low anxiety/high risk-taking population during puberty that will search for the rewarding properties of cocaine later in life and a high anxiety/low risk-taking population with low preference for cocaine during adulthood. This study clearly underscores the importance of early detection of behavioural traits opening the possibility of timely intervention to avoid the devastating consequences of drug addiction later in life. Moreover, studying individual differences of drug responsiveness is a key strategy to understand the underlying molecular mechanisms of vulnerability or resilience to the establishment of substance use disorders following drug exposure.

WTH08-14

Neonatal exposure to estradiol valerate increases morphine-induced locomotor activity and accumbal dopamine release in adult rats

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Neonatal exposure to sex hormones reprograms reproductive and non-reproductive tissues such as the brain in adult rats. Our lab has demonstrated that neonatal administration to Estradiol Valerate (EV) and Testosterone Propionate (TP) increases the content and release of dopamine (DA) in brain areas related to reward and locomotion in adult rats. On the other hand, it has been shown that sex hormones can modulate the expression of the mu-opioid receptor in different brain areas. Therefore, neonatal reprogramming with hormones could alter morphine response during adulthood in rats and predispose to addiction.

Our results show that locomotor activity induced by morphine is higher compared to locomotor activity induced by saline. However, neonatal reprogramming with EV significantly increases morphine-induced locomotor activity compared to control male rats. This increase in locomotor activity induced by morphine in EV rats is associated to a greater NAcc DA release induced by morphine compared to control male rats.

These results demonstrate that neonatal reprogramming with EV increases sensitivity to morphine effects possibly through increasing expression of mu-opioid receptors in GABAergic interneurons of the ventral tegmental area.

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WTH08-15

Lack of the SEZ6 protein attenuates cocaine relapse

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A significant problem of cocaine dependency is the propensity to relapse to cocaine-use even after extended periods of abstinence. A novel protein that influences cocaine relapse is Seizure-related gene 6 (Sez6). Sez6 is a neuron-specific protein, highly conserved between mice and humans, which plays an essential role in dendritic branching, dendritic spine formation and excitatory synaptic transmission. In the mature brain, Sez6 is strongly expressed in the dorsal striatum and nucleus accumbens (NAc), integral brain structures of the mesocorticolimbic dopamine pathway, which is pathologically altered by repeated exposure to cocaine. To investigate the function of Sez6 in cocaine dependence, mice with conditional deletion of Sez6 in all CaMKIIα-expressing forebrain projection neurons (Sez6 cKO) underwent investigator-administered (cocaine conditioned place preference (CPP)) and intravenously self-administered (IVSA) cocaine conditioning paradigms. Sez6 cKO and control mice

demonstrated equivalent cocaine CPP and stable self-administration of cocaine. However, following extinction of cocaine CPP and a subsequent low-dose cocaine prime, Sez6 cKO mice did not reinstate cocaine-seeking behaviour, unlike control mice. This behaviour was correlated with a reduced number of mature dendritic spines on NAc core medium spiny neurons of Sez6 cKO mice, compared to controls, immediately after cocaine-primed reinstatement. Similarly, after 1 month of abstinence from stable cocaine IVSA, Sez6 cKO mice did not relapse to cocaine-seeking upon presentation of drug-associated cues to the same extent as control mice. Together, these data indicate that Sez6 plays an important role in the synaptic changes that underpin cocaine relapse.

WTH08-16

Synthetic oxytocin-like treatment decreased the sensitized locomotor response to repeated methamphetamine exposure in male rats

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Background: Synthetic Oxytocin-Like Compound-1 (SOC-1) is a new compound that is currently being investigated for it's oxytocin-like effects in reducing drug dependence and social deficits. Repeat administration of the psychostimulant methamphetamine (METH) is associated with progressively increasing locomotor activity; a phenomenon described as behavioural sensitisation. As SOC-1 has been minimally investigated for it's modulation of METH-related behaviours, the aim of this study was to determine whether SOC-1 could reduce the heightened locomotor activity evident in METH sensitised rats.
Methods: Male Sprague–Dawley rats (n = 44) were subjected to daily METH (1 mg/kg on days 2 and 8, 5 mg/kg on days 3-7; i.p.) or saline (0.9%; i.p.) injections for 7 days. After 5 weeks of withdrawal, behavioural changes were examined over 4 challenge days where SOC-1 (0, 2.5, 5, and 10 mg/kg; i.p.) was administered 5 min prior to METH (1 mg/kg) or saline, after which locomotor activity was measured for 60 min.
Results: Rats pretreated with METH showed a significant sensitised locomotor response on the veh + METH challenge day when compared to saline controls. Additionally, SOC-1 dose-dependently reduced locomotor activity in METH sensitised rats when compared with saline. A significant difference in locomotor activity was also evident when comparing the 2.5 mg/kg and 10 mg/kg SOC-1 doses.
Conclusion: These results show that all doses of SOC-1 were able to reduce locomotor activity in METH-sensitised rats and may have specific effects to reduce behaviours associated with chronic METH abuse.

WTH08-17

Redox regulation via alpha-lipoic acid influences cocaine reinstatement behaviour

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Substance use disorders impose a heavy burden on affected individuals and society yet current treatment options are limited. There is thus a pressing need to more closely examine pathophysiological mechanisms underlying these disorders if new therapeutic options are to be developed. Elevated dopaminergic neurotransmission is the primary means by which drugs of abuse elicit a feeling of reward. However, this enhanced dopaminergic signalling is a source of reactive oxygen species and alters redox status with downstream effects on drug-induced neuroplasticity, signalling and behaviour. We therefore sought to determine whether targeting redox regulation with the antioxidant alpha-lipoic acid (ALA) may moderate cocaine extinction and/or reinstatement behaviour using a self-administration model and place preference paradigm. Male Sprague–Dawley rats were randomly assigned to receive either ALA (n = 7) or PBS (n = 6) and were trained to self-administer cocaine in operant chambers using a fixed ratio schedule. After successful acquisition of cocaine self-administration, rats underwent 3 weeks of extinction training followed by cue-primed reinstatement. The number of presses on the cocaine-paired lever was recorded across the experiment. To investigate whether ALA is intrinsically rewarding, we employed a compressed 7-day conditioned place preference paradigm with rats divided into 4 treatment groups: PBS (n = 8), ALA (n = 9), ALA and the μ opioid receptor agonist Naloxone (n = 8), or ALA and the dopamine type 1 receptor antagonist SCH23390 (n = 9). Analysis of the self-administration data indicated that ALA and PBS groups did not differ in their acquisition of cocaine self-administration; however, ALA reduced active lever pressing during reinstatement ($t(11)=4.298$, p = 0.001). Rats did not display any preference for the ALA-paired chamber in the place preference paradigm, and rats that received SCH23390 or Naoloxone did not differ to their ALA-treated counterparts. Combined these results suggest that ALA reduces the motivation to consume cocaine without eliciting reward itself. Moreover, these results provide further evidence that redox regulation of drug-induced pathophysiology is a valid target in the search for new therapies.

WTH09 Mechanism of Neuroprotection

WTH09-01

Cognitive-enhancing effects of beta-sitosterol in vanadium-induced neurotoxicity in mice

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Environmental discharge of vanadium causes physiological, cognitive and behavioural impairments in humans and animals via production of reactive oxygen species leading to lipid peroxidation and alteration in antioxidant defence system. The current study was carried out to investigate the cognitive-enhancing ability of beta-sitosterol in vanadium-induced neurotoxicity.

Forty eight mice were divided into four groups (A-D). Group A (control) received distilled water, B (standard group); α-tocopherol (500 mg/kg) every 72 hrs orally and sodium metavanadate (3 mg/kg) intraperitoneally (i/p), C; a single oral dose of β-sitosterol (100 μg) and sodium metavanadate (3 mg/kg) i/p while group D received sodium metavanadate (3 mg/kg) only i/p. All experimental groups received treatment for 7 consecutive days.

Cognitive, locomotor and antioxidant activities were evaluated by behavioural tests (Morris water maze, open field and hanging wire tests), antioxidant enzymes assay (catalase, SOD, GPx, GSH), and oxidative stress markers (MDA, NO and H_2O_2) measurements respectively. Immunohistochemical expression of Myelin Basic Protein (MBP) in the brain was also studied.

Beta-sitosterol significantly attenuated spatial learning deficits; improved motor coordination and reduced anxiety in vanadium neurotoxicity even better than the standard. Significant ($\alpha \leq 0.05$) increase in free radicals formation, decreased antioxidant enzyme activities, structural damage to myelin sheaths and decrease expression of MBP were observed in the sodium metavanadate only group, co-administration of beta-sitosterol however decreased these pathologic features and immunohistochemistry features were not significantly different from control mice.

The present study revealed that β-sitosterol possesses potent cognitive-enhancing, antioxidant and myelo-protective activities.

WTH09-02

LPS preconditioning attenuates neuroinflammation via gene reprogramming in rat model of epilepsy

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Background: Neuroinflammation seems to contribute to epileptogenesis, and reciprocally, prolonged seizures induce inflammation. Based on this, modulation of inflammation in the brain can be offered as one of the therapeutic approach to attenuate epileptic seizures. Preconditioning with sub-lethal dose of lipopolysaccharide (LPS) represented a state of neuroprotection to attenuate brain damage in rodent models. The purpose of this study was to clarify the effect of single brain LPS preconditioning on inflammatory profile induced by epileptic seizure in Pentylenetetrazol (PTZ) model of epilepsy.

Method: To determine the neuroprotective effect of brain LPS preconditioning on inflammatory profile induced by PTZ, all animals were evaluated for seizure duration. In addition, the effect of the LPS preconditioning on neuronal damage is observed in the hippocampal regions by histological assessments using nissl staining. Finally, the molecular assays were applied to analyze gene and protein expression associated with different cascades induced by LPS preconditioning such as TLR4 and inflammatory signaling pathways.

Result: Preconditioned animals performed significantly better on the behavioral tests to decrease seizure duration. Furthermore, this data were also consistent with the histological assessments. Additionally, molecular analysis showed that LPS preconditioning was accompanied by a reduction in pro-inflammatory mediators, whereas the expression of anti-inflammatory markers increased during tolerance. Additionally, expression of NFκB inhibitors such as SHIP1 and TOLLIP, which are known for their function in the reduction of pro-inflammatory reaction, was also enhanced. These findings were confirmed by western blot analysis.

Conclusion: Reduction in inflammatory response after LPS preconditioning may contribute to the induction of tolerance to epileptic seizures. This neuroprotection parallel the reprogramming strategy that leads to the synthesis of new markers to change molecular response against brain lesions. Altogether, our findings demonstrate that LPS preconditioning has a therapeutic effect on the modulation of neuroinflammation and this could suggest a promising therapeutic strategy for various neuronal disorders such as epilepsy.

WTH09-03

Dihydroprogesterone treatment restores myelin lipid profile in rat cerebral cortex

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Diabetes causes functional and structural changes in the nervous system leading to complications known as peripheral neuropathy, and encephalopathy. While the causes of diabetic peripheral neuropathy have been studied, the impact of diabetes on central nervous system (CNS) and its myelin compartment remains elusive. CNS myelin is a specialized membrane with high lipid to protein ratio to sustain CNS structure and function. Studies performed in an experimental model of diabetes using streptozotocin (STZ) injection revealed that the gene expression of some myelin proteins was significantly decreased by the pathology. Instead, myelin is highly enriched in lipids, such as cholesterol, glycosphingolipids and

plasmalogens contributing to myelin maintenance. Despite their important role, the effect of diabetes on myelin lipid profile of CNS has been poorly studied.

Data obtained by mass spec experiments showed that 3 months of diabetes induced an extensive impact on the levels of phosphatidylcholines (Ctrl: 376 ± 55 pg/μg of protein vs. STZ: 205 ± 41 pg/μg of protein, $p < 0.05$) and phosphatidylethanolamines (Ctrl: 602 ± 21 pg/μg of protein vs. STZ: 292 ± 46 pg/μg of protein, $p < 0.001$), plasmalogens (Ctrl: 153 ± 11 pg/μg of protein vs. STZ: 66 ± 12 pg/μg of protein, $p < 0.001$) as well as phosphatidylserines (Ctrl: 119 ± 23 pg/μg of protein vs. STZ: 45 ± 11 pg/μg of protein, $p < 0.001$) and phosphatidylinositols (Ctrl: 9.7 ± 0.2 pg/μg of protein vs. STZ: 4.03 ± 0.9 pg/μg of protein, $p < 0.01$). In addition, the levels of cholesterol (Ctrl: 6.1 ± 1.5 ng/μg of protein vs. STZ: 2.9 ± 0.55 ng/μg of protein, $p < 0.01$) and myelin basic protein were also decreased in the myelin of the same brain area. Interestingly, 1-month treatment with a neuroprotective molecule such as dihydroprogesterone, a metabolite of progesterone, restored the lipid and protein myelin profiles to the levels observed in non-diabetic animals.

Given the key functional and structural roles of lipid and protein in myelin, our data indicate, for the first time, that cerebral cortex myelin is severely compromised in diabetic status.

WTH09-04

Tetrahydroisoquinoline in ayurvedic medicine is neuroprotective in experimental Parkinson's disease

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Ancient '*Ayurvedic'* medicine from India could be an unexplored treasure for treating the incurable and debilitating neurodegenerative Parkinson's disease (PD). *'Kampavata'* the Vedic analogue of PD indicates *Ayurvedic* herbal formulation that could be neuroprotective. Tetrahydroisoquinoline (TIQ) an identified *Ayurvedic* alkaloid was assessed for its potential therapeutic effect *in vitro* and *in vivo* models for PD. *In vitro*, murine neuronal (Neuro2a), microglial (EOC20) and astrocytic (C8D30) cells exposed to MPP^+ dopaminergic neurotoxin were treated with TIQ (0.1–10 μM) for 24 h. Using indirect contact of dbcAMP-differentiated dopaminergic Neuro2a cells with astrocytes + microglia, mitochondrial superoxide radicals and survival within neurons was determined by Live Dead assay and MitoSOX flow cytometry. TIQ (10 μM) treatment significantly attenuated (37%) MPP^+-induced loss of live (Calcein AM-positive) differentiated neurons compared to MPP^+ alone. Further, MPP^+-induced mitochondrial accumulation of toxic superoxide radicals in dopamine neurons was significantly reduced (30%) by TIQ (10 μM). *In vivo,* adult C57/BL6 MPTP-PD mice (acute intraperitoneal MPTP 16 mg/kg dose, 4 times at 2 h intervals) were administered TIQ (per-oral 200 mg/kg body weight, bi-daily, 7 days post MPTP). Control mice were PBS injected or gavaged with TIQ alone. TIQ ameliorated dopaminergic neurotoxicity in mice causing a significant 16% increase in striatal dopamine level on the 7th day post-MPTP as detected by HPLC electrochemistry. Western blot for striatal expression of tyrosine hydroxylase (TH) the rate limiting enzyme of dopamine synthesis revealed 1.2-fold upregulation from its diminished expression post MPTP intoxication. This study indicates the anti-parkinsonian neuroprotective potential of TIQ in MPP^+-exposed cell co-culture and in MPTP mice. TIQ-mediated neuroprotection reflects through reduction in MPP^+-induced toxic buildup of mitochondrial superoxide radicals along with recovery in striatal dopamine levels and tyrosine hydroxylase expression. Knowing the molecular basis for the neuroprotective efficacy and safety of TIQ shall assist in translational therapeutic benefit in PD patients.

WTH09-05

Astrocyte-derived exosomes reduce infarct volume and improve neurological recovery in an *in vivo* model of focal cerebral ischemia

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Stroke is the second cause of death worldwide and induces permanent disabilities in surviving patients. Nowadays, the endogenous mechanisms that participate in neuronal rescue after stroke are not fully elucidated. Therefore, we aimed to assess how different cellular components of the brain interact to promote tissue recovery by establishing pathways of intercellular communication driven by exosomes. It has been proposed that exosomes derived from different cell types can mediate cellular responses able to reduce damage in neurological diseases, but there is limited information about their role in the protection against ischemic stroke. Here, we explored whether astrocytes contribute to protect neurons after ischemia by releasing exosomes. For this, we cultured rat primary cortical astrocytes under normoxia conditions for 48 h and collected the exosomes released to the medium. We also collected exosomes from astrocytes subjected to 6 h of hypoxia followed by 48 h of recovery. We tested whether theses vesicles induce a protective effect in the brain of rats subjected to experimental stroke, induced by a 90 min occlusion of the middle cerebral artery (tMCAO) in adult rats. Exosomes suspensions collected under normoxia and hypoxia were injected into the lateral ventricle of stroked rats 30 min after reperfusion. After 24 h of stroke, we analyzed the effect of exosomes administration on the infarct volume, neuronal survival, blood-brain barrier (BBB) integrity and neurological outcome. Notably, exosomes derived from astrocytes cultured under normoxia significantly reduced the infarct volume evaluated by triphenyl-tetrazolium chloride staining, attenuated BBB permeability alterations and improved neurological scores. Conversely, exosomes derived from astrocytes subjected to hypoxia were not as affective at reducing infarct volume, improving neurological recovery or limiting the BBB permeability alterations. These results show how the environment and molecular cues generated by support cells contribute to rescue neurons under ischemic conditions. Supported by PAPIIT-DGAPA IN226617 and CONACYT 219542.

WTH09-06

Melatonin attenuates βAPP processing VIA PIN1/NF-κB pathway in Aβ42-induced cellular model of Alzheimer's disease

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Alzheimer's disease (AD) is the most common cause of age-related dementia. AD-affected brain present extracellular deposits, which are the senile plaque composed of a set of hydrophobic peptides call amyloid β-peptide (Aβ). Soluble Aβ oligomers are the primary pathogenic factor leading to neuronal dysfunction in AD. Aβ-induced neurotoxicity exacerbates a vicious cycle of amyloidogenic processing stimulation and inflammation. Aβ is a normal product of β-amyloid precursor protein (βAPP) process resulting from the sequential cleavage of β-secretase (BACE1) and γ-secretase (PS1). Melatonin due to its incomparable functional versatility has multiple effects relevant to intervention in AD. Our previous studies demonstrated that melatonin regulated the non-amyloidogenic and amyloidogenic processing of βAPP by stimulating the α-secretases and down regulating both β- and γ-secretases. In the present study we proposed an Aβ-induced cellular model of AD to evaluate the therapeutic potential of melatonin and its underlying mechanisms of action. We confirmed this model in $A\beta_{42}$ treated SH-SY5Y neuroblastoma cell cultures by analyzing the levels of ADAM 10, BACE1 and PS1 expression. Pre-treatment with melatonin alleviated $A\beta_{42}$-induced alterations in the βAPP processing secretases. Considering neuroinflammation induced by Aβ played a critical role in the pathogenesis of AD, we observed the signaling pathway of Aβ-induced enhancement of nuclear factor-κB phosphorylation (pNF-κB) levels. The results indicated that $A\beta_{42}$ induced increased NF-κB p65 and up-regulated BACE1 possibly via Pin1/NF-κB signaling pathway. Pin1 is a key player in the pathogenesis of AD since the levels are compromised leading to increased Aβ formation. We showed that $A\beta_{42}$ led to Pin1 reduction causing destabilization of the p65/Rel subunit of NF-κB further resulting in its activation. Overall our present study demonstrated that melatonin prevented $A\beta_{42}$ induced alterations in βAPP processing secretases via Pin1/NF-κB signaling pathway.

WTH09-07

Investigating neuroprotective actions of irisin in the central nervous system

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Recent studies have pointed out that irisin, an exercise-induced myokine first identified as a regulator of adipocyte metabolism, may play important roles in brain function. Irisin was recently reported to stimulate hippocampal BDNF expression, which, in turn, promotes neuronal survival, synaptic plasticity and memory. However, it is still unclear whether peripheral irisin crosses the blood brain barrier (BBB) and what are the mechanisms underlying its effects in the brain. To address these questions, we have produced recombinant irisin and tested its ability to modulate signaling pathways relevant to brain homeostasis. First, we detected a slight increase in brain irisin levels after intravenous injection of recombinant irisin in mice. To investigate the signaling pathway underlying the neuronal actions of irisin, neuronal hippocampal cultures were treated with irisin, followed by quantification of the expression of BDNF and of genes related to the unfolded protein response (UPR), as these factors play key pathogenic roles in several neurodegenerative diseases. Irisin significantly increased BDNF and reduced UPR-related gene expression. Our results support the notion that irisin has neuromodulatory effects in the hippocampus, which could be of relevance to learning and memory processes. We are currently investigating additional effects irisin might have in the central nervous system and if peripherally delivered irisin triggers similar responses.

WTH09-08

Neuroprotective effect of whey protein concentrate (WPC) during aging

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Aging is a progressive multifactorial process exclusively marked by loss of cellular, molecular, and physiological functionality. The anatomical and physiological changes in the brain are fairly correlative with advancing of age. Moreover, aging causes substantial molecular to morphological changes in brain, the brain cells being more susceptible toward oxidative stress mediated damages due to the presence of high lipid content and higher oxygen consumption. It has been well documented that brain aging is also associated with decreased cellular uptake of L-cysteine, an amino acid essential for glutathione biosynthesis. Glutathione plays a critical role in protecting cells from oxidative stress and maintaining the thiol redox state in the central nervous system. The level of glutathione in brain is relatively lower than other organs. Whey protein concentrate (WPC) is a rich source of sulfur-containing amino acids such as methionine and cysteine and consumed as a functional food with wide range of nutritional attributes. Thus, the attempts were made to investigate the neuroprotective, anti-inflammatory and antioxidant efficacy of dietary supplementation of whey protein in brain tissue of old aged rats. Young (4 months) and old (24 months) male Wistar rats were supplemented with WPC (300 mg/kg b.w.) for 28 days. The data demonstrated that WPC augmented the decreased levels of FRAP, total thiol and acetyl cholinesterase in brain of old aged rats as compared to young control rats. Furthermore, WPC treated groups exhibited significant ($p < 0.001$) reduction in levels of lipid peroxide, protein carbonyls, reactive oxygen species and nitric oxide in aged rats. WPC supplementation also down-regulated the expression of inflammatory markers such as IL-1β and TNF-α, whereas up-regulated the expression of autophagy markers such as Atg3, LC3B and Beclin-1 in old aged rats. Taken together, the data confirmed the anti-aging and neuroprotective role of cysteine rich WPC that may also offers a better strategy to counteract age-dependent changes in brain.

WTH09-09

Quercetin attenuates the cadmium induced neurotoxicity by altering the autophagy via PI3K/AKT/MTOR signaling pathway

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Quercetin, a polyphenolic flavonoid has widely been present in varieties of foods has been regarded as nutraceutical. Having the potent antioxidant properties, protective role of quercetin in various neurodegenerative diseases like Alzheimer's has also been established. Further, Dysregulation of autophagy led to neuronal cell death in neurodegenerative diseases. Although studies carried out previously demonstrated the mechanism of cadmium induced neurotoxicity, however, the cellular and molecular mechanism underlying the role of autophagy in cadmium mediated neuronal death has not been fully understood. So, in order to gain insight into signaling cascade involved in cadmium mediated autophagy, the present study has been carried out to understand the protective role of quercetin in cadmium mediated increase in autophagy flux that led to neuronal death. We observed that exposure to cadmium altered the expression of autophagy proteins as LC3-II, Beclin1 and other Atg like proteins and GFP-LC3 puncta cells. Cadmium exposure in presence of bafilomycin A1 increased the levels of LC3-II and SQSTM1 levels in cholinergic rich areas of the brain. Further, interesting to it, combine treatment of cadmium with rapamycin (pharmacological activator of autophagy) mitigated such effects and in presence of 3MA (Pharmacological inhibitor of autophagy) cadmium induced neurotoxicity aggravated. Cadmium treatment activated the PI3K/Akt led to the down regulation in mTOR pathway. Further, cadmium also resulted in mitochondrial loss, bioenergetics deficits, and increased ROS generation leading to neuronal death. However, simultaneous treatment with quercetin ameliorated such changes. The results of the present study exhibit that cadmium-mediated neurotoxicity is associated with impaired autophagy through the PI3K/Akt/mTOR Signaling and these changes were further protected by quercetin.

WTH09-10

Neuroprotective effect of apelin against retinal ganglion cell death induced by retinal ischemia-reperfusion injury

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Glaucoma is a neurodegenerative optic neuropathy characterized by the loss of retinal ganglion cells, resulting in irreversible blindness. It is suggested that the loss of retinal ganglion cell is caused by several factors including glutamate excitotoxicity via *N*-methyl-D-aspartate (NMDA) receptors and retinal ischemia-reperfusion injury. We have recently reported that apelin, the oligopeptide ligand for the G protein-coupled receptor APJ, protects retinal ganglion cells from NMDA-induced excitotoxicity in mice. In this study, we investigated whether apelin protects against retinal ganglion cell death induced by retinal ischemia-reperfusion injury. Retinal ischemia-reperfusion injury was performed in apelin knockout mice and wild type mice using the high intraocular pressure method. Retinal ganglion cell death was assessed by counting the number of cells in the retinal ganglion cell layer in retinal sections stained with hematoxylin and eosin. TUNEL assay was conducted to detect apoptotic cells in the retinal ganglion cell layer. Electroretinography was performed to assess the electro-responses of retinal ganglion cells. Apelin deficiency in mice facilitated the decrease of cells in the retinal ganglion cell layer in retinas following retinal ischemia-reperfusion injury. Apelin deficiency also accelerated apoptosis in the retinal ganglion cell layer induced by retinal ischemia-reperfusion injury. Consistent with the retinal histopathological findings, electroretinography revealed that apelin deficiency enhanced the reduction of electro-responses in the retinas after retinal ischemia-reperfusion injury. These results suggest that endogenous apelin protects against retinal ganglion cell death induced by retinal ischemia-reperfusion injury.

WTH09-11

Protective role of aqueous extract of terminalia arjuna against cerebral ischemia induced mmp mediated blood brain barrier damage

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Compromised Blood brain barrier (BBB) following focal cerebral ischemia plays key role in infiltration of inflammatory cells which results in reperfusion injury and brain edema. Activated Matrix Metalloproteinases (MMPs) mediated degradation of tight junction (TJ) proteins is critical in disruption of BBB. To combat the multi-directional cerebral ischemic cascade events, a holistic approach is needed. *Terminalia arjuna,* a traditional medicinal plant well known cardiotonic was documented for its effective role in cardioprotection. Its antioxidant, hypolipidemic, anti-inflammatory, neuroprotective and hypertensive properties claim its effects. The present study intended to assess the ameliorative effect of *Terminalia arjuna* (*aeTA*) against transient focal cerebral ischemia. Adult male Sprague–Dawley rats divided into three groups ($n = 9$): Sham, Lesion and pretreated groups of 15 days *aeTA* were subjected to 2 hrs of Middle cerebral artery occlusion (MCAO) for 2 hrs followed by reperfusion. After 24 hrs of reperfusion the rats were assessed for behavioral deficits by Modified neurological severity scoring (mNSS), Evans blue (EB) extravasatation evaluation for BBB status, Triphenyl tetrazolium chloride (TTC) stain for Infarct measurement, Western blot analysis of MMPs (MMP2 & MMP9) and TJ proteins (Claudin5 & Occludin). The pretreatment with *aeTA* for 15 days exhibited significant improvement in neurological behavioral outcome and reduced infarct volume compared with the MCAO rats. Also the *aeTA* treatment was found to reduce BBB breach confirmed through decreased EB extravasations, significant reduction in matrix degrading MMPs (MMP2 & MMP9) and maintaining the levels of tight junction proteins (Claudin5 & Occludin). Thus pretreatment with *aeTA* treatment could reduce the BBB breach against focal cerebral ischemia and improve neurobehavioral outcome.

WTH09-12

Melatonin ameliorates cognitive deficits and synaptic dysfunction in streptozotocin-induced hyperglycemic state in rats

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Imperative evidences have indicated that deregulated glucose metabolism is a risk factor for cognitive dysfunction and developing Alzheimer's disease (AD). However, the underlying mechanisms are not fully elucidated. Melatonin is a neurohormone whose levels has been associated with the development of diabetes and AD patients. Melatonin exerts multiple complementary mechanisms of action against AD in animal models. Therefore, the present study examined whether melatonin can exert beneficial effects upon hippocampal-dependent cognitive function and synaptic plasticity in rats induced hyperglycemia by injection of streptozotocin (STZ). Adult Wistar rats were administered with one injection of STZ at (60 mg/kg; i.p.) and melatonin at (10 mg/kg/day, i.p.) for 42 consecutive days. Morris water maze (MWM) assay, western blotting and immunofluorescence staining in the hippocampus were performed to evaluate the effects of melatonin administration. The efficacious effect of melatonin was manifested in significantly ameliorated the spatial learning and memory impairment and alterations in expression of synaptic proteins (synaptophysin and PSD95) in STZ-induced rats. These results demonstrated that melatonin administration ameliorated memory deficit in hyperglycemic rats by up-regulating the plasticity-related proteins in hippocampus. However, further investigations are needed to explore the underlying mechanism of melatonin regulation in hyperglycemic rats.

WTH09-13

Melatonin improves cerebral blood flow decrease in aged mice

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Age-related degeneration of the brain vasculature may reduce the blood flow and stem cells' neurogenic potentials, which leads to reduced cognition. In our previous study, we found that the blood flow in old mice's brains is lower than that in young mice and the old mice had more curved pial arteries and fewer pial artery junctions than young mice. This decreased of cerebral blood flow and vascular alterations may reduce the arteriolar vasodilatory capacity and distensibility, thus contribute to the vascular cognition disease in seniors. Melatonin, which is a widely known and potent free radical scavenger and antioxidant. Furthermore, melatonin has been reported that vasoconstriction cerebral arterioles via activation of either MT_1 and MT_2 G-protein-linked membrane receptors. In this study, we examined the effects of melatonin treatment on cerebral blood flow and vascular structure. 12 months male Balb/c mice drank water with melatonin (10 mg/mL with ethanol; dilute in water) for 4 months and measured of cerebral blood flow every month. Using indocyanine green (ICG) fluorescence tracer, we measured and compared the normal aging mice (sham control) and melatonin-treated aging mice. The mean transit time (MTT) and T_{rising} (the time between of the first appearance of ICG fluorescence) were significantly faster in melatonin-treated aging mice than in normal aging mice. Moreover, blood flow index (BFI) was higher in the melatonin-treated aging mice than in the normal aging mice. These data suggest that melatonin may improve cerebral blood flow in aging animals.

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WTH09-14

Beneficial effects of enzyme-treated asparagus extract and buckwheat hull extract on memory functions in Sprague–Dawley rats

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Enzyme-Treated Asparagus extract (ETAS) is extracted from the residual lower parts of Asparagus (*Asparagus officinalis* L.) grown in Hokkaido, Japan. ETAS has been shown to have various benefical effects on health, such as anti-oxidative, anti-inflammatory or anti-stress activity. Buckwheat hull extract (BWHE) is a mixture of some flavonoids including rutin. We have shown that BWHE has protective effects on toxicant-induced hippocampal injury in rats. The objective of this study is to investigate the beneficial effects of ETAS, BWHE and rutin on spatial memory functions in rats. Male SD rats aged 4-weeks were fed chow containing 0.75% (w/w) ETAS, BWHE or rutin for 33 days. The rats were subjected to the Morris water maze task to examine memory acquisition and memory retrieval. Our results showed that ETAS supplementation facilitated memory acquisition in an early stage. On the other hand, BWHE supplementation seemed to facilitate memory retrieval. Rutin supplementation shown to have little effect both on memory acquisition and retrieval. In conclusion, these flavonoids have beneficial effects on different stages of memory formation processes in rats.

WTH09-15

Prevention of excitotoxicity associated changes in GLUN2B and TRKB levels by NMDA receptor inhibitors *in vivo*

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Excitotoxicity due to uncontrolled activation of NMDA receptor (NMDAR) causes neuronal death in various neurodegenerative diseases. Molecular mechanisms leading to cell death under excitotoxic conditions are still not clearly understood. Monosodiumglutamate (MSG) is known to cause excitotoxicity *in vivo*. In this

study we are observing the changes at the level of protein expression as well as their post translational modifications under excitotoxic conditions in the MSG treated rat model system. Activation of calcium signalling via NMDAR is expected upon chronic treatment with MSG. Briefly, methodology includes 15 days of MSG injection via i.p route in 100–150 gm adult male rat, which were given orally either vehicle or an NMDAR inhibitory plant extract. A group of animals were also fed with a known NMDAR inhibitory drug, dextromethorphan. Analysis by Morris water maze (MWM) test showed that the behavioural impairment caused by MSG administration could be ameliorated by simultaneous treatment with one of the NMDAR inhibitors, either the plant extract or dextromethorphan. Reduced GluN2B level was observed by immunoblotting in hippocampal and cortical tissues in MSG treated animals, which was reversed in the group fed with NMDAR inhibitory plant extract. Changes in the level of, TrkB upon MSG treatment was also found to be prevented in the group fed with NMDAR inhibitoray plant extract. Other proteins related to calcium signalling and cell death such as p-GluA1-Ser831, BDNF, p-PP1 and Bcl2 are also being analysed. Elucidation of the pathways that are altered would reveal the mechanism of action of neuroprotection by the NMDAR inhibitory plant extract.

WTH09-16

Lotus, a neural circuit formation factor, blocks PIRB-mediated axon growth inhibition

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The inability of damaged axons in the adult mammalian central nervous system (CNS) to regenerate is attributed to axonal growth inhibitors such as Nogo proteins, myelin-associated glycoprotein (MAG) and oligodendrocyte myelin glycoprotein (OMgp). Nogo receptor-1 (NgR1) is a common receptor for these inhibitors. Recently, paired immunoglobulin-like receptor B (PirB), which originally regulates the immune system, has also been identified as a novel receptor for these inhibitors. We previously identified lateral olfactory tract usher substance (LOTUS) as a novel key molecule for axonal bundling of lateral olfactory tract by antagonizing NgR1. However, another function of LOTUS remains unknown. In this study, we found that LOTUS interacted not only with NgR1 but also with PirB. Overexpression of LOTUS with PirB in COS7 cells interfered with the binding of Nogo to PirB. Soluble form of LOTUS suppressed Nogo-induced growth cone collapse and neurite outgrowth inhibition in cultured dorsal root ganglion neurons from *ngr1*-deficient mice. These results suggest that LOTUS also exerts the antagonistic activity on PirB as well as NgR1, raising the possibility that LOTUS may enable injured CNS neurons to re-elongate their axons and thereby to overcome the limitation of axonal regeneration.

WTH09-17

The role of lysophosphatidic acid (LPA) and in the maintenance of the blood retina barrier and photoreceptor function

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Retinal degeneration may be mediated by loss of outer blood retinal barrier (BRB) integrity, leading to injury of the retinal pigment epithelium and photoreceptor death. Lysophosphatidic acid (LPA), and autotaxin (ATX), have been implicated in inflammation, angiogenesis and apoptosis. Given that ATX is a signature RPE marker, we hypothesize that it plays an important role in maintaining the microenvironment of the retina.

Human pluripotent stem cell (hPSC) lines were differentiated into RPE cells (Lidgerwood et al., 2016), and were shown to express high levels of *ATX* and *LPA* receptors (*LPARs*) by qRTPCR. Measurements of endogenous LPA and ATX were determined using LC-MS and western blot, respectively, and indicate that RPE cells secrete low basal amounts of LPA (0.1-0.4 nM), but secrete large amounts of functional ATX (apical). Addition of exogenous LPA to hPSC-RPE cultures did not affect RPE markers or cell morphology, suggesting RPE-derived LPA serves as a paracrine signal for neighbouring cells. hPSC-RPE cells were co-cultured with 661W photoreceptors, and indicate that high doses of LPA (> 10 μM) resulted in cytoskeletal changes in photoreceptors. CD4 + and CD8 + T cells treated with LPA have reduced activation, suggesting RPE-derived LPA may play a role in the maintenance of the immunoprivaleged retina.

WTH09-18

Evaluation of guanosine effects in 6-OHDA model of non-motor symptoms of Parkinson's disease

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Parkinson's disease (PD) is a progressive neurodegenerative disorder associated with dopaminergic neurodegeneration in dorsolateral striatum (DLS) and *substantia nigra pars compacta* (SNpc).

Degeneration in SNpc affects other brain areas including the prefrontal cortex (PFC), which has been associated with anhedonia and depression. 6-hydroxydopamine (6-OHDA) is a neurotoxin-model of PD and undergoes a non-enzymatic auto-oxidation, generating reactive oxygen species (ROS) and inhibition of mitochondrial complex I. A bilateral injection of 6-OHDA (10 μg/hemisphere) in the DLS induced a partial degeneration (about 60%) of dopaminergic neurons in the SNpc and non-motor impairments, mimicking an early premotor stage of PD. In this study, we investigated the effects of the neuroprotective nucleoside guanosine (GUO) treatment in a temporal evaluation of behavioral tasks after 6-OHDA-induced damage. 6-OHDA infusion induced anhedonic-like behavior in the splash test (after 8 days), but it did not alter the sucrose consumption preference (after 5-8 days). 6-OHDA also increased immobility time in the forced swimming test (FST, after 21 days) and induced short memory impairment in the object recognition test (ORT, after 14-15 days). However, no alterations in olfactory discrimination (after 3 days), motor performance in the open field (after 14 days), anxiety-like behavior in Y-maze test (after 20 days) and social interaction (after 22 days). Biochemical analyses were performed in cortical, striatal and hippocampal slices of rats 22 days after 6-OHDA. ROS levels and mitochondrial membrane potential were not changed 22 days after 6-OHDA infusion. GUO presented a partial effect on anhedonic-like behavior and prevented the development of increased immobility time associated with depressive-like behavior. GUO present no alterations in biochemical assays. In summary, these results provide for the first time the GUO effects on anhedonic-like and defense behaviors relevant to depression in 6-OHDA-lesioned rat, a model of non-motor symptoms associated with PD.

WTH09-19

Intracellular calcium homeostasis and signal transduction in brain cortex in conditions of hypokinesia

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Lack of intracellular calcium ([Ca^{2+}]in) has a great role in signal transduction disturbances implicated in the pathogenesis of the circulatory and metabolic disturbances in brain. Our objective was investigation of [Ca^{2+}]in homeostasis and phosphatidylinositol cycle (PIC) signaling pathway in brain cortex in conditions of hypokinesia (HK). Male mature white rats were used. HK was modeled in individually cramped cages. Labeled [14C]-arachidonic acid (AA) and 45CaCl2 were used for radioisotopic measurement of 45Ca^{2+} influx/translocation and membrane-dependent phospholypase (PLase)activity. Our results evidence enhancement of inward current of labeled 45Ca 2 + ions with elevation of intracellular Ca^{2+} concentration in conditions of HK, as well as activation of the Ca^{2+}-dependent enzymes, such as PLaseA1, A2, C, activation of the PIC signaling pathway which was manifested in elevation of diacylglycerol - one of the secong messenger of PIC and elevation of free AA contentin brain cortical synaptosomes. PIC stimulation leads to the Ca-induced Ca-release from the endoplasmic reticulum (ER) which contributes to the additional rise in cytosolic Ca^{2+}. This phenomenon is confirmed by our results testifying that the content of $Ca^{+}2$ ions within the ER is changed insignificantly. Moreover, Ca^{2+} ions are mainly accumulated within the mintochondrial fraction (MF). Thus, HK is involved in the pathogenesi of ischemic brain injury due to ceasing of protective influx of the elevated cytosolic Ca^{2+} into ER and facilitating their accumulation within the MF, promoting irreversible damage of synaptosome in brain cortex, which was contributed also by accumulation of the free AA thereby leading to alteration of presynaptic function of neurocytes of the brain cortex.

WTH09-20

Effects of polyamines on protein elongation and autophagy in neurons

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During aging neurons have to face multiple challenges including lesions in their nucleic acids or oxidative stress. On top of that impaired autophagy and the accumulation of misfolded, and, therefore, non functional or even toxic proteins impair the physiological functions of neuronal cells. Bearing this in mind, a rescue of autophagy and the restoring of protein homeostasis is considered to be essential in term of healthy aging.

Supplementation of polyamines results in eliminating of so called stalled ribosomes (a phenomenon occuring when protein elongation is disturbed) most likely due to a higher amount of hypusinylated factor eIF5A.

We characterized synaptoneurosomes prepared from 3, 18 and 26 month old mice using metabolic labeling of nascent proteins via BONCAT and subsequent Western Blot analysis. First results show a clear restoration of *de novo* synthesized synaptic proteins including Shank2, IRS1, and also of Homer1. Furthermore, we also use a polysome profiling approach to characterize ongoing translation in young and aged primary neuronal cell cultures without treatment or under supply of polyamines.

As polyamines also positively effect autophagy in aged neurons, visualization of autophagosomes via staining with Monodansylcadaverine in fixed cells an live staining revealed a distinct but difficult to interpret effect of the supplementation of polyamines. In a more generell sense, autophagosome formation via the LC3- or the GABARAP-system seems to be influenced in aging too, whether this process is affected by polyamines is still unclear.

Our results underline a potential therapeutic benefit of polyamines with respect to protein homeostasis and autophagy.

WTH09-21

In-vivo modulation of transferrin receptor protein-1 by a complex vitamin molecule reverses Alzheimer's-type pathology

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Metal ions are crucial for normal neurochemical signalling, while perturbations in their regulation are associated with

neurodegenerative processes in Alzheimer's disease (AD). Hypothesizing that metal chelators, antioxidants and anti-inflammatory agents could improve disease outcomes via modulation of transferrin receptor protein-1 (TfP-1)-a metal ion regulator, we investigated the efficacy of a complex vitamin supplement (CVS) formulated with several B-vitamins and ascorbic acid in reversing AD-type neurodegeneration. Forty Wistar rats (8 weeks-old) were assigned into five groups ($n = 8$), including controls and those administered CVS (400 mg/kg/day) orally for 2 weeks before or after aluminium chloride ($AlCl_3$; 100 mg/kg)-induced neurotoxicity. Rats were assessed for standard behavioural functions related to cognition, learning, memory and anxiety. The prefrontal cortex (PFC), hippocampus and amygdala were prepared for spectrophotometry, histology, histochemistry and immunohistochemistry. Our data showed that CVS significantly reversed reduction of exploratory/working memory ($p < 0.05$), frontal-dependent motor deficits ($p < 0.01$), cognitive decline ($p < 0.005$), memory dysfunction ($p < 0.05$) and anxiety ($p < 0.01$) compared to $AlCl_3$ (neurotoxic) group. These findings correlated with CVS-dependent modulation of TfP-1 expression within the PFC, hippocampus and amygdala that were accompanied by significant reversal of neural oxidative stress in expressed superoxide dismutase, nitric oxide, catalase, glutathione peroxidase and malondialdehyde. Through modulation of TfP-1, CVS inhibited neural bioenergetics dysfunction as increased labelling of glucokinase within PFC and hippocampus (but not amygdala) correlated with increased glucose-6-phosphate dehydrogenase and decreased lactate dehydrogenase expressions. These further relates to inhibition of over-expressed acetylcholinesterase and increased total protein synthesis. H&E and Nissl staining of thin sections corroborated roles of CVS in reversing $AlCl_3$-induced AD-like pathology and were accompanied by related changes in astrocytes and neurofilaments (cytoskeleton) immunohistochemical analyses. Summarily, we showed that CVS modulates neural TfP-1 overexpression, thereby normalising neurochemical signalling pathways linking concurrent progression of oxidative stress, bioenergetics deficits, synaptic dysfunction, cytoskeletal dysregulation and cellular hypertrophy in AD.

WTH09-22

Garcinol reduces streptozotocin-induced Alzheimer's like phenotypes and neuropathology in rats

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Sporadic Alzheimer's disease (sAD) is a progressive neurodegenerative disorder with multi-factorial pathophysiology. It involves cholinergic malfunction and mitochondrial dysfunction, and probably oxidative stress plays a significant role in its progressive nature. There is keen interest in identifying compounds related to nutraceuticals that can attenuate oxidative stress and amyloidosis in sAD that can improve memory. Streptozotocin (STZ) intracerebroventricular (ICV) administration has been used for developing sAD model in rats. The aim of the current study is to illustrate the potential role of garcinol (ICV infusion) a polyisoprenylated benzophenone in this rodent sAD model in relation to mitochondrial impairment, oxidative stress and memory functions. Cognitive abilities were assessed by Morris water maze (MWM) and elevated plus maze (EPM). Reactive oxygen species (ROS) generation, caspase-3, mitochondrial complex-I and V activities were estimated by employing spectrophotometry and fluorometry respectively. Further, we investigated the protein expression of choline acetyltransferase, glial fibrillary acidic protein (GFAP), and mitochondrial dynamin regulated proteins (Drp1) by Western blot. STZ-ICV animals exhibited memory deficits, which was effectively recovered by garcinol ICV treatment (1 and 10 μg), and was comparable to Tacrine treated STZ-ICV rats. Furthermore, garcinol significantly attenuated STZ-ICV-mediated choline acetyltransferase, mitochondrial complexes I and V activities sAD animals. Garcinol prevented STZ-ICV-induced elevations in ROS production, caspase-3 over activation, and over expression of GFAP and DRP1 proteins. Garcinol 10 μg inhibited amyloidosis and cell death in STZ intoxicated animals. Our results demonstrated that garcinol could be a potent memory enhancer due to its mitochondrial rejuvenating effect and antioxidant capabilities. Our findings suggest that garcinol could be dominant nutraceutical used in AD specifically related to modify defective cholinergic functions, mitochondrial functions, caspase–3 over activation and oxidative stress.

WTH09-23

Administration of L-tyrosine with levodopa could be neuroprotective in Parkinson's disease

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Introduction: The 20 canonical amino acids (AAs) account for less than 2% of all AAs in Nature. Most non-protein amino acids (NPAAs) are made by plants and their natural toxicity is often utilised to protect against predation or inhibit the growth of competing plants. In some cases toxicity arises from the ability of a NPAA to be charged onto tRNA in place of a structurally similar protein AA and become inserted into a polypeptide chain. L-DOPA (levodopa), which is present in mucuna plants, replaces L-tyrosine in protein synthesis and effectively kills larvae. We have shown that L-DOPA and can induce protein aggregation and apoptosis in human neurons in vitro[1] and proteins containing incorporated DOPA are present in brain and plasma of L-DOPA-treated patients[2]. Here we test the ability of L-tyrosine to prevent L-DOPA incorporation into proteins *in vivo*.

Methods: Rats ($n = 22$) were administered L-DOPA (6.5 mg/kg) IP, twice daily with or without L-tyrosine (100 mg/kg). After sacrifice at 21 days, proteins were extracted from the motor cortex (MC), substantia nigra (SN) and striatum (CPu) and levels of DOPA-containing proteins measured by HPLC.

Results: Plasma tyrosine levels were 3 fold higher in the L-tyrosine-treated group 0.5 h after IP injection but returned to control levels after 2.5 h. DOPA in hydrolyzed proteins increased 5 fold in the CPu of DOPA-treated rats, and this was significantly reduced in the group of rats that received L-tyrosine with L-DOPA ($p < 0.01$).

Discussion: Mischarging of tRNAtyr with L-DOPA and incorporation into neuronal cell proteins is a mechanism of L-DOPA toxicity that has been largely overlooked. In the present study we demonstrate that after a short exposure to L-DOPA, proteins containing incorporated L-DOPA are detectable in the rat brain and incorporation can be significantly reduced with L-tyrosine, suggesting a potentially protective effect *in vivo*.

1. Rodgers, KJ., et al., J Neurochem (2006). 2. Chan, S. W., et al. Exp Neurol 238, 29-37, 9 (2012).

WTH09-24

Dibenzoylthiamine, a lipophilic thiamine precursor, protects against oxidative damage in neuroblastoma cells

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Recent evidence suggests that thiamine (vitamin B1) and some of its derivatives can exert prominent neuroprotective effects in the mammalian brain, particularly in mouse models of Alzheimer's disease and tauopathies. As orally administered thiamine crosses intestinal and blood-brain barriers only slowly, precursors with higher bioavailability e.g. sulbutiamine, benfotiamine and dibenzoylthiamine, have been developed. We investigated the protective effects of thiamine and those precursors in neuroblastoma cells cultured in a medium containing minimal amounts of thiamine (10 nM), but sufficient to sustain normal growth. We induced oxidative stress by incubating the cells (24 h) in the presence of the neurotoxic agent paraquat (0.25 mM). This treatment reduced cell viability by 40%. When thiamine or the precursors were present simultaneously, we observed protective effects by the precursors while free thiamine was ineffective. Dibenzoylthiamine was most efficient, affording complete protection of cells at 10-20 μM. It also caused the highest increase in intracellular thiamine, suggesting that the protection from oxidative damage is linked to increased levels of free thiamine (rather than thiamine disphophate) in the neuroblastoma cells. The mechanism of this protective effect is presently under investigation. These results and others from our laboratory raise the possibility that dibenzoylthiamine might useful as a neuroprotective agent in neurodegenerative disease.

WTH09-25

Apelin inhibits *N*-methyl-D-aspartate-induced retinal ganglion cell death by activating AKT and ERK via APJ receptor in mice

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Retinal ganglion cell death is a hallmark of retinal diseases including glaucoma and diabetic retinopathy. The loss of retinal ganglion cells in these diseases has been suggested to be caused by excessive glutamate-induced excitotoxicity as mediated via *N*-methyl-D-aspartate (NMDA) receptors. It has been reported that apelin, the bioactive peptide, has neuroprotective properties against excitotoxicity in the cultured-neuronal cells prepared from hippocampus via APJ receptors, the G protein-coupled receptors. In this study, we investigated whether apelin inhibits retinal ganglion cell death induced by NMDA in mice. The number of retinal ganglion cells was counted in retinal sections stained by hematoxylin-eosin. TUNEL assay was performed to detect apoptotic cells in retinal ganglion cell layer. APJ expression in the retina was identified by immunohistochemistry. Activations of Akt and extracellular signal-regulated kinase (ERK) were assessed by western blotting. APJ immunoreactivities were observed in retinal ganglion cells immunostained with Brn-3a antibody. Apelin injection into the vitreous body suppressed the decrease of retinal ganglion cells induced by following an intravitreal injection of NMDA. This protective effect was blocked by co-injection of ML-221, an APJ antagonist. An intravitreal injection of apelin induced activations of Akt and ERK in retinas, and these activations by apelin were completely inhibited by ML221. Inhibitors of Akt and ERK signaling pathways blocked the protective effect of apelin on NMDA-induced apoptosis in retinal ganglion cell layer. These results suggest that apelin inhibits NMDA-induced retinal ganglion cell death by activating Akt and ERK via APJ receptors.

WTH09-26

Rapamycin induced activation of autophagy protects hippocampal neurons of aging rat brain through MTOR/AKT1/CREB pathway

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Autophagy is a highly conserved catabolic process involved in continuous removal of toxic protein aggregates and cellular organelles to maintain cellular homeostasis and functional integrity. The mechanistic understanding of autophagy mediated neuroprotection during aging remains elusive. Here, we investigated the potential role of rapamycin-induced activation of autophagy and mTOR/Akt1/CREB pathway(s) in the neuroprotection of hippocampal neurons of aging rat brain. In hippocampus region of aging rat brain, we found impaired redox balance, synaptic neurotransmission and cognitive functions, and suppressed pro-survival signaling as compared to normal young control rats. Rapamycin (0.5 mg/kg b.w., oral, daily for 30 days) administration caused a significant reduction of mTOR complex 1 phosphorylation at Ser2481 and a significant increase in levels of autophagy markers such as microtubule-associated protein-1 light chain-3 (LC3), Beclin-1, sequestosome-1/p62, unc-51-like kinase 1 (ULK1). In addition, rapamycin-induced the activation of autophagy that further activated p-Akt1 (Ser473) and p-CREB (Ser183) expression in aged rats. The activated autophagy markedly reversed age-associated impaired redox homeostasis by decreasing the levels of pro-oxidants such as ROS generation, intracellular Ca^{2+} flux and lipid peroxidation, and increasing the levels of antioxidants such as superoxide dismutase, catalase and reduced glutathione. The activated autophagy also provided significant neuroprotection against age-associated synaptic dysfunction by increasing the expression of synapsin-I, synaptophysin and PSD95, neurotransmission dysfunction by increasing the levels of CHRM2, DAD2 receptor, NMDA receptor and AMPA receptor, and ultimately improved cognitive ability in aged rats. Moreover, wortmannin (an autophagy inhibitor) administration significantly reduced the expression of autophagy markers, p-Akt1 and p-CREB as well as the autophagy mediated neuroprotective effect. Our study demonstrates that autophagy can be an integrated part of pro-survival (mTOR/Akt1/CREB) signaling and autophagic activation restores the oxidative defense mechanism(s) and maintains the integrity of synapse and neurotransmission in aging rat brain.

WTH09-27

Neuroprotective effect of spermidine as a caloric restriction mimetics in accelerated senescence model of rat

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Brain aging is a degenerative process that undergoes a progressive decline and opens a new window of vulnerability towards oxidative damages. Caloric restriction (CR) has been well documented to have health-promoting and lifespan extending effects. However, long-term CR would be highly problematic because of compliance challenge and other unpleasant side effects. Thus, to develop or explore the molecules, either natural or synthetic that mimic the beneficial effects of CR, is attracting the more attention and interest. Such candidate's molecules are usually known as CR mimetics (CRMs). Caloric restriction, through autophagy induction, is widely accepted as a lifespan-prolonging and health-promoting measure. Spermidine is a polyamine found in a variety of food and also known as potential CRM. The present study aimed to investigate the potential anti-aging effects of Spermidine in an accelerated senescence aging rat model. Thus, the attempts were made to investigate the anti-aging effects of spermidine (10 mg/kg b.w., oral) on brain tissues in D-galactose (500 mg/kg b.w., subcutaneous 45 days) induced aging model as well as naturally aged. We found that D-galactose significantly increased the level oxidative stress biomarkers lipid peroxidation (LPO) and protein oxidation (protein carbonyl content, PCO) along with simultaneous decrease in ferric reducing antioxidant potential (FRAP), reduced glutathione (GSH), ion channels (Na^+/K^+ ATPase and Ca^{2+}ATPase activity) and acetylcholinesterase (AChE) activity in brain. Spermidine reverses the effects of D-galactose by significantly ($p < 0.01$) decreasing the level of LPO, PCO closer to the control value, and significantly increasing FRAP, GSH, ion channels (Na^+/K^+ ATPase activity, Ca^{2+}ATPase) and AChE activity. Moreover, spermidine also restored the level of antioxidative markers. The RT-PCR and western blot data for expression of Beclin-2, LC-3, Atg-3, IL-6, TNF-α, Sirt-1, Sirt-2 and synaptophysin further confirmed a neuroprotective and anti-aging effect of spermidine in aging rats. Thus, data confirmed that spermidine is a promising compound for preventing neurodegeneration during aging brain.

WTH09-28

Biphasic responses of trans-resveratrol on proliferation of neural progenitor cells and aged rat hippocampal neurogenesis

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The plethora of literature has supported the potential benefits of Resveratrol (RV) as a life-extending as well as an anticancer compound. However, these two functional discrepancies resulted at different concentration ranges. Likewise, the role of Resveratrol on adult neurogenesis still remains controversial and less understood despite its well documented health benefits. To gather insight into the biological effects of RV on neurogenesis, we assessed the potential effects of the compound on the proliferation and survival of neural progenitor cells (NPCs) in culture, and in the hippocampus of aged rats. Resveratrol exerted biphasic effects on NPCs; low concentrations (10 μM) stimulated cell proliferation mediated by increased phosphorylation of extracellular signal-regulated kinases (ERKs) and p38 kinases, whereas high concentrations (> 20 μM) exhibited inhibitory effects. Administration of Resveratrol (20 mg/kg body weight) to adult rats significantly increased the number of newly generated cells in the hippocampus, with upregulation of p-CREB and SIRT1 proteins implicated in neuronal survival and lifespan extension respectively. We have successfully demonstrated that Resveratrol exhibits dose dependent discrepancies and at a lower concentration can have a positive impact on the proliferation, survival of NPCs and aged rat hippocampal neurogenesis implicating its potential as a candidate for restorative therapies against age related disorders.

WTH09-29

Neuroprotection of ketamine against cell death caused by oxidative stress during epileptogenesis in the mouse pilocarpine model

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Oxidative stress contribute to epileptogenesis and constitute an extremely deleterious event considered to be a cause of prolonged seizures.The mechanism contributing to hyperexcitability induced oxidative stress is not very well-defined and the mechanism understanding can lead to a novel anti-epileptogenic therapies. The objective of this study was to investigate the effect of ketamine on oxidative stress during the epileptogenesis phase in a mouse model of temporal lobe epilepsy induced by pilocarpine. In our study, animals received intraperitoneally either 0.9% saline (control group), or pilocarpine (100 mg/kg) every 20 min still the beginning of status epilepticus (epileptic group),or Ketamine 10 mg/kg (ketamine group),or a combination of pilocarpine and ketamine (epileptic/ketamine group)(ketamine was administered 15 min after pilocarpine injection), or a combination of pilocarpine and ketamine (ketamine/epileptic group) (ketamine was administered 15 min before pilocarpine injection). Mice were sacrificed 10 days after treatment. The status of oxidative stress was investigated by measuring malondialdehyde (MDA), nitric oxide (NO) content and free iron (Fe) in brains animals. Brain antioxidants enzymes activities levels were also determined. Data showed that in the epileptic group, we obtained a highly significant increase of lipid peroxidation, nitric oxide and free iron but we noted a highly significant decrease of catalase (CAT), superoxide dismutase (SOD) and peroxidase (POD) activities. Ketamine treated alone decreased significantly MDA, increased significantly NO but has no effect on free iron. Ketamine increased highly significant the 3 antioxidants enzymes activities. Administration of Ketamine before or after the epileptic induction showed that there is a highly significant decrease of the parameters MDA, NO and Fe. Regarding antioxidants enzymes activities, we noted a decrease of CAT and POD but a

highly significant increase of SOD. So ketamine administration increasing SOD activity and decreasing POD and NO content. SOD and CAT activity are involved in mechanisms responsible for eliminating oxygen free radicals during the establishment of epileptogenesis. Ketamine play an antioxidant role in the brain during epileptogenesis.

WTH09-30

Autophagy induction in brain by severe hypoglycemia and its modulation by the ketone body beta-hydroxybutyrate

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During hypoglycemia alternative substrates to glucose such as the ketone bodies (KB), acetoacetate (AcAc) and β-hydroxybutyrate (BHB) can be used as energy in brain. Furthermore diverse studies have shown that KB prevent neuronal death in different injury models. Nevertheless, the mechanisms by which KB prevent neuronal damage are still not well understood. Previous studies from our group have suggested that autophagy, a lisosomal-dependent degradation process activated during energy failure, participates in neuronal death induced by glucose deprivation in cultured cortical neurons. In these conditions, D-BHB, stimulates the autophagic flux and prevents neuronal death (J.Neurosci.Res. 41:600). In the present study we aimed to investigate whether autophagy is activated *in vivo* during insulin-induced hypoglycemia and glucose reperfusion and whether the neuroprotective effect of D-BHB, is related to autophagy. We analyzed the changes in the content of the autophagy proteins, LC3-II, used as index of autophagosome formation, and p62/SQSTM1, involved in autophagic degradation by western blot, in the cortex and hippocampus of hypoglycemic rats treated or not with BHB. Results show that autophagosome accumulation is promoted after 2 h of severe hypoglycemia in all studied cerebral regions as evidenced by the increased levels. 6 h after glucose reperfusion LC3-II content decreased to basal levels, suggesting less autophagosome formation or stimulated autophagosome degradation. However, after 24 h a second increase in LC3-II was observed in rats exposed to the hypoglycemic coma, while in those treated with D-BHB a significant decrease in LC3-II content was observed, suggesting that less autophagosomes are formed. No changes in p62/SQSTM1 were observed in the cortex, while in the hippocampus, a significant decrease in p62/SQSTM1 content was observed at 24 h in animals treated with D-BHB, suggesting the stimulation of the autophagic flux. Altogether these results suggests that D-BHB prevent autophagosome formation and stimulates the autophagic flux during *in vivo* hypoglycemia.

WTH09-31

Anti-amyloid antibody SAR228810 is neuroprotective against oligomeric ABETA-42-induced toxicity in mouse primary neuronal cultures

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Alzheimer's disease (AD) is characterized by the accumulation of extracellular deposition of beta-amyloid peptide in senile plaques and the intracellular aggregation of tau protein in neurofibrillary tangles associated with cognitive deficit. Although the initial neurotoxic event leading to onset of AD remains to be defined, it is considered to be highly linked in experimental models for AD to the presence of protofibrillar forms of amyloid-β peptide 42 (Aβ42) in the brain. Two monoclonal antibodies developed against protofibrillar forms of Aβ42 have been evaluated in an *in vitro* model of neurotoxicity. In neuronal cultures isolated from mouse cerebral cortex, we showed that oligomeric Aβ42 preparations induced a marked increase in caspase-3/7 enzymatic activity assessed by caspase 3/7 assay (+649 ± 124%) and a reduction in neurite outgrowth (-47 ± 5%) assessed by image analysis of immuno-stained MAP2-positive neurons. SAR228810, a humanized anti-amyloid antibody, and SAR255952, its murine version, significantly inhibited the oAβ42-induced neurotoxicity in a concentration-dependent manner. The concentration of 1 μM SAR228810 showed a near full reversion of the oAβ42-induced increase in caspase 3/7 activity and reduction of neurite outgrowth. Nor SAR228810 or SAR255952 did exhibit any significant effect in the absence of oAβ42, nor IgG isotype controls on neuronal cultures. Our results demonstrate that the anti-amyloid monoclonal antibodies, SAR228810 and SAR255952, have neuro-protective activity against oAβ42-induced neurotoxicity *in vitro*. These data are in support of a therapeutic use of antibodies directed against protofibrillar amyloid b peptide in AD.

WTH09-32

Melatonin attenuates learning and memory impairment in mice after methamphetamine administration

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Methamphetamine (METH) is an psychostimulant drug which is highly abuse. It has been shown that METH is directly neurotoxic and can cause changes of brain structure and function in brain. The cognitive skills including memory and working memory impairment in correlate to METH use haves been reported. The previous studies have shown that melatonin (MEL), a neurohormone secretes by pineal gland, has beneficial roles in cognitive decline patients with mild cognitive impairment. Thus, in this study, the effect of MEL on METH-induced cognitive impairment were investigated. Adult male ICR mice were received METH (1 mg/kg body weight) or saline

subcutaneously (s.c.) once daily for 14 consecutive days. One day after last METH injection, METH-treated mice were further administered with saline or MEL (5 or 10 mg/kg body weight) s.c. once daily for 30 days. After that Morris water maze (MWM) test was used to evaluate the effect of MEL on METH-induced learning and memory impairment. Stereotypey behaviors were used to observed the behavior change between groups on the day before and after each drugs treatment. During the training trial, all mice gradually learned to locate the platform. However, mice treated with METH spend longer time to reach the platform when compare with the control and other groups. The escape latency in probe trial task increased in the METH-treated animals when compare with control saline-treated group. Mice treated with both at 5 mg/kg and 10 mg/kg MEL after induced by METH spent less time of the escape latency when compare with METH-treated animals, whereas MEL alone showed no effect in the time spend to reach the platform. Taken together, these results suggested that exposure to METH causes learning and memory impairment, which can be attenuated by treated with MEL. This work was supported by the Thailand Research Fund (TRF) under the TRF Research Career Development Grant (RSA5980041) to SM.

WTH09-33

Melatonin modulates permeability transition pore by inhibition of mitochondrial KATP channels in isolated brain mitochondria

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There is increasing recognition of the importance of mitochondria in neurodegenerative disorders. Mitochondria play a key role in apoptotic and necrotic cell death. Melatonin (Mel), an indoleamine produced in several organs including the pineal gland has been known for its neuroprotective actions. In our study, we have investigated whether the mitochondrial ATP sensitive potassium (mtK_{ATP}) channel blocker 5-hydroxydecanoate (5-HD) and calcium (Ca^{2+}) affects permeability transition pore (PTP) alterations in isolated brain mitochondria treated with melatonin (Mel) and cyclosporin A (CsA). Mitochondrial swelling, mitochondrial membrane potential ($\Delta\psi m$), ROS measurement and mitochondrial respiration were evaluated in isolated brain mitochondria. In our results, mitochondrial swelling stimulated by exposing Ca^{2+} ions and 5-HD associated by mPTP opening as depicted by modulation of CsA and Mel. In addition, Ca^{2+} and 5-HD decreased $\Delta\psi m$, depleted intracellular ROS, and inhibition of mitochondrial respiration (state 3 and state 4) in isolated brain mitochondria. Addition of Mel and CsA has shown significant restoration in mitochondrial swelling, $\Delta\psi m$, intracellular ROS measurement and mitochondrial respiration in isolated brain mitochondria. Therefore, we speculate the modulatory effect of Mel and CsA in mitochondria treated with 5-HD and Ca^{2+} hinders the mPTP-mediated mitochondrial dysfunction and cellular oxidative stress. We conclude that inhibition of mPT is one likely mechanism of CsA's and its neuroprotective actions. Development of neuroprotective agents including Mel targeting the mPTP therefore bears hope for future treatment of severe neurodegenerative diseases.

WTH09-34

Kynurenic acid prevents cytoskeletal disorganization induced by quinolinic acid in mixed cultures of rat striatum

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Kynurenic acid (KYNA) is a neuroactive metabolite of tryptophan known to modulate a number of mechanisms involved in neural dysfunction.Although its activity in the brain has been widely studied, the effect of KYNA counteracting the actions of quinolinic acid (QUIN) remains unknown. The present study aims at describing the ability of 100 μM KYNA preventing cytoskeletal disruption provoked by QUIN in astrocyte/neuron/microglia mixed culture.KYNA totally preserved cytoskeletal organization, cell morphology andredox imbalance in mixed cultures exposed to QUIN. However, KYNA only partially prevented morphologic alteration in isolated primary astrocytes, and failed to protect the morphology of neurons in which redox equilibrium was not disrupted by QUIN exposure.Moreover, KYNA prevented QUIN-induced microglial activation andupregulation of Iba-1, and partially preserved TNF-α level in mixed cultures. TNF-α level was also partially preserved in astrocytes.In addition to the mechanisms dependent on redox imbalance and microglial activation, KYNA prevented downregulation of connexin-43 and the loss of functionality of gap junctions (GJs), preserving cell-cell contact,cytoskeletal organization and cell morphologyin QUIN-treated cells.Furthermore, the toxicity of QUIN targeting the cytoskeleton of mixed cultureswas not mediated by *N*-methyl-D-aspartate (NMDA) signaling. We suggestthat KYNA protects the integrity of the cytoskeleton of mixed cultures modulating microglial activation, which in turn is upstream of oxidative imbalance andmisregulated GJs leading to disrupted cytoskeleton in QUIN-treated cells.This study contributes to elucidation of the molecular basis of KYNA protection against QUIN-induced cell damage directed to the cytoskeleton of neural cells.

WTH10 Cell Death

WTH10-01

Class IV semaphorin SEMA4A from CSF of multiple sclerosis patients induces oligodendrocyte cell death

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Multiple Sclerosis (MS) is a progressive demyelinating disease of the central nervous system (CNS) whose causes are not yet well understood. It is thought to be caused by demyelination followed by a lack of or suboptimal re-myelination by oligodendrocytes in the CNS, however the extrinsic and intrinsic factors relating to oligodendrocyte death in MS are also not well understood. Identifying how alterations in oligodendrocyte biology contribute to MS pathogenesis is an essential prerequisite for developing better intervention strategies in MS treatment.

We have previously shown a dose-dependent cytotoxic effect of the Class IV Semaphorin Sema4A on primary rodent oligodendrocytes. In rodents, Sema4A binds to Tim-2, a member of the T-cell immunoglobulin domain and mucin containing domain (Tim) family, a receptor that is expressed on activated T cells as well as oligodendrocytes. We recently discovered that Tim-2 is also the primary receptor for the iron delivery protein H-ferritin (Hft) and that oligodendrocytes are unique in the brain for taking up iron via this protein. However, the receptor for binding has yet to be identified in human oligodendrocytes as the gene for Tim-2 has not been detected in humans. We currently show that both recombinant Sema4A protein and Sema4A in the CSF of MS patients have a dose-dependent cytotoxic relationship with oligodendrocytes. Our data demonstrates that cell death following exposure to Sema4A is likely mediated via apoptosis. Our data also suggest that Sema4A levels in the CSF of MS patients could be used in combination with current standards of biomarkers for disease progression. Additionally, we have data that suggests addition of H-ferritin can prevent Sema4A cytotoxicity. Together, the data strongly suggest that identifying the receptor for both H-ferritin and Sema4A will provide novel targeting for potential therapeutic treatments of demyelinating disorders.

WTH10-02

Prognostic significance and survival statistics of astrocytoma patients' in a cohort with assessment of glioblastoma with oligodendroglial component

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Astrocytoma constitutes the most noted malignancies of central nervous system with worse clinical outcomes in Grade IV astrocytoma or glioblastoma multiformae. In present studies, we have seen anatomic distribution of astrocytoma subtypes in a cohort of 479 patients and correlated it with survival outcomes. Anatomic location was confirmed by MRI (magnetic resonance imaging) images. We have also looked into overall survival particulars in astrocytoma subtypes and further prognostically assessed significance of glioblastoma with oligodendroglial component (GBMO). Pattern of MIB1 and p53 expression was evaluated by immunohistochemistry studies in respective cases. Our findings highlights that in total cases, tumor location was dominated by frontal and temporal lobes. Survival analysis in high grade (Grade III, $p = 0.03$) (Grade IV, $p = 0.01$) astrocytic tumors confirms poor outcomes with temporal, parietal and occipital location as compared to frontal lobe. Overall survival studies demonstrates glioblastoma multiformae (GBM) was associated with worse prognosis as compared to astrocytoma subtypes ($p < 0.0001$). In high grade astrocytomas, anaplastic astrocytoma was found with 19 months of median survival age while 12 months in case of glioblastoma multiformae. These was statistically significant difference among survival of glioblastoma patients'. Glioblastoma multiformae patients with oligodendroglial component was found to have median survival of 16 months (Chi square= 11.09, $p = 0.0009$, 95% CI= 1.37 to 3.4). In conclusion, we state that in our cohort, parietal, temporal and occipital lobes were found with significantly poor prognosis in grade II, grade III and grade IV astrocytoma patients. Among astrocytoma subtypes, patients with glioblastoma multiformae were associated with worse survival outcomes. Glioblastoma multiformae patients with oligodendroglial components found to have significantly longer survival than glioblastoma patients' and respond well to chemo and radiotherapy.

WTH10-03

Autophagy fails to prevent energy stress-induced neuronal death due to calpain-mediated lysosomal dysfuction

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Autophagy is triggered during nutrient and energy deprivation in a variety of cells as a homeostatic response to metabolic stress. In the CNS deficient autophagy has been implicated in neurodegenerative diseases and ischemic brain injury. However, its role in hypoglycemic damage is poorly understood and the dynamics of autophagy during the hypoglycemic and the glucose reperfusion periods has not been fully described. In the present study we analyzed the changes in the content of the autophagy proteins BECN1, LC3-II and p62/SQSTM1 by Western blot and autophagosome formation was followed through time-lapse experiments, during glucose deprivation (GD) and glucose reintroduction (GR) in cortical cultures. According to the results, autophagosome formation rapidly increased during GD, and was followed by an active autophagic flux early after glucose replenishment. However, cells progressively died during GR and autophagy inhibition reduced neuronal death. Neurons undergoing apoptosis during GR did not form autophagosomes, while those surviving up to late GR showed a second weave of autophagosome formation. Calpain activity strongly increased after GR and remained elevated during progressive neuronal death. Its activation led to lysosome permeabilization

and the loss of lysosomal cathepsin B. Calpain inhibition increased cell viability and the number of neurons containing lysosomes, autophagosomes and cathepsin B immunoreactivity. Taken together, the present results suggest that calpain-mediated lysosome dysfunction during GR turns an adaptive autophagy response to energy stress into a defective autophagy pathway which contributes to neuronal apoptosis. In these conditions, autophagy inhibition results in the improvement of cell survival.

WTH10-04

Bax activation blocks self-renewal and induces apoptosis of human glioblastoma stem cells

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Glioblastoma (GBM) is characterized by a poor response to conventional chemotherapeutic agents, attributed to the insurgence of drug resistance mechanisms and to the presence of a subpopulation of glioma stem cells (GSCs)[1]. GBM cells and GSCs present, among others, an overexpression of anti-apoptotic proteins and an inhibition of pro-apoptotic ones, which help to escape apoptosis. Among pro-apoptotic inducers, the Bcl-2 family protein Bax has been recently emerged as a promising new target in cancer therapy along with first BAX activators (BAM7, Compound 106 and SMBA1)[2]. Herein, a derivative of BAM-7, named BTC-8[3], was employed to explore the effects of Bax activation in different human GBM cells and in their stem cell subpopulation. BTC-8 inhibited GBM cell proliferation, arrested cell-cycle arrest and induced apoptotis through the induction of mitochondrial membrane permeabilization. Most importantly, BTC-8 blocked proliferation and self-renewal of GSCs, and induced their apoptosis. Noteworthy, BTC-8 was demonstrated to sensitize both GBM cells and GSCs to the alkylating agent temozolomide. Overall, our findings shed light on the effects and on the relative molecular mechanisms related to Bax activation in GBM, and suggest Bax-targeting compounds as promising therapeutic tools against the GSC reservoir.

[1]*EMBO Mol. Med. 2014;6:1359-70.*
[2]*Nature 2008;455:1076-81.*
[3]*J. Med. Chem. 2015;58:2135-48.*

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WTH11 Autonomic-Autonomic/Neuroendocrine Systems

WTH11-01

Integrative microrna-mrna expression changes in vmh is associated with neuronal maladaptive response to hypoglycemia

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The brain is highly sensitive to proper fuel availability as evidenced by the neuronal maladaptive function during ischemic attacks and recurrent episodes of hypoglycemia (RH). Studies have demonstrated that RH induces the changes in gene (or mRNA) expression involved in neuronal glucose sensing, which limits the ability of brain to sense subsequent hypoglycemia (i.e. impaired glucose sensing), thereby compromises the neuroendocrine regulation. The current study tests the hypothesis that impaired neuronal glucose sensing in RH is mediated by coordinated changes in microRNAs (negatively regulate expression of genes at posttranscriptional level) and mRNAs expression in ventromedial hypothalamus (VMH), a well characterized glucose sensing brain region. In brief, adult male Sprague–Dawley rats were administered with either subcutaneous insulin (2-1.2 U/kg) or saline injections for 3 days to induce RH (30-50 mg/dl, $n = 10$) and control (recurrent saline, RS; $n = 10$) group respectively. On 4th day, both groups were subjected to either 1) hypoglycemic (40-50 mg/dl) clamps to assess neuroendocrine response to hypoglycemia, or 2) were euthanized to obtain RNA from the punch biopsy of VMH to determine genome-wide microRNAome and transcriptome profiling changes with a high-throughput RNA-sequencing and bioinformatics approach. Results indicate that RH leads to an impaired neuronal glucose sensing as demonstrated by blunted neuroendocrine (i.e. epinephrine) response to hypoglycemia. In RNA-sequencing analysis, a total of 205 microRNAs and 1013 mRNAs were differentially expressed between groups, in which, 82 microRNAs pair to 402 mRNAs at false discovery rate (FDR) < 0.05, analyzed by microRNA target filtering using Ingenuity Pathway Analysis software. A microRNA-mRNA integrative network analysis of RH induced genomic changes, identified miR-23a-3p and miR-7a-5p based on their large predicted network and association with mRNA target changes involved in neuronal processes, specifically *Clcn3* and *Mknk2* (gene products involved in GABAergic synaptic vesicles release and neurite outgrowth, respectively). These finding suggest that miR-23a-3p and miR-7a-5p might be potential mediators or regulators of the neuronal maladaptive response occurring in repetitive hypoglycemia.

WTH11-02

Gonadotropin-releasing hormone neurons are regulated by gut peptides at the level of the neuroterminals in the median eminence

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Gonadotropin-releasing hormone (GnRH) is produced in neurons in the preoptic area which project to the median eminence (ME), where the peptide is secreted into hypophysial portal blood. The neurosecretory terminals of GnRH neurons are outside the blood brain barrier in the external zone of the ME and hypothalamic factors such as kisspeptin control secretion at this level. Given that the GnRH neurosecretory terminals are freely accessible to blood-borne factors, gut hormones could also modulate GnRH secretion. We aimed to determine the effects of NPY_{13-36}, ghrelin, GLP-1 (and its agonist Exendin-4) on GnRH secretion in sheep. Ovariectomised ewes ($n = 6$/group), received guide tubes directed to 2 mm above the ME. Microinjections of each peptide (0.5-1 nmol) were made into the ME during continuous blood sampling to measure pulsatile luteinising hormone secretion. NPY_{13-36}, has similar properties to PYY and, when injected into the ME, where the neuroterminals of GnRH are found, it suppressed LH levels (reflecting an effect on GnRH secretion). Mean levels were reduced from 1.7 ± 0.23 to 1.1 ± 0.18 ng/mL ($p < 0.05$). An i.v. injection of NPY_{13-36} also reduced LH secretion. Immunostaining and *in situ* hybridisation showed that GnRH neurons do not express Y2 receptor, so the effect may be via the Y5 receptor. Microinjection of 0.5 nmol ghrelin into the ME also lowered LH levels from 3.3 ± 0.52 to 2.6 ± 0.49 ng/mL ($p < 0.05$). Ghrelin receptors were colocalised to GnRH neurosecretory terminals, by immunocytochemistry. To test effects of GLP-1 and Exendin-4, OVX ewes were treated with estrogen and progesterone to suppress GnRH/LH levels. Doses of 0.5 nmol of GLP-1 or Exendin-4 increased LH levels (from 0.5 ± 0.11 to 0.8 ± 0.17 ng/mL; $p < 0.05$ for GLP-1 and from 0.9 ± 0.22 to 2.3 ± 0.12 ng/mL; $p < 0.005$ for Exendin-4). Exendin-4 induced a more sustained elevation in LH secretion than GLP-1. We conclude that reproductive function is affected by gut peptides acting on GnRH neurosecretory terminals in the ME.

WTH11-03

A neuroanatomical evaluation of cholinergic, catecholaminergic, serotonergic and orexinergic neural systems in mammals pertaining

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One of the few remaining mysteries in mammalian phylogeny is the issue of chiropteran phylogeny. In order to further investigate the diphyletic hypothesis that states that megachiroptera evolved from primate-like gliders and that microchiroptera evolved from insectivores, the cholinergic, catecholaminergic, serotonergic and orexinergic systems were analyzed in five insectivores and three prosimian primates.

Brains were coronally sectioned and stained according to standard immunohistochemical methods and the presence or absence of 93 nuclei within the neuromodulatory systems was entered into modern cladistics software.

The three shrews lacked the cholinergic parabigeminal and Edinger-Westphal nuclei, had a mediodorsal arch of the cholinergic laterodorsal tegmental nucleus, lacked the catecholaminergic A4 and A15d nuclei and presented with an incipient ventral division of the substantia nigra which is identical to previously studied microchiroptera. All three prosimians presented with a central compact division of catecholaminergic locus coeruleus (A6c) surrounded by a shell of less densely packed (A6d) tyrosine hydroxylase immunopositive neurons. This combination of compact and diffuse divisions of the locus coeruleus complex is only found in primates and megachiropterans of all the mammalian species studied to date.

Our neuroanatomical analysis suggests a phylogenetic relationship between the Soricidae (shrews) and the microchiropterans, supports the phylogenetic grouping of primates with megachiropterans, and suggests that primates are phylogenetically closer to megachiroptera than to any members of the Euarchontoglires. The cladistic analysis confirmed the neuroanatomical analysis.

WTH11-04

Mode of action of RB150, an aminopeptidase a inhibitor, as a central-acting antihypertensive agent in DOCA-salt hypertensive rats

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In the brain, angiotensin III (AngIII) generated from angiotensin II (AngII) by aminopeptidase A (APA) exerts a tonic stimulatory action on the control of blood pressure (BP) in hypertensive rats. RB150, a prodrug of the specific and selective APA inhibitor is the prototype of a new class of centrally-acting antihypertensive agents responsible for the inhibition of brain APA activity leading to a decrease in BP in hypertensive rats. Since following brain AngIII formation blockade by RB150, no AngII accumulation was observed, we aimed to delineate if RB150 induces the activation of another metabolic pathway of brain AngII, in particular angiotensin converting enzyme type 2 (ACE2) which converts AngII into angiotensin 1-7 (Ang1-7). For this purpose, we used a model of salt-dependent hypertension, the DOCA-salt rat. RB150 and MLN4760, an ACE2 inhibitor were administered by intracerebroventricular (ICV) route and mean arterial BP (MABP) was recorded in alert hypertensive rats. Maximal MABP decrease and area under the curve (AUC) of the variations in MABP were calculated. ICV administration of RB150 (100 μg) significantly decreased MABP compared to vehicle (-30.41 ± 5.68 mmHg vs. -8.05 ± 6.14 mmHg, $p < 0.01$; AUC: 43.35 ± 10.25 vs. 3.87 ± 9.82 mmHg.min, $p < 0.01$). Administration of MLN4760 (10 μg) did not induce any significant change in MABP compared to vehicle (-14,19 ± 5.42 mmHg, $p > 0.05$; AUC: 9.39 ± 5.97 mmHg.min, $p > 0.05$). The combination of MLN4760 with RB150 co-administered by ICV route partially inhibited the RB150-induced antihypertensive effect (-16.26 ± 5.66 mmHg, $p < 0,01$; AUC: 23.84 ± 14.81 mmHg.min $p < 0,05$, compared to RB150 alone). These results suggest that inhibition of brain APA by RB150 triggers ACE2 metabolic pathway, resulting in the formation of Ang 1-7, which, by its action on the Mas receptor, could participate to the blood pressure decrease in the hypertensive rat.

WTH13 Sensory Systems

WTH13-01

Function of presynaptic TRPV1 receptors in the spinal cord dorsal horn is modulated by chemotherapeutic drug paclitaxel

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Neuropathic pain is major treatment-limiting factor accompanying cancer treatment with paclitaxel (PAC). We have reported previously (Li *et al.*, J. Neurosci., 35:13487–13500, 2015) that acute application of PAC (50 nM) modulated miniature excitatory postsynaptic currents (mEPSC) frequency and diminished tachyphylaxis of TRPV1 (transient receptor potential vanilloid 1) receptors mediated response after repeated capsaicin application via TLR4 receptors activation. Here we studied the signaling pathways involved in PAC-induced modulation. Whole-cell patch clamp recordings of mEPSC from spinal cord neurons in lamina I/II from adult male mice were used. Von Frey filament measurements were used to evaluate the presence of mechanical allodynia. PAC-neuropathy was induced by single dose application of PAC (8 mg/kg, *i.p.*). In naïve animals mEPSCs frequency evoked by second capsaicin application was reduced to 33% of the first one. After acute PAC-treatment the second response was 91% of the first one. Our data show that the second capsaicin response tachyphylaxis was diminished also 1 day (72%) and 8 days (83%) after single systemic *in-vivo* PAC-treatment. Effect of PAC treatment on tachyphylaxis was significantly reduced by PI3-Kinase antagonist wortmannin or LY294002 and by wide-spectrum kinases inhibitor staurosporine. These results suggest that PI3-Kinase and other kinases may play important role in the signaling between TLR4 and TRPV1 receptors in the spinal cord dorsal horn and may be involved in the development of painful states after PAC treatment. Targeting these molecules may represent a possible option for analgesic treatment in states of paclitaxel induced neuropathic pain. Our work was supported by grant support: GAUK 138215, LQ1604 BIOCEV-FAR, GACR 15-11138S, LH15279, GACR P304/12/G069, CZ.1.05/1.1.00/02.0109, RVO67985823.

WTH13-02

Peptidergic modulation of pain and anxiety: forebrain relaxin-3/RXFP3 networks and descending control of nociception in mice

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Persistent pain can hinder normal function and behaviour, with a negative impact on quality-of-life. In persistent pain conditions, patients develop conditions such as anxiety, which worsens pain sensation, creating a feedback loop between pain and this comorbid state. Anxiety is linked to altered function in brain areas innervated by relaxin-3 neurons. Indeed, activation of its receptor, Relaxin/Insulin Family Peptide Receptor 3 (RXFP3), can alter arousal, stress- and anxiety-related and reward-seeking behaviours in rodents. These data suggest a possible link between RXFP3 activity and control of pain sensitivity. Thus, these studies assessed the effect of RXFP3 activation/inhibition on the control of mechanical and thermal pain sensitivity in normal and persistent pain conditions in mice. Intracerebroventricular (icv) administration of RXFP3 agonist peptide reduced mechanical, but not thermal, pain sensation in C57BL/6J mouse model of inflammatory pain ($n = 5$ mice/group, $p < 0.01$). These effects were associated with decreased activity of nociceptive neurons in spinal cord ($n = 6$ mice, $p < 0.05$). In addition, RXFP3 antagonist augmented mechanical and thermal pain sensitivity ($n = 7$ mice/group, $p > 0.05$). These data suggest that relaxin-3 provides a tonic drive to maintain mechanical and thermal pain thresholds. In parallel, we sought to identify the neuronal circuits responsible for the observed effects. Using neural tract-tracing, we identified brain areas that receive relaxin-3 inputs, which in turn innervate the rostroventral medulla (RVM), a region that gates descending pain control. These regions include anterior cingulate cortex, central amygdala, bed nucleus of the stria terminalis and hypothalamus, which are functionally related to pain sensation and comorbidities. Together, these data suggest RXFP3 as a therapeutic target for pain management, and further studies of the specific circuits and mechanisms involved are warranted.

WTH13-03

Bifurcate spinal dorsal neurons projecting simultaneously to supraspinal centers by the dorsal column and the anterolateral system

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Sensory information arriving at the spinal dorsal horn (SDH) neurons can be transmitted to the supraspinal centers by two main systems: the dorsal column-medial lemniscal (DC-ML) or by the antero-lateral system (AL). These systems are well recognized and studied and the main conclusion is their independence or lack of interactions. To our knowledge no study has proposed their interactions as well as their bilateral projections. In order to test this hypothesis, we perform some neurochemical (retrograde neuronal tracing) and electrophysiological experiments in rats. We study principal SDH neurons at the L2-L5 segments having the characteristic of sending projections to the *Gracilis* nuclei (GRA) from the DC-ML or to the ventral postero lateral thalamic (VPL) nuclei from the AL. True Blue (TB) tracer was placed into the left GRA and Fluoro Gold (FG) or Fluor Rubi (FR) tracer was injected in the right VPL. After 13 days, the animals were perfused to localized the stained cells in spinal cord (SC). In order to test the functionality of the neurons founded we used concentric bipolar

electrodes to apply electric stimulation at the left GRA and at the right VPL, and recordings (using glass micropipettes) of the single unit SC cells (left side at L2-L5 SC level). The neurons were classified as SDH wide dynamic range (WDR) cells. A collision test was performed as follows: spontaneous or evoked spikes by receptive field SC stimulation triggered the GRA or VPL stimulation, evoking an antidromic spike. Our data showed that some spinal dorsal horn neurons presented a double staining with TB and FG. These double stained neurons were found in the left and right side of the dorsal horn of SC. The main projection was to the left side (3 to 1). Regarding the electrophysiological experiments, we found neurons showing C-fibers activation which also presenting collision activities for either GRA or VPL antidromic electric activation. Our data show bilateral SC cells projecting to the GRA and VPL. It is possible that this neuronal arrangement serves to assure the alarm system of nociception and generate a diffuse descending response.

WTH13-04

Non-inflammatory autoantibody-induced glia activation in a passive transfer-trauma mouse model of complex regional pain syndrome

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Complex Regional Pain Syndrome (CRPS) is a severe chronic pain condition after small injuries. Abnormal immune response against sensory nerve-derived antigens and complex neuro-immune interactions are suggested to be responsible for the symptoms, but the mechanisms are unclear and the therapy is not satisfactory. In our passive-transfer translational mouse model, we investigated the behavioural, immunological and neurochemical changes characteristic of the disease.

Plantar incision was performed in C57Bl/6 mice daily treated i.p. with purified serum-IgG of CRPS patients or healthy volunteers for 3-13 days. Paw mechanonociceptive threshold was measured by aesthesiometry, volume by plethysmometry, neutrophil/macrophage myeloperoxidase activity by luminescence *in vivo* imaging, sensory neuropeptides and cytokines by immunassays, glia markers in pain-related brain regions with immunochemistry.

CRPS IgG significantly increased and prolonged hyperalgesia and swelling of the incised paw compared to healthy IgG particularly in the second week, when swelling resolved. Myeloperoxidase activity and substance P-like immunoreactivity moderately, but significantly increased in CRPS IgG-treated mice transiently on days 2 and 7, but calcitonin gene-related peptide and inflammatory cytokines were not altered. CRPS IgG significantly increased the density of astrocyte-related glial fibrillary acidic protein (GFAP) and microglia-staining Iba1 in L4-L5 spinal dorsal horn, periaquaductal gray and somatosensory cortex. GFAP increased in the first week on both sides, Iba1 elevated only ipsilaterally mainly later.

The main symptoms of CRPS with special emphasis on hyperalgesia can be reliably transferred from patients to mice by IgG. Peripheral inflammation is not likely to play a crucial role in this pathological pain, but glia-mediated central sensitization is more likely to be involved.

WTH13-05

Microrna deregulation in the spared nerve injury mouse model for neuropathic pain

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Micro-RNAs (miRNAs) are small non-coding RNAs that regulate gene expression by interacting with the 3′-untranslated region of mRNAs. During the past few years, miRNAs emerged as potential biomarkers and an increasing number of miRNAs are found deregulated and associated with signatures of neuropathic pain, induced by experimental nerve injury. In relevant models, the neurons in the dorsal root ganglia (DRG) exhibit increased neuronal excitability, which results in augmented nociceptive signaling from the periphery to the central nervous system. Therefore, we set out to explore signatures of nociceptor hyperexcitability and correlate them with miRNA expression patterns.

Neuropathic pain was induced in mice by using the spared nerve injury model (SNI). Seven days post lesion, a combination of techniques (miRNA and RNA sequencing, *in vitro* electrophysiology, qRT-PCR, *in situ* hybridization) was employed for the identification of pain-related miRNAs, their *in silico* predicted mRNA targets and related functional alterations. Several up-regulated miRNAs, and down-regulated mRNAs, were found to be associated with the pain-phenotype displayed by SNI-treated mice. Targeting these miRNAs could potentially provide novel therapeutic approaches in the management of neuropathic pain.

WTH13-06

Oxytocin induce thermal analgesia via vasopressin-1A receptor by modulating TRPV1 and K-conductance

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Recent studies have provided several lines of evidence that peripheral administrations of oxytocin (OT) induces analgesia in human and rodents. However, the exact underlying mechanism of the analgesia still remains unclear. In the present study, we aimed to identify the receptor which mediates analgesic property of OT and its cellular mechanisms in thermal pain behavior. When we examined whether intraperitoneal (IP) injection of specific antagonist for oxytocin and vasopressin (AVP) receptors reverse the oxytocin-induced analgesia in Hargreaves' thermal pain behavioral tests in rats. We found that oxytocin-induced analgesia was reversed by AVP1a-RA, the antagonist for AVP1a receptor, but not by OTRA, oxytocin receptor antagonist. Single cell RT-PCR analysis revealed that while AVP1a receptor, compared to OT, AVP1b, and AVP2 receptors, was more profoundly expressed in DRG neurons,

expression of AVP1a receptor was predominant in TRPV1-expressing DRG neuons. The Fura-2 based Calcium imaging experiments showed that capsaicin-induced calcium transient was significantly inhibited by oxytocin, which was again reversed by AVP1a-RA, AVP1a receptor antagonist. These results suggest that AVP1a receptor in the DRG cells mediate oxytocin-induced thermal analgesia. Additionaly, the whole cell patch clamp recording demonstrated that oxytocin increasespotassium channel conductance via AVP1a receptor in the DRG cells.

Taken together, our findings suggests that the analgesic effects produced by peripheral administration of oxytocin are attributable to the activation of AVP1a receptor amongst oxytocin receptors, which results in the regulation of TRPV1 channels and increase of potassium conductance in DRG neurons.

WTH13-07

Mechanisms of pain hypersensitivity in a pharmacological mouse model of attention-deficit/hyperactivity disorder

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Aim: The 6-hydroxy-dopamine (6-OHDA) neonatal lesion induces brain deficits influencing cognitive and motor behaviors. It is accepted as a pharmacological model of ADHD with good face and construct validity. Recent clinical evidence pointed to pain hypersensitivity of ADHD patients. Here, we investigated the effects of neonatal dopamine depletion (P5) in mouse on pain thresholds at adulthood (P40).

Methods: We analyzed nociceptive behavioural responses to thermal and mechanical stimuli in 6-OHDA lesioned adult mice. Neuronal activity to mechanical stimulus was further examined *in vivo* by unit recording of spinal nociceptive neurons.

Results: 1). Neonatal dopamine (DA) depletion resulted in behavioral characteristics similar to those seen in patients with ADHD. At P24, ADHD-like mice exhibit hyperactivity. At P40, ADHD-like mice show anxiety, antisocial behavior, increased aggressiveness, mildly impaired latent inhibition and short-term memory. We also demonstrated attention deficit and increased impulsivity in ADHD-like mice. 2). Mice with neonatal dopamine depletion exhibited a marked increase in both thermal (heat and cold) and mechanical sensitivity. Dopamine depletion also increased pain sensitivity in persistent pain conditions, i.e. at 4 days after Complete Freund's Adjuvant injection in the hindpaw. Interestingly, ADHD symptoms were not modified in inflammatory conditions suggesting that ADHD influences pain sensitivity while the reverse was not true. 3). Electrophysiological recordings showed increased activity of spinal neurons in response to both innocuous and noxious stimuli only in the ADHD-like group. Moreover, our data indicated that ACC neurons are hyper-activated in ADHD-like mice. Finally, we found that the electrical stimulation of controlateral ACC (100 Hz; 10, 20, and 30 V; 10 s) increases the responses (amplitude and mean spike frequency) of WDR neurons to innocuous and noxious stimuli.

Conclusion: Our results demonstrated the validity of the neonatal 6-OHDA model to mimic ADHD syndrome. Taken together, our data demonstrate that ADHD conditions induce WDR spinal cord neurons hyperactivation and pain hypersensitivity. We also suggest that the deregulation of ACC may be the trigger for spinal neuron dysfunction

WTH13-08

Immunohistochemical and physiological analyses of histamine receptors on ganglion cells in the developing gerbil retina

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Histamine is an important neurotransmitter in the central nervous system. There are efferent nerve terminals from the brain to the retina, and histamine is reported to be released to the retina. Also, mammalian retinal neurons have been reported to express several types of histamine receptors. In order to confirm the presence of histaminergic pathway in the developing and adult retina, calcium-imaging and immunohistochemical analyses were used in the gerbil (*Meriones unguiculatus*). First, the activity of histamine receptors was examined by fura-2 based calcium-imaging technique. A bath application of 100 μM histamine or histamine agonists increased the intracellular calcium concentration in some retina ganglion cells. Next, we examined the localizations of H1, H2 and H3 receptors in the gerbil retinae from 1 to 350 postnatal days. We found that H1, H2 and H3 receptors expressed respectively on retinal ganglion cells. H1 receptor expresses through the retinal maturation. On the other hand, the expressions of H2 and H3 receptors became maximum from 14 to 21 postnatal days. Histidine decarboxylase, which produces histamine from histidine, also expressed in retinal ganglion cells, and moreover, each of histamine receptors and histidine decarboxylase were co-localized at the same retinal ganglion cells. Since the gerbil opens the eyes at 3 weeks old, it is considered that the H2 and H3 receptors play some specific roles at the formation of the early visual system. Colocalization of histidine decarboxylase and histamine receptors in retinal ganglion cells suggest that retinal ganglion cells may interact with each other via histamine. Therefore, histamine may be one of the important neurotransmitters and/or neuromodulators in the visual information processings of the mammalian retina.

WTH13-09

The role of PAR2 receptors in modulation of nociceptive transmission at spinal cord level under inflammatory conditions

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Modulation of synaptic transmission in the spinal cord dorsal horn plays a key role in the development of pathological pain states. Protease-activated receptors (PARs) are family of four G-protein-coupled receptors (PAR1-4) activated by proteases that cleave the N-terminal domain of the receptor unmasking a tethered peptide ligand sequence. The role of PAR2 in pain perception is closely related to their presence in a subpopulation of dorsal root ganglion

(DRG) neurons, where they are often co-expressed with TRPV1 receptors. Our previous work documented an important role of PAR2 receptors on the presynaptic endings of primary afferents in the spinal cord (Mrozkova et al.,PLoS ONE, 2016, 11, 10, e0163991). The present work aimed to study the role of PAR2 and the possible interaction with TRPV1 receptors at the spinal cord level under inflammatory conditions.

Whole-cell patch clamp recordings of miniature excitatory postsynaptic currents (mEPSCs), spontaneous (sEPSCs) and dorsal root stimulation evoked (eEPSCs) were made from superficial dorsal horn neurons in lumbar spinal cord slices of young (21 days) Wistar rats. Peripheral inflammation was induced by application of 3% carrageenan into the paw 24 h before the experiment. Application SLIGKV-NH2 (PAR2 AP, 100 μM) was used to activate PAR2 receptors.

Application of PAR2 agonist increased frequency of mEPSCs (136.8 ± 10.0%; $p < 0.01$), sEPSCs (127.2 ± 9.5%; $p < 0.05$) and also amplitude of the evoked EPSCs (154.4 ± 12.6%; $p < 0.01$). Administration of TRPV1 antagonist (SB366789) together with the PAR2-AP prevented the frequency (mEPSC, sEPSC) and amplitude (eEPSC) increases.

Our results suggest that presynaptic PAR2 receptors and their interaction with TRPV1 receptors may play an important role in modulation of nociceptive synaptic transmission in the spinal cord dorsal horn, particularly under the conditions of peripheral inflammation.

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WTH14 Limbic and other Systems

WTH14-01

Signaling by striatal cholinergic interneurons in mice is required for cue detection and behavioural flexibility

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While the role striatal dopamine plays in controlling goal-directed behaviour has been extensively studied, less is known about the importance of striatal cholinergic interneurons (CINs). CINs control striatal circuits by releasing two different neurotransmitters: acetylcholine (ACh) and glutamate (Glu). Initial findings suggest that both ACh and Glu released by CINs can increase and decrease the release of striatal dopamine, respectively, which could contribute to the control of goal-directed behaviour by CINs. To test this possibility, we generated three genetically modified mouse lines with a specific deletion of the vesicular acetylcholine transporter (VAChT) and/or vesicular glutamate transporter 3 (VGLUT3), necessary for the release of ACh and Glu from CINs. In VAChT-deficient mice, we found deficits in two different behavioural paradigms in which habit and goal like-behaviour were examined. Moreover, we reproduced these deficits by AAV virus-mediated deletion of VAChT limited to the dorsal striatum. Using fast cycling voltammetry we found a significant decrease of dopamine release in the dorsomedial but not in the dorsolateral striatum of VAChT-deficient mice. In contrast to these findings, the VGLUT3-deficient mice did not show a deficit in cognitive flexibility and they even performed slightly better than wildtype mice in some of the task parameters. We also found that the mice with a deletion of both VAChT and VGLUT3 from CINs performed worse in motivational tasks. However, the deficit did not seem to be caused by decreased motivation to work for a reward, but rather by an impaired ability to find salience in the reward-related stimulus. We conclude that modulation of striatal circuits by CINs is necessary for intact goal-directed behavior and that ACh released by CINs is specifically important for cognitive flexibility.

WTH14-02

Chronic relaxin-3 receptor activation in ventral hippocampus and medial amygdala reciprocally modulates anxiety-like behaviour

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The neuropeptide, relaxin-3, preferentially activates the $G_{i/o}$-protein-coupled receptor, RXFP3. Relaxin-3/GABA neurons constitute a conserved ascending neural network in mammalian brain, enriched in limbic areas involved in stress, arousal and emotion-related behaviours, such as amygdala, ventral hippocampus (vHip), medial and lateral septum, and the prefrontal cortex. We have previously observed differential effects of *acute* RXFP3 activation on conditioned fear in rats, which were associated with the site of administration (icv vs. local) and presumed differential sites and extent of action. In this study, we characterized the effects of *chronic* RXFP3 activation in two key limbic regions on 'affective' behaviours, including anxiety and social avoidance. Adeno-associated viral vectors driving local secretion of the RXFP3 agonist, R3/I5, were bilaterally injected into the vHip *or* medial amygdala (MeA) of adult male, Sprague–Dawley rats (7-10/group). Chronic vHip RXFP3 activation *decreased* time and distance travelled in the open arms of the elevated plus maze (EPM) *and* the aversive light zone of the light-dark box (LDB); and decreased social interaction with a conspecific stranger, compared to control (all $p < 0.05$). This was associated with a significant *decrease* in somatostatin (SOM) immunoreactivity in neurons in the 'virally-transduced' region. Conversely, chronic RXFP3 activation in the MeA *increased* time and distance travelled in EPM open arms *and* centre of a large open field (LOF), but did not alter behavior in the LDB or social assays. We are currently assessing effects of chronic RXFP3 activation on neuronal SOM levels in MeA; and the neurochemical identity of RXFP3-expressing neurons in the vHip and MeA. Our data suggest 'topographic' and 'disruptive' effects of persistent RXFP3 signalling on anxiety-related behaviour, related to precise site(s) and timing of the exogenous relaxin-3 actions, which likely contrast to those of endogenous peptide inputs, and receptor activation patterns. These studies provide a better understanding of the neurochemical basis of anxiety-related behaviour, with the potential for identifying novel therapeutic targets for anxiety disorders.

WTH14-03

Nucleus accumbens D2-dependent increase in motivation: impact of selective manipulation of local microcircuits

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The nucleus accumbens (NAc) is a key brain region of the reward circuit, playing an important role in reward processing, reinforcement and motivation toward such reward. Because of its biological function, NAc dysfunction has been implicated in various neurological and neuropsychiatric disorders, that include depression, obsessive-compulsive disorder, anxiety, and in addiction. Around 95% of the NAc neurons are medium spiny neurons (MSNs), mainly divided into those expressing dopamine receptor D1 (D1R, D1-MSNs) and those expressing dopamine receptor D2 (D2R, D2-MSNs). The remaining 5% are different subtypes of interneurons. Previous work from our group identified a relevant involvement of D2-MSNs in motivation and positive reinforcement; however, the intrinsic accumbal microcircuit wiring behind this positive effect is still unclear. In order to understand what type of neurons were involved in this behavioural effect, we combined optogenetic activation of NAc D2-MSNs with *in loco* pharmacological delivery

of specific neurotransmitter antagonists to show that accumbal D2-MSN-mediated enhancement in motivation requires dopamine release from VTA terminals in a cholinergic-mediated manner, that acts in both D1R and D2R.

In conclusion, this work highlights the fact that activation of D2-MSNs during cue exposure increases motivation, and that a concerted action of different neurotransmitter systems is required for this behavioural effect.

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Keyword Index

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14th Annual Meeting of the Australasian Neuroscience Society

MARCH 24–28, 2018 | RIVERSIDE, CA, USA
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11th FENS Forum of Neuroscience

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48th Annual Meeting of the Society for Neuroscience

AUGUST 4–8, 2019 | MONTREAL, CANADA
ISN-ASN MEETING

OCTOBER 19–23, 2019 | CHICAGO, ILLINOIS, USA
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